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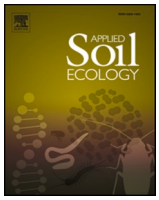

Research paper

Cessation of manure application diminishes the dissemination potential of antibiotic resistance genes by altering bacterial interaction patterns in soil–lettuce systems

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ABSTRACT

The application of livestock manure is a common waste utilization measure that can increase soil fertility and crop yields, but the antibiotics and resistance genes involved result in a potential threat to human health and animal welfare. Here, lettuce, a widely popular edible vegetable, was selected as a model with which to investigate the effects of long-term application (FM) and temporary cessation (cessation) of fresh chicken manure on the distribution and movement of antibiotic resistance genes (ARGs) in the soil-root-plant system to elucidate the bottleneck in assessing the health risks of manure application. ARGs associated with 13 antibiotics and 384 subtypes in soil were quantitatively analyzed via high-throughput qPCR, and the results revealed that cessation treatment significantly affected the patterns of bacteria, mobile genetic elements (MGEs) and ARGs in the soil, leaves and roots in the soil–lettuce cropping system compared with FM treatment. Cessation of manure application reduced the abundance of ARGs by 34.0 %, 53.7 %, and 23.9 % in the bulk soil, rhizosphere soil, and leaves, respectively. Correlation network and source-tracking analyses of ARGs and bacteria within leaves and roots revealed that cessation treatment reduced the diffusion of ARGs and bacteria within leaves and roots into adjacent sites, and partial least squares path model (PLSPM) analysis indicated that FM treatment indirectly affected the pattern of ARGs in soil by influencing the bacterial community and soil properties, which play key roles in the distribution of ARGs. In summary, we investigated the driving mechanism of the effects of manure on the microbial community and ARG spectrum in a soil–lettuce planting system, and the results can support strategies for managing the spread of ARGs in the soil.

1. Introduction

The spread and reproduction of antibiotic resistance genes (ARGs) and antibiotic resistance bacteria (ARB) in the environment threatens human health as well as the future use of antibiotics (Forsberg et al., 2012; Qian et al., 2022). Antibiotic uses increased by 65 % between 2000 and 2015, and based on current application rates, global antibiotic use is expected to increase by another 15 % by 2030 (Klein et al., 2018). In recent years, many veterinary antibiotics have been commonly used in animal husbandry as antibacterial agents and growth promoters (Palma et al., 2020). Livestock production uses almost twice as many antibiotics as humans do worldwide. However, most antibiotics have polar groups and high solubility in water, and many antibiotics used in livestock production are poorly absorbed in the animal intestine, resulting in 30 to 90 % of the residues being excreted in feces or urine (Sarmah et al., 2006; Zhou et al., 2013). Residual antibiotics induce the generation of ARB and ARGs (Andersson and Hughes, 2014; Gullberg et al., 2011), and even at low levels, antibiotics can increase the emergence and transmission of ARGs by triggering genetic mutations and recombination in microorganisms (Gullberg et al., 2011; López and

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Blázquez, 2009), leading to ecological and environmental problems.

Soil microbial communities contribute to the development of aboveground plant bacterial communities, and the recruitment of plant-associated bacterial communities is influenced by the biotic reservoirs found in the soil (Bai et al., 2015). Moreover, bacterial interactions encompass all forms of relationships between bacteria, which can range from mutualistic and symbiotic to antagonistic. In the soil environment, these interactions play a crucial role in shaping the structure and function of microbial communities, influencing nutrient cycling, pathogen suppression, and the transfer of genetic material, including ARGs (Molina-Santiago et al., 2021). The ARGs originating from soil resistomes may enter the food chain through contaminated crops and be transferred to human pathogens via horizontal gene transfer (HGT), posing a potential threat (Koonin et al., 2001; Wolters et al., 2016). In addition to the intrinsic resistance that may exist in some soil microbes, ARGs in soil originate primarily from anthropogenic activities such as irrigation with reclaimed wastewater and the application of manure and sewage sludge (Wang et al., 2022a). These activities not only facilitate the horizontal transfer of ARGs through mobile genetic elements such as transposons, plasmids, and integrons but also promote the proliferation of antibiotic-resistant bacteria and ARGs in soil through selective pressure, exacerbating soil ARG contamination. Reports have revealed an overlap of microbiomes and resistant groups in plant ecosystems, indicating the potential for soil-to-plant transmission of ARGs and their hosts (Chen et al., 2017; Wang et al., 2023). Therefore, it is necessary to understand the characteristics of environmental ARGs and their interactions with microorganisms to develop potentially effective strategies to minimize their spread.

The edible parts of vegetables serve as one of the primary pathways through which ARB and ARGs are transferred from the natural environment to humans (Wang et al., 2022a). In agricultural systems, microbial activity and movement from the soil to the edible parts significantly impact human food safety. The plant leaves are considered significant reservoirs of ARGs, as the high potential for biofilm formation on leaf surfaces also represents an active HGT zone (Wang et al., 2015). ARGs in leaves have multiple sources, such as soil, fertilizers, irrigation water, and air. ARGs exist as free DNA or are carried by ARB, which accumulate on leaves through internal or external transfer pathways (Zhou et al., 2019). Internally, the separation of root surface cells provides a pathway for soil-associated ARB to migrate from the rhizosphere to endophytic roots and leaves (Bulgarelli et al., 2013). In contrast, external transfer pathways involve the direct deposition of ARB from soil, air, or water onto the plant layer. However, a detailed investigation of the transfer of ARGs within the soil–plant system is not feasible. Therefore, obtaining information on the transfer characteristics within the soil–plant system and their contribution to ARG accumulation in leaf layers is crucial for controlling ARG dissemination in plants and transmission to humans through the food chain.

In previous studies, we investigated the dynamic effects of fresh manure, compost and mineral fertilizer on the soil microbiome and resistome in ARG-contaminated soils (Li et al., 2022). However, in many regions of China, untreated chicken manure produced on farms is directly applied to agricultural fields to enhance soil fertility, while the severe pollution from ARGs is overlooked. Currently, we are implementing several initiatives to mitigate ARG contamination in soils and plants. In this study, we conducted a field experiment involving long-term application and temporary cessation of fresh chicken manure, with lettuce used as the model plant, to investigate 1) the response of ARGs in soil–lettuce systems to terminated manure inputs; 2) changes in ARG hosts in microbial communities and their relationships with resistance genes; and 3) the mechanisms that drive the distribution of ARGs. The application of high-throughput qPCR was used to characterize ARGs and mobile genetic elements (MGEs) in both soil and lettuce, along with bacterial community network analysis in the present study, which could provide valuable insights into the mobility and distribution of ARGs under different fertilization practices. This study contributes to our understanding of how fertilization strategies influence ARG dynamics, offering potential solutions for mitigating ARG pollution in agricultural systems.

2. Materials and methods

2.1. Field experiment and sample collection

The field trial was performed in long-term amended soil with chicken manure in Jiangsu Province, China (32°66′N, 120°70′E), which has a typical northern subtropical monsoon climate with an average annual temperature and precipitation of 14.5 °C and 1025 mm, respectively. Two field treatments were selected for this study: 1) cessation of application of manure (cessation) and 2) continuous fresh chicken manure application (FM). In the long-term experimental field, fertilization was carried out according to local management practices. Over the past 30 years, two to three seasons of vegetable crops have been rotated annually, with a fresh chicken manure application rate of 75 tons per hectare for each season. This experiment followed a two-season lettuce planting schedule. In the first season (March to June), fresh chicken manure was applied as the base fertilizer in early February, which is 30 days before lettuce transplanting, at a rate of approximately 30 tons per hectare. Meanwhile, for autumn planting (September to December), the same amount of fresh chicken manure was applied in early August. The fresh chicken manure used in this study was directly collected from the waste discharge area of a local poultry farm. No pretreatment, such as composting, was conducted prior to its use in the experiment. The antibiotic residues in the chicken manure are shown in Table S1 of the Supplementary materials. The concentrations of aminoglycoside, tetracyclines, sulfonamides, Macrolide-Lincosamide-Streptogramin B and fluoroquinolones in chicken manure were quantified using the high-performance liquid chromatography (HPLC) method developed by Xie et al. (2016). The fresh chicken manure has a moisture content of 75 %, and its nutrient levels are as follows: nitrogen (N) 21.7 $g{\cdot}kg^{-1}$, phosphorus (P_2O_5) 17.7 $g{\cdot}kg^{-1}$, and potassium (K_2O) 46.5 $g{\cdot}kg^{-1}$. In the fertilization cessation treatment, only chemical fertilizers providing equivalent nutrition to the fresh manure treatment were used, with urea as the nitrogen source, superphosphate as the phosphorus source, and potassium sulfate as the potassium source. The treatments were replicated in three plots, each of which measured 10 m × 4 m. A total of 400 plants were planted in each plot. The amount of fresh chicken manure used in this experiment was 120 kg, and fertilizer was applied to the soil prior to planting.

Lettuce was planted from September to December 2021, and soil and plant samples were collected during the harvest period. Each treatment was randomly sampled three times, and each replicate consisted of three composite samples of soil cores and lettuce plants. For bulk soil (BS) sample collection (Li et al., 2021b), soil were collected to a depth of 20 cm. After the soil samples passed through a 2-mm sieve and were thoroughly mixed, a portion of each soil sample was air-dried for chemical property measurements, and the other portion was stored at −80 °C for further DNA extraction. The aboveground (leaves, L) and belowground (root, R) parts of the lettuce were separated with ethanol-sterilized scissors and collected separately in sampling bags, and the soil attached to the lettuce roots was collected as rhizosphere soil samples, which were collected according to Tao's method (Tao et al., 2020). Briefly, the root samples were shaken to remove bulk soil and rinsed with sterile stroke–physiological saline solution. Then, the supernatant of the mixture was separated via centrifugation at 10,000 ×g for 10 min, and the precipitate was defined as rhizosphere soil (RS). Prior to DNA extraction, the soil and plant samples were stored in sterile plastic bags at −80 °C and 4 °C, respectively (Fatima et al., 2014; Liu et al., 2015).

2.2. DNA extraction and sequencing

All the soil samples were prepared for 16S rRNA gene sequencing.

According to the manufacturer's instructions for the Soil Genomic DNA Extraction Kit (TIANGEN Biotech Co., Ltd. China), microbial DNA was extracted from 0.5 g of rhizosphere soil. A NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) was used to determine the concentration and quality of the DNA samples. Lettuce leaf and root DNA was extracted according to the methods of previous studies (D'Amico et al., 2008). To ensure consistency across treatments, root and leaf samples were collected from the same anatomical locations of each plant for all treatment groups. Root samples were obtained from the primary root system, while leaf samples were taken from mature, healthy leaves of similar developmental stages. Prior to DNA extraction, surface contaminants were removed from root samples, while the majority of leaf veins and damaged areas were excluded from the leaf samples. Briefly, the lettuce leaves and root surfaces were first washed with 75 % ethanol, sterilized by soaking in 75 % ethanol, 2.5 % sodium hypochlorite, and 75 % ethanol, and finally rinsed with sterile water. The roots and leaves were cut into small pieces, ground with liquid nitrogen and extracted via a Plant Genomic DNA Extraction Kit (TIANGEN Biotech Co., Ltd. China). Total microbial DNA was extracted from the samples, after which the total amount and purity of the DNA samples were tested.

Bacterial sequencing libraries were constructed according to the MiSeq Reagent Kit Preparation Guide (Illumina, San Diego, CA, USA) as described previously (Caporaso et al., 2012). The paired-end amplicons were sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) according to standard protocols at Magigene Technology Co., Ltd. (Guangdong, China). The forward primer 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and the reverse primer 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) were used to amplify the V4-V5 hypervariable region of the bacterial 16S rRNA gene. The raw sequence data of all the samples were submitted to the NCBI Sequence Read Archive database (https://www.ncbi.nlm.nih.gov/) under accession number PRJNA1091716.

2.3. Quantification of ARGs, MGEs, and bacterial taxa

With the SmartChip Real-Time PCR System (Wafergen Inc., Fremont, CA, USA), the corresponding qPCR primers designed with 384 resistance genes (319 ARGs, 57 MGEs, 7 taxonomic genes, and 16S rRNA genes) were packaged into a thin-layer metal alloy nanopore chip to obtain a high-throughput qPCR chip, which was used to detect and quantify target genes with high efficiency, high throughput, high precision and high sensitivity in soil and lettuce samples (Zhu et al., 2013, 2017). The DNA samples and reagents for qPCR were added to a 384-well plate as a sample source plate, while the primers and reagents for qPCR were added to another 384-well plate as an assay source plate. High-throughput automatic microsampling equipment was used to add the reagents of the sample plate and the primer plate to the nanowells of the high-throughput qPCR chip, and qPCR and fluorescence signal detection were carried out with the SmartChip Real-Time PCR System. An amplification curve and solubilization curve were generated automatically.

According to the Ct value of each gene in each sample given by the SmartChip Real-Time PCR System and Canco software, the relative copy number of each gene and 16S rRNA was calculated according to the following formula: relative copy number $= 10^{\wedge(31\text{-}Ct)/(10/3)}$. The absolute quantitative information of each gene in each sample was obtained by converting the absolute quantitative information of 16S rRNA genes according to the following formula: 16S relative quantification/16S absolute quantification = gene relative quantification/gene absolute quantification.

2.4. Soil chemical properties

The determination of the soil chemical properties was conducted according to the previously described methods (Li et al., 2021b). Specifically, nitrate and ammonium concentrations were measured in the filtered supernatant via a Lachat QuikChem 8500 flow injection analyzer (Hach, Loveland, CO). The soil pH was determined with a glass electrode in a 1:2 slurry of air-dried soil in 0.01 M $CaCl_2$. Soil EC was measured for the water extracts containing the 1:5 soil/water ratios (w/v). Total nitrogen (TN) and soil organic carbon (SOC) were determined in oven-dried soil via dry combustion using an elemental analyzer (Costech ECS 4010, Valencia, CA, USA). The soil available phosphorus (AP) was extracted with $NaHCO_3$ solution and finally measured via molybdenum antimony spectrophotometry. Available potassium (AK) was analyzed via the NH_4OAc extraction and flame photometry methods.

2.5. Statistical analysis

Bacterial raw sequences were processed by trimming adaptors and primer sequences using cutadapt (https://github.com/marcelm/cutadapt) (Xiong et al., 2020). The trimmed sequences were then analyzed with the UPARSE pipeline (Edgar, 2013). Sequences with a quality score below 0.5 or shorter than 200 bp were excluded. After removing singletons, bacterial sequences were clustered into OTUs at 97 % similarity (Edgar et al., 2011). Taxonomic classification of bacterial OTUs was performed using the RDP database version 11.5 (http://rdp.cme.msu.edu/) (Wang et al., 2007).

Alpha diversity was assessed using the Shannon index, and beta diversity was evaluated by principal coordinate analysis (PCoA) based on the Bray-Curtis distance. These analyses were performed using the 'vegan' package in R version 4.0.4 (Oksanen et al., 2020). SourceTracker (v. 1.0), which is based on the Bayesian approach, was used to estimate the sources of plant-associated bacterial communities in the different plant compartments (Knights et al., 2011). The R package "plspm" was used for partial least squares path modeling (Tang et al., 2023). The two-sample *t*-test was performed using SPSS 25.0 (SPSS, Chicago, Ill., USA) to explore significant differences in variables and the relative abundances of microbial taxa and ARGs were explored between treatments. Gephi (v. 5.2.2011) was used to visualize the co-occurrence network (Bastian et al., 2009). Spearman correlation analyses between the relative abundances of microbial communities and ARGs were performed via the 'psych' package in R version 4.0.4 (https://mirrors.tuna.tsinghua.edu.cn/CRAN/src/base/R-4/R-4.0.4.tar.gz).

3. Results

3.1. Content and composition of ARGs in soil–lettuce cropping systems

A total of eight ARGs and four MGEs, which consisted of 235 subtypes, were detected in all the samples via high-throughput PCR (Figs. 1; S1). Aminoglycoside resistance genes with 45 subtypes were most frequently identified, accounting for 22.26 % of the total abundance of ARGs. Multidrug resistance genes of 35 subtypes were identified and recognized as the most abundant resistance genes in all the soils, as they accounted for 27.88 % of the total abundance of ARGs (Fig. 1a, b). In addition, the antibiotics corresponding to the top five most abundant ARG groups (Fig. 1b) (excluding multidrug resistance) corresponded to antibiotics (tetracyclines, aminoglycosides, sulfonamides, MLSB and fluoroquinolones) that all were detected in fresh manure (Table S1).

At the overall level of ARGs, PCoA revealed that the composition of ARGs significantly differed ($P < 0.01$) in different parts of the soil and lettuce (Fig. 1c). Among the 235 subtypes of ARGs detected, 77 subtypes were found in all four parts of the lettuce-soil system: leaves, roots, rhizosphere soil, and bulk soil. Additionally, 9 unique subtypes were identified in the leaves, 7 in the roots, 10 in the bulk soil, and 30 in the rhizosphere soil.

The cessation of manure application significantly affected the abundance of ARGs and MGEs in the soil and lettuce (Fig. S2). The results revealed that, compared with FM, cessation significantly reduced

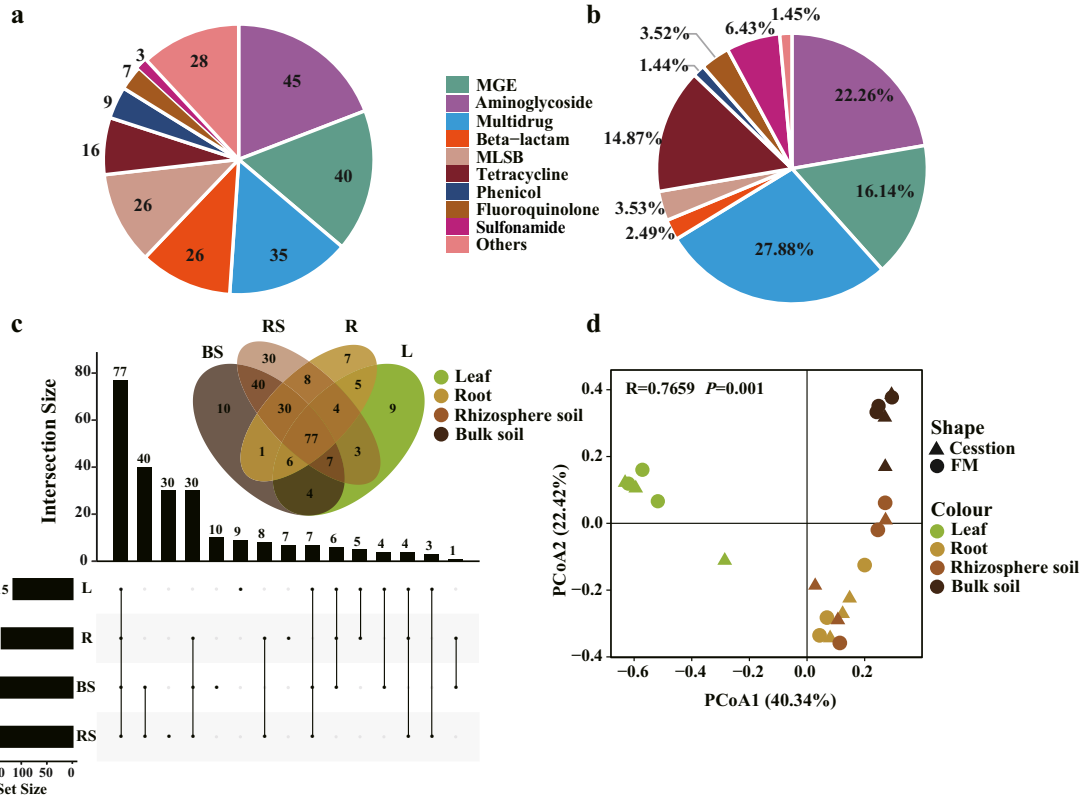

Fig. 1. Number (a) and normalized (b) abundance of ARGs in all the soil samples detected via high-throughput quantitative PCR methods. Venn diagram showing common and specific ARGs at different sites (c). Principal coordinate analysis (PCoA) plots showing the composition of ARG subtypes among different sites and treatments based on the Bray–Curtis distance matrix (d). MLSB: Macrolide-Lincosamide-Streptogramin B.

the absolute abundances of ARGs and MGEs in soil and lettuce by 32.2 % and 44.5 %, respectively. To explore the effects of the two treatments on ARGs in the roots and leaves of lettuce, we compared the total abundance of ARGs (Fig. S2) and the abundance of specific broad categories of resistance genes (Fig. 2). The results revealed that in lettuce leaves, cessation treatment significantly reduced the abundance of ARGs by 23.9 %; specifically, the abundance of fluoroquinolone resistance genes was reduced by 33.3 %, that of multidrug resistance genes was reduced by 38.2 %, and that of tetracycline resistance genes was reduced by 35.2 %. There was no significant difference in the total abundance of ARGs in the roots, but the cessation treatment reduced the abundance of multidrug resistance genes, tetracycline resistance genes, sulfonamide resistance genes, and β-lactamase resistance genes in the roots.

3.2. *Cessation of manure application affects the transmission of ARGs in soil–lettuce systems*

Source tracking revealed that the ARG subtypes in rhizosphere soil were derived from soils with different fertilization practices. However, the percentage of endophytic ARGs differed between the two fertilization treatments (Fig. 3a). In the FM treatment, 33.35 % of leaf ARGs originated from the roots, and 36.01 % of those in the roots originated

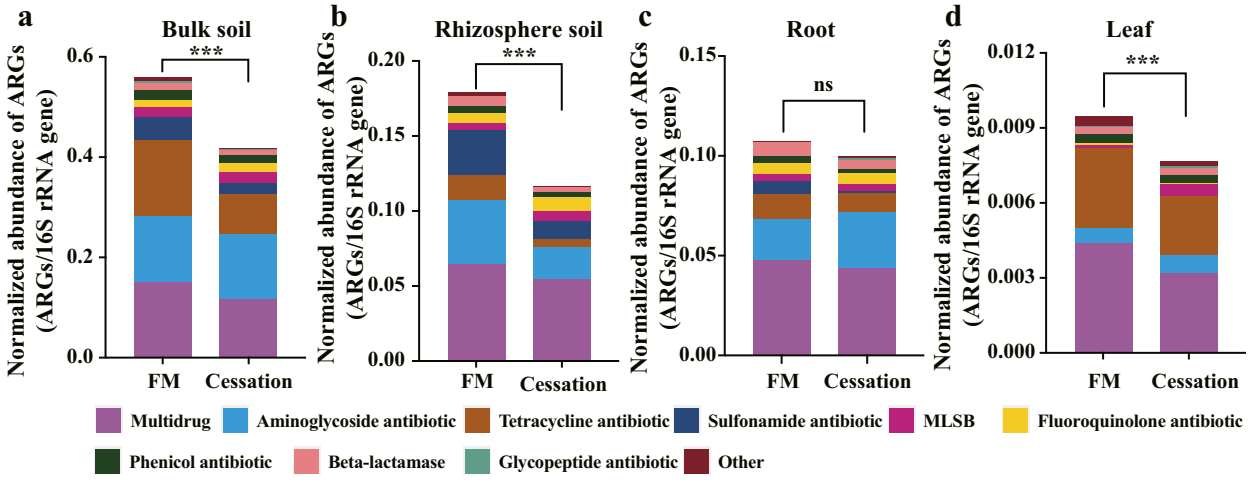

Fig. 2. Stack bar chart showing the normalized abundance (ARGs/16S rRNA) of ARG types in different parts of the soil–lettuce system (*t*-test, mean SD, $n = 3$; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). MLSB: Macrolide-Lincosamide-Streptogramin B.

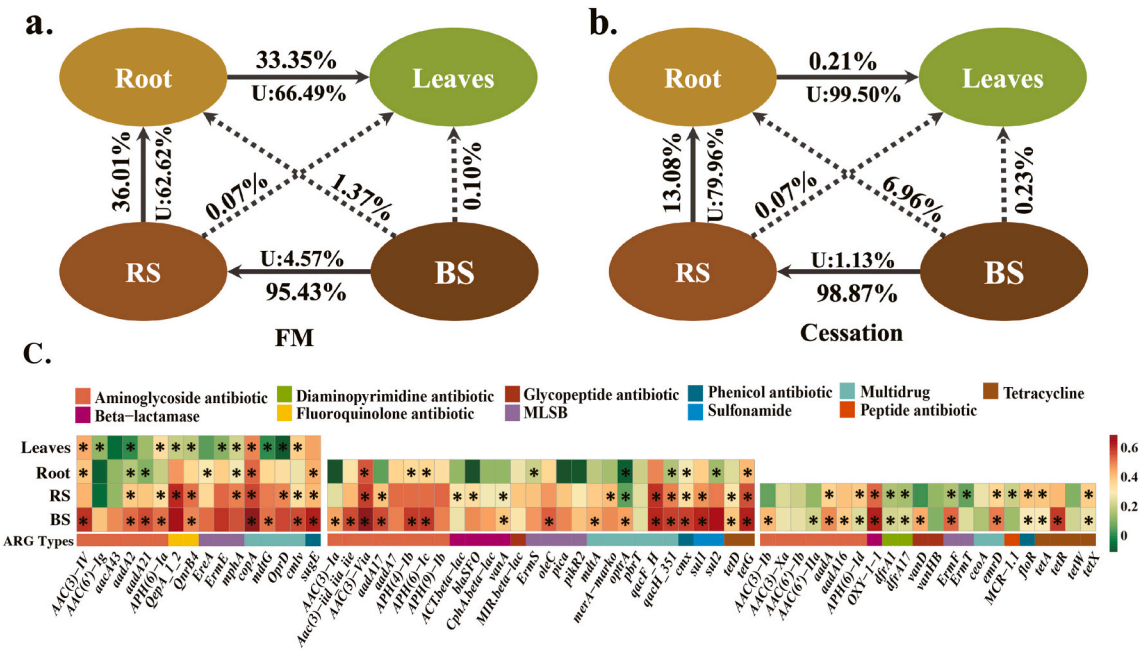

Fig. 3. Potential sources of plant-associated microbiome ARGs (a, b) under the two fertilization regimes. BS, bulk soil; RS, rhizosphere soil. U represents the unknown source, and the percentages represent the direct source contributions. Transfer of ARGs in the soil and lettuce (c). The symbol "*" indicates that the relative abundance of ARGs was significantly greater in the FM treatment group than in the cessation treatment group (*t*-test, mean ± SD, n = 3; $*p < 0.05$). MLSB: Macrolide-Lincosamide-Streptogramin B.

directly from the rhizosphere soil, whereas in the cessation treatment, 0.21 % and 13.08 % of the ARGs in the leaves and roots respectively originated from adjacent compartments (Fig. 3b), indicating that cessation treatment reduced the diffusion of ARGs within the leaves and roots into adjacent compartments.

Compared with the absence of manure application, continuous manure application significantly influenced the transmission subtypes of ARGs in the soil–lettuce system (Fig. 3c). Sixteen subtypes of ARGs, including those conferring resistance to aminoglycoside, multidrug, Macrolide-Lincosamide-Streptogramin B (MLSB), phenicol and fluoroquinolone, were continuously present from the soil to the leaves. After the cessation of manure treatment, the accumulation of major ARG subtypes in the leaves, such as *AAC(3)-IV*, *QnrB4*, *copA*, *mdtG*, *cmlv*, and *mphA*, decreased. Concurrently, ARGs influenced by fertilization in other compartments of the soil–lettuce system present varying degrees of significant discrepancy, with aminoglycoside resistance, multidrug resistance, MLSB resistance and fluoroquinolone resistance predominantly represented.

3.3. The impact of cessation fertilization on the relative abundance of bacterial communities in the soil–lettuce system

The doses (level) of fertilization significantly affected the abundance of microbial families within the soil–lettuce system (Fig. 4). Due to cessation of fertilization, the relative abundances of Xanthomonadaceae and Pseudomonadaceae were significantly reduced in the plant leaves, roots, and rhizosphere soil. Additionally, the relative abundance of Rhizobiaceae in the lettuce roots, rhizosphere, and bulk soil in the cessation treatment was significantly lower than in the FM treatment. Continuous manure application significantly increased the relative abundance of Bacillaceae in lettuce roots and bulk soil. In both the roots and rhizosphere soil, the relative abundances of Erwiniaceae and Gaiellaceae significantly decreased following the cessation of fertilization, whereas Cellvibrionaceae exhibited the opposite trend.

The connections among bacteria in the soil–lettuce system were revealed by constructing a symbiotic network (Fig. S3). Co-occurrence network networks revealed that microbial interactions were also affected by fertilization. The point-to-point covariance in the network reveals the space of ecological niches shared by members of the microbial community, and each model in the symbiotic network may have an ecological niche and function. The network topology indicated that the number of nodes and connections of the bacterial network in the soil with chicken manure (FM) input was greater than that in the cessation treatment, and the average weighted degree (AWD) showed an identical trend. Within the leaves of lettuce, the density (D) of the bacterial symbiotic network was greater in the FM treatment than in the cessation treatment.

3.4. Potential derivations of bacteria and ARGs in soil–lettuce systems

To explore the variations in potential bacterial hosts of ARGs shared between leaves and other compartments, we constructed a correlation network between bacterial families and ARGs, retaining portions that both presented significant positive correlations within the network (Fig. 5). In both leaves and roots, the shared microbiota Rhizobiaceae, Moraxellaceae, Bacillaceae and Paenibacillaceae were significantly positively correlated with aminoglycoside resistance. Additionally, the shared microbiota, such as Pseudonocardiaceae, Burkholderiaceaes and Lachnospiraceae, among the roots, rhizosphere and bulk soils were significantly correlated with the *mdG* value. Some microbiota, such as Devosiaceae and Clostridiaceae, were unique to leaves, which may be related to microbial community selection in the environment (Figs. 5 and S4A). In addition, indirect relationships between ARGs and microorganisms in lettuce leaves and those in the rhizosphere and bulk soils were also observed (Fig. S4B). Microorganisms within leaves, such as Moraxellaceae, Rhizobiaceae, and Microbacteriaceae, which are also present in the soil, are significantly related to shared ARGs. Notably, the abundances of Moraxellaceae, Rhizobiaceae, and Bacillaceae in the fertilization treatment were significantly greater than those in the cessation treatment, and similar patterns were also observed for the shared ARGs. Additionally, we found a significant positive correlation between detected zoonotic pathogens and ARGs in chicken manure (Fig. S6).

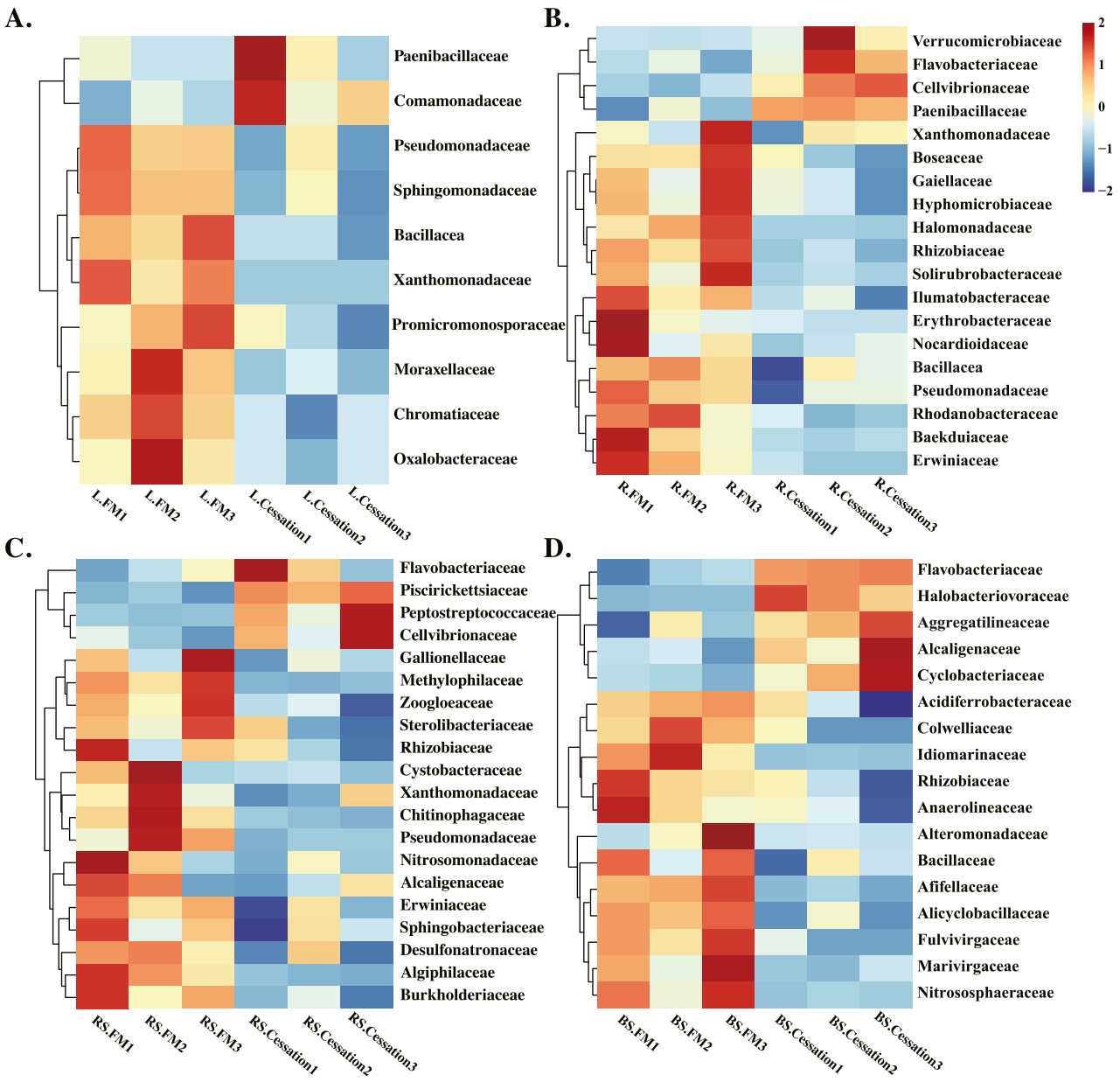

Fig. 4. The degree of fertilization significantly affects the microbial community at the family level within the soil–lettuce system, including A: within lettuce leaves, B: within roots, C: in the rhizosphere soil, and D: in the bulk soil. An independent samples *t*-test was employed to compare the differences between the two treatments ($p < 0.05$). L, leaves; R, root; RS, rhizosphere soil; BS, bulk soil.

3.5. Effect of cessation of fertilization on soil properties and bacterial communities is a factor influencing the patterns of soil ARGs and MGEs

As indicated in Table 1, after the cessation of continuous manure application, the soil EC significantly increased. In addition, the cessation of fertilization also significantly affected soil pH, available nutrients, including nitrate nitrogen, ammonium nitrogen, available potassium, and available phosphorus, all of which markedly decreased. Nevertheless, no significant effect on TN or SOC was observed.

Furthermore, the partial least squares path model (PLSPM) showed direct and indirect effects of multiple factors: the effects of cessation of fertilization on soil properties and bacterial communities influenced the patterns of soil ARGs and MGEs (Fig. 6). The cessation of fertilization exerts a primary direct negative effect on soil properties (negative pathway). Soil properties indirectly regulate the accumulation of ARGs in soil by influencing the bacterial community and bacterial diversity. The bacterial community positively promotes MGEs, which in turn directly and positively regulate the ARGs in soil. In plants, the cessation of fertilization indirectly affects ARGs in plants through soil ARGs. Additionally, the bacterial community and bacterial diversity in soil play critical roles in the regulation of ARGs in plants.

4. Discussion

4.1. Significant effects of ceasing manure application on the transmission potential of ARGs

In this study, the number of ARGs and MGEs was greater in soils with long-term manure application than in soils and plant leaf layers where manure application had ceased. This suggests that fresh chicken manure serves as a reservoir for various ARGs, posing a potential risk of introducing exogenous ARGs into agricultural fields and crops (Zhou et al., 2019). This is consistent with our findings, where the antibiotics corresponding to the top five most abundant ARGs in the soil-lettuce system

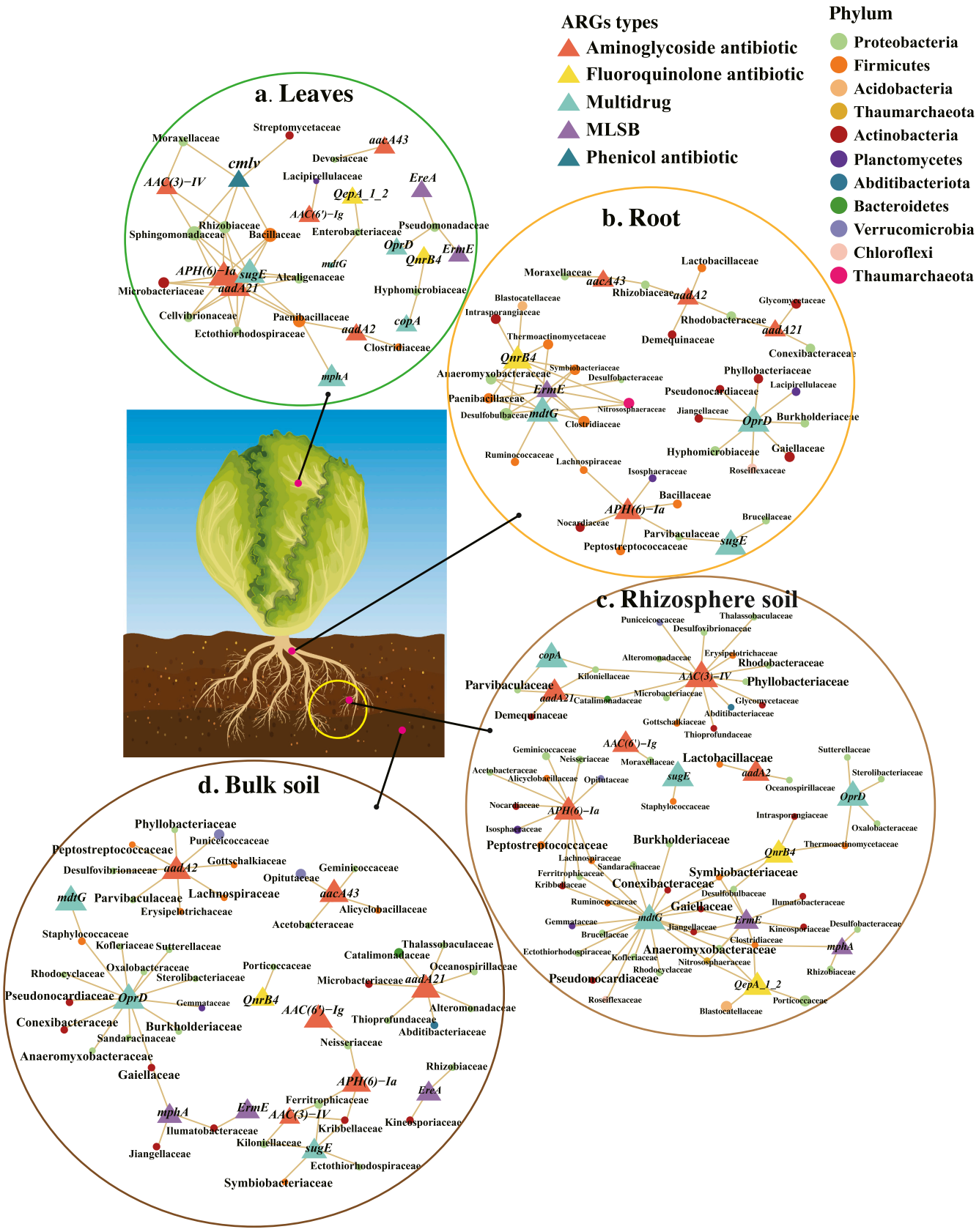

Fig. 5. Distribution of shared ARGs and potential microbial hosts in the soil–lettuce system. The internal network explained the positive Spearman correlation between microbes and ARGs (rho >0.8) and was statistically significant ($p < 0.01$). Microorganisms at the same taxonomic family level are displayed at different sites. MLSB: Macrolide-Lincosamide-Streptogramin B.

Table 1
The impact of continuous fertilization and cessation of fertilization on soil chemical properties.

Item	pH	EC (μS/cm)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	AP (mg/kg)	AK (mg/kg)	TN (g/kg)	SOC (g/kg)	C/N
Cessation	7.39	685.00*	28.73	12.62	80.53	156.61	0.84	14.46	17.49
FM	7.68*	521.33	35.65*	20.99*	118.18*	239.31*	1.01	17.56	17.48

An independent samples *t*-test was employed to compare the differences between the two treatments (*p < 0.05). EC = electrical conductivity, AP = available phosphorus, AK = available potassium, TN = total nitrogen, SOC = soil organic carbon.

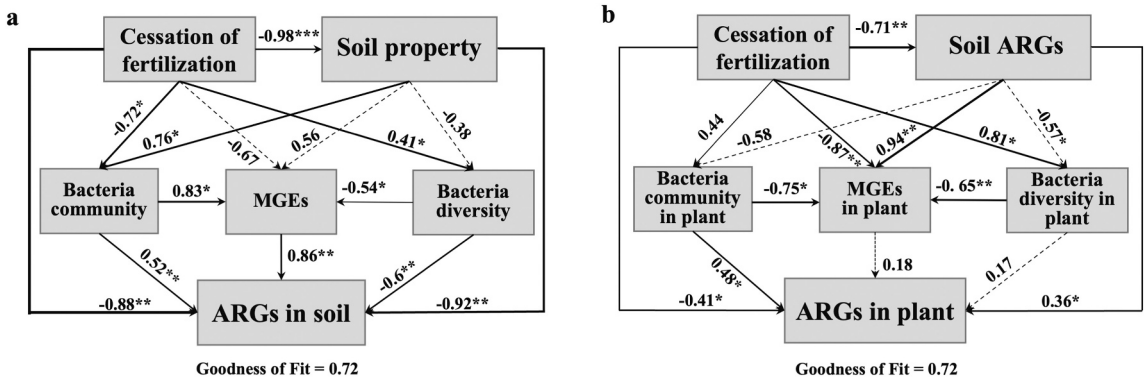

Fig. 6. The PLSPM showed direct and indirect effects of multiple factors: the composition of antibiotic-resistant groups in soil (a) and lettuce (b). The solid and dotted lines indicate direct and indirect effects on the target, respectively (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

were detected in fresh chicken manure (Fig. S5). Previous studies have shown that the long-term application of manure significantly increases the quantity and abundance of antibiotic resistance genes in both soil and cultivated plants (Liu et al., 2021; Wang et al., 2015; Xie et al., 2018). Our study revealed that cessation of manure inputs significantly reduced the overall content of ARGs in soil and lettuce leaves. Notably, among the ARGs shared between the leaf interior and other parts, aminoglycoside and multidrug resistance genes were more abundant than other types of ARGs, which may be related to their widespread use in agriculture (Zhao et al., 2018) and the fact that reduced inputs of manure reduce their accumulation in the soil and lettuce plants.

Source-tracking analysis was used to trace and determine the impact of different fertilization practices on ARGs in the soil–lettuce system. The analysis revealed that the cessation of manure application reduced the spread of ARGs from the rhizosphere to the root interior and from the root interior to the leaf interior. The rhizosphere of plants is a major hotspot for bacterial activity in the soil. Enhanced nutrient inputs and water fluxes within the rhizosphere have been implicated in promoting conjugative plasmid transfers among microbial inhabitants (Xiao et al., 2023). Additionally, the plant phyllosphere has been demonstrated to similarly facilitate conjugative plasmid transfer (Van Elsas et al., 2003). Recent studies have further suggested that the horizontal gene pool in the phytosphere is highly mobile and directly associated with host fitness, as reflected by the successful colonization of successive microbial populations on developing plants (Kläui et al., 2024; Wang et al., 2022b). Cessation of manure application not only halts the input of external sources but also alters the physicochemical properties and microbial community structure of the soil, thereby influencing the mobility of ARGs within the soil–lettuce system.

4.2. The cessation of fresh chicken manure affected the bacterial communities within the soil–lettuce system

We found that the cessation of manure application led to specific changes in the soil and plant endophytic microbial communities, which is consistent with previous findings tracking responses to different fertilization management practices (Liu et al., 2017). Compared with continuous manure application, the cessation of manure input decreased both the relative abundance of soil–lettuce-associated microbes and the complexity of microbial networks, suggesting that shifts in bacterial community composition and abundance have significant functional implications (Bonanomi et al., 2016).

In this study, we found that the cessation of manure application resulted in a significant decrease in the relative abundance of Pseudomonadaceae and Bacillaceae in both soil and lettuce. Research indicates that *Bacillus* and *Pseudomonas* are two of the predominant bacterial genera within plant microbiomes (Wei et al., 2019). Their beneficial effects on plant resistance against fungal and bacterial pathogens have been extensively studied, with mechanisms including direct antagonism and indirect activation through the induction of plant defense responses (ISRs) (Kloepper et al., 2004). Many well-known antibiotic-producing bacteria, particularly those in the genera *Pseudomonas* and *Bacillus*, are capable of producing a variety of antibiotics and secondary metabolites with broad-spectrum antimicrobial activity that inhibits the growth of other microorganisms (Molina-Santiago et al., 2021). Interestingly, we found that the population densities of Pseudomonadaceae and Bacillaceae in the lettuce leaves were positively correlated with each other and were significantly positively coincided with ARGs, including multidrug resistance genes, aminoglycoside resistance genes, and MLSB resistance genes, which is consistent with the findings of previous studies (Li et al., 2024; Thacharodi and Lamont, 2022). Additionally, following the cessation of manure application, there was a notable reduction in the abundance of Xanthomonadaceae in the soil–lettuce system. Although members of this family, especially the genus *Xanthomonas*, are more commonly recognized as plant pathogens than major antibiotic producers, their decrease is noteworthy (Timilsina et al., 2020). In addition to bacterial communities, symbiotic networks revealed that microbial interactions were also affected by fertilization. The point-to-point covariance in the network reveals the space of ecological niches shared by members of the microbial community, and each model in the symbiotic network may have an ecological niche and function (Barberán et al., 2012). Our results revealed that cessation of manure application reduced the number of microbial nodes and their density in the soil–lettuce system. Previous research has indicated that organic fertilization enhances the overall bacterial concentration, likely due to the elevated nutrient levels associated with sustained organic manure input,

which can support increased microbial biomass (Cui et al., 2024; Tian et al., 2022). Furthermore, exogenous microorganisms introduced through manure application may also contribute to the regulation of indigenous soil microbial communities (Bonanomi et al., 2016; Li et al., 2021a).

4.3. Drivers of ARG models for soil–lettuce systems

Organic fertilizers are considered a major source of ARGs in soil, and soil-derived ARGs are an important source of resistance genes in vegetables (Xie et al., 2018; Zhang et al., 2018). The changes in the distribution of ARGs in soil and within leaves may be closely related to alterations in soil chemical properties following the cessation of fertilization. We observed an increase in soil pH after the cessation of fertilization; an increase in pH reduces antibiotic adsorption in the soil, which aids in the removal of antibiotic residues and, consequently, decreases the abundance of corresponding ARGs (Lin et al., 2020). Soil nutrient factors, such as soil organic carbon (SOC) and total nitrogen (TN), may also have a synergistic effect on the composition of ARGs in soil (Sun et al., 2019). The cessation of continuous fertilization significantly altered the microbial communities and MGEs in both the soil and rhizosphere. MGEs play a key role in ARG transfer between microorganisms in the environment, and previous studies have shown a strong positive correlation between bacteria and MGEs in various ecosystems (Liu et al., 2020). The increased interactions between bacterial taxa detected in this study may be explained the increased abundance of MGEs in the manure input treatment.

Our study showed a possible pathway for ARGs spreading from soil to plant systems, which may directedly ingested by animals and humans and result in a serious threaten to public health. However, it is worth to mention that our findings only displayed a potential risk. The relationship between ARGs dissemination and human health still needs more observation and future verification works. For example, further research involving the collection of samples from a wider range of environments, the identification of long-read ARG sequences through methods such as nanopore metagenomic sequencing, and the application of machine learning tools to track antibiotic resistomes is needed. Overall, further studies on the linkages between the structure and composition of soil microbial communities and ARGs will provide deeper insights into the distribution and dissemination of ARGs within the organic fertilizer-soil–plant system.

5. Conclusions

In summary, we identified changes in ARGs, bacterial communities, and the topological role of microbial symbiotic networks in soil–lettuce systems and revealed the effects of manure on ARG patterns, microbial communities, and microbe–microbe interactions. This study further confirms that the cessation of manure application significantly affects the distribution of ARGs and the microbial community, which in turn influences the spread of antibiotic resistance genes. This study integrates field-scale cessation trials with ARGs mobility tracking, filling critical gaps in sustainable manure management and providing a more comprehensive assessment of ARG risks in agroecosystems.

CRediT authorship contribution statement

Ruochen Li: Writing – original draft, Visualization, Validation, Methodology, Conceptualization. **Xin Pei:** Resources, Data curation. **Ming Zhang:** Validation, Data curation. **Xuhui Deng:** Writing – review & editing, Conceptualization. **Chengyuan Tao:** Writing – review & editing, Methodology. **Jiabao Wang:** Writing – review & editing, Methodology. **Xueli Chen:** Writing – review & editing. **Nicholas Clarke:** Writing – review & editing. **Lidia Sas-Paszt:** Writing – review & editing. **Zongzhuan Shen:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Rong Li:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Qirong Shen:** Writing – review & editing, Supervision, Investigation, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2025.106100.

Data availability

The raw sequence data of all samples were submitted to the NCBI Sequence Read Archive database (https://www.ncbi.nlm.nih.gov/) with the accession number PRJNA1091716.

References

Andersson, D.I., Hughes, D., 2014. Microbiological effects of sublethal levels of antibiotics. Nat. Rev. Microbiol. 12, 465–478. https://doi.org/10.1038/nrmicro3270.

Bai, Y., Müller, D.B., Srinivas, G., Garrido-Oter, R., Potthoff, E., Rott, M., Dombrowski, N., Münch, P.C., Spaepen, S., Remus-Emsermann, M., Hüttel, B., McHardy, A.C., Vorholt, J.A., Schulze-Lefert, P., 2015. Functional overlap of the Arabidopsis leaf and root microbiota. Nature 528, 364–369. https://doi.org/10.1038/nature16192.

Barberán, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. ISME J. 6, 343–351. https://doi.org/10.1038/ismej.2011.119.

Bastian, M., Heymann, S., Jacomy, M., 2009. Gephi: an open source software for exploring and manipulating networks. ICWSM 3, 361–362. https://doi.org/10.1609/icwsm.v3i1.13937.

Bonanomi, G., De Filippis, F., Cesarano, G., La Storia, A., Ercolini, D., Scala, F., 2016. Organic farming induces changes in soil microbiota that affect agro-ecosystem functions. Soil Biol. Biochem. 103, 327–336. https://doi.org/10.1016/j.soilbio.2016.09.005.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Van Themaat, E.V.L., Schulze-Lefert, P., 2013. Structure and functions of the bacterial microbiota of plants. Annu. Rev. Plant Biol. 64, 807–838. https://doi.org/10.1146/annurev-arplant-050312-120106.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624. https://doi.org/10.1038/ismej.2012.8.

Chen, Q.-L., An, X.-L., Zhu, Y.-G., Su, J.-Q., Gillings, M.R., Ye, Z.-L., Cui, L., 2017. Application of struvite alters the antibiotic resistome in soil, rhizosphere, and phyllosphere. Environ. Sci. Technol. 51, 8149–8157. https://doi.org/10.1021/acs.est.7b01420.

Cui, E., Zhou, Z., Cui, B., Fan, X., Ali Abid, A., Chen, T., Gao, F., Du, Z., 2024. Effects of nitrogen fertilization on the fate of high-risk antibiotic resistance genes in reclaimed water-irrigated soil and plants. Environ. Int. 190, 108834. https://doi.org/10.1016/j.envint.2024.108834.

D'Amico, M., Frisullo, S., Cirulli, M., 2008. Endophytic fungi occurring in fennel, lettuce, chicory, and celery — commercial crops in southern Italy. Mycol. Res. 112, 100–107. https://doi.org/10.1016/j.mycres.2007.11.007.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998. https://doi.org/10.1038/nmeth.2604.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381.

Fatima, F., Pathak, N., Rastogi Verma, S., 2014. An improved method for soil DNA extraction to study the microbial assortment within rhizospheric region. Mol. Biol. Int. 2014, 1–6. https://doi.org/10.1155/2014/518960.
Forsberg, K.J., Reyes, A., Wang, B., Selleck, E.M., Sommer, M.O.A., Dantas, G., 2012. The shared antibiotic resistome of soil bacteria and human pathogens. Science 337, 1107–1111. https://doi.org/10.1126/science.1220761.
Gullberg, E., Cao, S., Berg, O.G., Ilbäck, C., Sandegren, L., Hughes, D., Andersson, D.I., 2011. Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog. 7, e1002158. https://doi.org/10.1371/journal.ppat.1002158.
Kläui, A., Bütikofer, U., Naskova, J., Wagner, E., Marti, E., 2024. Fresh produce as a reservoir of antimicrobial resistance genes: a case study of Switzerland. Sci. Total Environ. 907, 167671. https://doi.org/10.1016/j.scitotenv.2023.167671.
Klein, E.Y., Van Boeckel, T.P., Martinez, E.M., Pant, S., Gandra, S., Levin, S.A., Goossens, H., Laxminarayan, R., 2018. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc. Natl. Acad. Sci. USA 115. https://doi.org/10.1073/pnas.1717295115.
Kloepper, J.W., Ryu, C.-M., Zhang, S., 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology 94, 1259–1266. https://doi.org/10.1094/PHYTO.2004.94.11.1259.
Knights, D., Kuczynski, J., Charlson, E.S., Zaneveld, J., Mozer, M.C., Collman, R.G., Bushman, F.D., Knight, R., Kelley, S.T., 2011. Bayesian community-wide culture-independent microbial source tracking. Nat. Methods 8, 761–763. https://doi.org/10.1038/nmeth.1650.
Koonin, E.V., Makarova, K.S., Aravind, L., 2001. Horizontal gene transfer in prokaryotes: quantification and classification. Ann. Rev. Microbiol. 55, 709–742. https://doi.org/10.1146/annurev.micro.55.1.709.
Li, H., La, S., Zhang, X., Gao, L., Tian, Y., 2021a. Salt-induced recruitment of specific root-associated bacterial consortium capable of enhancing plant adaptability to salt stress. ISME J. 15, 2865–2882. https://doi.org/10.1038/s41396-021-00974-2.
Li, R., Gao, Y., Chen, Q., Li, Z., Gao, F., Meng, Q., Li, T., Liu, A., Wang, Q., Wu, L., Wang, Y., Liu, Z., Zhang, M., 2021b. Blended controlled-release nitrogen fertilizer with straw returning improved soil nitrogen availability, soil microbial community, and root morphology of wheat. Soil Tillage Res. 212, 105045. https://doi.org/10.1016/j.still.2021.105045.
Li, T., Li, Ruochen, Cao, Y., Tao, C., Deng, X., Ou, Y., Liu, H., Shen, Z., Li, Rong, Shen, Q., 2022. Soil antibiotic abatement associates with the manipulation of soil microbiome via long-term fertilizer application. J. Hazard. Mater. 439, 129704. https://doi.org/10.1016/j.jhazmat.2022.129704.
Li, Ruochen, Zhang, N., Deng, X., Tao, C., Pei, X., Yue, Y., Xu, X., Wang, J., Shen, Z., Shen, Q., Li, Rong, 2024. Tomato bacterial wilt disease outbreaks are accompanied by an increase in soil antibiotic resistance. Environ. Int. 190, 108896. https://doi.org/10.1016/j.envint.2024.108896.
Lin, H., Sun, W., Yu, Q., Ma, J., 2020. Acidic conditions enhance the removal of sulfonamide antibiotics and antibiotic resistance determinants in swine manure. Environ. Pollut. 263, 114439. https://doi.org/10.1016/j.envpol.2020.114439.
Liu, J.D., Goodspeed, D., Sheng, Z., Li, B., Yang, Y., Kliebenstein, D.J., Braam, J., 2015. Keeping the rhythm: light/dark cycles during postharvest storage preserve the tissue integrity and nutritional content of leafy plants. BMC Plant Biol. 15, 92. https://doi.org/10.1186/s12870-015-0474-9.
Liu, P., Jia, S., He, X., Zhang, X., Ye, L., 2017. Different impacts of manure and chemical fertilizers on bacterial community structure and antibiotic resistance genes in arable soils. Chemosphere 188, 455–464. https://doi.org/10.1016/j.chemosphere.2017.08.162.
Liu, C., Chen, Y., Li, X., Zhang, Y., Ye, J., Huang, H., Zhu, C., 2020. Temporal effects of repeated application of biogas slurry on soil antibiotic resistance genes and their potential bacterial hosts. Environ. Pollut. 258, 113652. https://doi.org/10.1016/j.envpol.2019.113652.
Liu, W., Ling, N., Guo, J., Ruan, Y., Wang, M., Shen, Q., Guo, S., 2021. Dynamics of the antibiotic resistome in agricultural soils amended with different sources of animal manures over three consecutive years. J. Hazard. Mater. 401, 123399. https://doi.org/10.1016/j.jhazmat.2020.123399.
López, E., Blázquez, J., 2009. Effect of subinhibitory concentrations of antibiotics on intrachromosomal homologous recombination in *Escherichia coli*. Antimicrob. Agents Chemother. 53, 3411–3415. https://doi.org/10.1128/AAC.00358-09.
Molina-Santiago, C., Vela-Corcía, D., Petras, D., Díaz-Martínez, L., Pérez-Lorente, A.I., Sopeña-Torres, S., Pearson, J., Caraballo-Rodríguez, A.M., Dorrestein, P.C., De Vicente, A., Romero, D., 2021. Chemical interplay and complementary adaptative strategies toggle bacterial antagonism and co-existence. Cell Rep. 36, 109449. https://doi.org/10.1016/j.celrep.2021.109449.
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., 2020. Vegan: Community Ecology Package Version 2.5-7. 2020.
Palma, E., Tilocca, B., Roncada, P., 2020. Antimicrobial resistance in veterinary medicine: an overview. Int. J. Mol. Sci. 21, 1914. https://doi.org/10.3390/ijms21061914.
Qian, H., Zhang, Z., Zhang, Q., Lu, T., Zhu, Y.-G., 2022. Abundance cannot represent antibiotic resistance risk. Soil Ecol. Lett. 4, 291–292. https://doi.org/10.1007/s42832-022-0133-7.
Sarmah, A.K., Meyer, M.T., Boxall, A.B.A., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65, 725–759. https://doi.org/10.1016/j.chemosphere.2006.03.026.
Sun, Y., Qiu, T., Gao, M., Shi, M., Zhang, H., Wang, X., 2019. Inorganic and organic fertilizers application enhanced antibiotic resistome in greenhouse soils growing vegetables. Ecotoxicol. Environ. Saf. 179, 24–30. https://doi.org/10.1016/j.ecoenv.2019.04.039.
Tang, S., Ma, Q., Marsden, K.A., Chadwick, D.R., Luo, Y., Kuzyakov, Y., Wu, L., Jones, D. L., 2023. Microbial community succession in soil is mainly driven by carbon and nitrogen contents rather than phosphorus and sulphur contents. Soil Biol. Biochem. 180, 109019. https://doi.org/10.1016/j.soilbio.2023.109019.
Tao, C., Li, R., Xiong, W., Shen, Z., Liu, S., Wang, B., Ruan, Y., Geisen, S., Shen, Q., Kowalchuk, G.A., 2020. Bio-organic fertilizers stimulate indigenous soil Pseudomonas populations to enhance plant disease suppression. Microbiome 8, 137. https://doi.org/10.1186/s40168-020-00892-z.
Thacharodi, A., Lamont, I.L., 2022. Aminoglycoside-modifying enzymes are sufficient to make Pseudomonas aeruginosa clinically resistant to key antibiotics. Antibiotics 11, 884. https://doi.org/10.3390/antibiotics11070884.
Tian, S., Zhu, B., Yin, R., Wang, M., Jiang, Y., Zhang, C., Li, D., Chen, X., Kardol, P., Liu, M., 2022. Organic fertilization promotes crop productivity through changes in soil aggregation. Soil Biol. Biochem. 165, 108533. https://doi.org/10.1016/j.soilbio.2021.108533.
Timilsina, S., Potnis, N., Newberry, E.A., Liyanapathiranage, P., Iruegas-Bocardo, F., White, F.F., Goss, E.M., Jones, J.B., 2020. Xanthomonas diversity, virulence and plant–pathogen interactions. Nat. Rev. Microbiol. 18, 415–427. https://doi.org/10.1038/s41579-020-0361-8.
Van Elsas, J.D., Turner, S., Bailey, M.J., 2003. Horizontal gene transfer in the phytosphere. New Phytol. 157, 525–537. https://doi.org/10.1046/j.1469-8137.2003.00697.x.
Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267. https://doi.org/10.1128/AEM.00062-07.
Wang, F.-H., Qiao, M., Chen, Z., Su, J.-Q., Zhu, Y.-G., 2015. Antibiotic resistance genes in manure-amended soil and vegetables at harvest. J. Hazard. Mater. 299, 215–221. https://doi.org/10.1016/j.jhazmat.2015.05.028.
Wang, J., Wang, L., Zhu, L., Wang, Jun, Xing, B., 2022a. Antibiotic resistance in agricultural soils: source, fate, mechanism and attenuation strategy. Crit. Rev. Environ. Sci. Technol. 52, 847–889. https://doi.org/10.1080/10643389.2020.1835438.
Wang, T., Sun, S., Xu, Y., Waigi, M.G., Odinga, E.S., Vasilyeva, G.K., Gao, Y., Hu, X., 2022b. Nitrogen regulates the distribution of antibiotic resistance genes in the soil–vegetable system. Front. Microbiol. 13, 848750. https://doi.org/10.3389/fmicb.2022.848750.
Wang, Y.-Z., Zhou, S.-Y.-D., Zhou, X.-Y., An, X.-L., Su, J.-Q., 2023. Manure and biochar have limited effect on lettuce leaf endophyte resistome. Sci. Total Environ. 860, 160515. https://doi.org/10.1016/j.scitotenv.2022.160515.
Wei, Z., Gu, Y., Friman, V.-P., Kowalchuk, G.A., Xu, Y., Shen, Q., Jousset, A., 2019. Initial soil microbiome composition and functioning predetermine future plant health. Sci. Adv. 5, eaaw0759. https://doi.org/10.1126/sciadv.aaw0759.
Wolters, B., Widyasari-Mehta, A., Kreuzig, R., Smalla, K., 2016. Contaminations of organic fertilizers with antibiotic residues, resistance genes, and mobile genetic elements mirroring antibiotic use in livestock? Appl. Microbiol. Biotechnol. 100, 9343–9353. https://doi.org/10.1007/s00253-016-7742-y.
Xiao, R., Huang, D., Du, L., Song, B., Yin, L., Chen, Y., Gao, L., Li, R., Huang, H., Zeng, G., 2023. Antibiotic resistance in soil-plant systems: a review of the source, dissemination, influence factors, and potential exposure risks. Sci. Total Environ. 869, 161855. https://doi.org/10.1016/j.scitotenv.2023.161855.
Xie, W.-Y., Yang, X.-P., Li, Q., Wu, L.-H., Shen, Q.-R., Zhao, F.-J., 2016. Changes in antibiotic concentrations and antibiotic resistome during commercial composting of animal manures. Environ. Pollut. 219, 182–190. https://doi.org/10.1016/j.envpol.2016.10.044.
Xie, W.-Y., Yuan, S.-T., Xu, M.-G., Yang, X.-P., Shen, Q.-R., Zhang, W.-W., Su, J.-Q., Zhao, F.-J., 2018. Long-term effects of manure and chemical fertilizers on soil antibiotic resistome. Soil Biol. Biochem. 122, 111–119. https://doi.org/10.1016/j.soilbio.2018.04.009.
Xiong, W., Song, Y., Yang, K., Gu, Y., Wei, Z., Kowalchuk, G.A., Xu, Y., Jousset, A., Shen, Q., Geisen, S., 2020. Rhizosphere protists are key determinants of plant health. Microbiome 8, 27. https://doi.org/10.1186/s40168-020-00799-9.
Zhang, Y., Jewett, C., Gilley, J., Bartelt-Hunt, S.L., Snow, D.D., Hodges, L., Li, X., 2018. Microbial communities in the rhizosphere and the root of lettuce as affected by Salmonella-contaminated irrigation water. FEMS Microbiol. Ecol. 94. https://doi.org/10.1093/femsec/fiy135.
Zhao, W., Wang, B., Yu, G., 2018. Antibiotic resistance genes in China: occurrence, risk, and correlation among different parameters. Environ. Sci. Pollut. Res. 25, 21467–21482. https://doi.org/10.1007/s11356-018-2507-z.
Zhou, L.-J., Ying, G.-G., Liu, S., Zhang, R.-Q., Lai, H.-J., Chen, Z.-F., Pan, C.-G., 2013. Excretion masses and environmental occurrence of antibiotics in typical swine and dairy cattle farms in China. Sci. Total Environ. 444, 183–195. https://doi.org/10.1016/j.scitotenv.2012.11.087.
Zhou, S.-Y.-D., Zhu, D., Giles, M., Yang, X.-R., Daniell, T., Neilson, R., Zhu, Y.-G., 2019. Phyllosphere of staple crops under pig manure fertilization, a reservoir of antibiotic resistance genes. Environ. Pollut. 252, 227–235. https://doi.org/10.1016/j.envpol.2019.05.098.
Zhu, Y.-G., Johnson, T.A., Su, J.-Q., Qiao, M., Guo, G.-X., Stedtfeld, R.D., Hashsham, S.A., Tiedje, J.M., 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proc. Natl. Acad. Sci. USA 110, 3435–3440. https://doi.org/10.1073/pnas.1222743110.
Zhu, Y.-G., Zhao, Y., Li, B., Huang, C.-L., Zhang, S.-Y., Yu, S., Chen, Y.-S., Zhang, T., Gillings, M.R., Su, J.-Q., 2017. Continental-scale pollution of estuaries with antibiotic resistance genes. Nat. Microbiol. 2, 16270. https://doi.org/10.1038/nmicrobiol.2016.270.