

Research article

Soil co-occurring bacterial communities serve as assembly hubs of antibiotic resistance determinants under organic fertilization



Ya-Lan Hong ^{a,1}, Wei-Ming Xi ^{a,1}, Ya-Ting Wang ^a, Yi Yuan ^a, Zong-Zhuan Shen ^a, Ming Tian ^b, Jihong Liu Clarke ^c, Wan-Ying Xie ^{a,*} Fang-Jie Zhao ^a

^a Jiangsu Key Laboratory for Organic Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, 210095, China

^b Institute of Animal Husbandry, Heilongjiang Academy of Agricultural Sciences, Harbin, 150086, China

^c Norwegian Institute of Bioeconomy Research (NIBIO), Ås, N-1431, Norway

ARTICLE INFO

Keywords:

Antibiotic resistance genes
Organic fertilization
Water management
Half-life
Dissemination
Pathogenic hosts

ABSTRACT

Environmental transmission of antibiotic resistance poses a significant threat to human health by undermining the efficacy of therapeutic interventions against bacterial infections. Agricultural practices, particularly the application of organic fertilizers derived from animal manure, are major contributors to the spread of antibiotic resistance determinants (ARDs) in soil ecosystems. However, the fates of ARDs and their bacterial hosts in soil following organic fertilization as well as the impact of water management regimes remain poorly understood. We investigated the attenuation and persistence of ARDs in soil following organic fertilization under water management practices of upland, continuous flooding, and intermittent flooding. Most ARDs introduced via the organic fertilizer exhibited significant attenuation, with half-lives ranging from 19 to 50 days, primarily due to the decline of fertilizer-derived bacterial hosts. Specific ARDs, such as *aph(3')-IIIa* and *tetO*, persisted across all treatments. Upland conditions accelerated the attenuation of ARDs and their pathogenic hosts compared to flooding conditions, which prolonged their survival and promoted horizontal gene transfer. The divergent responses of ARD composition and soil bacterial communities to the environmental variables revealed a unique dissemination pattern, wherein the soil co-occurring bacterial communities served as critical hubs for the dissemination of ARDs and their bacterial hosts from organic fertilizers. The soil co-occurring bacterial communities exhibited strong interspecies interactions and high sensitivity to environmental changes. Targeted strategies to disrupt these assembly hubs may provide an effective way to mitigate the spread of antibiotic resistance from organic fertilizers to soil ecosystems.

1. Introduction

Soil ecosystem is an important reservoir of antibiotic resistance determinants (ARDs), encompassing a broad spectrum of antibiotic resistance genes (ARGs) that confer resistance to antibiotics and mobile genetic elements (MGEs) that facilitate the horizontal transmission of ARGs (Forsberg et al., 2012; Wu et al., 2021). Organic fertilization has been adopted worldwide to maintain soil fertility and biodiversity (Diacono and Montemurro, 2011). However, applications of organic fertilizers can introduce substantial quantities of ARDs from animal production into soil environments, due to the extensive use of veterinary antibiotics in the treatment and prevention of diseases and even in the

growth promotion (Xie et al., 2018a; Odetokun et al., 2023). The enrichment and diversification of ARDs in soil ecosystem due to organic fertilization have been widely observed (Zhu et al., 2013; Johnson et al., 2016; Xie et al., 2018b), raising significant concerns for both ecological safety and human health, given the linkage role of soil ecosystem in the dissemination of antibiotic resistance from anthropogenic facilities to the broader environment, and vice versa.

Extensive research has demonstrated that organic fertilization, especially long-term practices, can have profound impacts on the composition, diversity, abundance and dissemination of ARDs in agricultural soils (Xie et al., 2018a, 2018b; Zhao et al., 2020). However, while the effects of organic fertilization on ARDs are well-documented,

* Corresponding author.

E-mail address: wyxie@njau.edu.cn (W.-Y. Xie).

¹ These authors contributed equally to this work.

the dynamics of ARDs in response to other environmental changes, such as soil water management, and the interaction between ARDs and bacterial communities during these changes have been scarcely investigated. Soil water status, i.e. upland or flooding, has been shown to significantly influence the composition, structure and function of the soil bacterial communities (Sun et al., 2020; Gao et al., 2024; Zhang et al., 2025). Given that the phylogeny of bacterial community is a key determinant of resistome structure, soil water status may exert a considerable impact on the soil resistome (Forsberg et al., 2014). Notably, submerged soils have been suggested to harbor a higher abundance of human bacterial pathogens (Li et al., 2024), which are more likely to acquire and disseminate antibiotic resistance, especially clinically relevant resistance, due to their higher probability of encountering antibiotics than non-pathogenic bacteria during infections (Gipson et al., 2020). In addition, previous studies have reported significant differences in the composition of ARGs between upland and paddy soils, with certain ARG subtypes being more abundant under wetter or submerged conditions (Kang et al., 2018; Wang et al., 2018; Li et al., 2024). These findings suggest that water management practices may significantly influence the soil resistome. However, the extent to which water management practices contribute to the temporal dynamics of soil resistome and bacterial communities, especially under the circumstances of organic fertilization, remains unclear. Concomitantly, key ARDs and their bacterial hosts that persist in soil environment, as well as the dissemination patterns of ARDs in soil bacterial communities following fertilization, have yet to be fully elucidated. Addressing these knowledge gaps and unraveling the underlying interactions between ARDs and bacterial communities are essential for developing effective strategies in the remediation of ARD-loaded soils.

In the present study, we employed real-time quantitative PCR and high-throughput 16S rRNA gene sequencing to quantitatively and comparatively investigate the temporal dynamics of ARDs and bacterial

communities in soil upon organic fertilization under different water managements. We hypothesize that 1) different ARDs may have distinct fates and half-lives in soil after organic fertilization, 2) water management can exert significant impact on both soil ARDs and bacterial communities, and 3) dissemination of ARDs from organic fertilizers to soil environment is driven by specific members in bacterial communities.

2. Materials and methods

2.1. Soil collection and incubation experiment

An upland agricultural soil (Fluwo-aquic soil, pH = 7.93 ± 0.02, Total carbon = 11.5 ± 0.6 g/kg) was collected from Haian, Jiangsu Province, China (32°66'N, 120°70'E). For the incubation experiment, the soil was thoroughly mixed after the removal of small stones and plant debris. A pig-manure-composted organic fertilizer (OF) which was rich in ARDs was supplemented at a weight ratio of 0.52 % (approximately 12,000 kg/ha considering the 15-cm surface soil as the plough layer) (Tang et al., 2015) into the soil to simulate the process of fertilization and increase the initial abundance of soil ARDs (Fig. 1A, Fig. S1). The OF-supplemented soil was then divided into three groups, with each group receiving 600 g of soil placed in black plastic pots ($n = 3$) on Day 0. Soil water content was maintained at 60 % of the soil water-holding capacity. On Day 3, different water management strategies were implemented to create three distinct treatments: continuous flooding (CF + OF), intermittent flooding (IF + OF), and Upland incubation (Upland + OF) (Fig. 1A, Fig. S1). In the treatments of CF + OF and IF + OF, sterilized water was used to flood the soil, with the water level maintained approximately 3 cm above the soil surface. In the treatment of IF + OF, water was drained through a hole at the bottom of the pot, which was used to re-flood the soil at the indicated time points (Fig. S1).

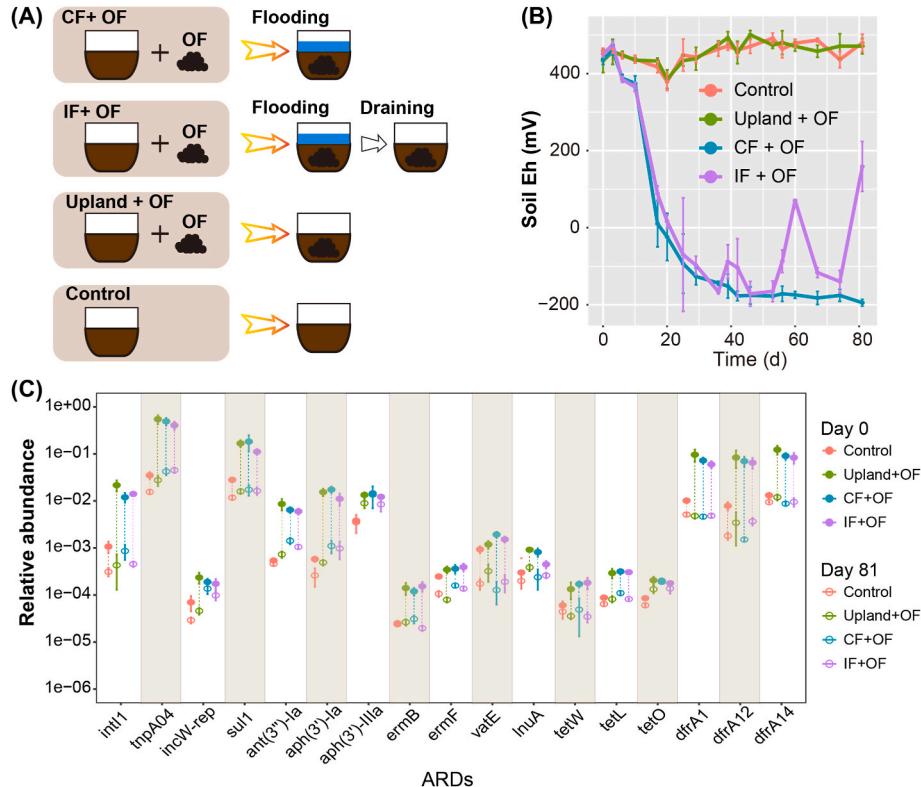


Fig. 1. Incubation treatments, soil Eh and abundance comparison between ARDs on Day 0 and Day 81. (A) Experimental treatments of soil incubation. Detailed incubation and sampling scheme is available in Fig. S1. (B) Soil Eh during the incubation. (C) Abundance comparison between ARDs on Day 0 and Day 81. Only ARDs that showed significant increase in abundance upon the fertilization are presented here. Dashed lines indicate significant abundance reductions ($p < 0.05$). OF, organic fertilizer. CF, Continuous Flooding. IF, Intermittent Flooding.

In the treatment of Upland + OF, soil water content was kept at 60 % of the water-holding capacity throughout the entire incubation period. Soil without OF supplementary was designated as Control and was incubated following the same procedure as Upland + OF treatment. Each treatment and the Control contained three biological replicates. All soils were incubated at 28 °C under dark conditions for 81 days. At the indicated time points (Days 0, 3, 6, 10, 17, 20, 25, 29, 36, 39, 42, 46, 53, 56, 60, 67, 74 and 81), soil redox potential (Eh) was recorded with combined Pt/Ag-AgCl electrodes (Fig. 1B, Fig. S1). Soil samples collected on Days 0, 3, 17, 36, 53, 60, 74 and 81, representing characteristic soil Eh values observed during the incubation, underwent DNA extraction and ARD quantification (Fig. S1). DNA samples on Days 0, 3, 17, 53, 60, 74 and 81 were further used for 16S rRNA gene amplicon sequencing (Fig. 1B, Fig. S1).

2.2. DNA extraction

DNA was extracted from fresh soil samples and the organic fertilizer ($n = 3$) using DNeasy PowerSoil Pro Kits (Qiagen, Germany) according to the manufacturer's instructions. The DNA concentrations and absorption spectra of the DNA solutions were measured using a NanoDrop 2000C spectrophotometer (Thermo Scientific, Wilmington, USA).

2.3. Abundance quantification of ARDs

Representative ARGs (44 subtypes) that are prevalently detected in agricultural environments, encompassing resistance to sulfonamides, aminoglycosides, beta-lactams, tetracyclines, phenicols, fluoroquinolones, colistin, vancomycin, macrolides, lincosamides and streptogramin B and multidrug, along with ten MGEs including marker genes for integrons, transposons and plasmids, were initially determined in the organic fertilizer (Table S1). The genes observed in the organic fertilizer were subsequently quantified in the soil samples. Gene quantification was performed on the platform of QuantStudio™ Real-Time PCR System (384 wells, Applied Biosystems, Thermo Fisher Scientific) as previously described (Xie et al., 2022). Absolute abundances were calculated as gene copies per gram of dry weight for both soil samples and the organic fertilizer. Relative abundances were calculated by normalizing the absolute abundances of ARDs to those of the 16S rRNA gene.

2.4. Amplicon sequencing of 16S rRNA gene

The primer pair of 515F (5'-GTGCCAGCMGCCGCGTAA-3') and 907R (5'-CCGYCAATTYMTTTRAGTT-3') was utilized to amplify the 16S rRNA gene for sequencing on the Illumina NovaSeq 6000 platform at Biozeron (Shanghai, China) (Sun et al., 2013). The PCR reaction was conducted as previously described (Xie et al., 2018b). For quality control and sequence merging, Fastp (<https://github.com/OpenGene/fastp, version 0.20.0>) and FLASH (<http://www.cbcn.umd.edu/software/flash, version 1.2.7>) were employed, respectively. Subsequently, QIIME2 was used to identify amplicon sequence variants (ASVs) according to DADA2 algorithm. The taxonomy classification of the ASVs was performed using the RDP classifier (<http://rdp.cme.msu.edu/>, version 2.2) against the SILVA 16S rRNA database with a threshold of 70% confidence. Pathogenic risk of the ASVs was evaluated using the multiple bacterial pathogen detection pipeline (MBPD) with a sequence identity of > 99% (Yang et al., 2023). The pathogenicity of specific ASVs was manually verified by searching for the reports associated with pathogenicity or diseases using the corresponding genera or species on Web of Science. The raw sequencing data can be accessed in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under the accession number of PRJNA1196183.

2.5. Bioinformatic and data analysis

The distribution of each data set during statistical analyses was assessed using the Shapiro-Wilk normality test in R. The half-lives ($t_{1/2}$) of ARDs and bacteria were calculated using a first-order kinetic model in SPSS 18, based on the relative abundances of the genes or ASVs (Wang et al., 2016). The removal rate constant k (day⁻¹) was first calculated using the equation:

$$\ln\left(\frac{A}{A_0}\right) = -kt$$

where A represents the relative abundance at time t and A_0 represents the relative abundance at Day 0. The half-life $t_{1/2}$ was then calculated using the equation:

$$t_{1/2} = \frac{\ln(2)}{k}$$

The parameter $-k$ (the slope of the regression) was calculated using a simple linear regression model in R, and the performance of the fitting was evaluated using ANOVA. Differences in $-k$ between treatments were compared in R utilizing the "simba" package (Jurasinski and Retzer, 2015). The co-occurrence of the bacterial ASVs in soils was calculated based on their presence (value = 1) or the absence (value = 0) using the "cooccur" package in R as previously described (Griffith et al., 2016; Adair et al., 2018). ASVs exhibiting a probability of co-occurrence at a frequency greater than ($p_{gt} < 0.05$) or less than ($p_{lt} < 0.05$) the observed frequency were identified as members of significantly co-occurring bacterial communities (BC), with the remaining ASVs classified as members of randomly-occurring BC. A co-association network of ASVs and ARDs based on relative abundances were constructed using Gephi (Version 0.10.1) to identify the potential bacterial hosts of ARDs, employing spearman correlation coefficients obtained with the "psych" package in R. An ASV was identified as a potential bacterial host of ARDs if it showed significantly positive correlations ($r > 0.7, p < 0.0001$) with any ARD in the co-association network analysis. Analyses of non-metric multidimensional scaling (NMDS), partial mantel test, variance partitioning analysis (VPA), and permutational multivariate analysis of variance (PERMANOVA) were all conducted in R using the "vegan" package (Oksanen et al., 2022).

3. Results

3.1. Attenuation and persistence of ARDs in soil during incubation

Soil Eh of the Control and Upland + OF was between 378 and 500 mV during the incubation (Fig. 1B). Flooding gradually reduced the soil Eh from above 400 mV to less than -150 mV in 39 days. Draining for three days (from Day 17 to Day 20 and from Day 36 to Day 39) in the treatment of IF + OF showed little influence on the soil Eh. Comparatively, draining for seven days (from Day 53 to Day 60 and from Day 76 to Day 81) in IF + OF significantly increased soil Eh for more than 235 mV (Fig. 1B).

Among the 54 ARDs measured, 31 were identified in the organic fertilizer, including six MGEs and 25 ARGs (Table S1). Twenty-seven ARDs exhibited significantly higher abundances in the organic fertilizer compared to the control soil on Day 0 (Fig. S2). Following the application of organic fertilizer, three MGEs (*intI1*, *tnpA04* and *incW-rep*) and fourteen ARGs (*sul1*, *dfrA1*, *dfrA12*, *dfrA14*, *ant(3')-Ia*, *aph(3')-Ia*, *aph(3')-IIIa*, *ermB*, *ermF*, *vatE*, *tetW*, *tetL*, *tetO* and *lnuA*) in the soil samples showed significant increases in relative abundance, compared to the unfertilized Control (Fig. 1C, Table S2). Most of these genes, with the exception of *incW-rep*, *aph(3')-IIIa* and *tetO*, significantly (ANOVA, $p < 0.05$) attenuated with a reduction rate exceeding 40% in all three OF-supplemented treatments, by the comparison of relative abundances between Day 81 and Day 0 (Fig. 1C, Table S3). Genes such as *intI1*,

trpA04, *sul1*, *dfrA1*, *dfrA12*, *dfrA14*, *ant(3'')*-Ia and *aph(3'')*-Ia, displayed the most pronounced attenuation, with reduction rates from 78.0% to 98.0% in the OF-supplemented treatments (Table S3). At the end of the incubation, the relative abundances of *intI1*, *sul1*, *dfrA1*, *dfrA12*, *dfrA14*, *ermB*, *vatE*, *tetW* and *lnuA* in the OF-supplemented treatments all exhibited no significant difference compared to the unfertilized Control (Table S2). However, the three OF-supplemented treatments still contained significantly ($p < 0.05$) more abundant *ant(3'')*-Ia than the Control. In addition, *trpA04* and *ermF* in CF + OF and IF + OF, and *aph(3'')*-Ia and *tetL* in CF + OF were still significantly more abundant than those in the Control (Table S2). Among the three genes that showed no general attenuation, *aph(3'')*-IIIa and *tetO* persisted in all three OF-supplemented treatments, whereas *incW*-rep persisted solely in the flooding treatments (CF + OF and IF + OF) (Fig. 1C, Table S2).

Eleven ARDs in soil showed no significant increase in relative abundance following the addition of organic fertilizer, in comparison to the unfertilized Control (Fig. S3, Table S2). These genes generally displayed less variation over the incubation period compared to those that increased significantly with fertilization (Figure S3, Fig. 1C). Among these eleven genes, only *aac(6'')*-Ib3 and *bla-OXA* showed significant attenuation (reduction rate $> 40\%$, $p < 0.05$) across all three OF-supplemented treatments, when comparing the relative abundances between Day 81 and Day 0 (Table S3). By the end of the incubation, *tetX* and *tetG* exhibited significantly ($p < 0.05$) higher relative abundances in CF + OF than those in the Control. In contrast, the remaining nine genes showed no significant difference in relative abundance among the Control and three OF-supplemented treatments (Fig. S3, Table S2).

Inspecting from the overall detected ARGs and MGEs, the total relative abundances of all detected ARGs in the three OF-supplemented treatments all decreased to a status that showed no significant difference from the Control after the incubation (Fig. S4A). In addition, the total relative abundance of all detected MGEs in Upland + OF also decreased to the same level observed in the Control after the incubation (Fig. S4B). However, the abundances of MGEs in the two flooding treatments (CF + OF and IF + OF) remained significantly higher ($p < 0.05$) than that in the Control (Fig. S4B).

3.2. Half-lives of ARDs during soil incubation

When considering all the sampling time points, most of the ARDs exhibited significant attenuation ($p < 0.05$) according to a first-order kinetic model. The half-lives of ARDs were successfully calculated (Table 1, Table S4). When calculated on the summed abundances of all detected ARGs and MGEs, the half-lives ranged from 27 to 28 days for ARGs and from 19 to 25 days for MGEs in the OF-supplemented treatments, respectively (Fig. 2). The water management showed no significant effect on the attenuation of ARGs based on total abundance (Fig. 2A). However, the half-life of total MGEs appeared to be the shortest in the Upland + OF treatment (Fig. 2B).

With the exception of *incW*-rep, *aph(3'')*-IIIa and *tetO*, all ARDs that significantly increased upon fertilization exhibited better fittings (higher R^2) to the attenuation model in the three OF-supplemented treatments compared to those in the Control (Table 1, Table S4). Furthermore, fertilization clearly shortened the half-lives of these genes in soil, suggesting a pronounced attenuation process for the exogenously introduced ARDs from the organic fertilizer (Table 1, Fig. S5). Specifically, *intI1*, *trpA04*, *sul1*, *dfrA1*, *dfrA12*, *dfrA14*, *ant(3'')*-Ia, *aph(3'')*-Ia, *ermB*, *tetW*, and *tetL* had half-lives less than 50 days across the OF-supplemented treatments. This indicates a relatively rapid attenuation of these genes in soil environment (Table 1, Fig. S5). Gene *vatE* underwent a significant and rapid attenuation in the Control, whereby the fertilization had minimal impact on its attenuation process in soil. The half-lives of *intI1*, *sul1*, *dfrA1*, *dfrA12*, *dfrA14*, *ermB*, *vatE*, *tetW* and *tetL* all showed no significant difference among the three OF-supplemented treatments. Nevertheless, *trpA04*, *ant(3'')*-Ia, *aph(3'')*-Ia and *ermF* exhibited the shortest half-lives in the Upland + OF treatment (Table 1).

Table 1

Half-lives of ARDs in soils.

ARDs ¹	Control		Upland + OF		CF + OF		IF + OF	
	$t_{1/2}$ (day)	MC ²	$t_{1/2}$ (day)	MC	$t_{1/2}$ (day)	MC	$t_{1/2}$ (day)	MC
<i>intI1</i>	53	a	16	b	17	b	17	b
<i>trpA04</i>	89	a	19	c	25	bc	25	b
<i>sul1</i>	63	a	24	b	26	b	28	b
<i>dfrA1</i>	67	a	22	b	20	b	22	b
<i>dfrA12</i>	35	a	18	b	17	b	18	b
<i>dfrA14</i>	160	a	27	b	24	b	26	b
<i>ant(3'')</i> -Ia	NA	-	26	b	38	a	33	ab
<i>aph(3'')</i> -Ia	79	a	15	c	21	b	19	b
<i>ermB</i>	NA	-	36	ns	35	ns	29	ns
<i>ermF</i>	70	a	37	b	67	a	52	a
<i>vatE</i>	28	a	28	ab	22	ab	21	b
<i>tetW</i>	NA	-	49	ns	40	ns	43	ns
<i>tetL</i>	116	a	47	b	50	b	41	b
<i>lnuA</i>	NA	-	50	ns	61	ns	NA	-
<i>incW</i> -rep	73	a	37	b	141	a	104	a
<i>aph(3'')</i> -IIIa	NA	-	NA	-	NA	-	102	-
<i>tetO</i>	131	ns	145	ns	NA	-	108	ns
<i>aac(6'')</i> -Ib3	83	ns	66	ns	51	ns	51	ns
<i>bla</i> -OXA	86	ns	87	ns	71	ns	72	ns
<i>tetX</i>	42	b	52	ab	61	a	40	b
<i>tetG</i>	97	ns	163	ns	132	ns	102	ns
<i>tetM</i>	124	a	113	a	104	ab	66	b
<i>sul2</i>	74	ns	NA	-	NA	-	100	ns
<i>ermT</i>	NA	-	NA	-	NA	-	NA	-
<i>lnuB</i>	73	ab	NA	-	132	a	58	b
<i>catB3</i>	129	ns	152	ns	NA	-	171	ns
<i>MOBy</i>	114	ns	NA	-	NA	-	86	ns

1: Gene names in bold indicate genes significantly increased by fertilization. Calculation of the half-life of *intI2* is not available since the gene was undetectable in some treatments during the incubation.

NA, half-life is not available because of the insignificance ($p > 0.05$) of the gene in the attenuation model.

2: MC, Multiple comparison of $t_{1/2}$ among treatments. Different lowercase letters indicate significant difference between treatments at a level of 0.05. ns, difference is not significant. "-": Difference of $t_{1/2}$ cannot be compared for genes without significant attenuation.

OF, organic fertilizer. CF, Continuous Flooding. IF, Intermittent Flooding.

For the three genes that were significantly increased by fertilization but exhibited no general improvement in their fittings to the attenuation model in OF-supplemented treatments (Table 1, Table S4, Fig. S6), *incW*-rep showed a short half-life of 36 days in Upland + OF, but had long half-lives in the Control ($t_{1/2} = 73$ d), CF + OF ($t_{1/2} = 141$ d) and IF + OF ($t_{1/2} = 104$ d>). *aph(3'')*-IIIa significantly attenuated only in IF + OF, but with a long half-life of 102 days. *tetO* showed no significant attenuation in CF + OF and had long half-lives in the Control ($t_{1/2} = 131$ d), Upland + OF ($t_{1/2} = 145$ d) and IF + OF ($t_{1/2} = 108$ d) (Table 1, Table S4). For the genes that did not exhibit significant increases following fertilization, there was no notable effect of fertilization on their half-lives in the Upland treatment, compared to the Control. Additionally, flooding, whether continuously or intermittently, exhibited no significant effect on the half-lives of these genes in comparison to the Upland treatment (Table 1, Table S4, Fig. S7).

3.3. Variation of bacterial communities during soil incubation

The bacterial composition displayed divergent differences between the organic fertilizer and soil samples (Fig. S8). Among the 129 ASVs that accounted for 97.1% of the relative abundance in the organic fertilizer, only 17 ASVs were present with a relative abundance of 2.0% in the control soil on Day 0. In the organic fertilizer, there was a clear dominance of Actinomycetota (64.3% of the relative abundance),

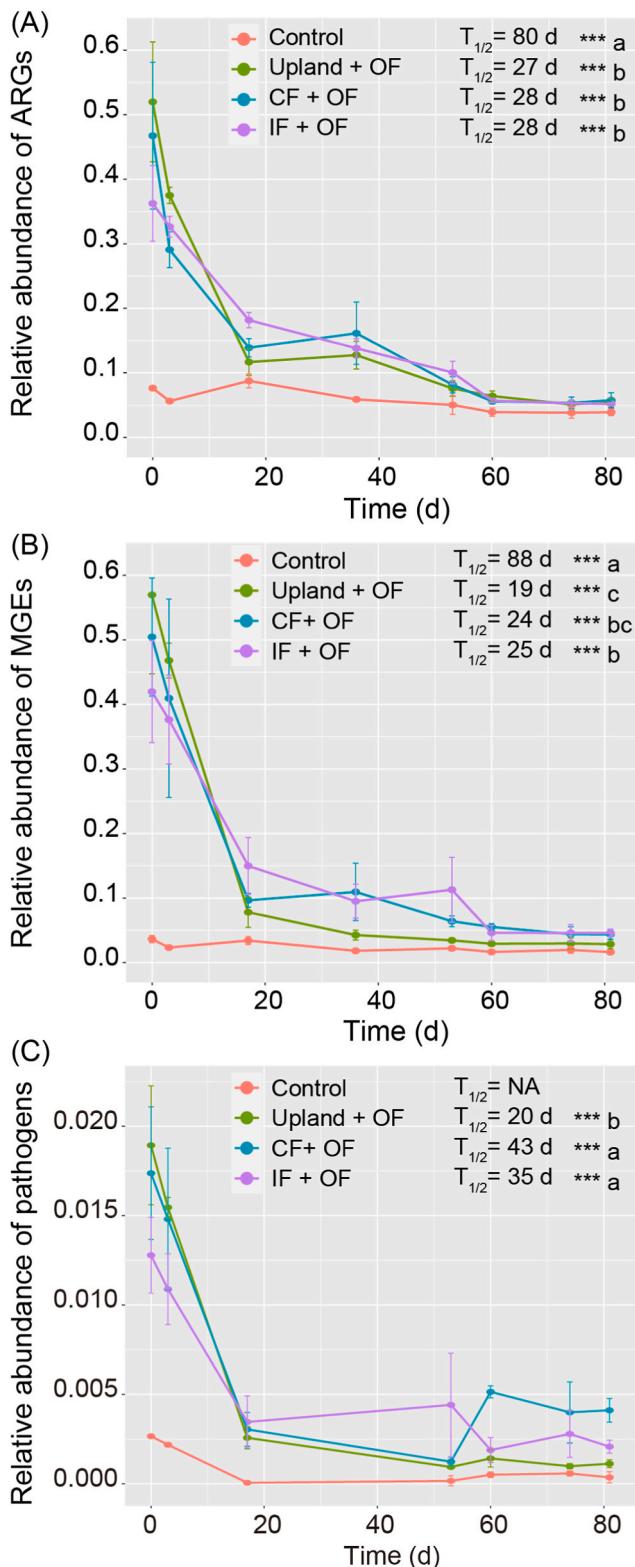


Fig. 2. Temporal variation and half-lives of total ARGs, total MGEs and pathogens increased by fertilization. (A) Temporal variation and half-lives of total ARGs. (B) Temporal variation and half-lives of total MGEs. (C) Temporal variation and half-lives of pathogens increased by fertilization. *** indicates the p value of the fitting to the attenuation model is < 0.001 . Different lowercase letters following *** indicate significant difference of half-lives between treatments. NA: Half-life is not available because of the insignificance ($p > 0.05$) in the attenuation model. OF, organic fertilizer. CF, Continuous Flooding. IF, Intermittent Flooding.

Bacillota (20.7%), and Chloroflexota (11.4%). In contrast, the main phyla in soil samples, including Actinomycetota, Pseudomonadota, Chloroflexota, Bacillota, Planctomycetota and Acidobacteriota were more evenly distributed (Fig. S8). Fertilization significantly ($p < 0.05$) increased the relative abundance of Actinomycetota in the soil of all three OF-supplemented treatments on Day 0 and Day 3 (Table S5). This increment was consistent across the OF-supplemented treatments, with no significant inter-treatment differences ($p > 0.05$; Table S6). No similar increase occurred in other soil phyla (Fig. S8, Table S5, Table S6). During the incubation, temporal variations occurred for Actinomycetota, Pseudomonadota, Chloroflexota and Bacillota in the OF-supplemented soils, and for Chloroflexota and Bacillota in the Control (Table S5). Critically, only Actinomycetota in the OF-supplemented soils displayed a clear and significant decreasing temporal trend, evident both in combined and separate comparisons with Control (Table S5, Table S7).

The overall soil bacterial communities (BC) were categorized into two modules based on ASV co-occurrence patterns: significantly co-occurring communities showing structured interactions and randomly-occurring communities lacking such interactions (Fig. S9). The co-occurring BC and randomly-occurring BC comprised 23.2%–33.0% (relative abundance, the same below) and 31.1%–41.5% of the overall soil communities, respectively (Fig. 3). Strikingly, a total of 70.6% of the organic fertilizer-derived bacteria integrated into the soil co-occurring BC, compared to only 0.3% that fell into the randomly-occurring counterparts (Fig. 3). The effect of fertilization on the abundance increase of Actinomycetota was more pronounced in the co-occurring BC compared to that in the randomly-occurring BC (Fig. 3, Tables S8 and S9). A significant ($p < 0.05$) temporal variation, specifically an abundance reduction of Actinomycetota, was observed in the co-occurring BC of the OF-supplemented treatments, with no significant ($p > 0.05$) differences among the three OF-supplemented treatments. In contrast, the randomly-occurring BC exhibited no such temporal variation (Tables S8 and S9).

MBPD analysis classified 13.4% of the bacterial community in organic fertilizer as potential pathogens. In soils, pathogen abundance ranged from 6.1% to 9.1% during the incubation, with no overall attenuation observed across treatments (Fig. S10A). Pathogens detected in control soil but not in the organic fertilizer (4.0%–5.0% of soil bacterial communities) were soil-originated and exhibited stable persistence (half-lives > 326 days) unaffected by fertilization (Fig. S10B). Conversely, pathogens detected in the organic fertilizer but not in the control soil (0.15%–0.25% of bacterial communities in OF-amended soils on Day 0) were OF-originated and showed significant attenuation with half-lives of 24 days (Upland + OF), 39 days (CF + OF), and 53 days (IF + OF) (Fig. S10C). Pathogens shared between the organic fertilizer and control soils (11.1% of the bacterial community in OF, 0.5% in control soil, and 1.6%–2.5% in OF-amended soils on Day 0) increased initially in OF-amended treatments but subsequently declined, with half-lives of 29 days (Upland + OF), 47 days (CF + OF), and 51 days (IF + OF) (Fig. S10D). When considering only the pathogens that significantly increased upon fertilization, significant attenuation occurred with half-lives of 20 days, 43 days and 35 days in Upland + OF, CF + OF and IF + OF, respectively (Fig. 2C). Notably, compared to upland treatment, flooding significantly prolonged the survival of pathogens increased by fertilization and those originated from the organic fertilizer (Fig. 2C, Fig. S10C & S10D).

3.4. Different responses of ARDs and bacterial communities to environmental factors

Incubation time, soil water status (upland, flooding and drained) and fertilization all exerted significant ($p < 0.05$) effects on both the compositions of ARDs and bacterial communities. Nevertheless, ARDs and bacterial communities displayed different responses to these environmental factors (Fig. 4). According to the results of PERMANOVA and

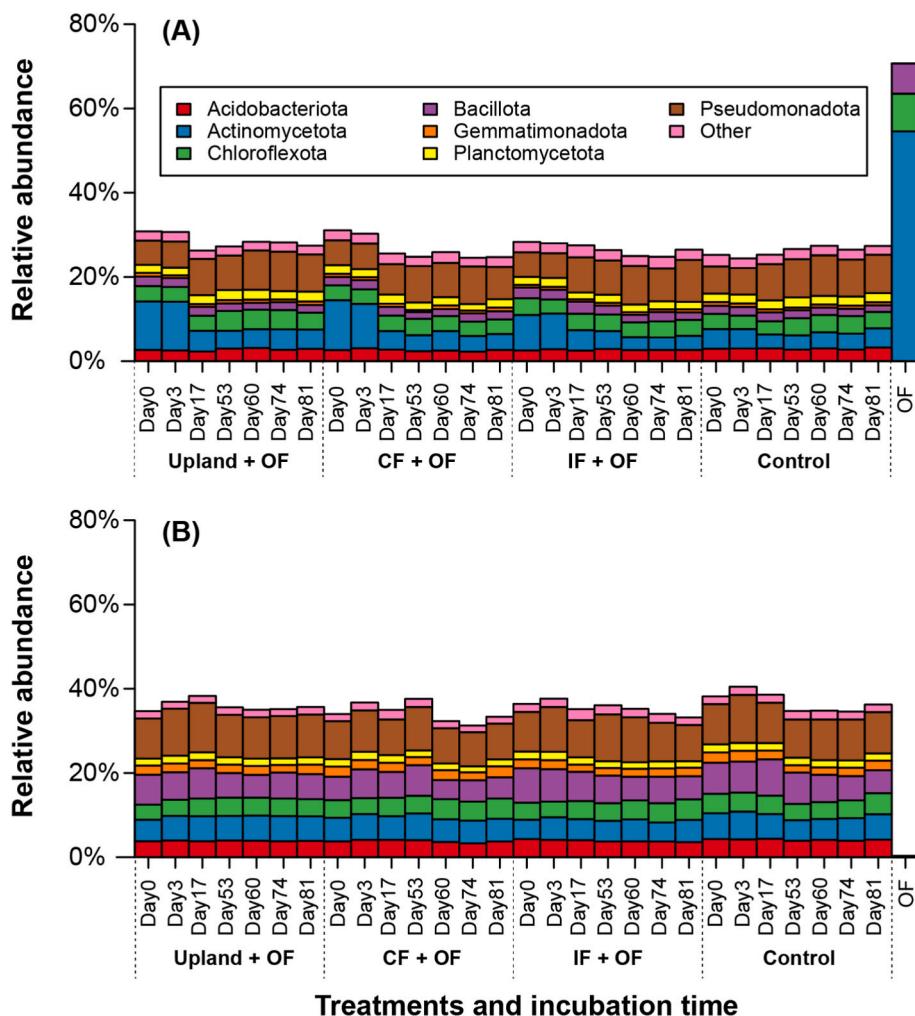


Fig. 3. Relative abundance of (A) significantly co-occurring bacterial communities and (B) randomly occurring bacterial communities in soil samples and the corresponding relative abundances in the organic fertilizer. The co-occurrence pattern of bacterial ASVs was analyzed only in soil samples. OF, organic fertilizer. CF, Continuous Flooding. IF, Intermittent Flooding.

MRPP analyses, the compositional structure of ARDs was primarily influenced by incubation time (PERMANOVA, $R^2 = 0.391$; MRPP, $A = 0.446$) and to a lesser extent by organic fertilization (PERMANOVA, $R^2 = 0.133$; MRPP, $A = 0.102$) (Fig. 4, Table S10). Soil water status showed a relatively minor effect on the compositional structure of ARDs (PERMANOVA, $R^2 = 0.025$; MRPP, $A = 0.085$). Between the two groups of ARD members, MGEs were more significantly influenced by soil water status compared to ARGs (Table S11). Comparatively, the compositional structure of the bacterial communities was less affected by the three environmental factors (Fig. 4, Table S10). Among these factors, incubation time had the most substantial contribution (PERMANOVA, $R^2 = 0.177$; MRPP, $A = 0.130$), followed by soil water status (PERMANOVA, $R^2 = 0.074$; MRPP, $A = 0.047$). Organic fertilization (PERMANOVA, $R^2 = 0.028$; MRPP, $A = 0.023$) had the smallest impact (Fig. 4, Table S10). Furthermore, compared to the randomly-occurring BC, the co-occurring BC was more susceptible to being influenced by environmental factors (Table S12).

3.5. Relationship between ARDs and bacterial communities

Significant relationships were observed between ARGs and MGEs, as well as between ARGs and bacterial communities in terms of the overall, co-occurring and randomly-occurring BC, respectively (Fig. 5A–C). Notably, the co-occurring BC exhibited a stronger relationship with ARGs ($r = 0.258$, $p = 0.0001$) compared to the randomly-occurring BC

($r = 0.139$, $p = 0.0004$) (Fig. 5B & C). While MGEs did not show a significant relationship ($p > 0.05$) with the overall BC or the randomly-occurring BC (Fig. 5A & C), they did demonstrate a significant relationship with the co-occurring BC ($p = 0.0001$) (Fig. 5B). The results of VPA indicated that the co-occurring BC accounted for 24.8% of the variation in ARDs, whereas the randomly-occurring BC explained only 3.3% of the variation (Fig. 5D).

Network analysis revealed prominent associations among different ARD members (Fig. 6). Notably, the network visually illustrated a closer association between ARDs and the co-occurring BC compared to that between ARDs and the randomly-occurring BC (Fig. 6). Specifically, there were 146 edges representing significant correlations ($r \geq 0.7$ or $r \leq -0.7$, $p < 0.0001$) between ARDs and the co-occurring BC, whereas only 2 edges were observed between ARDs and the randomly-occurring BC. In addition, significant correlations among ASV members were observed much more frequently in the co-occurring BC (137 edges) than in the randomly-occurring BC (6 edges).

In the co-occurring BC, both positive (14 ASVs) and negative (six ASVs) correlations were observed between ASVs and ARDs (Fig. 6). The 14 ASVs that exhibited positive correlations ($p < 0.0001$) with ARDs totally accounted for 9.8%, 9.6% and 6.7% of the relative abundances in Upland + OF, CF + OF and IF + OF on Day 0, respectively, which were all significantly higher ($p < 0.05$) than that observed in the Control (1.5%). These ASVs belonged to various taxa, including nine ASVs from Actinobacteria and two ASVs from Acidimicrobia within the phylum

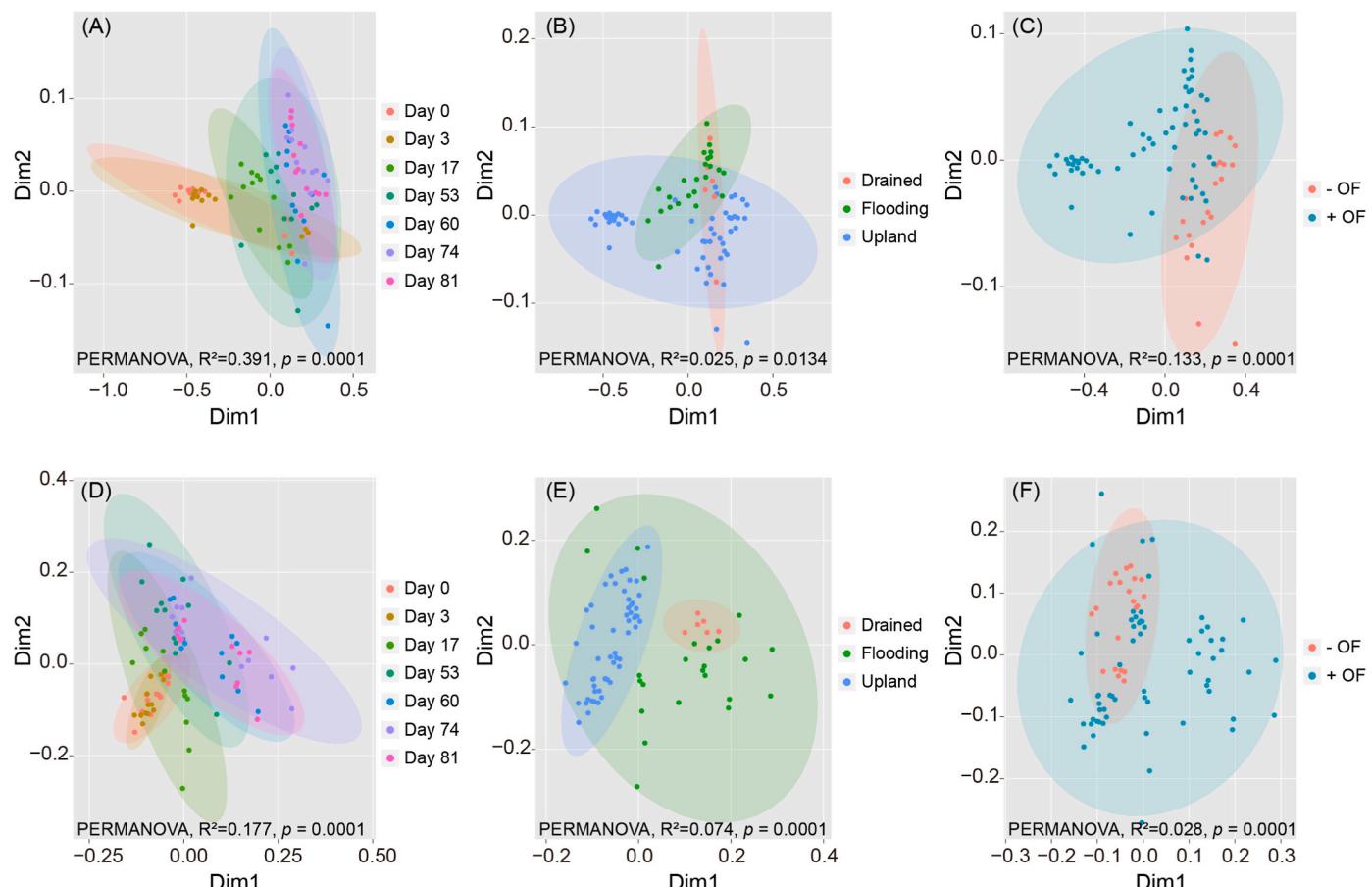


Fig. 4. Non-metric multidimensional scaling (NMDS) of ARDs and bacterial communities in soil samples. (A)–(C) NMDS of ARDs. (D)–(F) NMDS of bacterial communities. Groups are classified according to sampling time (A)&(D), soil water status (B)&(E) and fertilization (C)&(F).

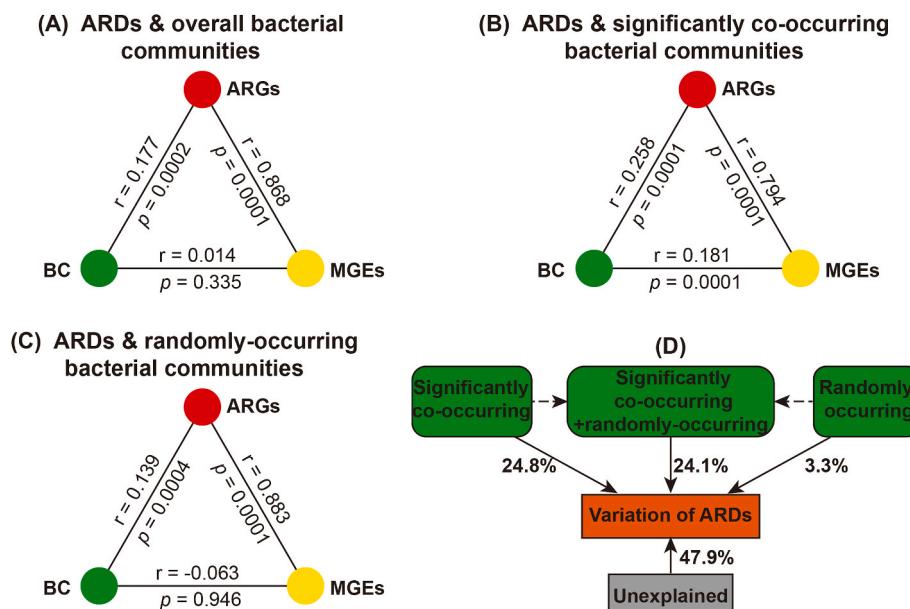


Fig. 5. Relationship between ARDs and bacterial communities. (A) Partial mantel test among ARGs, MGEs and the overall bacterial communities. (B) Partial mantel test among ARGs, MGEs and the significantly co-occurring bacterial communities. (C) Partial mantel test among ARGs, MGEs and the randomly-occurring bacterial communities. (D) Variance partitioning analysis showing the contributions of significantly co-occurring and randomly occurring bacterial communities to the variation of ARDs. BC, bacterial communities.

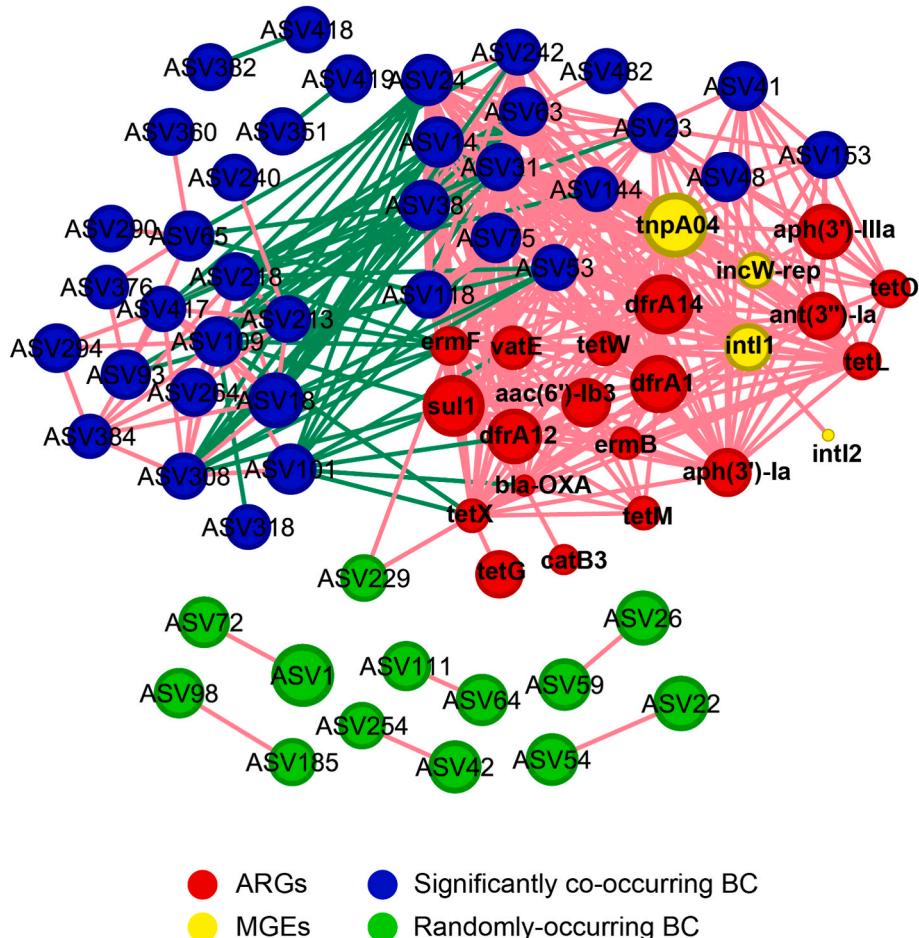


Fig. 6. Network showing the correlations among ARGs, MGEs, significantly co-occurring BC and randomly occurring BC. The network was constructed based on the spearman correlations between the relative abundances of ARGs, MGEs and ASVs. Correlation coefficients ($p < 0.0001$) greater than 0.7 (pink edges) or less than -0.7 (green edges) are presented in the network. The node size is proportioned to the log10-transformed average relative abundance of the gene or ASV. BC, bacterial communities.

Actinomycetota, two ASVs from Clostridia in the phylum Bacillota, and one ASV from Chloroflexia in the phylum Chloroflexota (Fig. 7A, Table S13). The identified genera included *Ornithinicoccus*, *Georgenia*, *Brachybacterium*, *Mycobacterium*, *Dietzia*, *Pseudactinotalea*, *Aeromicrobium*, *Corynebacterium*, *Clostridium sensu stricto 1*, and *Terrisporobacter* (Table S13). Each of these 14 ASVs was detected with individual relative abundances ranging from 1.1% to 10.7%, contributing to a total relative abundance of 69.2% in the organic fertilizer (Fig. 7A). These ASVs are likely the potential bacterial hosts of ARDs that can be transmitted from the organic fertilizer into the soils. It is noteworthy that ASV31, ASV38, ASV41, ASV48, ASV63, ASV153, ASV242 were potential pathogens or from bacterial genera that were reported to prevalently contain pathogenic species (Fig. 7A, Table S13).

The total relative abundance of the potential ARD hosts significantly attenuated ($p < 0.001$) in the OF-supplemented treatments throughout the incubation period, exhibiting half-lives of 20 days, 28 days and 28 days in Upland + OF, CF + OF and IF + OF, respectively (Fig. 7B). Both continuous and intermittent flooding significantly ($p < 0.05$) extended the half-lives of these ASVs in total, compared to the Upland + OF treatment. ASV23, ASV41, ASV48, ASV153 were detectable throughout the entire incubation period in all three OF-supplemented treatments. Among these ASVs, ASV23 (family JG30-KF-CM45) exhibited significant attenuation across all three OF-supplemented treatments, with the shortest half-life of 30 days observed in Upland + OF (Fig. 7C). Notably, ASV41 (genus *Clostridium sensu stricto 1*), ASV48 (*Mycobacterium thermostresistibile*) and ASV153 (genus *Terrisporobacter*) showed significant

attenuation exclusively in Upland + OF (Fig. 7D–F). In addition, ASV23 strongly correlated ($r > 0.7, p < 10^{-13}$) with *trpA04*, *incW-rep*, *ant(3'')-Ia*, *aph(3')-Ia*, and *ermF*, which all demonstrated the shortest half-life in Upland + OF, as well as with *tetO*, which persisted across all OF-supplemented treatments during the incubation (Table S14). Similarly, ASV41, ASV48 and ASV153 also exhibited strong correlations ($r > 0.7, p < 10^{-13}$) with *trpA04*, *incW-rep*, *ant(3'')-Ia*, *aph(3')-Ia* as well as with *tetO* and *aph(3'')-IIIa*, both of which showed little attenuation in all OF-supplemented treatments during the incubation (Table S14).

Six ASVs displayed an increase in abundance during the incubation period, comprising three from γ -Proteobacteria, two from Actinobacteria, and one from Chloroflexia. These ASVs displayed negative correlations with both ARDs and the ARD hosts (Fig. 6, Fig. S11), and they formed a distinct cluster separate from that of the ARD hosts (Fig. 6). Notably, none of these ASVs were detected in the organic fertilizer, suggesting that they may be indigenous soil species. Additionally, neither fertilization nor water management had a significant effect ($p > 0.05$) on their abundances in the soil (Fig. S11).

4. Discussion

4.1. Effect of water management on the persistence and attenuation of ARDs

The current study investigated the fates of ARDs and bacterial communities in soil after organic fertilization and characterized the

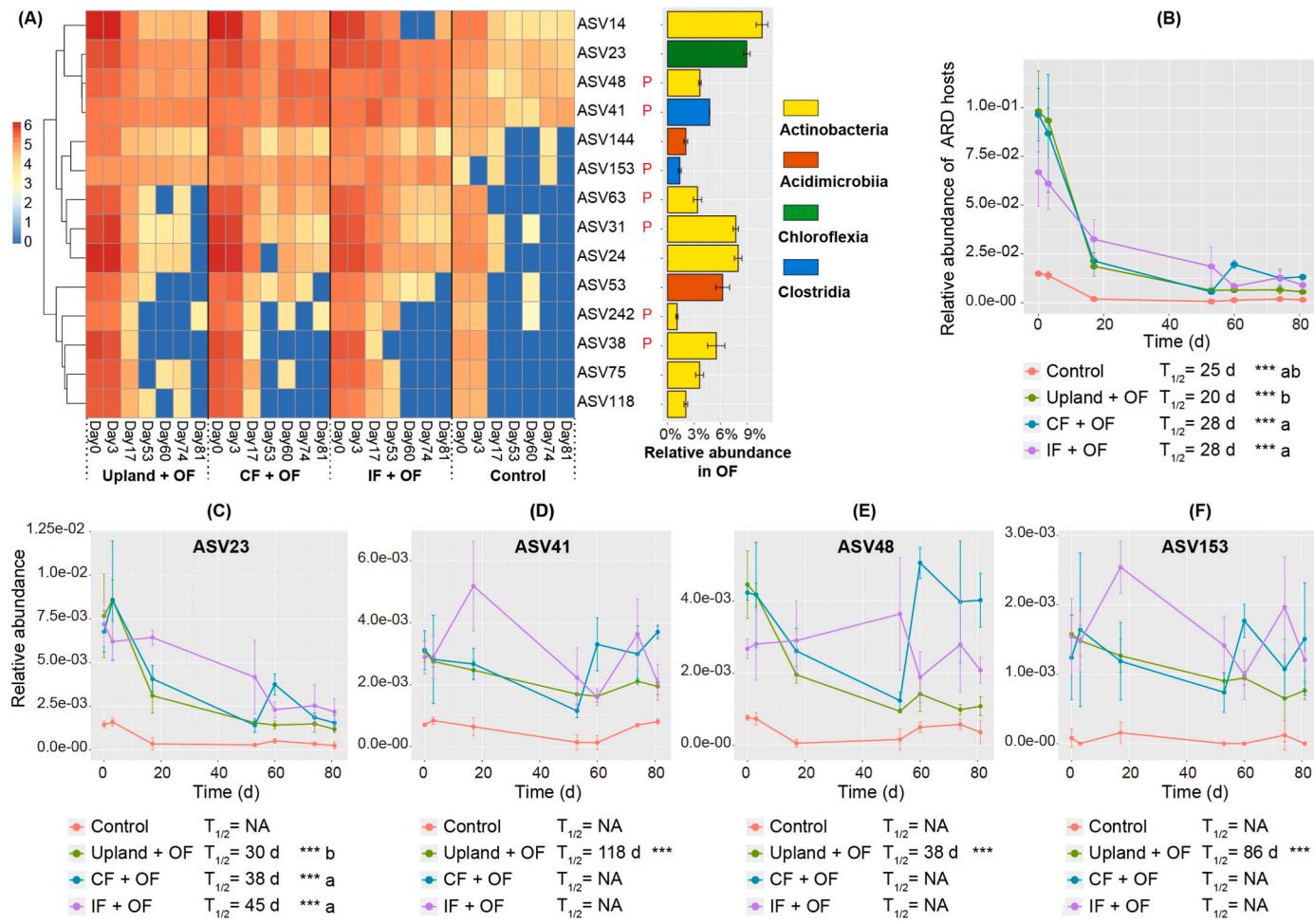


Fig. 7. Dynamics of ARD hosts. (A) Relative abundances of the potential ARD hosts in soil during the incubation. A red P indicates that the ASV is a potential pathogen. (B) Temporal variation and half-lives of potential ARD hosts in total. (C)–(F) shows the temporal variation and half-lives of ASV23, ASV41, ASV48 and ASV153, respectively. NA: Half-life is not available because of the insignificance ($p > 0.05$) of the ASV in the attenuation model or because the ASV was undetectable in the Control. *** indicates the p value of the fitting to the attenuation model is < 0.001 . Different lowercase letters following *** indicate significant difference of half-lives between treatments. OF, organic fertilizer. CF, Continuous Flooding. IF, Intermittent Flooding.

attenuation and persistence of ARDs as well as the succession of soil bacterial communities under different water managements. The ARDs detected in the present study showed divergent fates following organic fertilization during the soil incubation. Most of the ARDs that significantly increased in relative abundance following organic fertilization can attenuate with half-lives below 50 days across all OF-supplemented soils, with no significant distinction observed between upland and flooding soils (Table 1). ARDs from the organic fertilizer typically undergo a process of attenuation due to the unfitness of most fertilizer-borne ARD hosts in soil environment, and to some extent, due to the degradation of extracellular ARDs from the organic fertilizer during the soil incubation (Sun et al., 2015; Liu et al., 2024). The unfitness of most of the fertilizer-borne ARD hosts in soil was supported by the divergent bacterial compositions between the organic fertilizer and the soils, as well as the declining trends of the potential ARD hosts (Fig. 7 & S8). The half-lives of ARGs (27–28 days), MGEs (19–25 days) and ARD hosts (20–28 days) in OF-supplemented soils generally fell within a similar range, indicating that the reduction of ARD hosts primarily drove the attenuation of the ARDs (Figs. 2 & 7B). The emergence and proliferation of indigenous soil bacteria exhibiting strong negative correlations with ARDs ($r < -0.7$, $p < 0.0001$) suggested their potential advantage over the ARD hosts from the organic fertilizer (Fig. 6, Fig. S11).

Our findings demonstrate accelerated attenuation of ARDs in upland soils compared to flooded treatments, particularly for *tspA04*, *incW-rep*,

ant(3")-Ia, *aph(3')-Ia*, and *ermF* (Tables 1 and S2). This aligns with prior evidence that flooding promotes persistence of specific ARD subtypes in manured soils (Wang et al., 2018; Xiang et al., 2023; Li et al., 2024). The anaerobic-reducing conditions in flooded soils, resembling the gut environments of livestock, likely enhance the survival of ARD hosts originating from organic fertilizers, as evidenced by prolonged host half-lives under flooding (Fig. 7B–F). Notably, key pathogenic hosts, including *Clostridium sensu stricto 1* (ASV41), *Mycobacterium thermoresistibile* (ASV48), and *Terrisporobacter* (ASV153), exhibited no significant post-fertilization attenuation in flooded soils (Fig. 7D–F). These genera are well-documented as zoonotic or human pathogens (Songer and Anderson, 2006; Cassir et al., 2016; Vishkautsan et al., 2016; Kang et al., 2024; Subramaniam et al., 2024), and their persistence under flooding was further corroborated by elevated survival rates of organic fertilizer-derived and fertilization-induced pathogens (Fig. 2C & S10). Critically, MGEs such as the transposase gene *tspA04* and plasmid marker *incW-rep* displayed slower attenuation in flooded soils, likely due to their strong association with the aforementioned pathogenic hosts (ASV41, ASV48, ASV153) (Tables 1 and S14). MGEs center in the assembly and horizontal transfer of multiple ARGs among bacterial species, especially among pathogenic taxa (Ghaly and Gillings, 2021; Lang et al., 2025). Our results collectively highlight dual risks of soil antibiotic resistance under flooding: (1) extended persistence of ARD hosts, especially pathogenic ones, from organic fertilizers, and (2) enhanced

horizontal gene transfer of ARDs via MGEs.

Special attention should be paid to genes such as *aph(3')-IIIa* and *tetO*, given their persistence in both upland and flooding soils (Table 1, Tables S2 and S3, Fig. S6). *aph(3')-IIIa* encodes a plasmid-mediated aminoglycoside phosphotransferase that is prevalently found in enteric Gram-positive bacteria (Trieu-Cuot and Courvalin, 1983; Zaidi et al., 2023). Similarly, *tetO* has been documented to be associated closely with conjugative plasmids in bacteria residing in animal guts (Sougakoff et al., 1987; Avrain et al., 2004). The persistence of these two genes in OF-supplemented soils could be attributed to their close associations to both plasmids and the ARD hosts that exhibited prolonged survival in soil environments, such as ASV41 and ASV153 (Fig. 7D & F). Specifically, field studies show that *tetO* introduced through long-term pig manure fertilization persists for at least one year in soil (Peng et al., 2017). Consequently, these genes could accumulate readily in soils under various water management practices, particularly in systems subjected to long-term organic fertilization.

ARDs that showed no significant change upon the fertilization generally varied less during the soil incubation. These genes might have broad occurrence in the microbial communities of both animal wastes and soils, allowing them to respond less dramatically to environmental changes such as fertilization and water management practices.

4.2. ARDs and bacterial communities respond divergently to the changes of environmental factors

The compositions of both ARDs and bacterial communities were predominantly differentiated by incubation time rather than by fertilization or soil water status (Fig. 4), highlighting temporal dynamics as the primary driver of variability in ARDs and soil bacterial communities. This temporal dominance was reinforced by significant reductions in ARDs, their host taxa and the fertilization-increased bacteria over time (Table 1, Fig. 7, Table S5–S7). These findings emphasize that the time elapsed between agricultural interventions (e.g., fertilization, land use conversion) and soil sampling is a critical consideration, as microbial effects, especially the transient ones, may be confounded by temporal attenuation (Wang et al., 2018; Xie et al., 2018b).

Soil water status exerted a stronger influence than fertilization on bacterial community variation (Fig. 4E & F, Table S10), consistent with prior observations that organic fertilization minimally alters bacterial diversity metrics (e.g., Shannon/Chao1 indices) in upland soils (Sun et al., 2015; Xie et al., 2018b). This contrasts with ARD composition, which responded more sensitively to fertilization than to hydrological conditions. Organic fertilizers, particularly animal waste-derived amendments, directly introduce substantial ARDs, driving immediate and persistent shifts in soil resistome abundance, diversity, and structure (Xie et al., 2018b, 2022).

The discrepancy between bacterial communities (water status-driven) and ARDs (fertilization-driven) in terms of their responses to environmental factors implies distinct dissemination pathways for exogenous ARDs that are relatively independent of the overall bacterial communities. Specifically, redox dynamics under varying water regimes primarily govern native microbial assembly (Peralta et al., 2014; Meng et al., 2019; Santos-Medellín et al., 2021), while fates of ARDs are predominantly determined by initial input from organic fertilizers. Consequently, pre-treatment mitigation of ARD loads in animal waste may probably prove more effective for resistome control than post-application water management. These findings underscore the need for source-targeted strategies to disrupt ARD transmission from organic amendments to agricultural ecosystems.

4.3. Co-occurring bacterial communities in soil are assembly hubs in the harboring and dissemination of ARDs

Soil bacterial communities were partitioned into two distinct modules based on ASV co-occurrence patterns: (1) co-occurring communities

with strong positive/negative correlations (indicative of ecological interactions) (Veech, 2012; Griffith et al., 2016), and (2) randomly-occurring communities lacking structured associations (Fig. 6 & S9). Network analysis revealed that robust correlations were concentrated within co-occurring communities (Fig. 6), aligning with their heightened sensitivity to environmental perturbations compared to random counterparts (Fig. 3, Table S12). Notably, nearly all fertilizer-derived ASVs that persisted in soils were integrated into the co-occurring communities (Figs. 6 & 7A), suggesting these modules act as preferential reservoirs for exogenous bacteria, including ARD hosts (Fig. 7A). This niche prioritization explains why post-fertilization ARDs were almost exclusively linked to the co-occurring communities (Fig. 6).

The potential of horizontal gene transfer of ARGs was significantly elevated within soil co-occurring bacterial communities compared to randomly-occurring counterparts (Fig. 5A–C). The dense co-association networks observed within soil co-occurring bacterial communities (Fig. 6) suggest frequent microbial interactions, potentially through close physical proximity or contact, that facilitate ARG dissemination (Tecon et al., 2018). Consequently, it is plausible that the transmission of ARGs in soil bacterial communities is primarily concentrated within the co-occurring communities. Given the enhanced survival capacity of bacteria originating from organic fertilizers in the soil co-occurring bacterial communities, it is conceivable that a “dissemination highway” for antibiotic resistance exists, linking ARD hosts in the organic fertilizers to the co-occurring bacterial communities in soil. Along this pathway, pathogenic bacterial species play a significant role in carrying and disseminating ARDs (Fig. 7). Disrupting this “highway” or clearing away its pathogenic travelers could be crucial in halting the rapid spread of ARDs from animal industries to soil ecosystems and beyond.

5. Conclusion

This study provides new insights into the fates of ARDs and the dynamics of soil bacterial communities under different water management practices following organic fertilization. Our findings show that despite the rapid attenuation of most ARDs along with their bacterial hosts introduced via the organic fertilizer, particular attention should be given to resistance determinants such as *aph(3')-IIIa* and *tetO*, which exhibit persistence across different water management regimes and have the potential to accumulate in soil. Water management significantly influences the persistence and attenuation of ARDs and pathogens, with flooding conditions favoring the survival of pathogens, specific ARD hosts and accelerating horizontal gene transfer through MGEs. Our results reveal the dissemination pattern that orchestrates the fates of ARDs from organic fertilizers to soil environments. The soil co-occurring bacterial communities, characterized by strong interspecies interactions and high sensitivity to environmental shifts, serve as the preferential assembly hubs for fertilizer-originating ARD hosts and facilitate the horizontal transfer of ARGs. Breaking this dissemination pathway between organic fertilizers and soil by targeting pathogenic ARD hosts or disrupting their interactions within the soil microbial network represents a promising strategy to mitigate the dissemination of antibiotic resistance from animal industries to soil ecosystems.

CRediT authorship contribution statement

Ya-Lan Hong: Writing – original draft, Visualization, Methodology, Investigation, Data curation. **Wei-Ming Xi:** Writing – original draft, Visualization, Software, Methodology, Investigation, Data curation. **Ya-Ting Wang:** Writing – review & editing, Validation, Software, Methodology, Investigation, Data curation, Conceptualization. **Yi Yuan:** Writing – review & editing, Methodology, Investigation. **Zong-Zhuan Shen:** Writing – review & editing, Resources. **Ming Tian:** Writing – review & editing, Resources. **Jihong Liu Clarke:** Writing – review & editing, Resources, Funding acquisition. **Wan-Ying Xie:** Writing – review & editing, Writing – original draft, Visualization, Validation,

Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Fang-Jie Zhao: Writing – review & editing, Resources, 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.

Acknowledgements

This work was supported by National Natural Science Foundation of China (grant number 42090062) and the Research Council of Norway, China-Norway Collaborative and Knowledge-building Project (SiNor-AMR, grant number 336168).

Appendix B. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2025.126708>.

Data availability

Data will be made available on request.

References

- Adair, K.L., Wilson, M., Bost, A., Douglas, A.E., 2018. Microbial community assembly in wild populations of the fruit fly *Drosophila melanogaster*. ISME J. 12, 959–972.
- Avrain, L., Vernozy-Rozand, C., Kempf, I., 2004. Evidence for natural horizontal transfer of *terO* gene between *Campylobacter jejuni* strains in chickens. J. Appl. Microbiol. 97, 134–140.
- Cassir, N., Benamar, S., La Scola, B., 2016. *Clostridium butyricum*: from beneficial to a new emerging pathogen. Clin. Microbiol. Infection 22, 37–45.
- Diacomo, M., Montemurro, F., 2011. Long-term effects of organic amendments on soil fertility. In: Lichtfouse, E., Hamelin, M., Navarrete, M., Debaeke, P. (Eds.), Sustainable Agriculture Volume 2. Springer, Netherlands, Dordrecht, pp. 761–786.
- Forsberg, K.J., Patel, S., Gibson, M.K., Lauber, C.L., Knight, R., Fierer, N., Dantas, G., 2014. Bacterial phylogeny structures soil resistomes across habitats. Nature 509, 612–616.
- Forsberg, K.J., Reyes, A., Wang, B., Selleck, E.M., Sommer, M.O., Dantas, G., 2012. The shared antibiotic resistome of soil bacteria and human pathogens. Science 337, 1107–1111.
- Gao, A.X., Chen, C., Gao, Z.-Y., Zhai, Z.-Q., Wang, P., Zhang, S.-Y., Zhao, F.-J., 2024. Soil redox status governs within-field spatial variation in microbial arsenic methylation and rice straighthead disease. ISME J. 18, wrae057.
- Ghaly, T.M., Gillings, M.R., 2021. New perspectives on mobile genetic elements: a paradigm shift for managing the antibiotic resistance crisis. Phil. Trans. Biol. Sci. 377.
- Gipson, K.S., Nickerson, K.P., Drenkard, E., Llanos-Cheia, A., Dogiparthi, S.K., Lanter, B., Hibbler, R.M., Yonker, L.M., Hurley, B.P., Faherty, C.S., 2020. The great ESKAPE: exploring the crossroads of bile and antibiotic resistance in bacterial pathogens. Infect. Immun. 88, e00865.
- Griffith, D.M., Veech, J.A., Marsh, C.J., 2016. Cooccur: probabilistic species co-occurrence analysis in R. J. Stat. Software 69, e02.
- Johnson, T.A., Stedtfeld, R.D., Wang, Q., Cole, J.R., Hashsham, S.A., Loft, T., Zhu, Y.G., Tiedje, J.M., 2016. Clusters of antibiotic resistance genes enriched together stay together in swine agriculture. mBio 7, e02214.
- Jurasinski, G., Retzer, V., 2015. R package “simba”: A collection of functions for similarity analysis of vegetation data. Version 0.3-5.
- Kang, I., Youm, D.-J., Kim, I., Choi, J., Yoon, J., Yoon, S.Y., Lim, C.S., Cho, M.-C., 2024. Identification of *Terrisporobacter muris* isolated from human blood using whole-genome sequencing: a case report. Heliyon 10, e38284.
- Kang, Y., Li, Q., Mei, L., Zhao, H., Bai, Y., Shen, M., Hu, J., 2018. Tetracycline resistance genes are more prevalent in wet soils than in dry soils. Ecotoxicology and environmental safety 156, 337–343.
- Lang, A.S., Buchan, A., Burrus, V., 2025. Interactions and evolutionary relationships among bacterial mobile genetic elements. Nat. Rev. Microbiol. 23, 423–438.
- Li, X., Zhu, L., Zhang, S.-Y., Li, J., Lin, D., Wang, M., 2024. Characterization of microbial contamination in agricultural soil: a public health perspective. Sci. Total Environ. 912, 169139.
- Liu, W., Xie, W.-Y., Liu, H.-J., Chen, C., Chen, S.-Y., Jiang, G.-F., Zhao, F.-J., 2024. Assessing intracellular and extracellular distribution of antibiotic resistance genes in the commercial organic fertilizers. Sci. Total Environ. 929, 172558.
- Meng, D., Li, J., Liu, T., Liu, Y., Yan, M., Hu, J., Li, X., Liu, X., Liang, Y., Liu, H., Yin, H., 2019. Effects of redox potential on soil cadmium solubility: insight into microbial community. Journal of Environmental Sciences 75, 224–232.
- Odetokun, I.A., Mulchandani, R., Wang, Y., Gilbert, M., Van Boeckel, T.P., 2023. Global trends in antimicrobial use in food-producing animals: 2020 to 2030. PLOS Global Public Health 3, e0001303.
- Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoeics, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hillm, M.O., Lahti, L., McGlinn, D., Ouellette, M.H., Cunha, E.R., Smith, T., Stier, A., Ter Braak, C.J.F., Weedon, J., 2022. R package “vegan”: Community Ecology Package. Version 2.6-2.
- Peng, S., Feng, Y., Wang, Y., Guo, X., Chu, H., Lin, X., 2017. Prevalence of antibiotic resistance genes in soils after continually applied with different animal manure for 30 years. J. Hazard Mater. 340, 16–25.
- Peralta, A.L., Ludmer, S., Matthews, J.W., Kent, A.D., 2014. Bacterial community response to changes in soil redox potential along a moisture gradient in restored wetlands. Ecol. Eng. 73, 246–253.
- Santos-Medellin, C., Liechty, Z., Edwards, J., Nguyen, B., Huang, B., Weimer, B.C., Sundaresan, V., 2021. Prolonged drought imparts lasting compositional changes to the rice root microbiome. Nat. Plants 7, 1065–1077.
- Songer, J.G., Anderson, M.A., 2006. *Clostridium difficile*: an important pathogen of food animals. Anaerobe 12, 1–4.
- Sougakoff, W., Papadopoulou, B., Nordmann, P., Courvalin, P., 1987. Nucleotide sequence and distribution of gene *tetO* encoding tetracycline resistance in *Campylobacter coli*. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett. 44, 153–159.
- Subramanian, S., Kanhere, M., Shephard, L., Burke, A., Saxon, S., Geake, J., 2024. A case of severe *Mycobacterium thermoresistibile* pneumonia. Respiratory Case Reports 12, e01308.
- Sun, D.-L., Jiang, X., Wu, Q.L., Zhou, N.-Y., 2013. Intrageneric heterogeneity of 16S rRNA genes causes overestimation of prokaryotic diversity. Appl. Environ. Microbiol. 79, 5962–5969.
- Sun, M., Li, T., Li, D., Zhao, Y., Gao, F., Sun, L., Li, X., 2020. Conversion of land use from upland to paddy field changes soil bacterial community structure in mollisols of northeast China. Microb. Ecol. 81, 1018–1028.
- Sun, R., Zhang, X.-X., Guo, X., Wang, D., Chu, H., 2015. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. Soil Biol. Biochem. 88, 9–18.
- Tang, X., Lou, C., Wang, S., Lu, Y., Liu, M., Hashmi, M.Z., Liang, X., Li, Z., Liao, Y., Qin, W., Fan, F., Xu, J., Brookes, P.C., 2015. Effects of long-term manure applications on the occurrence of antibiotics and antibiotic resistance genes (ARGs) in paddy soils: evidence from four field experiments in south of China. Soil Biol. Biochem. 90, 179–187.
- Tecon, R., Ebrahimi, A., Kleyer, H., Erev Levi, S., Or, D., 2018. Cell-To-Cell Bacterial Interactions Promoted by Drier Conditions on Soil Surfaces, vol.115. Proceedings of the National Academy of Sciences, pp. 9791–9796.
- Trieu-Cuot, P., Courvalin, P., 1983. Nucleotide sequence of the *Streptococcus faecalis* plasmid gene encoding the 3'5'-aminoglycoside phosphotransferase type III. Gene 23, 331–341.
- Veech, J.A., 2012. A probabilistic model for analysing species co-occurrence. Global Ecol. Biogeogr. 22, 252–260.
- Vishkautsan, P., Reagan, K.L., Keel, M.K., Sykes, J.E., 2016. Mycobacterial panniculitis caused by *Mycobacterium thermoresistibile* in a cat. Journal of Feline Medicine Surgery Open Reports 2, 2055116916672786.
- Wang, F., Xu, M., Stedtfeld, R.D., Sheng, H., Fan, J., Liu, M., Chai, B., Soares de Carvalho, T., Li, H., Li, Z., Hashsham, S.A., Tiedje, J.M., 2018. Long-term effect of different fertilization and cropping systems on the soil antibiotic resistome. Environmental Science & Technology 52, 13037–13046.
- Wang, L., Chen, G., Owens, G., Zhang, J., 2016. Enhanced antibiotic removal by the addition of bamboo charcoal during pig manure composting. RSC Adv. 6, 27575–27583.
- Wu, S., Wu, Y., Cao, B., Huang, Q., Cai, P., 2021. An invisible workforce in soil: the neglected role of soil biofilms in conjugative transfer of antibiotic resistance genes. Crit. Rev. Environ. Sci. Technol. 1–29.
- Xiang, Q., Fu, C.-X., Lu, C.-Y., Sun, A.-Q., Chen, Q.-L., Qiao, M., 2023. Flooding drives the temporal turnover of antibiotic resistance gene in manure-amended soil-water continuum. Environ. Int. 179, 108168.
- Xie, W.Y., Shen, Q., Zhao, F.J., 2018a. Antibiotics and antibiotic resistance from animal manures to soil: a review. Eur. J. Soil Sci. 69, 181–195.
- Xie, W.Y., Wang, Y.T., Yuan, J., Hong, W.D., Niu, G.Q., Zou, X., Yang, X.P., Shen, Q., Zhao, F.J., 2022. Prevalent and highly mobile antibiotic resistance genes in commercial organic fertilizers. Environ. Int. 162, 107157.
- Xie, W.Y., Yuan, S.T., Xu, M.G., Yang, X.P., Shen, Q.R., Zhang, W.W., Su, J.Q., Zhao, F.J., 2018b. Long-term effects of manure and chemical fertilizers on soil antibiotic resistome. Soil Biol. Biochem. 122, 111–119.
- Yang, X., Jiang, G., Zhang, Y., Wang, N., Zhang, Y., Wang, X., Zhao, F.J., Xu, Y., Shen, Q., Wei, Z., 2023. MBPD: a multiple bacterial pathogen detection pipeline for one Health practices. iMeta 2, e82.
- Zaidi, S.-E.-Z., Zaheer, R., Poulin-Laprade, D., Scott, A., Rehman, M.A., Diarra, M., Topp, E., Domselaar, G.V., Zovoilis, A., McAllister, T.A., 2023. Comparative genomic analysis of enterococci across sectors of the one health continuum. Microorganisms 11, 727.

- Zhang, D., Sun, J., Peng, S., Wang, Y., Hua, Q., Wu, P., Lin, X., 2025. Paddy-upland rotation combined with manure application: an optimal strategy for enhancing soil multifunctionality. *J. Environ. Manag.* 373, 123788.
- Zhao, Y., Yang, Q.E., Zhou, X., Wang, F.-H., Muurinen, J., Virta, M.P., Brandt, K.K., Zhu, Y.-G., 2020. Antibiotic resistome in the livestock and aquaculture industries: status and solutions. *Crit. Rev. Environ. Sci. Technol.* 1–38.
- 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. *Proceedings of the National Academy of Sciences of the United States of America* 110, 3435–3440.