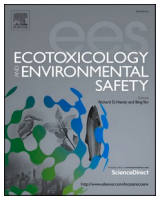
Ecotoxicology and Environmental Safety 266 (2023) 115551

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Insights into the reduction of antibiotic-resistant bacteria and mobile antibiotic resistance genes by black soldier fly larvae in chicken manure

Zhengzheng Zhao [a,b], Chongrui Yang [a,b], Bingqi Gao [a,b], Yushi Wu [a,b], Yue Ao [c], Shiteng Ma [a,b], Núria Jiménez [d], Longyu Zheng [a,b], Feng Huang [a,b], Jeffery K. Tomberlin [e], Zhuqing Ren [b,f], Ziniu Yu [a,b], Chan Yu [c], Jibin Zhang [a,b,**], Minmin Cai [a,b,*]

[a] State Key Laboratory of Agricultural Microbiology, National Engineering Research Center of Microbial Pesticides, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China
[b] Hubei Hongshan Laboratory, Wuhan 430070, Hubei, PR China
[c] State Key Laboratory of Biocatalysis and Enzyme Engineering, School of Life Sciences, Hubei University, Wuhan 430062, PR China
[d] Department of Chemical Engineering, Vilanova i la Geltrú School of Engineering (EPSEVG), Universitat Politècnica de Catalunya·BarcelonaTech, Vilanova i la Geltrú 08800, Spain
[e] Department of Entomology, Texas A&M University, TX, 77843, USA
[f] Key Laboratory of Agriculture Animal Genetics, Breeding and Reproduction of the Ministry of Education, College of Animal Science, Huazhong Agricultural University, Wuhan, Hubei, 430070, PR China

ARTICLE INFO

Editor: Bing Yan

Keywords:
Insect larvae
Antibiotic-resistant bacteria
Organic manure
Antimicrobial
Nutrition competition

ABSTRACT

The increasing prevalence of antibiotic-resistant bacteria (ARB) from animal manure has raised concerns about the potential threats to public health. The bioconversion of animal manure with insect larvae, such as the black soldier fly larvae (BSFL, *Hermetia illucens* [L.]), is a promising technology for quickly attenuating ARB while also recycling waste. In this study, we investigated BSFL conversion systems for chicken manure. Using metagenomic analysis, we tracked ARB and evaluated the resistome dissemination risk by investigating the co-occurrence of antibiotic resistance genes (ARGs), mobile genetic elements (MGEs), and bacterial taxa in a genetic context. Our results indicated that BSFL treatment effectively mitigated the relative abundance of ARB, ARGs, and MGEs by 34.9%, 53.3%, and 37.9%, respectively, within 28 days. Notably, the transferable ARGs decreased by 30.9%, indicating that BSFL treatment could mitigate the likelihood of ARG horizontal transfer and thus reduce the risk of ARB occurrence. In addition, the significantly positive correlation links between antimicrobial concentration and relative abundance of ARB reduced by 44.4%. Moreover, using variance partition analysis (VPA), we identified other bacteria as the most important factor influencing ARB, explaining 20.6% of the ARB patterns. Further analysis suggested that antagonism of other bacteria on ARB increased by 1.4 times, while nutrient competition on both total nitrogen and crude fat increased by 2.8 times. Overall, these findings provide insight into the mechanistic understanding of ARB reduction during BSFL treatment of chicken manure and provide a strategy for rapidly mitigating ARB in animal manure.

1. Introduction

Antimicrobial are commonly used as feed additives for livestock as growth promoters and to prevent infections (Zhang et al., 2015). However, approximately 30–90% of parent antimicrobial are excreted in manure (Sarmah et al., 2006), resulting in high levels of antibiotic residues in the environment (Burch et al., 2017; Shen et al., 2023). This can lead to the development of antibiotic-resistant bacteria (ARB) and an increased frequency of antibiotic resistance genes (ARGs) (Wichmann et al., 2014; Yan et al., 2023), which are recognized environmental

Abbreviations: BSFL, Black solider fly larvae; ARGs, Antibiotic resistance genes; MGEs, Mobile genetic elements; ARB, Antibiotic-resistant bacteria; HGT, horizontal gene transfer; HPB, Human pathogen bacteria; PCR, Polymerase chain reaction; RDA, Redundancy analysis; VPA, variation partitioning analysis.

* Corresponding author at: State Key Laboratory of Agricultural Microbiology, National Engineering Research Center of Microbial Pesticides, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China.
** Co-corresponding author.
E-mail addresses: zhangjb@mail.hzau.edu.cn (J. Zhang), cmm114@mail.hzau.edu.cn (M. Cai).

https://doi.org/10.1016/j.ecoenv.2023.115551
Received 11 July 2023; Received in revised form 20 September 2023; Accepted 4 October 2023
Available online 11 October 2023

pollutants (Pruden et al., 2006). Bacteria can acquire ARGs by mobile genetic elements (MGEs) via horizontal gene transfer (HGT) and become resistant to clinically used antimicrobial (Arias and Murray, 2009; Zhou et al., 2023).

Livestock manure is considered an important reservoir of ARB, ARGs, and MGEs (Guo et al., 2021). In China alone, 4 billion tons of livestock manure are produced every year (Kaufmann, 2015), and manure is often applied as fertilizer on farmland, leading to the dissemination of ARB and ARGs in soil and ultimately posing a potential risk to human health if these ARB and ARGs enter the food chain (Chen et al., 2020; Chen et al., 2023). It is also imperative to addressing antibiotic resistance (AR) from a sustainable agricultural development and One Health perspective (Durso and Cook, 2019). Antimicrobial resistance is a significant global health threat that has the potential to cause severe infections and mortality. According to Murray and Antibiotic Resistance Collaborators (Collaborators, 2022), bacterial antimicrobial resistance resulted in 1.27 million direct deaths in 2019, with *Escherichia coli* and *Pseudomonas aeruginosa* among the primary antibiotic-resistant pathogenic bacteria (ARPB). Consequently, safe and effective disposal methods that eliminate ARB and ARGs during the livestock manure recycling process are urgently needed. In recent years, several techniques have been proposed to remediate ARB and ARGs in manure, such as continuous thermophilic composting, thermophilic anaerobic digestion, disinfection, transformation into biochar or co-digestion with lignite (Chen et al., 2022; Guo et al., 2020; Mch et al., 2020; Pang et al., 2022; Qian et al., 2016). Nevertheless, these strategies are not widely applied due to their poor efficiency, complex operation, and high cost.

The use of black soldier fly (BSF, *Hermetia illucens* [L.]) larvae to bioconvert manure is an effective method for manure treatment, as these insects can overcome the aforementioned drawbacks. Furthermore, BSFL-treated compost is an excellent biofertilizer with high contents of organic matter, total phosphorus, and potassium (Guo et al., 2021). Moreover, BSFL can accelerate the bio-stabilization of heavy metals (Wu et al., 2020) and the biodegradation of antimicrobial (Cai et al., 2018b; Zhao et al., 2023) during the conversion of manure. In addition, a recent study suggests that BSFL has potential to lower the environmental impact of food (Grossule et al., 2023). BSFL conversion also favors the reduction of human pathogenic bacteria (HPBs) (Wu et al., 2021), with reported reductions on the relative abundance of HPB derived from pig manure by 89% (Wang et al., 2017), effectively mitigating the risks of these bacteria carrying ARGs (Cai et al., 2018a). The selective pressures from the BSFL gut and gut indigenous microbes could inhibit the invasion of exogenous microbiota (Zhang et al., 2022). In the process of bio-conversion, BSFL gut microbes could help reduce ARB and the occurrence risk of multidrug resistant-pathogens (Zhao et al., 2023). The immune system of BSFL is well developed and total of 50 antimicrobial peptide genes have been observed (Zhan et al., 2020). The antimicrobial peptides released from the larval gut could reduce the pathogenic microbes thus inhibits the potential antibiotic resistant- pathogenic bacteria (Xia et al., 2021).

Previous studies have provided more knowledge about the fate of partial ARGs in BSFL manure conversion systems by employing real-time and high-throughput quantitative PCR (Cai et al., 2018b). Nevertheless, the occurrence of ARB and resistome dissemination risk during BSFL conversion of chicken manure remains unclear because the limits of conventional culture methods of bacteria and many ARGs have never been detected during BSFL conversion.

In this study, we investigated BSFL conversion systems for chicken manure. Using metagenomic analysis, we tracked the hosts of ARGs and evaluated the resistome dissemination risk by investigating the co-occurrence of ARGs, MGEs, and bacterial taxa in a genetic context (Fresia et al., 2019; Liang et al., 2020; Zhang et al., 2020a; Zhang et al., 2020c). The objectives of this study included (i) evaluating the effects of BSFL treatment on the fate of ARB, ARGs, and MGEs; (ii) exploring the dynamics of nutrients and antimicrobial residues in chicken manure during BSFL treatment; (iii) identifying multiple factors accounting for ARB; and (iv) proposing the underlying mechanisms by which BSFL mitigates the risks of ARB in chicken manure.

2. Materials and methods

2.1. Source of insect larvae and chicken manure

BSFL were obtained from a colony maintained at Huazhong Agricultural University, Wuhan, China. This colony was established in November 2008 from eggs gathered at a poultry facility in Wuhan (Zheng et al., 2012). Six-day-old larvae were collected from the colony and used in the experiments. The manure was collected from a chicken facility operated by Chaotuo Ecological Agriculture Co. Ltd. in Wuhan, China.

2.2. Experimental overview and sampling

For this study, a BSFL chicken manure conversion system and control system (without BSFL) were established (Fig. S1). Each replicate included approximately 10,000 six-day-old BSFL inoculated into 10 kg of fresh chicken manure (moisture content: 65%, used directly within 12 h after collection from the chicken) held in a plastic container (60 cm length ×20 cm width × 10 cm height). Meanwhile, the initial chicken manure sample was named the Ch0 sample. A manure control group consisting of fresh chicken manure without BSFL was also produced. All experiments were carried out in triplicate in a greenhouse at 28 °C with 70% relative humidity. According to previous study (Li et al., 2021; Rehman et al., 2019), the BSFL conversion was terminated when the first prepupae emerged, which lasted for 12 days in this study. The samples were named ChL12 in the conversion group and ChC12 in the control group. The conversion residues were allowed to composting for 16 days after BSFL removal, and the samples were named ChL28 in the conversion group and ChC28 in the control group. The manure samples (50 g) were randomly collected via sterile methods at 0 (larval introduction), 12 (larval harvest), and 28 days (12 days larval growth + 16 days composting) and was extracted total microbial DNA within 24 h. The rest of samples was stored at − 80 °C for further chemical analysis.

2.3. DNA extraction, library construction, and sequencing

The total microbial DNA of manure samples was extracted using the phenol–chloroform method (Zhou et al., 1996). The concentrations of the extracted DNA were determined using a Nanodrop 2000 spectrophotometer (Thermo Scientific, USA). About 1 μg of DNA per sample was used as input material for the DNA sample preparations. Sequencing libraries were generated using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's recommendations, and index codes were added to attribute sequences to each sample. In brief, the DNA sample were fragmented by sonication to a size of 350 bp (Tang et al., 2023), and DNA fragments were end-polished, A-tailed, and ligated with the full-length adaptor for Illumina sequencing with further PCR amplification. Finally, PCR products were purified (AMPure XP system), and libraries were analyzed for size distribution by an Agilent 2100 Bioanalyzer and quantified using real-time PCR. The clustering of the index-coded samples was performed on a cBot Cluster Generation System according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina HiSeq platform, and paired-end reads were generated.

2.4. Nutrient and antimicrobial analysis

To explore the dynamics of essential nutrients during BSFL conversion, we measured the content of organic matter (OM), total nitrogen (TN), total phosphorus (TP), total potassium (TK), moisture content (MC), and crude fat (CF) in all samples. The contents of TN, TP, TK, and

OM in the manure samples were analyzed following the standard procedures specified in the NY 525–2012 Standardization Administration of the People's Republic of China. MC was measured as described in our previous study (Ao et al., 2021). CF was determined by Soxhlet extraction (Virot et al., 2007). A total of 15 antimicrobial commonly used in agriculture and animal husbandry were analyzed, including tetracyclines (oxytetracycline, chlortetracycline, and doxycycline), quinolones (norfloxacin, ciprofloxacin, and enrofloxacin), macrolides (erythromycin, roxithromycin, tilmicosin, and tylosin), β-lactams (penicillin G sodium and amoxicillin), sulfonamides (sulfadimethoxine), and amphenicol (chloramphenicol and thiamphenicol). These antimicrobial were chosen as indicators due to their environmental significance. Detailed information on the extraction method can be found in Supplementary Method S1.

2.5. Bioinformatic analysis

To obtain clean data for analysis, low-quality reads were filtered using Readfq (V8, https://github.com/cjfields/readfq). Possible host contamination was removed using Bowtie2.2.4 software (Bowtie2.2.4, http://bowtiebio.sourceforge.net/bowtie2/index.shtml). The clean reads were then assembled by MEGAHIT (v1.0.4-beta). MetaGeneMark (V2.10, http://topaz.gatech.edu/GeneMark/) software was used to predict open reading frames (ORFs), with length information shorter than 100 nt filtered from the predicted result using default parameters. CD-HIT software (V4.5.8, http://www.bioinformatics.org/cd-hit) was used to obtain the unique initial gene catalog. Unigenes were obtained by combining the clean data of each sample and the unique initial gene catalog using Bowtie2.2.4. Finally, DIAMOND software (V0.9.9, https://github.com/bbuchfink/diamond/) was used to blast the unigenes to the sequences of bacteria, fungi, archaea, and viruses, extracted from the NR database (Version: 2018–01–02, https://www.ncbi.nlm.nih.gov/) of NCBI.

ARG annotation of the unigenes was performed using the CARD database (https://card.mcmaster.ca/, blastp, e-value $\leq$ 1e-30). Resistance gene distribution in each sample, species attribution analysis of resistance genes, and analysis of the resistance mechanism of ARGs were also conducted. Potential bacterial hosts of ARGs (ARB) were identified by annotating ARGs and bacteria from the same unigenes, and the remaining bacteria that did not contain ARGs were defined as other bacteria (Liang et al., 2020; Liu et al., 2021). MGEs, including plasmids, insertion sequences (IS), and integrons, were identified using NCBI RefSeq plasmid databases, ISfinder databases, and INTEFRALL databases with default instructions (Guo et al., 2017). Unigenes were analyzed for the co-occurrence of ARGs and MGEs (Jia et al., 2019).

2.6. Statistical analyses and visualization

Student's *t* test and one-way ANOVA with Tukey's HSD test were performed with SPSS v20.0 (SPSS, USA). Heatmap analyses were generated through the "pheatmap" package of RStudio (R version 3.6.3). The Mantel test and Procrustes analysis were conducted using the "vegen" package of RStudio (R version 3.6.3) to assess the correlation between ARGs and MGEs, as well as between antimicrobial and ARB induction. Variation partitioning analysis (VPA) were performed using CANOCO 5.0 to determine the contribution of multiple factors to variations in ARB. Link test was performed to identify the nutrition competition between ARB and other bacteria. Spearman's correlation coefficients were calculated using the "psych" package in R with and were used to construct network analyses. The network analyses were conducted in RStudio (R version 3.6.3), and a network plot was created with Gephi (V 0.9.2) to determine the induction of antimicrobial on ARB, the antagonistic effect and nutritional competition of other bacteria on ARB. Microsoft Excel 2010 was used for the generation of other plots.

3. Results

3.1. Dynamics of antibiotic-resistant bacteria in chicken manure

Metagenomic assembly and annotation revealed 130 distinct ARB species across all samples combined (Fig. 1**A**). Following BSFL treatment, relative abundance of ARB decreased by 34.9% ($p < 0.05$), compared to the initial manure, while levels in the controls remained unchanged (Fig. 1**B**). BSFL treatment also resulted in a 19.8% (ChL12) and 26.6% (ChL28) reduction in ARB relative abundance, within 12 and 28 days, respectively, compared to the corresponding controls. BSFL conversion significantly reduced the relative abundance of multidrug- and vancomycin-resistant bacteria by 64.6% and 90.3% within 12 days, respectively, compared with initial manure ($p < 0.05$). After BSFL conversion, the relative abundances of tetracycline-, MLSB (Macrolide-Lincosamide-Streptogramin B) - and vancomycin-resistant bacteria were significantly decreased by 34.8%, 41.7%, and 60.3%, respectively ($p < 0.05$). Subsequent composting further reduced their relative abundances by 43.1%, 52.7%, and 59.5%, respectively, compared with the control. These results indicate that BSFL treatment is highly efficient in ARB attenuation.

Network analysis was conducted to evaluate the effects of BSFL on individual ARB. The network size (total number of nodes, *n*) and connectivity (total number of links, *L*) were used to evaluate the complexity of the network (Fig. 1C). Compared with the control, BSFL treatment reduced the network size and connectivity, indicating a reduction in the occurrence risks of ARB. The dominant ARB in the initial manure were *Escherichia coli, Lactobacillus reuteri*, and *Bacillus stratosphericus*, with a collective initial relative abundance of 35.2%. After BSFL treatment, their relative abundances significantly decreased in the larvae groups (15.1% in ChL12 and 3.8% in ChL28), which still lower by 17.0–18.5% than that in the controls. *Atopostipes suicloacalis*, the host of several ARGs, including *mexX*, *msrA*, and *OXA-131,* which were affiliated with multidrug, MLSB, and β-lactamase resistance genes, had a significantly decreased relative abundance by 79.8% and 69.0% in ChL12 and 12.7% in ChL28 compared with ChC12 and ChC28, respectively (Fig. S2). *Staphylococcus aureus*, hosted aminoglycoside resistance genes (*ANT4-Ib* and *apmA*) in our study, and had a decrease in relative abundance by 29.7% in ChL12 and 12.7% in ChL28 compared with ChC12 and ChC28, respectively. In addition, several pathogens (e.g., *Oblitimonas alkaliphile*, *Weeksella*, and *Bilophila wadsworthia*) could carry multiple subtypes of ARGs, and their relative abundance was significantly reduced after BSFL treatment ($p < 0.05$), indicating that BSFL could reduce the risks of drug resistance of pathogens.

3.2. Horizontal transfer risk of antibiotic resistance genes

A total of 491 distinct ARG subtypes were identified in all samples, and the highest relative abundance of ARGs (3467 ± 287 ppm) was found in the initial chicken manure (Fig. 2A and S3). BSFL conversion significantly reduced the relative abundance of ARGs in manure by 53.3% compared with the initial manure ($p < 0.05$). In addition, compared with the control, BSFL conversion also significantly reduced the richness of 33ARG subtypes (among which 7 subtypes of multidrug ARGs), but there was no significant difference in the relative abundance of ARGs. Most of the dominant ARG subtypes in the initial chicken manure, such as *tetW/N/W*, *ErmF*, *tetQ*, *floR*, *APH3-IIIa*, *APH3-Ib*, and *tet39*, displayed a significant decline after BSFL treatment. Importantly, BSFL treatment could effectively reduced the relative abundance of carbapenemase-encoding genes. For example, the relative abundance of *OXA-22*, *OXA-10*, *OXA-131* and *OXA-85* was reduced by 79.14%, 41.83%, 43.81% and 71.80%, respectively. Of all ARGs detected in the samples, the relative abundance of 193, 204, 227, and 241 subtypes of ARGs showed a decrease of 85% or more in ChC12, ChL12, ChC28, and ChL28, respectively. These results indicated that BSFL treatment effectively reduced the richness and relative abundance of ARGs in chicken

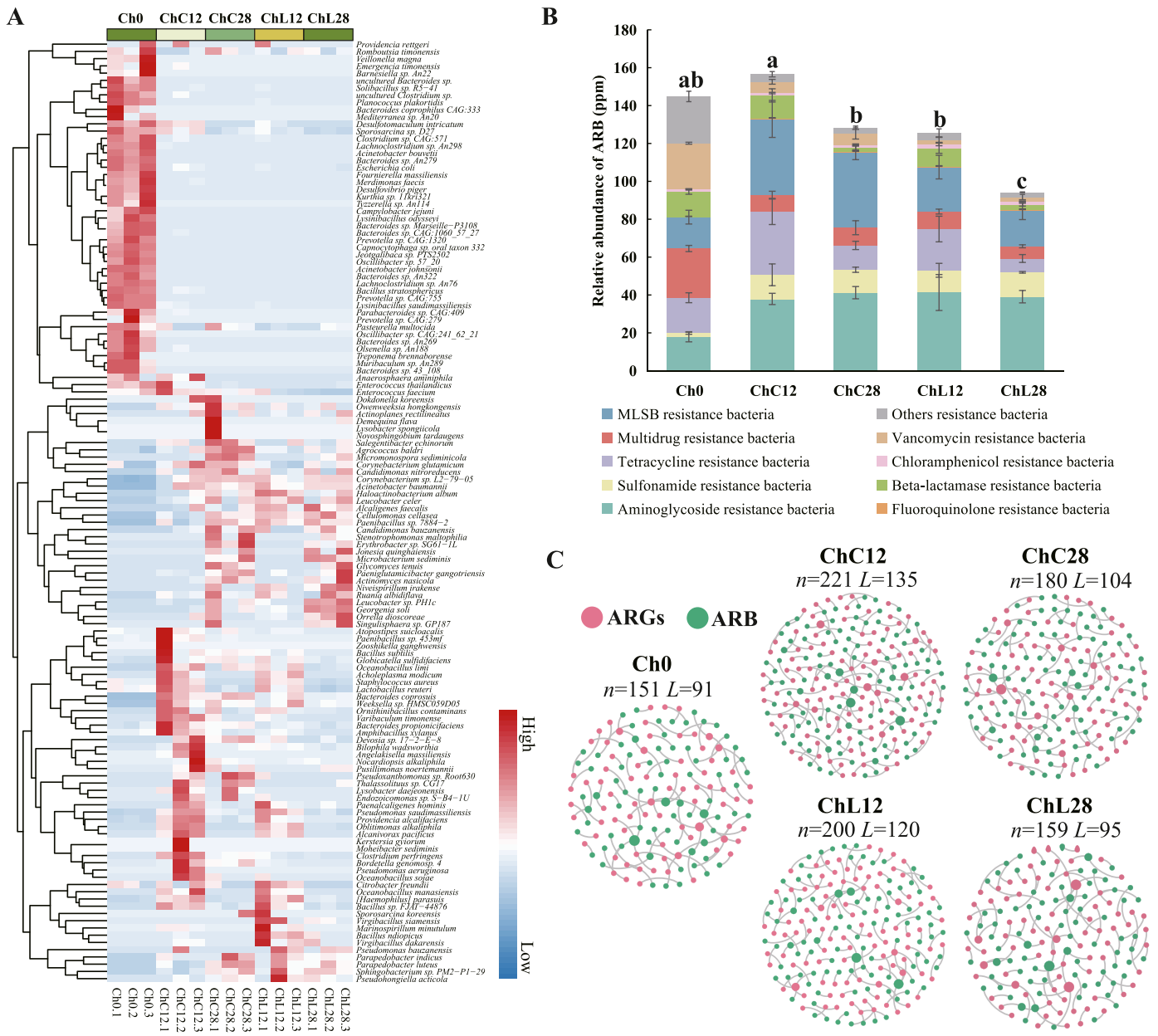


Fig. 1. Antibiotic-resistant bacteria (ARB) abundance and distribution and co-occurrence with ARGs (A) Heatmaps showing the distribution profiles of ARB across the different treatments. (B) Relative abundance of ARB detected across different treatments, with error bars indicating standard errors (n = 3). Different letters above the bars indicate a significant difference as defined by one-way ANOVA with Tukey's HSD test. (C) Network analysis of the co-occurrence patterns of ARGs and ARB (species level) based on genetic context in chicken manure. The node size is proportional to its degree and lines connect potential ARGs and bacteria found in the same unigene. Green and red nodes denote ARB and ARGs, respectively.

manure.

Compared with the initial manure, the plasmid richness decreased by 16.4% and 21.3%in ChC12 and ChL12, respectively, with a significant reduction in relative abundance of 29.4% and 41.8%, respectively (Fig. S3). The IS richness significantly decreased by 17.1%, and their relative abundance decreased by 32.4% in ChL12 relative to ChC12. Moreover, the richness and relative abundance of integrons significantly decreased by 16.8% and 46.6%, respectively, in ChL12 compared with ChC12. In contrast, the relative abundance of Tn significantly increased in both larvae and the control treatments, but ChL12 had 55.4% lower abundance, compared to ChC12 ($p < 0.05$). There was no significant difference in the relative abundance of MGEs between ChC28 and ChL28. The dominant plasmids, IS, integrons, and Tn were NZ-CP022019–1, NZ-CP013691–1, ISBbi1, ISVsa3, c6178, c1426, TnAs3, and TnAs1. Procrustes analysis and Mantel tests showed that total ARGs and MGEs passed a goodness-of-fit test (Fig. 2B, *Protest* $M^2 = 0.1768$, $p < 0.001$, 999 permutations; Mantel test $r = 0.69$, $p < 0.01$, 999 permutations) based on the Bray−Curtis dissimilarity metric, indicating possible horizontal transfer risks of ARGs. These results suggest that BSFL conversion can effectively reduce MGEs, thereby reducing the spread risks of ARGs in manure.

For each treatment, we calculated the relative abundance of transferable ARGs by determining the co-occurrence of ARGs and MGEs on the same unigene (Fig. 2C). We identified 159 unigenes containing ARGs and MGEs. The dominant transferable ARGs were MLSB (29), aminoglycoside (44), and tetracycline (23) resistance genes. Plasmids were the main medium for transferable ARGs, accounting for 129 out of 159 unigenes across all samples. The initial manure contained the highest abundance of transferable ARGs (1543 ± 10 ppm), indicating the highest risks of ARG horizontal gene transfer. After 12 days of

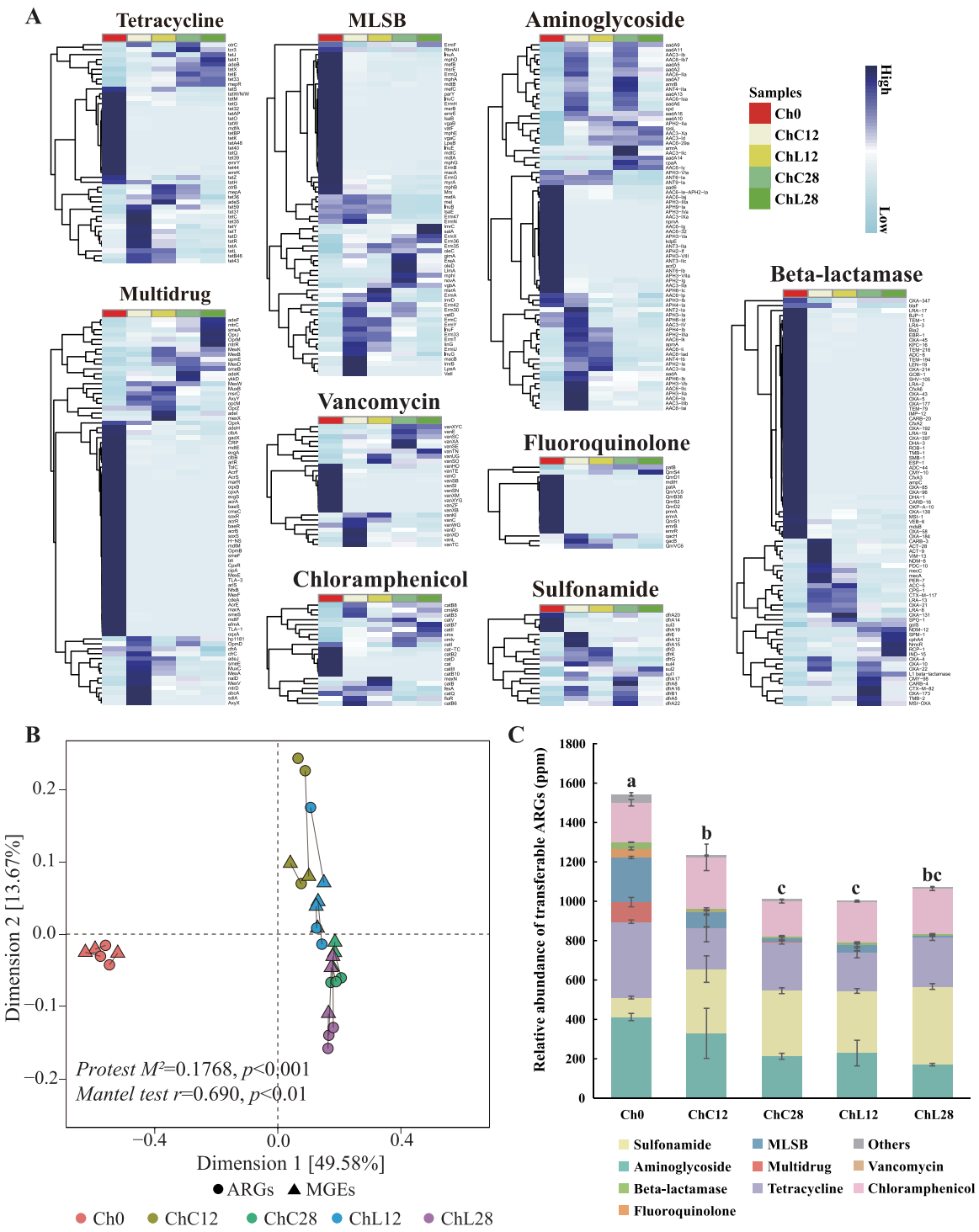


Fig. 2. Changes in antibiotic resistance genes (ARGs) and their co-occurrence patterns with mobile genetic elements (MGEs). (A) Heatmaps showing the distribution profiles of ARGs across the different treatments. (B) Procrustes analysis depicting a significant correlation between ARG abundance and MGEs (measured by Bray–Curtis distance) (*Protest* $M^2 = 0.1768$, $p < 0.001$, 999 permutations; Mantel test $r = 0.690$, $p < 0.01$, 999 permutations). (C) Relative abundance of transferred ARGs detected across different treatments, with error bars indicating standard errors (n = 3). Different letters above the bars indicate significant differences as defined by one-way ANOVA with Tukey's HSD test.

conversion, the relative abundance of transferable ARGs decreased significantly by 35.2% in ChL12 (1000 ± 70 ppm) compared with that in the initial manure sample (Ch0) and the corresponding control group (ChC12, 1230 ± 194 ppm, by 18.5%). The relative abundance of transferable ARGs in ChL28 (1067 ± 24 ppm) decreased significantly by 30.8%, compared to Ch0, but there was no significant difference when compared to the corresponding control group ChC28 (1004 ± 55 ppm).

Moreover, we characterized the coexistence of ARGs and MGEs among the assembled unigenes annotated as bacteria based on metagenomic analysis (Table S1). Collectively, 28 unigenes containing ARGs and MGEs were assigned as bacteria, with the highest abundance of coexisting unigenes in ChC12 (112 ± 16 ppm). In ChL12 (99 ± 31 ppm) and ChL28 (83 ± 1 ppm), the relative abundance of transferred ARB was 11.7% and 9.3% lower, respectively, than in the corresponding controls

(ChC12 and ChC28), but higher than in Ch0 (36.1 ± 5.5 ppm). For instance, the relative abundances of transferred ARB, including *Corynebacterium glutamicum, S. aureus, Enterococcus faecium, Enterococcus thailandicus, Lactobacillus reuteri, E. coli*, and *Pseudomonas aeruginosa,* were reduced by 52.6%, 29.7%, 52.2%, 100%, 28.8%, 52.6%, and 1.4%, respectively, in ChL12 compared to ChC12, and further decreased by 27.5%, 12.7%, 69.5%, 41.6%, 42.2%, 27.8%, and 48.6%, respectively, in ChL28 compared to ChC28.

3.3. The induction effect of antimicrobial on antibiotic-resistant bacteria

To investigate the characteristics of antibiotic degradation by BSFL treatment of chicken manure and explore the relationship between antibiotic degradation and ARB induction, we analyzed 15 antimicrobial of 6 categories in all experimental groups (Fig. 3A). Within 12 days of treatment, BSFL significantly decreased the total concentration of antimicrobial by 61.3% in manure compared to initial level ($p < 0.001$). The concentrations of β-lactams ($p < 0.001$), amphenicol ($p < 0.01$) and macrolides ($p < 0.001$) decreased by 64.8%, 91.7% and 96.5%, respectively, while the concentration of sulfonamides tetracycline increased significantly ($p < 0.05$) by 276%, in ChL12 group compared to the initial level. Compared to the corresponding control, the total concentration of antimicrobial in manure was significantly decreased ($p < 0.001$) by 45.1% after 12 days of BSFL conversion, and the concentration of β-lactams and amphenicol was significantly ($p < 0.01$) reduced by 47.4% and 91.9%, respectively.

After 16 days further composting, the total concentration of antimicrobial significantly decreased ($p < 0.001$) by 64.4% and 52.9% in larvae group, relative to initial manure and control groups. Compared to the initial manure, the concentration of β-lactams ($p < 0.001$), amphenicol ($p < 0.01$) and macrolides ($p < 0.001$) significantly decreased by 66.9%, 77.0% and 98.6%, while the concentration of sulfonamides significantly ($p < 0.05$) increased by 212.7% in the larvae group. The concentration of β-lactams, sulfonamides, quinolones, tetracycline and macrolides decreased by 56.6%, 15.7%, 25.4%, 14.5% and 26.2%, with the β-lactams ($p < 0.001$), quinolones ($p < 0.01$) had significant difference, in larvae group related to control group.

Further spearman analysis results (Fig. S4) showed that the significant positive correlation ($r = 0.62$, $p < 0.05$) between total ARB and antimicrobial, which was verified by a strong correlation in Mantel test ($r = 0.67$, $p < 0.001$, 999 permutations), indicating a close correlation between antimicrobial and ARB induction. The sulfonamides ($r = 0.73$, $p < 0.01$) antimicrobial significantly correlated with sulfonamides resistance bacteria. Interestingly, most of antimicrobial had significant correlations with ARB exhibiting resistance to another antimicrobial class. For example, β-lactams and macrolides showed strong positive

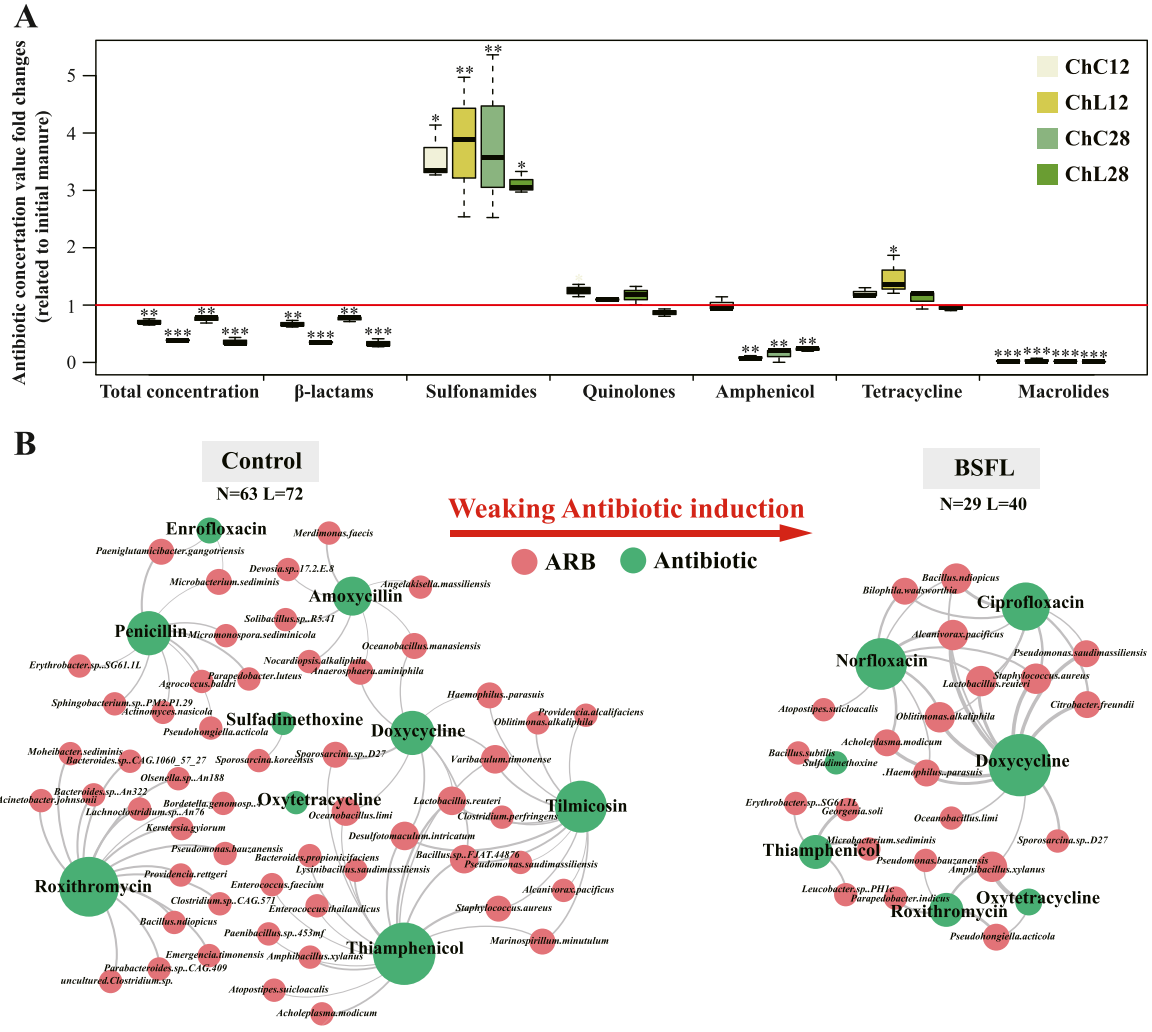


Fig. 3. Reduction of antimicrobial pressure and risk of ARB occurrence by BSFL treatment. (A) Fold changes (relative to the initial manure sample) in antibiotic concentration (n = 3) in chicken manure. (B) Network analysis showing co-occurrence patterns of antibiotics and ARB (species level) in manure, with node size proportional to node degree and edges representing interactions between nodes. Green and red nodes denote antimicrobial and ARB, respectively. Connections are based on spearman's correlation coefficient ($r > 0.6$, $p < 0.05$).

correlation ($r > 0.75$, $p < 0.01$) with multidrug and vancomycin resistance bacteria, while sulfonamide had strong ($r < -0.79$, $p < 0.001$) negative correlation with these ARB. Amphenicol had significant positive correlation ($r > 0.58$, $p < 0.05$) with tetracycline-, multidrug-, β-lactamase- and vancomycin-resistant bacteria.

Furthermore, the network analysis (Fig. 3B) showed that the degradation of antimicrobial caused by BSFL treatment could weaken the induction pressure on ARB. The number of links between antimicrobial and ARB was 44.4% lower in the BSFL treatment group than in the control group. In addition, while nine antimicrobial had strong positive correlation with 54 species of ARB in control group, only seven antimicrobial showed significantly positive correlation with 22 ARB in BSFL treatment group. Specifically, thiamphenicol and roxithromycin showed the highest induction effect in control group, which had strong positive correlation with 16 and 15 species of ARB, respectively. However, this induction was weakened after BSFL treatment which thiamphenicol and roxithromycin only showed induction on 4 ARB. Furthermore, there was no strong positive correlation of tilmicosin, doxycycline, penicillin, amoxicillin and enrofloxacin with ARB in BSF group, but its showed induction effect on 2–12 species of ARB in control.

3.4. Nutrient competition between ARB and non-ARB

In the present study, ARB constituted only a small fraction of the

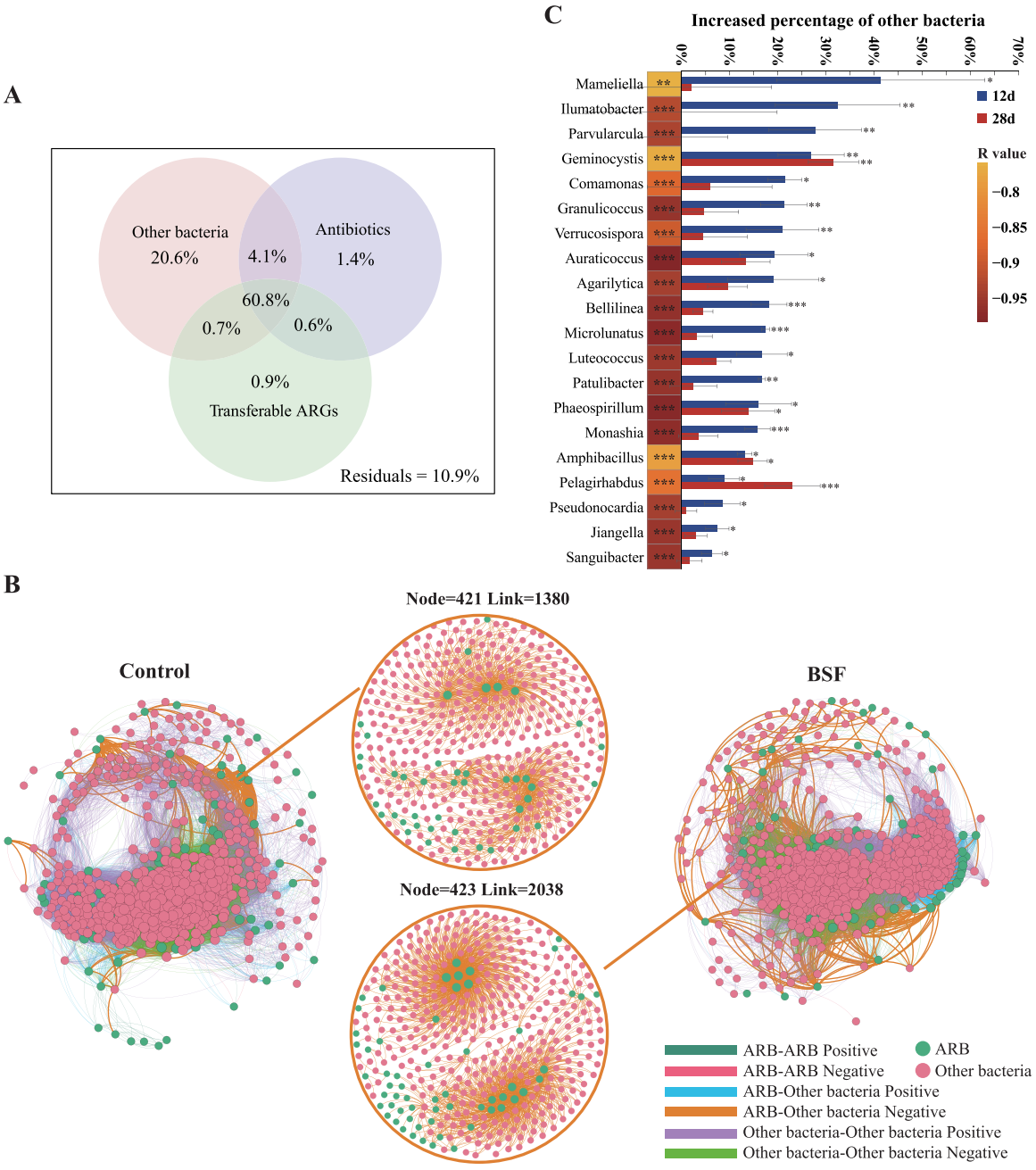


Fig. 4. Antagonism between ARB and other bacteria. (A) Variation partitioning analysis (VPA) showing the effects of the influencing factors on ARB. (B) Network analysis of the co-occurrence patterns of other bacteria and ARB (genus level) in manure, where node size is proportional to node degree and edges represent interactions between nodes. Nodes are colored for ARB and other bacteria. A connection was based on spearman's correlation coefficient (r > 0.6, p < 0.05). (C) The increased percentage of environmental microorganisms (TOP 20) in ChL12 and ChL28 compared with the corresponding controls (ChC12 and ChC28). The color in the box indicates the spearman correlation coefficients between ARB and environmental microorganisms. * : $0.01 < p \leq 0.05$; * *: $0.001 < p \leq 0.01$; * ** : $p \leq 0.001$.

total microbes in manure. Therefore, as vulnerable microbes in the manure ecosystem, ARB may be subject to competitive antagonism from other bacteria through niche or nutritional competition. Using variation partitioning analysis (Fig. 4A), we determined the contribution of other bacteria, antimicrobial, and transferable ARGs to variations in ARB. The selected factors accounted for 89.1% of the variation, with other bacteria being the major influencing ARB pattern (20.6%) and antimicrobial and transferable ARGs accounting for 1.4%, and 0.9%, respectively.

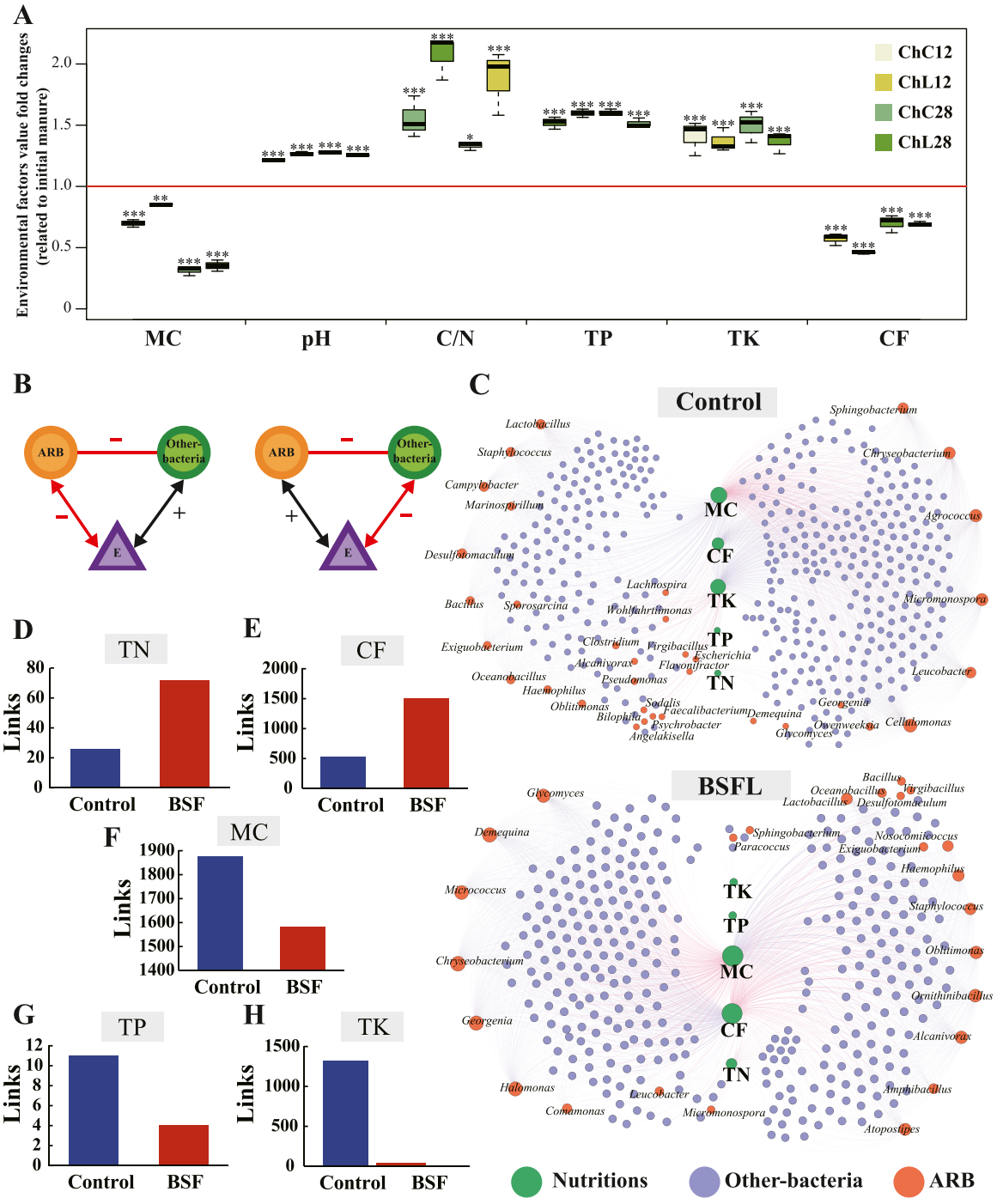


Fig. 5. Nutritional competition between ARB and other bacteria. (A) Fold changes (relative to the initial manure sample) in nutrients (n = 3) in chicken manure. (B) Conceptual diagram of the criteria for identifying potential negative links between ARB, other bacteria and environmental conditions. The links are formed due to covariations in the abundance of ARB and other bacterial with environmental conditions. ARB and other bacteria nodes are negatively (-) linked in the network. E can be any environmental variable measured, grouped by treatment and time. The link between ARB and other bacteria is identified as a negative ARB-other bacteria-environment link if the sign combination of the correlation coefficients of ARB-E, other bacteria-E, and ARB-other bacteria belongs to one of the categories shown. All links are based on Spearman correlation ($|r| > 0.6$, $p < 0.05$) that illustrates of other bacteria on nutrients. Links show the strong correlation ($|r| > 0.6$, $p < 0.05$) between other bacteria and (C) total nitrogen, (D) crude fat, (E) water, (F) total phosphorus, and (G) total potassium. (H) Network analysis of the competitive ARB-other bacteria-environment covariates, where node size is proportional to node degree and edges s represent interactions between nodes. Nodes are colored for ARB, other bacteria, and nutrients. Connections are based on Spearman's correlation coefficient ($|r| > 0.6$, $p < 0.05$). Lines are colored for the interactions (red represents positive interactions, whereas blue represents negative interactions).

To explore the interaction between among ARB, between ARB and other bacteria, as well as among other bacteria, we conducted a network analysis (Fig. 4B) and found that the total links in the BSFL treatment system decreased to 56,149 compared with those in the control (60,736), which might be due to the bacteriostatic of BSFL. Interestingly, the links of strong negative correlation (spearman, $r < -0.6$, $p < 0.05$) between other bacteria and ARB increased from 1380 (control group) to 2038 (BSFL treatment system), suggesting that BSFL treatment could strengthen the antagonistic effect between other bacteria on ARB. Further analysis (Fig. 4C) showed that various other bacteria displayed a strong significant negative correlation with ARB. BSFL treatment expedited the growth of those other bacteria, thereby enhancing the antagonistic effect between other bacteria and ARB and inhibiting the prevalence of ARB. For example, BSFL conversion significantly increased the abundances of other bacteria, namely, *Mameliella*, *Ilumatobacter*, and *Parvularcula,* by 41.4%, 32.4%, and 27.8%, respectively, in ChL12 compared with ChC12. Meanwhile, Spearman analysis showed that *Mameliella* ($r = -0.76$, $p < 0.01$), *Ilumatobacter* ($r = -0.92$, $p < 0.001$), and *Parvularcula* ($r = -0.93$, $p < 0.001$) had a strong significant negative correlation with ARB, indicating that BSFL could also eliminate ARB by increasing the abundance of these other bacteria.

Moreover, competition for nutrients such as carbohydrates, proteins, lipids, and water between other bacteria and ARB was also negligible, especially because these nutrients were rapidly consumed during BSFL treatment. To evaluate changes in nutrient profiles in chicken manure, we analyzed the following parameters: OM, TN, TP, TK CF, pH, and MC; Fig. 5A and Table S2. After 12 days of larval conversion, the pH significantly ($p < 0.001$) increased from 6.3 to 8.1, and the MC significantly ($p < 0.01$) decreased from 65.2% to 55.7%. The contents of TP, TK, and the C/N ratio significantly ($p < 0.001$) increased by 52.0%, 40.2%, and 54.5%, respectively, whereas those of TN ($p < 0.001$), OM ($p < 0.05$), and CF ($p < 0.001$) significantly decreased by 45.3%, 15.4%, and 42.8%, respectively, in chicken manure relative to initial levels. Compared with the control group, the contents of OM, TP, MC, and C/N increased by 3.6%, 5.2%, 22.2%, and 33.7%, respectively, whereas those of TN, TK, and CF decreased by 22.5%, 2.8%, and 19.6%, respectively, in the larvae conversion group. The contents of MC and the C/N ratio significantly ($p < 0.001$) increased, and TN ($p < 0.001$) and CF ($p < 0.05$) significantly decreased after larval conversion compared with the control group.

After 16 days of further composting, C/N ratio, TP, and TK significantly ($p < 0.001$) increased by 86.6%, 51.6%, and 36.5%, respectively, whereas those of OM ($p < 0.05$), TN ($p < 0.001$), MC ($p < 0.001$), and CF ($p < 0.001$) significantly decreased by 14.9%, 54.6%, 64.7%, and 30.8%, respectively, in chicken manure compared with the initial levels. Compared with the control group, the contents of OM, MC, and C/N ratio increased by 5.4%, 13.2%, and 39.9%, respectively, whereas TN, TP, TK, and CF decreased by 24.9%, 5.6%, 8.3%, and 1.0%, respectively, in the larvae conversion group. C/N ratio increased significantly ($p < 0.01$), while TN ($p < 0.01$) and TK ($p < 0.05$) decreased significantly in the larvae treatment group, compared with the control.

Network analysis and link tests for environmental factors were conducted to explore the potential nutrition competition between ARB and other bacteria. If the link between ARB and other bacteria is caused by their competition for nutrients (Fig. 5B), a strong correlation pattern among ARB, other bacteria (negative correlation), and the responsible environmental variable (positive correlation) should be observed (Fig. 5B). We tested all the links among ARB, other bacteria, and nutrients in the network (Fig. 5C), and found that 48.8% (2434 / 4988) of the links were considered competitive ARB-other bacteria-environment covariates in the BSFL treatment system, which was higher than that in the control (35.2%, 3114 / 8838). Specifically, water (Fig. 5F) was the nutrient for which ARB and other bacteria competed the most in both the control and BSFL conversion systems, due to the quick loss of water content in manure. After BSFL treatment, the competition of TN and CF between ARB and other bacteria intensified due to the rapid consumption of these nutrients by larvae, where the negative links between other bacteria and TN increased from 26 to 72 and CF increased from 529 to 1506 (Figs. 5D and 5E). The competition between ARB and other bacteria for TK decreased sharply after BSFL treatment, where the links between other bacteria and TK (Fig. 5H) decreased from 1323 to 2, possibly due to the potassium-enriching effect of BSFL. Collectively, the rapid reduction in antimicrobial pressure and ARG horizontal transfer risks and the increase in nutrient competition and antagonism from other bacteria contributed to the attenuation of ARB during BSFL treatment (Fig. 6).

4. Discussion

The present study demonstrated that BSFL treatment is a promising alternative for recycling chicken manure while attenuating ARB and ARGs. Previous studies have widely reported the co-occurrence of ARGs and microbes in manure, but evidence were based on statistical analysis and did not provide direct evidence for their co-occurrence (Wang et al., 2022; Xu et al., 2023; Yue et al., 2021). In contrast, our study used metagenomic assembly with gene arrangement analysis and showed that BSFL treatment significantly reduced the relative abundance of ARB and ARGs in the chicken manure samples by 34.9% and 53.3% within 28 days, respectively. By comparison, composting only reduced the total abundance of ARB by 10.2% within 42 days based on statistical analyses (Awasthi et al., 2022). Meanwhile, a culture-based study (Syafiuddin and Boopathy, 2021) reported that anaerobic digestion does not effectively reduce the richness of ARB in manure.

Previous research has reported the key role of MGEs in the mobility and acquisition of ARGs among different microbes via horizontal gene transfer (Gurmessa et al., 2020; Lee et al., 2020; Zou et al., 2020). It has been established that bacteria can acquire antimicrobial resistance through conjugation, transduction, and transformation by MGEs (Blair et al., 2015). RDA (Fig. S5) also confirmed that MGEs were beneficial for most ARB, including tetracycline-, beta-lactamase-, vancomycin-, and multidrug- resistant bacteria.

In addition, the present study showed that BSFL conversion effectively reduced the richness and abundance of MGEs, thereby eliminating the spread risks of ARGs, as proven by the strong significant Spearman correlation ($r = 0.78$, $p < 0.001$) and Mantel analysis ($r = 0.69$, $p < 0.01$) between total MEGs and total ARGs. Other studies have also reported the associations between ARGs and MGEs in manure through statistical analysis (Cao et al., 2020; Jia et al., 2015; Zhang et al., 2020b), but no direct evidence was provided to support their co-occurrence. In the present study, metagenomic assembly with gene

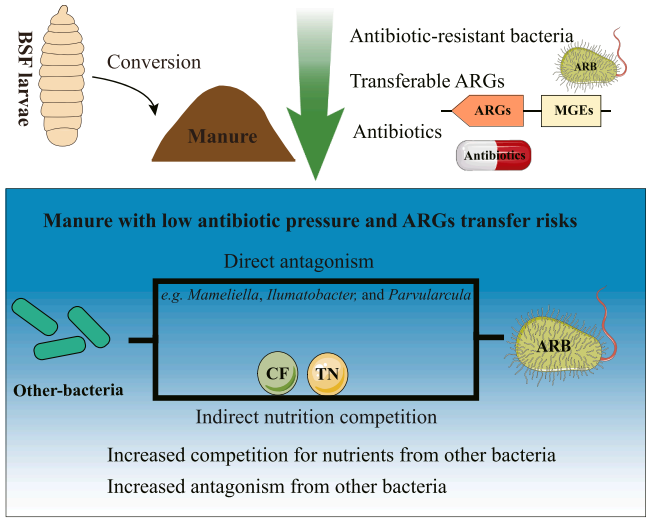


Fig. 6. Schematic of the mechanisms for ARB attenuation in chicken manure.

arrangement analysis clearly indicated that BSFL alleviated the risk of ARG horizontal transfer, as reflected by the significant decrease by 35.2% in the transferable ARGs within 12 days. Remarkably, we identified 28 unigenes harboring ARGs and MGEs and assigned them as bacteria.

Our results showed that BSFL conversion was effective in eliminating transferable ARB in manure, resulting in a significant reduction in opportunistic pathogens, such as *E. coli*, *S. aureus*, and *P. aeruginosa*. This decrease plays a crucial role in reducing the occurrence and spread risk of pathogen resistance. ARB were outcompeted during chicken manure conversion, accounting for approximately 2% of all microflora abundance. In an ecosystem, vulnerable microbes are often outcompeted by other dominant microbes in terms of niche and nutritional availability (Coyte and Rakoff-Nahoum, 2019). In our study, the addition of BSFL significantly reduced TN and CF in chicken manure and enhanced the competition between ARB and other bacteria for these nutrients.

Furthermore, BSFL also increased the abundance of beneficial ARB-antagonistic bacteria, which show a significant negative correlation with ARB. *Mameliella, Ilumatobacter, Parvularcula* were among the major antagonists, and the increase of their abundance may be related to the BSFL gut and the manure micro-environment altered by BSFL treatment. BSFL bio-conversion can rapidly consume nitrogen and fat in manure, resulting in oligotrophic environment which is conducive to the survival of alphaproteobacteria such as *Mameliella* and *Parvularcula* (Roth Rosenberg et al., 2021). In addition, previous study has showed the increase of *Parvularculaceae* in larval gut during BSFL bio-convert chicken manure, which may be the direct reason of the corresponding bacteria increased in the manure (Ao et al., 2021). *Mameliella*, for example, has been reported to degrade toxins and produce beneficial metabolites (Danish-Daniel et al., 2016). All these genera are related to the marine environment and specifically to marine biota (e.g. algae, fish, shrimp), and we hypothesize that they could have been introduced into chicken manure via feeding (Reyes et al., 2022; Wang et al., 2021; Yu et al., 2013). The enrichment of the intestinal symbiotic *Mameliella* in *Litopenaeus vannamei* may improve the intestinal health of *L. vannamei* by degrading of toxins and producing of beneficial metabolites, thus enhancing the immunity and digestive (Duan et al., 2019). In the gut of *Lates calcarifer* fed the Fe-enriched diet, enriched *Ilumatobacter* could reducing the inflammatory and carcinogenic and restorating intestinal barrier (Spilsbury et al., 2022). In this respect, fish and shrimp flour from by-products are commonly used as protein sources in poultry (Beski et al., 2015; Mounica et al., 2020). Taking this into account, not only BSFL larvae microflora but also microbiota associated to chicken feeding could play a major role in controlling the resistome in chicken manure. Some studies have focused in the protein income, digestability and nutritional value of different sources (Beski et al., 2015; Mounica et al., 2020), but, to our knowledge, so far none has studied the influence in excreted microbiota. This should be explored further.

RDA (Fig. S5) was conducted to investigate the relationships of BSFL with antimicrobial, MGEs, environmental factors, and ARB. RDA1 and RDA2 accounted for 65.9% and 17.0% of the total variation in ARB, respectively. The BSF larvae were negatively correlated with the majority of tetracycline-, beta-lactamase-, vancomycin-, and multidrug-resistant bacteria. The BSF larvae facilitated the degradation of MC, TN, and CF, as well as most antimicrobial (amphenicol, β-lactams, quinolones, and macrolides) and MGEs, all of which contributed to ARB. Additionally, the contribution of nutrient elements, MGEs, and antimicrobial to variations in ARB was determined via variation partitioning analysis (Fig. S5). The selected factors explained 88.5% of the variation, with most of the variance being attributed to the combined effects of nutrients, MGEs and antimicrobial. Individually, nutrient elements, MGEs, and antimicrobial explained 15.0%, 2.7%, and 7.8% of the total ARB, respectively, whereas jointly they accounted for 67%, suggesting that these factors influencing ARG patterns were interdependent (Liu et al., 2023; Shen et al., 2023).

Sub-inhibitory concentrations of antimicrobial are conducive to the maintenance and enrichment of MGEs to promote the effective response to external antimicrobial pressure and spread among bacteria (San Millan, 2018). MGEs may affect ARB in combination with nutrient elements and antimicrobial, the three of which can explain 67% of the dynamics of ARB. Sustained selective pressure of antimicrobial on bacteria can lead to the occurrence and prevalence of ARB (Tiedje et al., 2019). In this study, fifteen antimicrobial commonly used in animal husbandry and threatening the environment (e.g. soil, water and plant) were analyzed (Bártíková et al., 2016; Huang et al., 2021). We found a significant correlation between antimicrobial and ARB (spearman analysis, $r > 0.6$, $p < 0.05$), which confirmed the induction role of antimicrobial on ARB. Furthermore, network analysis showed that BSFL treatment could eliminate ARB by significantly depleting antimicrobial residues in chicken manure, thus weakening the antimicrobial pressure and ARGs induction. Some studies have demonstrated that antimicrobial degradation products can induce ARGs as well (Li et al., 2022). We did not determine the degradation products in our study. Nevertheless, given the strong positive correlation between ARB and antimicrobial, as well as between ARGs and ARB, it seems that the induction of ARGs in this case is mainly attributable to the parent compounds themselves. The occurrence and prevalence of ARB are influenced by changes in nutrients and environmental factors. Spearman analysis results (Fig. S4) indicated that pH, TP, and TK were strongly negatively correlated, and OM, TN, CF, and MC were strongly positively correlated with the main ARB. In the larvae conversion system, pH and contents of TP and TK increased, while OM, TN, CF, and MC decreased, suggesting that BSFL could alter the environment, thereby affecting the structure of the antibiotic-resistant bacterial community and ARG profiles. Both network analysis and link tests showed that BSFL conversion enhanced the nutrition competition between ARB and other bacteria. The degree of change in pH, MC, OM, TN, and CF in the larvae conversion system was significantly higher than that in the controls on the 12th day as a result of the large demand for nutrient elements (e.g., carbohydrates, proteins, and lipids) and water for rapid larval growth (Carvalho et al., 2012; Ogunwande et al., 2008). The nutritional competition between ARB with other bacteria and larvae might limit or even inhibit the growth of ARB.

Overall, the BSFL treatment is powerful in mitigating ARB, ARGs and MGEs in manure. Further studies would propose more effective methods that can strengthen the removal efficiency of antibiotic resistance by BSFL. The first is to screen for BSFL strains with high efficiency in removing antibiotic resistance by genetic breeding. The second is to add additives during BSFL treatment, such as biochar and lignite which have been shown to be effective in removing antibiotic resistance. The third is to screen antimicrobial degrading strains in larval gut for co-conversion with BSFL to reduce antibiotic selective pressure in manure. In addition, the earthworms or other insects can be considered to treat the residues of BSFL bio-conversion.

5. Conclusion

This study demonstrated that BSFL treatment can reduce the abundance of ARB, ARGs, MGEs and transferable ARGs in chicken manure. In addition, BSFL could rapidly degrade antimicrobial and thus weaken the antimicrobial induction effect. The consumption of total nitrogen and crude fat strengthen nutrient competition between other bacteria and ARB. Overall, the rapid degradation of antimicrobial and reduction of transferable ARGs resulted in the low antibiotic pressure and ARGs transfer risks on ARB occurrence in manure. Meanwhile, the direct antagonism and indirect nutrition competition from other bacteria also contributed to the attenuation of ARB during BSFL treatment. Thus, BSFL treatment is an effective measure for the reduction of antibiotic resistance in manure.

CRediT authorship contribution statement

Zhengzheng Zhao: Data curation, Formal analysis, Methodology, Visualization, Writing - original draft. **Chongrui Yang:** Methodology. **Bingqi Gao:** Formal analysis. **Yushi Wu:** Methodology. **Yue Ao:** Methodology. **Shiteng Ma:** Methodology. **Núria Jiménez:** Writing - review & editing. **Longyu Zheng:** Writing - review & editing. **Feng Huang:** Writing - review & editing. **Jeffery K. Tomberline:** Writing - review & editing. **Zhuqing Ren:** Writing - review & editing. **Ziniu Yu:** Writing - review & editing. **Chan Yu:** Methodology, Formal analysis. **Jibin Zhang:** Writing - review & editing. **Minmin Cai:** Funding acquisition; Investigation; Methodology; Project administration, Writing - review & editing. All authors read and approved the final manuscript.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

Acknowledgement

This work was funding by the National Natural Science Foundation of China (41977279), the Fundamental Research Funds for the Central Universities (2662020SKPY002 and 2662022SKYJ006), the Key Technology R & D Program of Hubei Province (2021BBA258) and the Major Project of Hubei Hongshan Laboratory (2022hszd013).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115551.

References

Ao, Y., Yang, C., Wang, S., Hu, Q., Yi, L., Zhang, J., Yu, Z., Cai, M., Yu, C., 2021. Characteristics and nutrient function of intestinal bacterial communities in black soldier fly (*Hermetia illucens* L.) larvae in livestock manure conversion. Micro Biotechnol. 14 (3), 886–896. https://doi.org/10.1111/1751-7915.13595.

Arias, C.A., Murray, B.E., 2009. Antibiotic-resistant bugs in the 21st century–a clinical super-challenge. N. Engl. J. Med 360 (5), 439–443. https://doi.org/10.1056/NEJMp0804651.

Awasthi, M.K., Liu, H., Liu, T., Awasthi, S.K., Zhang, Z., 2022. Effect of biochar addition on the dynamics of antibiotic resistant bacteria during the pig manure composting. Sci. Total Environ. 814, 152688 https://doi.org/10.1016/j.scitotenv.2021.152688.

Bártíková, H., Podlipná, R., Skálová, L., 2016. Veterinary drugs in the environment and their toxicity to plants. Chemosphere 144, 2290–2301. https://doi.org/10.1016/j.chemosphere.2015.10.137.

Beski, S.S.M., Swick, R.A., Iji, P.A., 2015. Specialized protein products in broiler chicken nutrition: a review. Anim. Nutr. 1 (2), 47–53. https://doi.org/10.1016/j.aninu.2015.05.005.

Blair, J.M.A., Webber, M.A., Baylay, A.J., Ogbolu, D.O., Piddock, L.J.V., 2015. Molecular mechanisms of antibiotic resistance revisited. Nat. Rev. Microbiol 13 (1), 42–51. https://doi.org/10.1038/s41579-022-00820-y.

Burch, T.R., Sadowsky, M.J., LaPara, T.M., 2017. Effect of different treatment technologies on the fate of antibiotic resistance genes and class 1 integrons when residual municipal wastewater solids are applied to soil. Environ. Sci. Technol. 51 (24), 14225–14232. https://doi.org/10.1021/acs.est.7b04760.

Cai, M., Ma, S., Hu, R., Tomberlin, J.K., Thomashow, L.S., Zheng, L., Li, W., Yu, Z., Zhang, J., 2018a. Rapidly mitigating antibiotic resistant risks in chicken manure by *Hermetia illucens* bioconversion with intestinal microflora. Environ. Microbiol 20 (11), 4051–4062. https://doi.org/10.1111/1462-2920.14450.

Cai, M., Ma, S., Hu, R., Tomberlin, J.K., Yu, C., Huang, Y., Zhan, S., Li, W., Zheng, L., Yu, Z., Zhang, J., 2018b. Systematic characterization and proposed pathway of tetracycline degradation in solid waste treatment by *Hermetia illucens* with intestinal microbiota. Environ. Pollut. 242, 634–642. https://doi.org/10.1016/j.envpol.2018.06.105.

Cao, R., Ben, W., Qiang, Z., Zhang, J., 2020. Removal of antibiotic resistance genes in pig manure composting influenced by inoculation of compound microbial agents. Bioresour. Technol. 317, 123966 https://doi.org/10.1016/j.biortech.2020.123966.

Carvalho, M., Sampaio, J.L., Palm, W., Brankatschk, M., Eaton, S., Shevchenko, A., 2012. Effects of diet and development on the *Drosophila lipidome*. Mol. Syst. Biol. 8, 600. https://doi.org/10.1038/msb.2012.29.

Chen, P., Yu, K., He, Y., 2023. The dynamics and transmission of antibiotic resistance associated with plant microbiomes. Environ. Int 176, 107986. https://doi.org/10.1016/j.envint.2023.107986.

Chen, Q.L., Hu, H.W., Zhu, D., Ding, J., Yan, Z.Z., He, J.Z., Zhu, Y.G., 2020. Host identity determines plant associated resistomes. Environ. Pollut. 258, 113709 https://doi.org/10.1016/j.envpol.2019.113709.

Chen, Z., Bao, H., Wen, Q., Wu, Y., Fu, Q., 2022. Effects of H_3PO_4 modified biochar on heavy metal mobility and resistance genes removal during swine manure composting. Bioresour. Technol. 346, 126632 https://doi.org/10.1016/j.biortech.2021.126632.

Collaborators, A.R., 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399 (10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0.

Coyte, K.Z., Rakoff-Nahoum, S., 2019. Understanding competition and cooperation within the mammalian gut microbiome. Curr. Biol. 29 (11), R538–r544. https://doi.org/10.1016/j.cub.2019.04.017.

Danish-Daniel, M., Han Ming, G., Noor, M.E., Yeong, Y.S., Usup, G., 2016. Draft genome sequence of *Mameliella alba* strain UMTAT08 isolated from clonal culture of toxic dinoflagellate *Alexandrium tamiyavanichii*. Genom. Data 10, 12–14. https://doi.org/10.1016/j.gdata.2016.08.015.

Duan, Y., Wang, Y., Liu, Q., Dong, H., Li, H., Xiong, D., Zhang, J., 2019. Changes in the intestine microbial, digestion and immunity of *Litopenaeus vannamei* in response to dietary resistant starch. Sci. Rep. 9 (1), 6464. https://doi.org/10.1038/s41598-019-42939-8.

Durso, L.M., Cook, K.L., 2019. One health and antibiotic resistance in agroecosystems. EcoHealth 16 (3), 414–419. https://doi.org/10.1007/s10393-018-1324-7.

Fresia, P., Antelo, V., Salazar, C., Giménez, M., D'Alessandro, B., Afshinnekoo, E., Mason, C., Gonnet, G.H., Iraola, G., 2019. Urban metagenomics uncover antibiotic resistance reservoirs in coastal beach and sewage waters. Microbiome 7 (1), 35. https://doi.org/10.1186/s40168-019-0648-z.

Grossule, V., Zanatta, S., Modesti, M., Lavagnolo, M.C., 2023. Treatment of food waste contaminated by bioplastics using BSF larvae: Impact and fate of starch-based bioplastic films. J. Environ. Manag. 330, 117229 https://doi.org/10.1016/j.jenvman.2023.117229.

Guo, H.G., Chen, Q.L., Hu, H.W., He, J.Z., 2020. Fate of antibiotic resistance genes during high-solid anaerobic co-digestion of pig manure with lignite. Bioresour. Technol. 303, 122906 https://doi.org/10.1016/j.biortech.2020.122906.

Guo, J., Li, J., Chen, H., Bond, P.L., Yuan, Z., 2017. Metagenomic analysis reveals wastewater treatment plants as hotspots of antibiotic resistance genes and mobile genetic elements. Water Res 123, 468–478. https://doi.org/10.1016/j.watres.2017.07.002.

Guo, Y., Qiu, T., Gao, M., Sun, Y., Cheng, S., Gao, H., Wang, X., 2021. Diversity and abundance of antibiotic resistance genes in rhizosphere soil and endophytes of leafy vegetables: focusing on the effect of the vegetable species. J. Hazard Mater. 415, 125595 https://doi.org/10.1016/j.jhazmat.2021.125595.

Gurmessa, B., Pedretti, E.F., Cocco, S., Cardelli, V., Corti, G., 2020. Manure anaerobic digestion effects and the role of pre- and post-treatments on veterinary antibiotics and antibiotic resistance genes removal efficiency. Sci. Total Environ. 721, 137532 https://doi.org/10.1016/j.scitotenv.2020.137532.

Huang, R., Guo, Z., Gao, S., Ma, L., Xu, J., Yu, Z., Bu, D., 2021. Assessment of veterinary antibiotics from animal manure-amended soil to growing alfalfa, alfalfa silage, and milk. Ecotox Environ. Safe 224, 112699. https://doi.org/10.1016/j.ecoenv.2021.112699.

Jia, S., Shi, P., Hu, Q., Li, B., Zhang, T., Zhang, X.X., 2015. Bacterial Community Shift Drives Antibiotic Resistance Promotion during Drinking Water Chlorination. Environ. Sci. Technol. 49 (20), 12271–12279. https://doi.org/10.1021/acs.est.5b03521.

Jia, S., Wu, J., Ye, L., Zhao, F., Li, T., Zhang, X.X., 2019. Metagenomic assembly provides a deep insight into the antibiotic resistome alteration induced by drinking water chlorination and its correlations with bacterial host changes. J. Hazard Mater. 379, 120841 https://doi.org/10.1016/j.jhazmat.2019.120841.

Kaufmann, T., 2015. Sustainable livestock production: Low emission farm - the innovative combination of nutrient, emission and waste management with special emphasis on Chinese pig production. Anim. Nutr. 1 (3), 104–112. https://doi.org/10.1016/j.aninu.2015.08.001.

Lee, K., Kim, D.W., Lee, D.H., Kim, Y.S., Bu, J.H., Cha, J.H., Thawng, C.N., Hwang, E.M., Seong, H.J., Sul, W.J., Wellington, E.M.H., Quince, C., Cha, C.J., 2020. Mobile resistome of human gut and pathogen drives anthropogenic bloom of antibiotic resistance. Microbiome 8 (1), 2. https://doi.org/10.1186/s40168-019-0774-7.

Li, S., Wu, Y., Zheng, H., Li, H., Zheng, Y., Nan, J., Ma, J., Nagarajan, D., Chang, J.S., 2022. Antibiotics degradation by advanced oxidation process (AOPs): recent advances in ecotoxicity and antibiotic-resistance genes induction of degradation products. Chemosphere 311 (Pt 2), 136977. https://doi.org/10.1016/j.chemosphere.2022.136977.

Li, X., Zhou, Z., Zhang, J., Zhou, S., Xiong, Q., 2021. Conversion of mixtures of soybean curd residue and kitchen waste by black soldier fly larvae (*Hermetia illucens L.*). Insects 13 (1). https://doi.org/10.3390/insects13010023.

Liang, J., Mao, G., Yin, X., Ma, L., Liu, L., Bai, Y., Zhang, T., Qu, J., 2020. Identification and quantification of bacterial genomes carrying antibiotic resistance genes and virulence factor genes for aquatic microbiological risk assessment. Water Res. 168, 115160 https://doi.org/10.1016/j.watres.2019.115160.

Liu, H., Wang, Y., Zhang, Z., Qi, H., Zhang, Y., Li, W., Shi, Q., Xie, X., 2023. Nutrient condition modulates the antibiotic tolerance of *Pseudomonas aeruginosa*. Sci. Total Environ., 166749 https://doi.org/10.1016/j.scitotenv.2023.166749.

Liu, S., Wang, P., Wang, C., Wang, X., Chen, J., 2021. Anthropogenic disturbances on antibiotic resistome along the Yarlung Tsangpo River on the Tibetan Plateau: ecological dissemination mechanisms of antibiotic resistance genes to bacterial pathogens. Water Res 202, 117447. https://doi.org/10.1016/j.watres.2021.117447.

Mch, A., Jd, A., Jeg, B., Ams, C., Bh, A., Dds, D., Kme, E., Xu, L.A., 2020. Antibiotic resistance genes in swine manure slurry as affected by pit additives and facility disinfectants. Sci. Total Environ. 761, 143287 https://doi.org/10.1016/j.scitotenv.2020.143287.

Mounica, N., Ramana, J.V., Rao, D.S., Suresh, J., Kavitha, P., 2020. Effect of replacing fish meal protein by shrimp waste meal protein with or without supplementation of amino acids on performance and cost economics of broilers. IJPS 55 (2), 129. https://doi.org/10.5958/0974-8180.2020.00019.7.

Ogunwande, G.A., Osunade, J.A., Adekalu, K.O., Ogunjimi, L.A., 2008. Nitrogen loss in chicken litter compost as affected by carbon to nitrogen ratio and turning frequency. Bioresour. Technol. 99 (16), 7495–7503. https://doi.org/10.1016/j.biortech.2008.02.020.

Pang, L., Xu, K., Qi, L., Chatzisymeon, E., Liu, X., Yang, P., 2022. Response behavior of antibiotic resistance genes to zinc oxide nanoparticles in cattle manure thermophilic anaerobic digestion process: a metagenomic analysis. Bioresour. Technol. 347, 126709 https://doi.org/10.1016/j.biortech.2022.126709.

Pruden, A., Pei, R., Storteboom, H., Carlson, K.H., 2006. Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. Environ. Sci. Technol. 40 (23), 7445–7450. https://doi.org/10.1021/es060413l.

Qian, X., Sun, W., Gu, J., Wang, X.-J., Zhang, Y.-J., Duan, M.-L., Li, H.-C., Zhang, R.-R., 2016. Reducing antibiotic resistance genes, integrons, and pathogens in dairy manure by continuous thermophilic composting. Bioresour. Technol. 220, 425–432. https://doi.org/10.1016/j.biortech.2016.08.101.

Rehman, K.U., Ur Rehman, R., Somroo, A.A., Cai, M., Zheng, L., Xiao, X., Ur Rehman, A., Rehman, A., Tomberlin, J.K., Yu, Z., Zhang, J., 2019. Enhanced bioconversion of dairy and chicken manure by the interaction of exogenous bacteria and black soldier fly larvae. J. Environ. Manag. 237, 75–83. https://doi.org/10.1016/j.jenvman.2019.02.048.

Reyes, G., Betancourt, I., Andrade, B., Panchana, F., Román, R., Sorroza, L., Trujillo, L.E., Bayot, B., 2022. Microbiome of penaeus vannamei larvae and potential biomarkers associated with high and low survival in shrimp hatchery tanks affected by acute hepatopancreatic necrosis disease. Front Microbiol 13, 838640. https://doi.org/10.3389/fmicb.2022.838640.

Roth Rosenberg, D., Haber, M., Goldford, J., Lalzar, M., Aharonovich, D., Al-Ashhab, A., Lehahn, Y., Segrè, D., Steindler, L., Sher, D., 2021. Particle-associated and free-living bacterial communities in an oligotrophic sea are affected by different environmental factors. Environ. Microbiol 23 (8), 4295–4308. https://doi.org/10.1111/1462-2920.15611.

San Millan, A., 2018. Evolution of plasmid-mediated antibiotic resistance in the clinical context. Trends Microbiol 26 (12), 978–985. https://doi.org/10.1016/j.tim.2018.06.007.

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. https://doi.org/10.1016/j.chemosphere.2006.03.026.

Shen, C., He, M., Zhang, J., Liu, J., Su, J., Dai, J., 2023. Effects of the coexistence of antibiotics and heavy metals on the fate of antibiotic resistance genes in chicken manure and surrounding soils. Ecotox Environ. Safe 263, 115367. https://doi.org/10.1016/j.ecoenv.2023.115367.

Spilsbury, F., Foysal, M.J., Tay, A., Gagnon, M.M., 2022. Gut microbiome as a potential biomarker in fish: dietary exposure to petroleum hydrocarbons and metals, metabolic functions and cytokine expression in juvenile lates calcarifer. Front Microbiol 13, 827371. https://doi.org/10.3389/fmicb.2022.827371.

Syafiuddin, A., Boopathy, R., 2021. Role of anaerobic sludge digestion in handling antibiotic resistant bacteria and antibiotic resistance genes - a review. Bioresour. Technol. 330, 124970 https://doi.org/10.1016/j.biortech.2021.124970.

Tang, B., Tang, L., Li, S., Liu, S., He, J., Li, P., Wang, S., Yang, M., Zhang, L., Lei, Y., Tu, D., Tang, X., Hu, H., Ouyang, Q., Chen, X., Yang, S., 2023. Gut microbiota alters host bile acid metabolism to contribute to intrahepatic cholestasis of pregnancy. Nat. Commun. 14 (1), 1305. https://doi.org/10.1038/s41467-023-36981-4.

Tiedje, J.M., Wang, F., Manaia, C.M., Virta, M., Sheng, H., Ma, L., Zhang, T., Topp, E., 2019. Antibiotic resistance genes in the human-impacted environment: a one health perspective. Pedosphere 29 (3), 273–282. https://doi.org/10.1016/S1002-0160(18)60062-1.

Virot, M., Tomao, V., Colnagui, G., Visinoni, F., Chemat, F., 2007. New microwave-integrated Soxhlet extraction. An advantageous tool for the extraction of lipids from food products. J. Chromatogr. A 1174 (1–2), 138–144. https://doi.org/10.1016/j.chroma.2007.09.067.

Wang, C., Su, B., Lu, S., Han, S., Jiang, H., Li, Z., Liu, Y., Liu, H., Yang, Y., 2021. Effects of glutathione on growth, intestinal antioxidant capacity, histology, gene expression, and microbiota of juvenile triploid oncorhynchus mykiss. Front Physiol. 12, 784852 https://doi.org/10.3389/fphys.2021.784852.

Wang, G., Kong, Y., Yang, Y., Ma, R., Li, L., Li, G., Yuan, J., 2022. Composting temperature directly affects the removal of antibiotic resistance genes and mobile genetic elements in livestock manure. Environ. Pollut. 303, 119174 https://doi.org/10.1016/j.envpol.2022.119174.

Wang, H., Sangwan, N., Li, H.Y., Su, J.Q., Oyang, W.Y., Zhang, Z.J., Gilbert, J.A., Zhu, Y.G., Ping, F., Zhang, H.L., 2017. The antibiotic resistome of swine manure is significantly altered by association with the *Musca domestica* larvae gut microbiome. Isme J. 11 (1), 100–111. https://doi.org/10.1038/ismej.2016.103.

Wichmann, F., Udikovic-Kolic, N., Andrew, S., Handelsman, J., 2014. Diverse antibiotic resistance genes in dairy cow manure. mBio 5 (2), e01017. https://doi.org/10.1128/mBio.01017-13.

Wu, N., Wang, X., Xu, X., Cai, R., Xie, S., 2020. Effects of heavy metals on the bioaccumulation, excretion and gut microbiome of black soldier fly larvae (*Hermetia illucens*). Ecotox Environ. Safe 192, 110323. https://doi.org/10.1016/j.ecoenv.2020.110323.

Wu, N., Wang, X., Yan, Z., Xu, X., Xie, S., Liang, J., 2021. Transformation of pig manure by passage through the gut of black soldier fly larvae (*Hermetia illucens*): Metal speciation, potential pathogens and metal-related functional profiling. Ecotoxicol. Environ. Saf. 211, 111925 https://doi.org/10.1016/j.ecoenv.2021.111925.

Xia, J., Ge, C., Yao, H., 2021. Antimicrobial peptides from black soldier fly (*Hermetia illucens*) as potential antimicrobial factors representing an alternative to antibiotics in livestock farming. Anim. (Basel) 11 (7). https://doi.org/10.3390/ani11071937.

Xu, Z., Wu, X., Zhang, J., Cheng, P., Xu, Z., Sun, W., Zhong, Y., Wang, Y., Yu, G., Liu, H., 2023. Microplastics existence intensified bloom of antibiotic resistance in livestock feces transformed by black soldier fly. Environ. Pollut. 317, 120845 https://doi.org/10.1016/j.envpol.2022.120845.

Yan, M., Zhu, C., Li, B., Su, S., Li, H., 2023. Manure application facilitated electrokinetic remediation of antibiotic-arsenic co-contaminated paddy soil. J. Hazard Mater. 441, 129897 https://doi.org/10.1016/j.jhazmat.2022.129897.

Yu, Z., Lai, Q., Li, G., Shao, Z., 2013. Parvularcula dongshanensis sp. nov., isolated from soft coral. Int J. Syst. Evol. Microbiol 63 (Pt 6), 2114–2117. https://doi.org/10.1099/ijs.0.044313-0.

Yue, Z., Zhang, J., Zhou, Z., Ding, C., Wan, L., Liu, J., Chen, L., Wang, X., 2021. Pollution characteristics of livestock faeces and the key driver of the spread of antibiotic resistance genes. J. Hazard Mater. 409, 124957 https://doi.org/10.1016/j.jhazmat.2020.124957.

Zhan, S., Fang, G., Cai, M., Kou, Z., Xu, J., Cao, Y., Bai, L., Zhang, Y., Jiang, Y., Luo, X., Xu, J., Xu, X., Zheng, L., Yu, Z., Yang, H., Zhang, Z., Wang, S., Tomberlin, J.K., Zhang, J., Huang, Y., 2020. Genomic landscape and genetic manipulation of the black soldier fly *Hermetia illucens*, a natural waste recycler. Cell Res 30 (1), 50–60. https://doi.org/10.1038/s41422-019-0252-6.

Zhang, H., Zhang, Q., Song, J., Zhang, Z., Chen, S., Long, Z., Wang, M., Yu, Y., Fang, H., 2020a. Tracking resistomes, virulence genes, and bacterial pathogens in long-term manure-amended greenhouse soils. J. Hazard Mater. 396, 122618 https://doi.org/10.1016/j.jhazmat.2020.122618.

Zhang, M., He, L.Y., Liu, Y.S., Zhao, J.L., Zhang, J.N., Chen, J., Zhang, Q.Q., Ying, G.G., 2020b. Variation of antibiotic resistome during commercial livestock manure composting. Environ. Int 136, 105458. https://doi.org/10.1016/j.envint.2020.105458.

Zhang, Q.Q., Ying, G.G., Pan, C.G., Liu, Y.S., Zhao, J.L., 2015. Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modeling, and linkage to bacterial resistance. Environ. Sci. Technol. 49 (11), 6772–6782. https://doi.org/10.1021/acs.est.5b00729.

Zhang, T., Fukuda, K., Topp, E., Zhu, Y.G., Smalla, K., Tiedje, J.M., Larsson, D.G.J., 2020c. Editorial: the environmental dimension of antibiotic resistance. FEMS Microbiol Ecol. 96 (8) https://doi.org/10.1093/femsec/fiaa130.

Zhang, Y., Xiao, X., Elhag, O., Cai, M., Zheng, L., Huang, F., Jordan, H.R., Tomberlin, J.K., Sze, S.H., Yu, Z., Zhang, J., 2022. *Hermetia illucens* L. larvae-associated intestinal microbes reduce the transmission risk of zoonotic pathogens in pig manure. Micro Biotechnol. 15 (10), 2631–2644. https://doi.org/10.1111/1751-7915.14113.

Zhao, Z., Yu, C., Yang, C., Gao, B., Jiménez, N., Wang, C., Li, F., Ao, Y., Zheng, L., Huang, F., Tomberlin, J.K., Ren, Z., Yu, Z., Zhang, J., Cai, M., 2023. Mitigation of antibiotic resistome in swine manure by black soldier fly larval conversion combined with composting. Sci. Total Environ. 879, 163065 https://doi.org/10.1016/j.scitotenv.2023.163065.

Zheng, L., Hou, Y., Li, W., Yang, S., Li, Q., Yu, Z., 2012. Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. Energy 47 (1), 225–229. https://doi.org/10.1016/j.energy.2012.09.006.

Zhou, J., Bruns, M.A., Tiedje, J.M., 1996. DNA recovery from soils of diverse composition. Appl. Environ. Microbiol 62 (2), 316–322. https://doi.org/10.1128/aem.62.2.316-322.1996.

Zhou, Y., Li, J., Huang, F., Ai, H., Gao, J., Chen, C., Huang, L., 2023. Characterization of the pig lower respiratory tract antibiotic resistome. Nat. Commun. 14 (1), 4868. https://doi.org/10.1038/s41467-023-40587-1.

Zou, Y., Xiao, Y., Wang, H., Fang, T., Dong, P., 2020. New insight into fates of sulfonamide and tetracycline resistance genes and resistant bacteria during anaerobic digestion of manure at thermophilic and mesophilic temperatures. J. Hazard Mater. 384, 121433 https://doi.org/10.1016/j.jhazmat.2019.121433.