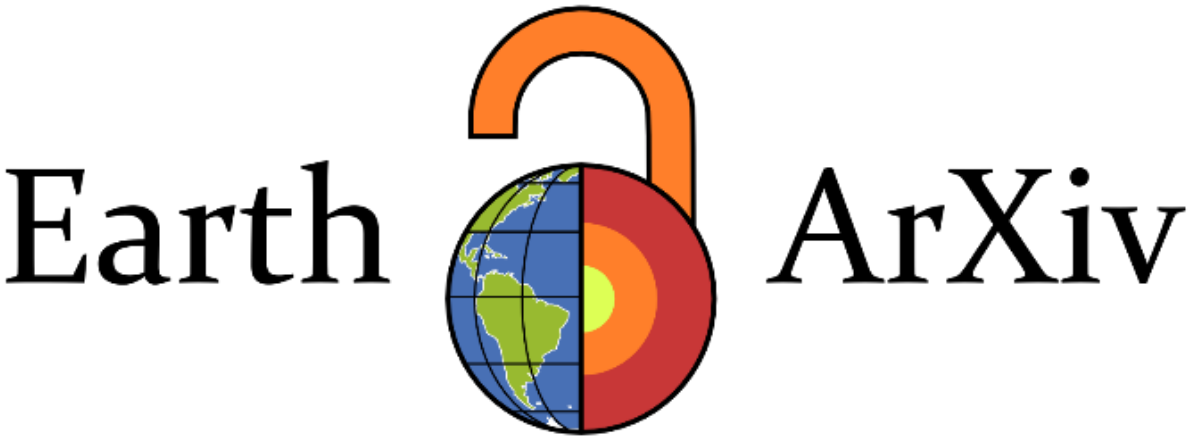

# Anthropogenic Impacts on Antibiotic Resistance Genes and Microbial Communities in Groundwater of Taopu Industrial Park, Shanghai

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[a]Key Laboratory of Geographic Information Science (Ministry of Education), School of Geographic Sciences, East China Normal University, Shanghai 200241, China

[&]These authors contributed equally to this work



[*]Corresponding Author:
Xinran Liu, Email: xrliu@geo.ecnu.edu.cn

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***Corresponding Author:**

Xinran Liu, Email: xrliu@geo.ecnu.edu.cn

## Abstract

Urban groundwater is increasingly recognized as an emerging reservoir and transport pathway for antibiotics, antibiotic-resistant bacteria (ARBs), and antibiotic resistance genes (ARGs), posing potential ecological and public-health risks. However, the distribution and transport mechanisms of antibiotics and ARGs in groundwater systems under complex anthropogenic pollution remain insufficiently understood. Here, we investigated groundwater in Shanghai's Taopu Industrial Park, a region characterized by multiple industrial contamination sources. Antibiotic concentrations were quantified using ultra-performance liquid chromatography–tandem mass spectrometry, while metagenomic sequencing and high-throughput quantitative PCR were employed to characterize ARG diversity and abundance and microbial community composition. Integrated analyses were performed to elucidate the distribution and transport patterns of antibiotics, microorganisms, and ARGs, and to identify key environmental drivers. Co-occurrence network analysis was further applied to infer potential ARB hosts.

Twenty antibiotics were detected in groundwater at concentrations ranging from 24.1 to 1161.1 ng $L^{-1}$, with sulfonamides dominating. Fluoroquinolones were more enriched in soil than groundwater, likely due to stronger sorption associated with their polar/ionic functional groups, whereas tetracyclines exhibited higher vertical mobility than sulfonamides. Antibiotic concentrations decreased exponentially with depth. Groundwater physicochemical parameters (dissolved organic carbon, salinity,

dissolved oxygen, and conductivity) together with co-occurring polycyclic aromatic hydrocarbons (PAHs) were the major determinants of antibiotic distribution.

Groundwater microbial communities were bacteria-dominated and of relatively low diversity, with Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes as the major phyla. Actinobacteria occurred at higher relative abundance than in other industrial groundwater systems, likely reflecting selective pressure from the combined presence of antibiotics and PAHs, consistent with their roles in PAH degradation, antibiotic production, and ARG hosting. Redundancy analysis indicated that contaminants—particularly antibiotics and PAHs—were the primary drivers of microbial community structure, exceeding the effects of physicochemical parameters (pH, dissolved organic carbon, and dissolved oxygen).

The composition and relative abundance of 21 ARG types were highly similar between soil and groundwater, with multidrug, macrolide, glycopeptide, tetracycline, and peptide resistance genes predominating in both media, suggesting potential ARG exchange and migration across compartments. In groundwater, contaminants (especially antibiotics and PAHs) were the dominant determinants of ARG profiles, followed by microbial community composition, while mobile genetic elements and physicochemical conditions further facilitated ARG dissemination. Network analysis identified several shared potential ARG-hosting genera in soil and groundwater, indicating that ARB-mediated vertical transport may represent an important pathway for ARG contamination in groundwater.

Overall, this study reveals the coupled occurrence, transport behavior, and

environmental drivers of antibiotics, microbial communities, and ARGs in urban groundwater under anthropogenic influence. The findings highlight the need for integrated management strategies that reduce source pollutants and selective pressure to mitigate ARG dissemination in subsurface environments.

**Keywords:** Ultra-performance liquid chromatography–tandem mass spectrometry, Metagenomics, HT-qPCR, Antibiotics, ARGs, Groundwater

## 1 Introduction

Since their initial discovery in the 1920s, antibiotics—owing to their bactericidal or bacteriostatic effects—have fundamentally transformed modern medicine and animal husbandry, and are now among the most extensively used pharmaceuticals worldwide (Wang et al., 2015a). However, a large proportion of antibiotics administered to humans and animals are not fully absorbed or metabolized, with 30–90% of veterinary antibiotics excreted via animal manure (Pan et al., 2011). The widespread overuse of antibiotics, coupled with incomplete absorption and gmetabolism, has led to substantial antibiotic release into the environment. This has resulted in pervasive contamination of soil, water bodies, and the atmosphere, exerting selective pressure on environmental microorganisms and thereby accelerating the proliferation of ARBs and ARGs. These developments pose a significant threat to human health and ecosystem stability (Zhang et al., 2017a; Yi et al., 2019).

As a novel class of environmental contaminants, ARGs can be utilized by bacteria, undergoing rapid proliferation and dissemination through the growth of ARBs and horizontal gene transfer mechanisms. This enables the potential transfer of ARB from common environmental bacteria to those recognized as human pathogens, thereby seriously compromising antibiotic efficacy and posing a detrimental impact on public health (Pehrsson et al., 2016). Owing to the selective pressure exerted by antibiotics on ARGs, current research has predominantly focused on environmental media with known antibiotic sources, such as agricultural soils (Li et al., 2021), wastewater (Jong

et al., 2020), sewage sludge (Wei et al., 2018), various sediments (Chen et al., 2022a), and the atmosphere (He et al., 2020). However, existing studies indicate that other contaminants, including heavy metals (Li et al., 2022c) and organic pollutants (Azhogina et al., 2022), also facilitate the spread and dissemination of ARGs. Consequently, it is imperative to investigate the contamination levels and influencing factors of ARGs in industrial parks characterized by multiple pollutant sources.

Groundwater constitutes a critical component of the urban water cycle and water supply systems, and its quality is closely linked to ecological security and human health. Previous research on antibiotics and ARGs has predominantly focused on surface soils and surface water bodies (Das et al., 2021; Hao et al., 2021), often overlooking the impact of intensive anthropogenic activities in urban industrial parks on the deeper soil layers and groundwater systems. While the vadose zone can provide a degree of buffering capacity for groundwater quality, thereby slowing the migration of contaminants (Garcia-Galan et al., 2011), the adsorption efficiency of the soil-groundwater system is influenced by both soil properties (e.g., pH, organic matter content, and soil composition) and contaminant characteristics (e.g., acid dissociation constants and water solubility). To date, limited research exists on the migration of antibiotics and ARGs across different environmental media, especially between soil and groundwater. Therefore, this study investigates the distribution and transport of antibiotics and ARGs within the soil-groundwater continuum, aiming to provide data support and a scientific foundation for the prevention, control, and remediation of antibiotic and ARG contamination in groundwater.

Based on the aforementioned background, this study selects the Shanghai Taopu Industrial Park, characterized by typical composite pollution, as the research area, with groundwater as the primary focus. The aim is to elucidate the distribution patterns, migration processes, and key influencing factors of antibiotics and ARGs within the soil-groundwater system. The findings are expected to provide a scientific basis for the remediation and ecological restoration of groundwater contamination in the region, as well as offer theoretical support for systematically assessing the ecological risks and transport mechanisms of ARGs in complex pollution environments.

## 2 Materials and methods

### 2.1 Study area and sampling

The Taopu Industrial Park is located in northwestern Shanghai and was established in the 1950s, with fine chemicals and pharmaceutical manufacturing historically serving as its leading industries. During its early development, the area hosted a concentration of pharmaceutical, chemical, pen manufacturing, and textile enterprises, resulting in a series of environmental issues. While existing studies have examined contamination by heavy metals and persistent organic pollutants in the region (Lu et al., 2006), the potential antibiotic and antibiotic resistance gene (ARG) pollution associated with the historical presence of numerous pharmaceutical companies—such as Shanghai Fangda Pharmaceutical Co., Ltd., Shanghai Jiuzhoutong Pharmaceutical Co., Ltd., and Shanghai Schering-Plough Pharmaceutical Co., Ltd.—has not yet been systematically

reported.

## 2.2 Sample collection

Groundwater sampling was conducted in the Taopu Industrial Park, Shanghai, between October and November 2020 (Fig. 1). An integrated soil-groundwater sampling device equipped with a bailer tube (Model: QY-60, Xitan, Jiangsu) was used to collect a total of 20 groundwater samples, labeled A1–5, B1–5, and C1–10. At each sampling point, 4 liters of groundwater were collected. Immediately after collection, the samples were stored in polyethylene bottles, appropriately labeled, placed in light-proof insulated containers with ice packs, and transported to the laboratory within 24 hours, where they were refrigerated at 4°C. During the pretreatment process, each water sample was divided into two portions: one portion was filtered through a 0.22 μm membrane to collect suspended particulate matter retained on the filter for subsequent DNA extraction; the other portion was filtered through a 0.45 μm membrane, and the filtrate was preserved for the analysis of antibiotics, PAHs, and physicochemical water quality indicators.

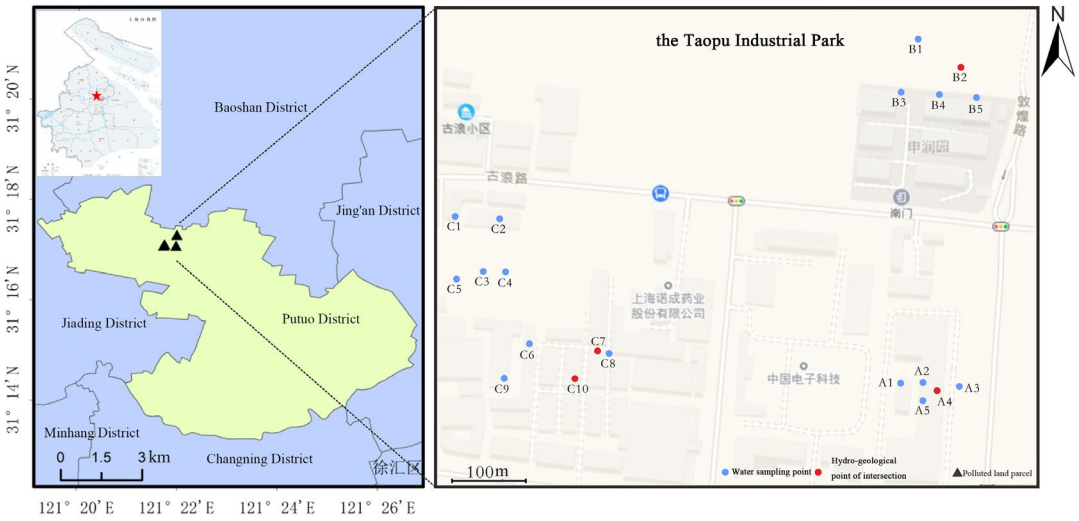



Fig. 1 Sampling sites in Taopu industrial park in Shanghai.

## 2.3 Analyses of physicochemical properties, metals and PAHs

On-site measurements of groundwater temperature, dissolved oxygen (DO), pH, and conductivity were performed using a portable multi-parameter water quality analyzer (Multi 3630 IDS, WTW, Germany). Concentrations of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were determined by a total organic carbon analyzer (TOC-V, Shimadzu, Japan). Prior to analysis, water samples were diluted 100-fold, with ultrapure water serving as the blank control.

Six heavy metals commonly associated with anthropogenic activities and potentially linked to the presence of ARGs in the environment—Mn, Cu, Zn, Ni, Pb, and Cr—were analyzed following a modified version of the method described by Yang et al. (2016a). Groundwater samples were filtered through 0.45 μm aqueous syringe filters (polyethersulfone membrane) and subsequently analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES). The spike recovery rates for

the target metals ranged from 64.4% to 92.7%.

The analysis of 16 PAHs in groundwater samples followed a previously established method (Wu et al., 2018). The specific procedure is summarized as follows: one liter of filtered water sample was spiked with 100 ng of deuterated terphenyl as a recovery indicator. PAHs were enriched and purified using an HC-18 solid-phase extraction cartridge (200 mg, 6 mL, CNW). The PAH fraction was eluted with 15 mL of a hexane–dichloromethane mixed solvent (3:7, v/v). The eluate was dehydrated with anhydrous sodium sulfate (high-purity grade) and then concentrated using rotary evaporation. The solvent was subsequently replaced with hexane and concentrated again to 1–2 mL before being transferred into a GC vial. After precise volume adjustment to 1 mL under a gentle nitrogen stream, 20 μL of an internal standard solution (10 mg/L) was added. The concentration of PAHs was determined using a gas chromatography–mass spectrometry system (GC–MS, Agilent 7890A/5977B, USA). The recoveries of target PAHs ranged from 60.1% to 125.5%; all sample concentrations were corrected based on recovery rates. For every ten samples, one method blank (using deionized water as a sample substitute) was processed, and the concentrations of target analytes in all blank samples were below the instrumental detection limit. In addition, 25% of the samples were randomly selected for triplicate analysis, with relative standard deviations all below 15.0%.

## 2.4 Quantification of Antibiotics

### 2.4.1 Antibiotic analysis

During the pretreatment step, 800 mL of membrane-filtered water sample was measured and transferred into an amber glass bottle. A total of 40 ng of surrogate standard (tetracycline-D6, TC-D6) was spiked into the sample. The pH of the sample was adjusted to 5.0 using high-purity hydrochloric acid and sodium hydroxide solution. Subsequently, analytical-grade ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) was added to achieve a final concentration of 1.0 g/L. After thorough mixing, the sample was left to stand for 1 hour to allow chelation of interfering metal ions, followed by loading onto a solid-phase extraction cartridge.

Prior to sample loading, the solid-phase extraction cartridge was sequentially preconditioned with 5 mL of methanol and 5 mL of ultrapure water at a flow rate of 1 $mL \cdot min^{-1}$. During the enrichment process, the water sample was passed through the cartridge at a controlled flow rate of 5 $mL \cdot min^{-1}$. After enrichment, the cartridge was rinsed with 10 mL of ultrapure water and dried under vacuum for at least 30 minutes. Target analytes were then eluted using 10 mL of a methanol–acetonitrile mixture (1:1, v/v). The eluate was concentrated by rotary evaporation to a volume of 1–2 mL. The solvent was then exchanged to methanol using 8 mL of methanol and concentrated again to 1–2 mL. The concentrate was transferred to an injection vial and evaporated under a gentle nitrogen stream to 0.5 mL. Finally, the sample was diluted to 1 mL with 0.02 $mol \cdot L^{-1}$ formic acid solution, filtered, and prepared for instrumental analysis.

This study selected 20 target antibiotic standards covering five major classes:

chloramphenicols (CPs), fluoroquinolones (FQs), macrolides (MLs), sulfonamides (SAs), and tetracyclines (TCs). The quantification of target antibiotics in the samples was performed using an ultra-performance liquid chromatography-tandem mass spectrometry system (UPLC-MS/MS, Waters Corp., Manchester, UK).

### 2.4.2 Quality assurance/quality control

A rigorous quality control and quality assurance (QC/QA) protocol was implemented throughout the experimental procedure. The specific measures included: (1) spiking 40 ng of TC-D6 as a recovery indicator into the groundwater samples, with the recoveries of the 20 target antibiotics ranging from 40.1% to 114.9%; (2) conducting method blank tests using deionized water in place of samples, and the limits of detection for target compounds were between 0.00 and 0.53 ng/L; and (3) performing triplicate analyses on 30% of randomly selected samples, all of which exhibited relative standard deviations below 15.0%.

Table 1 Basic information of 20 antibiotics

| Types of antibiotics | Chemical formula | CAS Number | Molecular weight |
|---|---|---|---|
| Sulfadiazine | $C_{10}H_{10}N_4O_2S$ | 68-35-9 | 250.28 |
| Sulfapyridine | $C_{11}H_{11}N_3O_2S$ | 144-83-2 | 249.29 |
| Sulfamethoxazole | $C_{10}H_{11}N_3O_3S$ | 723-46-6 | 253.28 |
| Sulfathiazole | $C_9H_9N_3O_2S_2$ | 72-14-0 | 255.32 |
| Sulfamerazine | $C_{11}H_{12}N_4O_2S$ | 127-79-7 | 264.30 |
| Sulfamethazine | $C_{12}H_{14}N_4O_2S$ | 200-346-4 | 278.33 |
| Sulfaquinoxaline | $C_{14}H_{12}N_4O_2S$ | 59-40-5 | 300.34 |
| Norfloxacin | $C_{16}H_{18}FN_3O_3$ | 70458-96-7 | 319.33 |
| Ciprofloxacin | $C_{17}H_{18}FN_3O_3$ | 85721-33-1 | 331.34 |
| Enrofloxacin | $C_{19}H_{22}FN_3O_3$ | 93106-60-6 | 359.40 |
| Ofloxacin | $C_{18}H_{20}FN_3O_4$ | 82419-36-1 | 361.37 |
| Tetracycline | $C_{22}H_{24}N_2O_8$ | 60-54-8 | 444.45 |

| | | | |
|---|---|---|---|
| Oxytetracycline | $C_{22}H_{24}N_2O_9$ | 79-57-2 | 460.43 |
| Doxycyclinehyclate | $C_{22}H_{24}N_2O_8$ | 564-25-0 | 444.44 |
| Chlorotetracycline | $C_{22}H_{23}ClN_2O_8$ | 57-62-5 | 478.88 |
| Erythromycin | $C_{37}H_{67}NO_{13}$ | 114-07-8 | 733.94 |
| Roxithromycin | $C_{41}H_{76}N_2O_{15}$ | 80214-83-1 | 837.05 |
| Chloramphenicol | $C_{11}H_{12}Cl_2N_2O_5$ | 56-75-7 | 323.13 |
| Thiamphenicol | $C_{12}H_{15}Cl_2NO_5S$ | 15318-45-3 | 356.22 |
| Florfenicol | $C_{12}H_{14}Cl_2FNO_4S$ | 73231-34-2 | 358.21 |

## 2.5 DNA extraction, metagenomic sequencing, and gene annotation

Two liters of groundwater were filtered through a 0.22 μm mixed cellulose ester membrane (Sangon Biotech, Shanghai) to collect particulate matter retained on the filter for DNA extraction. The filter membrane was aseptically cut into small pieces using sterilized scissors and forceps, then transferred into a lysis tube. DNA extraction was performed using the FastDNA® Spin Kit (MP Biomedicals, USA) strictly following the manufacturer's protocol. The extracted DNA was assessed for quality and concentration using a microvolume spectrophotometer (Thermo Fisher Scientific, USA), ensuring that the OD260/280 ratio fell within 1.8–2.0 to meet the requirements for subsequent analyses. Finally, the extracted DNA was aliquoted: one portion was used for metagenomic sequencing, and another portion was reserved for high-throughput quantitative PCR (HT-qPCR) analysis. All DNA aliquots were stored at -20°C to prevent degradation.

DNA extracted from the groundwater samples was shipped on dry ice to Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) for sequencing. Extracted DNA was fragmented to approximately 400 bp using a Covaris M220 (Gene Company Limited, China) for paired-end library preparation. Sequencing was performed on an

Illumina NovaSeq platform, and raw reads were saved in FASTQ format. Quality control was conducted using fastp (Chen et al., 2018) to obtain high-quality clean data, ensuring the reliability of subsequent analyses (https://github.com/OpenGene/fastp, version 0.20.0). Clean reads were assembled into contigs using MEGAHIT (Li et al., 2015b) (https://github.com/voutcn/megahit, version 1.1.2). Open reading frames (ORFs) were predicted from the assembled contigs with MetaGene (Version 2.10) (Noguchi et al., 2006) (http://metagene.cb.k.u -tokyo.ac.jp/). The predicted genes were clustered using CD-HIT (Fu et al., 2012) to construct a non-redundant gene catalog (http://www.bioinformatics. org/cd-hit/). Abundance information of genes in each sample was obtained by aligning high-quality reads to the non-redundant gene catalog using SOAPaligner (Li et al., 2009) with a default identity threshold of 95% (http://soap.genomics.org.cn/). The non-redundant gene set was functionally annotated against the NR database (http://www.diamondsearch.org/index.php, version 0.8.35) using DIAMOND (Buchfink et al., 2015; Buchfink et al., 2021) with the blastp algorithm and an E-value cutoff of 1e-5. ARGs were annotated using the Comprehensive Antibiotic Resistance Database (CARD) (Jia et al., 2017) (https://card.mcmaster.ca/, version 0.8.35) under the same parameters. Mobile genetic elements (MGEs) were identified by alignment to a curated MGE database (Parnanen et al., 2018) (https://github.com/KatariinaParnanen/MobileGenetic ElementDatabase, Version 2.1) using blastp with an E-value $\leq$ 1e-5. All sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA945244.

## 2.6 Statistical analyses

For data analysis and visualization, this study employed a variety of statistical and graphical tools. Statistical description and preprocessing of data on antibiotic resistance genes, heavy metals, and physicochemical parameters were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). To evaluate the direct and indirect effects of soil properties, pollutants, microbial abundance and diversity, and mobile genetic elements (MGEs) on the spatial distribution of ARGs, a structural equation model (SEM) was constructed.

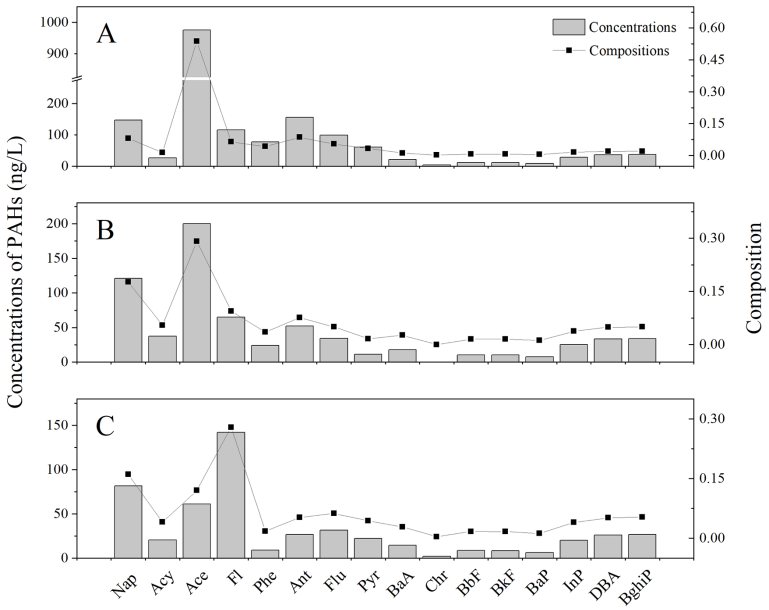
Within the R environment, redundancy analysis (RDA) and variation partitioning analysis (VPA) were conducted using the Vegan package to quantify the contributions of environmental factors to the variation in ARG distribution. Spearman correlation analysis was performed with the Hmisc package to identify co-occurrence patterns among ARG subtypes, microbial taxa, and MGEs, applying selection thresholds of Spearman's rho > 0.8/0.6 and a p-value < 0.01. Network visualization was generated using Gephi 9.1. The spatial distribution map of the study area and sampling points was created with ArcGIS 10.6. All bar charts, line graphs, and pie charts were produced in OriginPro 2019b.

## 3 Results

### 3.1 Physicochemical properties and PAH concentrations

Analysis of groundwater monitoring data from the Taopu Industrial Park in Shanghai reveals the following characteristics: the pH of the 20 collected samples ranged from 6.8 to 9.5, with an average of 7.4; dissolved oxygen (DO) concentrations varied between 2.3 and 11.8 mg/L, with the lowest value recorded at site A2; salinity ranged from 0.3‰ to 1.4‰; electrical conductivity, dissolved organic carbon (DOC), and total nitrogen (TN) exhibited significant variation, with ranges of 805.0–3060.0 μS/cm, 3.7–34.5 mg/L, and 1.2–35.8 mg/L, respectively; the average groundwater temperature was 19.4°C, with minimal variation observed within the same sampling area.

As illustrated in Fig. 3, significant spatial variability was observed in the concentrations of PAHs in the groundwater samples from the Taopu Industrial Park. The highest total PAHs concentration was recorded at site A2, reaching 5172.8 ng $L^{-1}$, while the lowest was observed at site C7 (250.5 ng $L^{-1}$), with an average concentration of 899.1 ng $L^{-1}$. Compared to groundwater in other regions both domestically and internationally, the total concentration of the 16 PAHs in this area is significantly higher than those reported for Taiyuan, China (135 ng $L^{-1}$), Jharkhand, India (22 ng $L^{-1}$), and Shenfu New District, Liaoning, China (467 ng $L^{-1}$). It is comparable to the level reported for Rawalpindi, Pakistan (763 ng $L^{-1}$), indicating that the groundwater in this industrial park is subjected to a relatively high level of PAHs contamination (Fig. 2).

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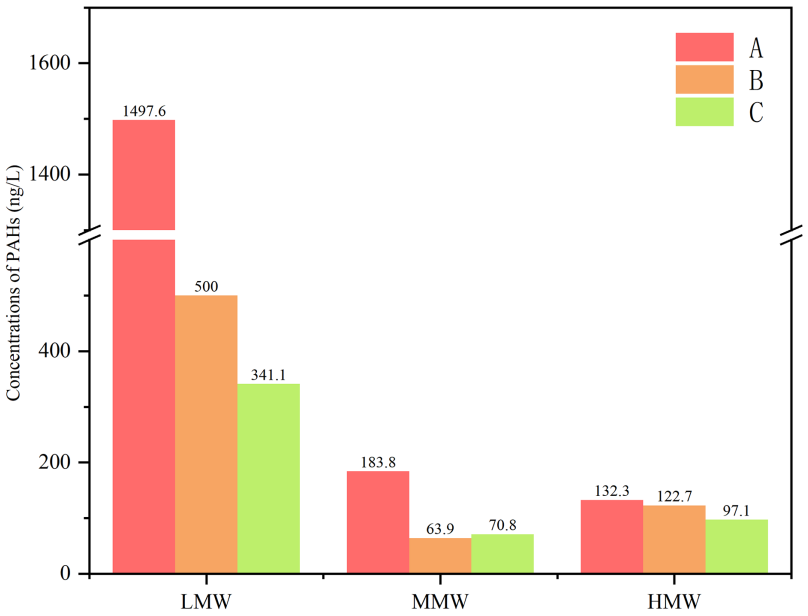
313 Fig. 2 Concentrations and composition of Individual PAHs in Groundwater in Taopu industrial
314 park in Shanghai.

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316 Fig. 3 Concentration of PAHs in Groundwater in Taopu industrial park in Shanghai.

317

## 3.2 The distribution of antibiotics in groundwater

### 3.2.1 The concentration of antibiotics in groundwater

Analysis of five major classes of antibiotics in 20 groundwater samples from the Taopu Industrial Park revealed that the total concentration of the 20 target antibiotics ranged from 24.1 to 1161.1 ng $L^{-1}$, with an average concentration of 263.3 ng $L^{-1}$. Compared with domestic and international studies, the antibiotic contamination in the groundwater of this industrial park is at a moderate level.

Sulfonamides (SAs) exhibited the highest concentration among all antibiotic classes, accounting for 68.8% of the total antibiotic concentration, followed by fluoroquinolones (FQs, 12.1%), tetracyclines (TCs, 10.7%), chloramphenicols (CPs, 7.0%), and macrolides (MLs, 1.4%). This distribution pattern is consistent with findings from previous studies (Chen et al., 2017d; Ma et al., 2021). The strong mobility and low degradation rates of SAs likely contribute to their enrichment in groundwater (Fig. 4).

It is noteworthy that all 20 target antibiotics were detected in every sample. Among them, two SAs (SD and SMT), one FQ (NFC), four TCs (TC, DXC, OTC, and CTC), two MLs (ETM and RTM), and one CP (CAP) were detected in 100% of the samples.

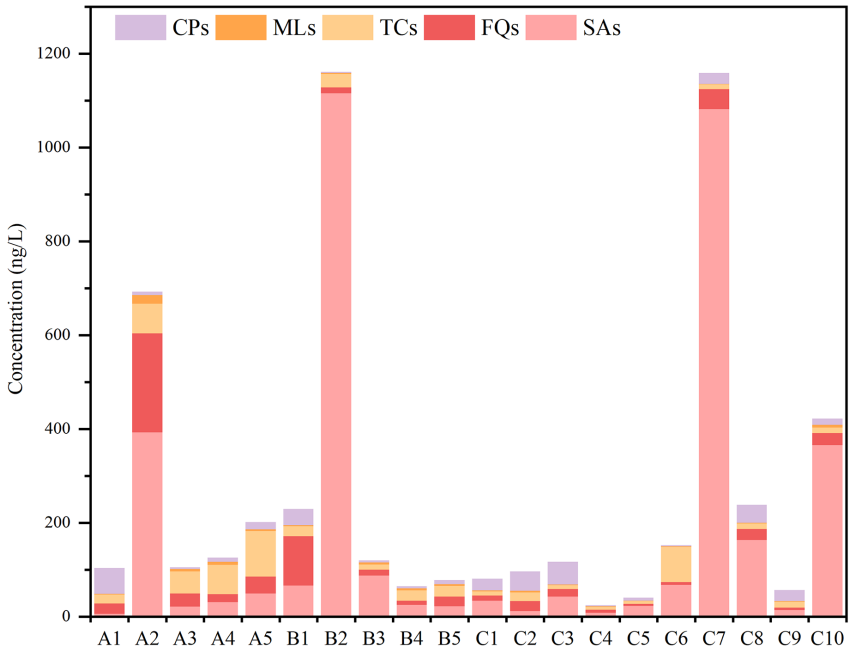



Fig. 4 The concentration of antibiotics in groundwater in Taopu industrial park in Shanghai.

### 3.2.2 Distribution and Transport of Antibiotics in the Soil-Groundwater System

Driven by anthropogenic activities, a significant portion of antibiotics used in human medicine, animal husbandry, and agriculture enters various environmental compartments through direct or indirect pathways due to incomplete absorption and utilization (Huang et al., 2019b; Norback et al., 2019; Seyoum et al., 2021).

Soil serves as a major reservoir for environmental antibiotics. Once introduced into soil, antibiotics undergo processes such as adsorption/desorption, degradation, and bioaccumulation, resulting in distinct distribution and transport patterns along the soil profile (Qiao et al., 2018; Zhao et al., 2017). In urban settings, intensive human activities further influence the distribution and migration of pollutants within the soil

profile (Bu et al., 2009).

Antibiotics released from anthropogenic activities are widely distributed in soil and groundwater environments. In our previous study conducted in the same area, the concentrations of total antibiotics, tetracyclines (TCs), and fluoroquinolones (FQs) in soil cores followed an exponential decay model, decreasing with soil depth. In contrast, the distribution of sulfonamides (SAs), macrolides (MLs), and chloramphenicols (CPs) along the soil profile did not conform to an exponential decay pattern, which may be attributed to their physicochemical properties, chemical degradation, and biodegradation. Although the vadose zone soil provides some retardation of antibiotic migration, the low temperatures, anoxic conditions, and lack of light in groundwater environments slow down antibiotic degradation, leading to their accumulation. Antibiotic contamination in the study area's groundwater has reached a moderate level, with sulfonamides (SAs)—a highly mobile antibiotic class—being the dominant component.

The distribution of antibiotics in groundwater is also significantly influenced by dissolved organic carbon (DOC), salinity, and dissolved oxygen (DO). Furthermore, the elevated levels of PAHs in the industrial park groundwater play an important role in shaping antibiotic distribution. Eight PAHs, including Ace, Flu, Pyr, BaA, Chr, BbF, BkF, and InP, as well as total PAHs (TPAH), showed significant correlations with antibiotic concentrations. This may be due to the selective pressure exerted by PAHs, which promotes the proliferation of antibiotic-producing microbial strains while simultaneously suppressing the degradation functions of PAH-degrading bacteria,

thereby facilitating the long-term coexistence of both pollutant groups in the environment.

This study demonstrates that the migration and transformation of antibiotics in the soil-groundwater system result from the combined effects of anthropogenic discharge, intrinsic antibiotic properties, environmental media characteristics, and regional composite pollution. Therefore, effective pollution prevention and control require an integrated strategy that accounts for cross-media interactions and multi-factor synergistic management (Fig. 5 and Fig. 6).

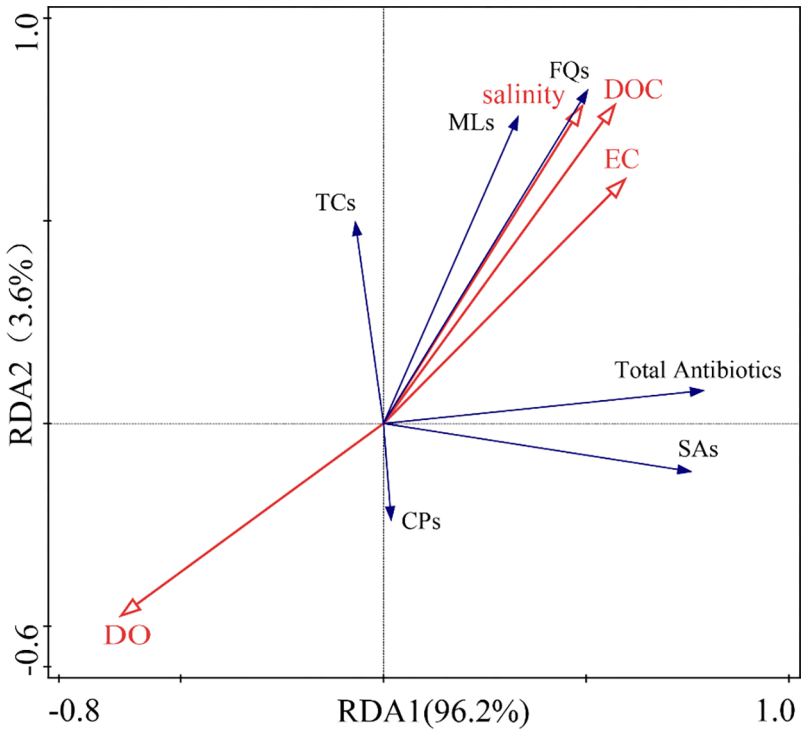



Fig. 5 Redundancy analysis (RDA) showing the influence from physical and chemical properties on antibiotics in groundwater samples.

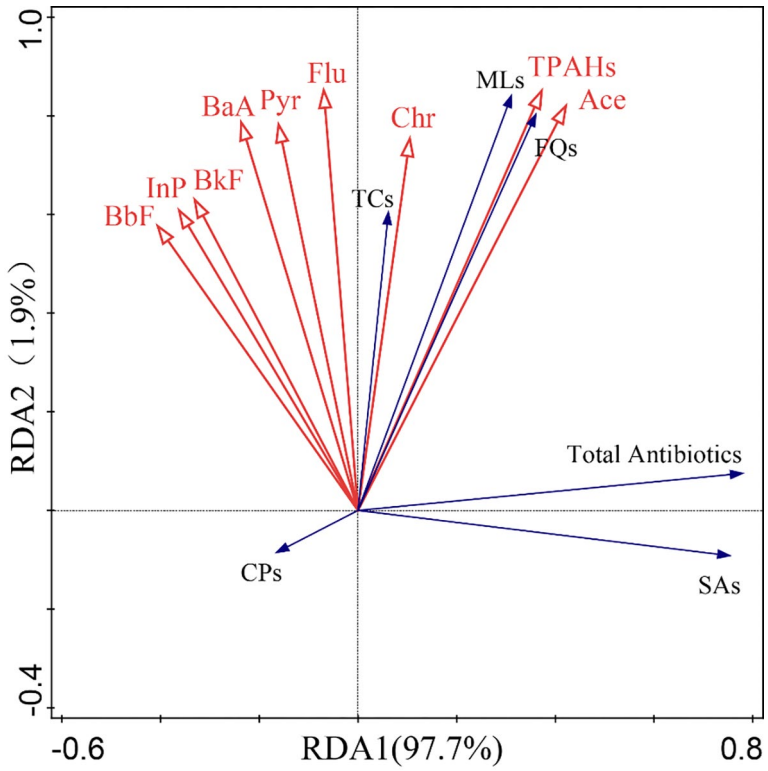



Fig. 6 Redundancy analysis (RDA) showing the influence from PAHs contents on antibiotics in groundwater samples.

### 3.3 ARG and MGE profiles

Based on antibiotic class, the ARGs detected via high-throughput quantitative PCR (HT-qPCR) in this study were categorized into eight groups: sulfonamide, tetracycline, multidrug, fluoroquinolone, macrolide-lincosamide-streptogramin B (MLSB), β-lactam, aminoglycoside, and other resistance genes.

Fig. 7 presents the number of distinct antibiotic resistance gene (ARG) subtypes detected via high-throughput quantitative PCR (HT-qPCR) across 20 groundwater samples. A total of 163 different ARG subtypes were identified, with their counts varying moderately among samples, ranging from 56 to 95 subtypes per sample and averaging 75 subtypes. The composition of the eight ARG classes was generally

consistent across all samples. Among them, aminoglycoside and multidrug resistance genes were the most abundant in terms of subtype diversity, with counts ranging from 18–25 and 15–24 subtypes, respectively.

The absolute and relative abundances of the eight ARG classes in each groundwater sample are shown in Fig. 8. The absolute abundance of ARGs exhibited considerable variation, ranging from $2.9\times10^4$-$4.0\times10^5$ copies/mL, with an average of $1.4\times10^5$ copies/mL. In contrast, the relative abundance of ARGs showed less variability, ranging from 0.1 to 1.1. In terms of compositional distribution, multidrug resistance genes accounted for the highest proportion across all samples (3.3–77.9%), followed by aminoglycoside resistance genes (6.1–47.6%) and sulfonamide resistance genes (0.3–34.8%). The remaining five ARG classes contributed comparatively smaller proportions.

Compared to other studies that employed the high-throughput quantitative PCR (HT-qPCR) method to quantify ARGs in aquatic environments, the absolute abundance of ARGs in the groundwater of this study area is higher than that reported in household tap water from Zhangzhou, China ($1.8\times10^7$ copies/L) (Huang et al., 2021) and aquaculture pond water in Huzhou, China ($1.2\times10^7$ copies/L) (Tiimub et al., 2022). Conversely, it is lower than the levels found in the Houxi River, Xiamen, China ($1.2\times10^{10}$ copies/L) (Zhou et al., 2020) and the drinking water source of the Jiulong River in Zhangzhou, China ($1.9\times10^{10}$ copies/L) (Huang et al., 2021). The ARG abundance in this study is comparable to that observed in reservoir surface water in southeastern China ($2.2\times10^6$-$4.5\times10^8$ copies/L) (Fang et al., 2022) and summer

inflowing river water of Taihu Lake, China (6.17–9.47 $Log_{10}$ copies/L) (Fernanda et al., 2022), indicating a moderate level of contamination.

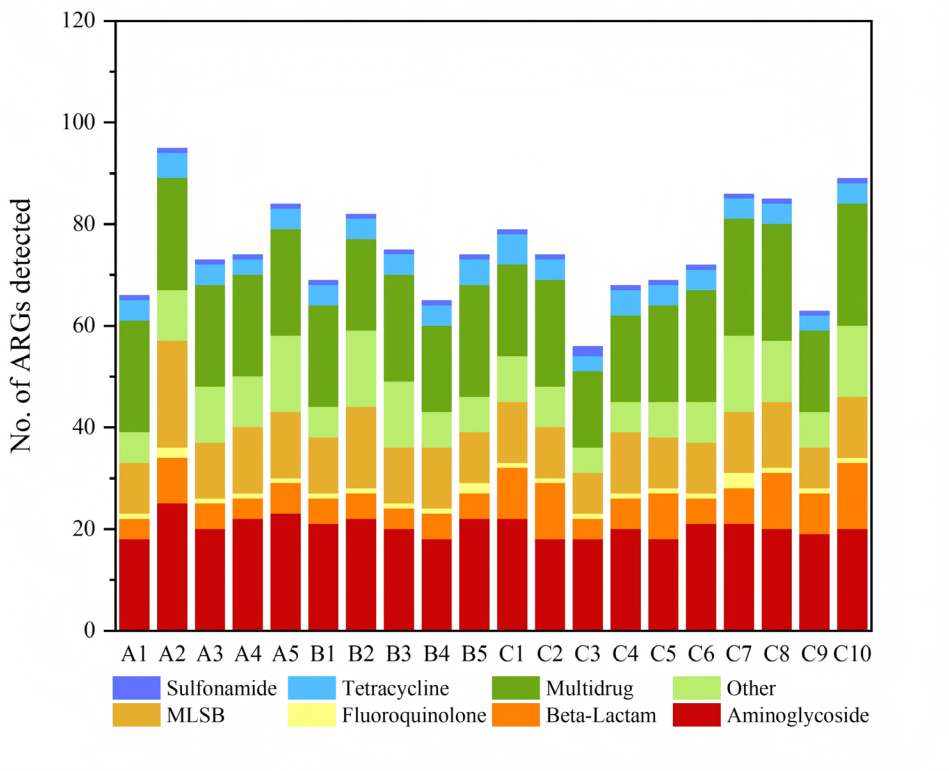
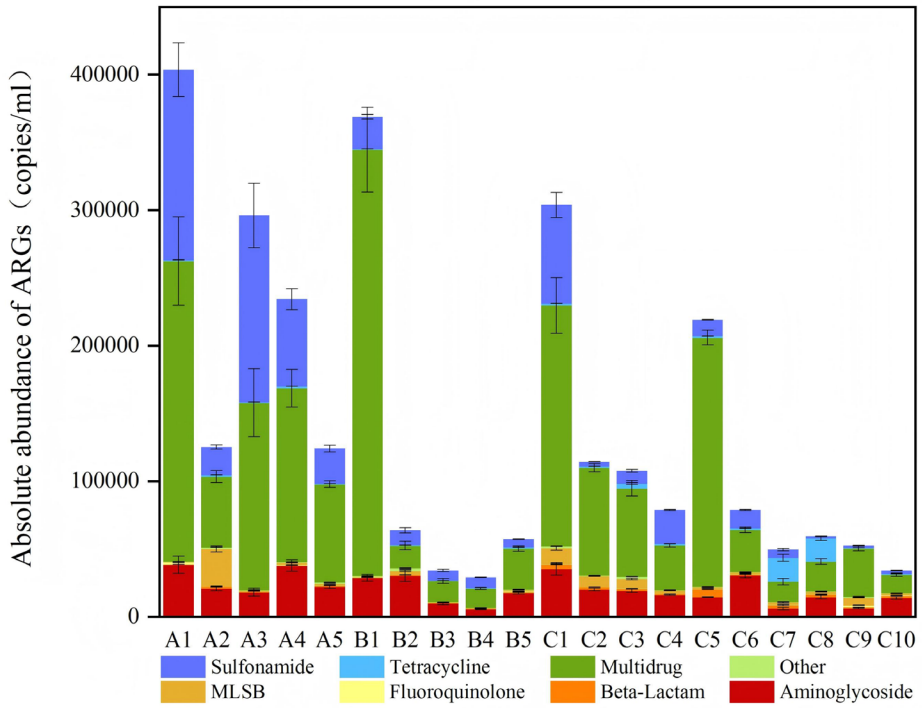



Fig. 7 The total number of ARGs detected in groundwater samples.

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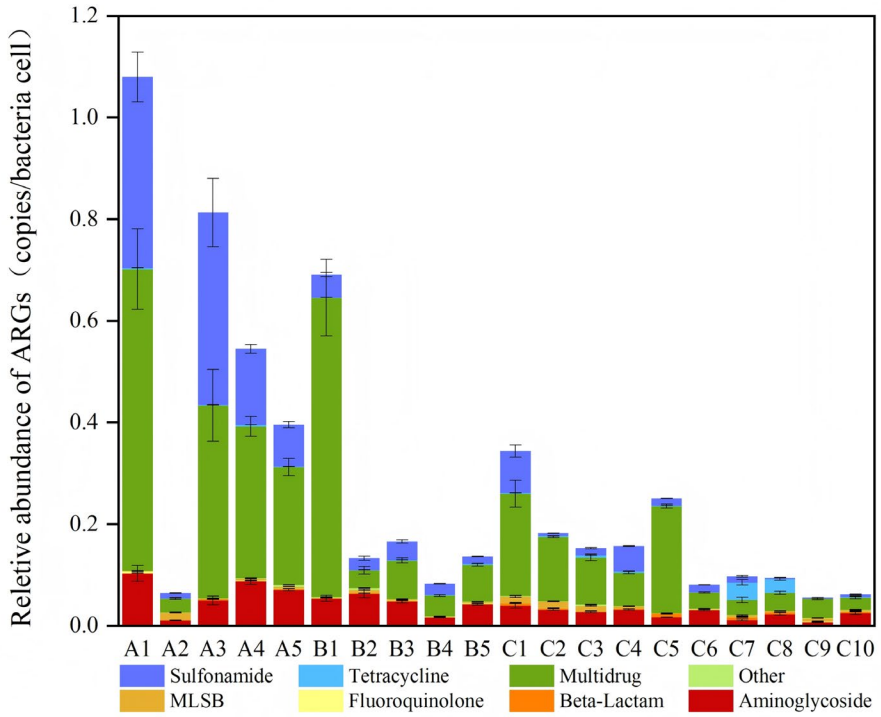



426

427 Fig. 8 Absolute abundance (a) and relative abundance (b) of ARGs detected in groundwater
428 samples.

Building upon the HT-qPCR method, we employed metagenomic sequencing to conduct a comprehensive analysis of ARGs in the groundwater of the study area. Fig. 9 illustrates the number of detected ARG subtypes and their normalized abundance across 20 groundwater samples using metagenomic sequencing.

A total of 1,139 distinct ARG subtypes were identified in the 20 groundwater samples. The number of ARG subtypes per sample varied slightly, ranging from 526 to 790, with an average of 665 subtypes (Fig. 9a). Among these, multidrug resistance genes were the most abundant in terms of subtype diversity, ranging from 167 to 180 subtypes, followed by β-lactam resistance genes (78–222 subtypes) and glycopeptide resistance genes (52–65 subtypes).

As shown in Fig. 9b, the total normalized abundance of the 21 ARG classes varied substantially across samples, ranging from 7,951.8 to 28,548.9 ppm, with an average of 16,545.2 ppm. The top 10 most abundant ARG subtypes in the groundwater samples were macB, evgS, tetA(58), msbA, novA, mtrA, baeS, cpxA, oleC, and Sris_parY_AMU. Among these, macB was the most dominant, accounting for an average of 5.7% of the total ARG abundance, followed by evgS (5.4%) and tetA(58) (3.1%).

Among the 21 classes of ARGs detected in groundwater, multidrug resistance genes accounted for the highest proportion, ranging from 33.0% to 55.8%, followed by glycopeptide resistance genes (8.4–12.0%), macrolide resistance genes (7.7–12.4%), tetracycline resistance genes (6.8–11.6%), and peptide resistance genes (3.7–8.4%). This distribution pattern is consistent with findings from previous studies (Yang et al.,

2018) (Fig. 9b).

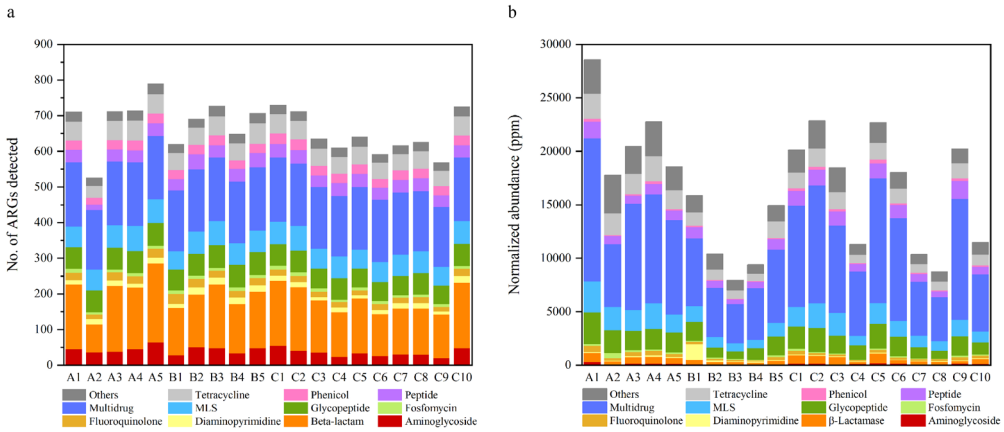



Fig. 9 The total number (a) and normalized abundance (b) of ARGs detected in groundwater samples

A total of 554 mobile genetic elements (MGEs) were identified across the 20 groundwater samples, comprising 370 transposons, 133 insertion sequences, 48 integrons, and 3 plasmids. The normalized abundance of MGEs ranged from 127.0 to 2199.3 ppm, with an average value of 711.6 ppm (Fig. 10). Among the different MGE categories, transposons exhibited the highest relative abundance, accounting for 65.9% to 88.5% of the total MGE composition.

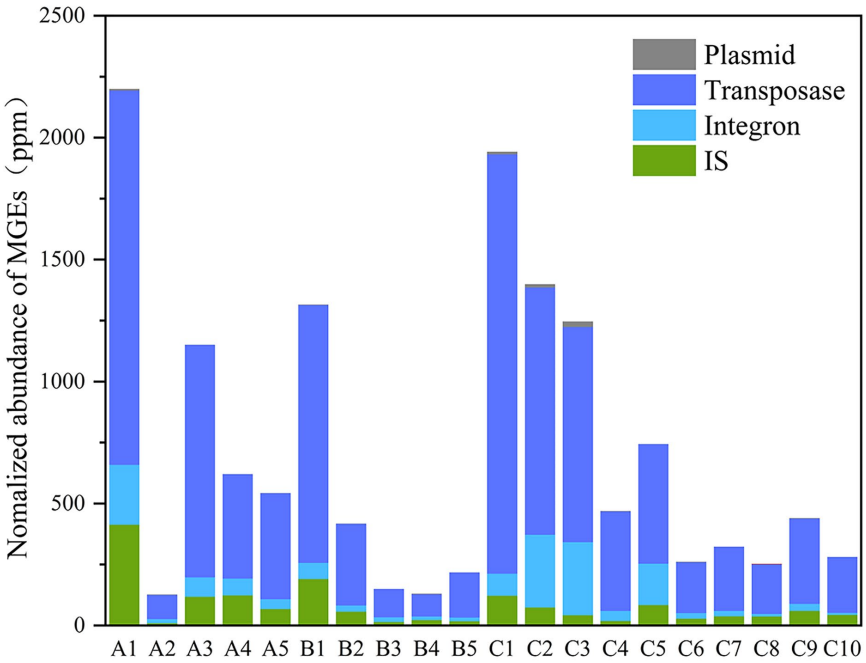



Fig. 10 Normalized abundance of MGEs in groundwater samples.

### 3.4 Profiles of host bacteria of ARGs and potential human pathogens

Across the 20 groundwater samples examined in this study, microorganisms belonging to five distinct domains were detected. Bacteria constituted the dominant microbial group, with their standardized relative abundance ranging from 74.8% to 99.8%. These were followed, in descending order of abundance, by Archaea (0.0–12.1%), Eukaryota (0.0–11.8%), Viruses (0.0–3.4%), and unclassified microorganisms (0.0–0.4%). Further taxonomic analysis revealed that the bacterial community encompassed 153 phyla, 245 classes, 421 orders, 830 families, and 3538 genera.

To investigate the associations between specific antibiotic resistance gene (ARG) subtypes and the microbial community, a co-occurrence network analysis was

480 performed using ARG subtypes with a standardized abundance ≥10 ppm and bacterial
481 genera with an abundance ≥1000 ppm (Fig. 11). Among the 32 bacterial genera
482 included in the network, 23 belonged to the phylum Proteobacteria, 6 to Bacteroidetes,
483 and one each to Firmicutes, unclassified Bacteria, and Candidatus_Colwellbacteria.
484 These findings indicate that Proteobacteria and Bacteroidetes are key microbial phyla
485 influencing the distribution and dissemination of ARGs in the groundwater of this study
486 area.This is consistent with the composition of the microbial community in the
487 groundwater of the industrial park reported in the study by Xu et al. (2019).

488 We performed a network analysis on ARG subtypes with a standardized abundance
489 of 10 ppm and bacterial genera with an abundance exceeding 1000 ppm, with the results
490 shown in Fig. 11. Among these, the bacterial genera exhibiting the highest
491 connectivity—unclassified Bacteroidales, Methylobacter, unclassified
492 Sphingomonadales, unclassified Thiotrichales, unclassified Colwellbacteria, and
493 unclassified Firmicutes—each demonstrated significant associations with 12 ARG
494 subtypes.

495 Methylobacter is a genus of methanotrophic bacteria that assimilates
496 formaldehyde via the ribulose monophosphate pathway and plays a significant role in
497 mitigating methane emissions. However, recent studies have isolated strains of this
498 genus from culture media supplemented with multiple antibiotics, indicating potential
499 multidrug resistance. Coupled with the co-occurrence relationships observed in this
500 study between Methylobacterium and multiple ARG subtypes, it is plausible that this
501 genus may act as a potential host for ARGs. Therefore, when utilizing

Methylobacterium for methane emission reduction, attention must be given to its potential role in disseminating antibiotic resistance genes.

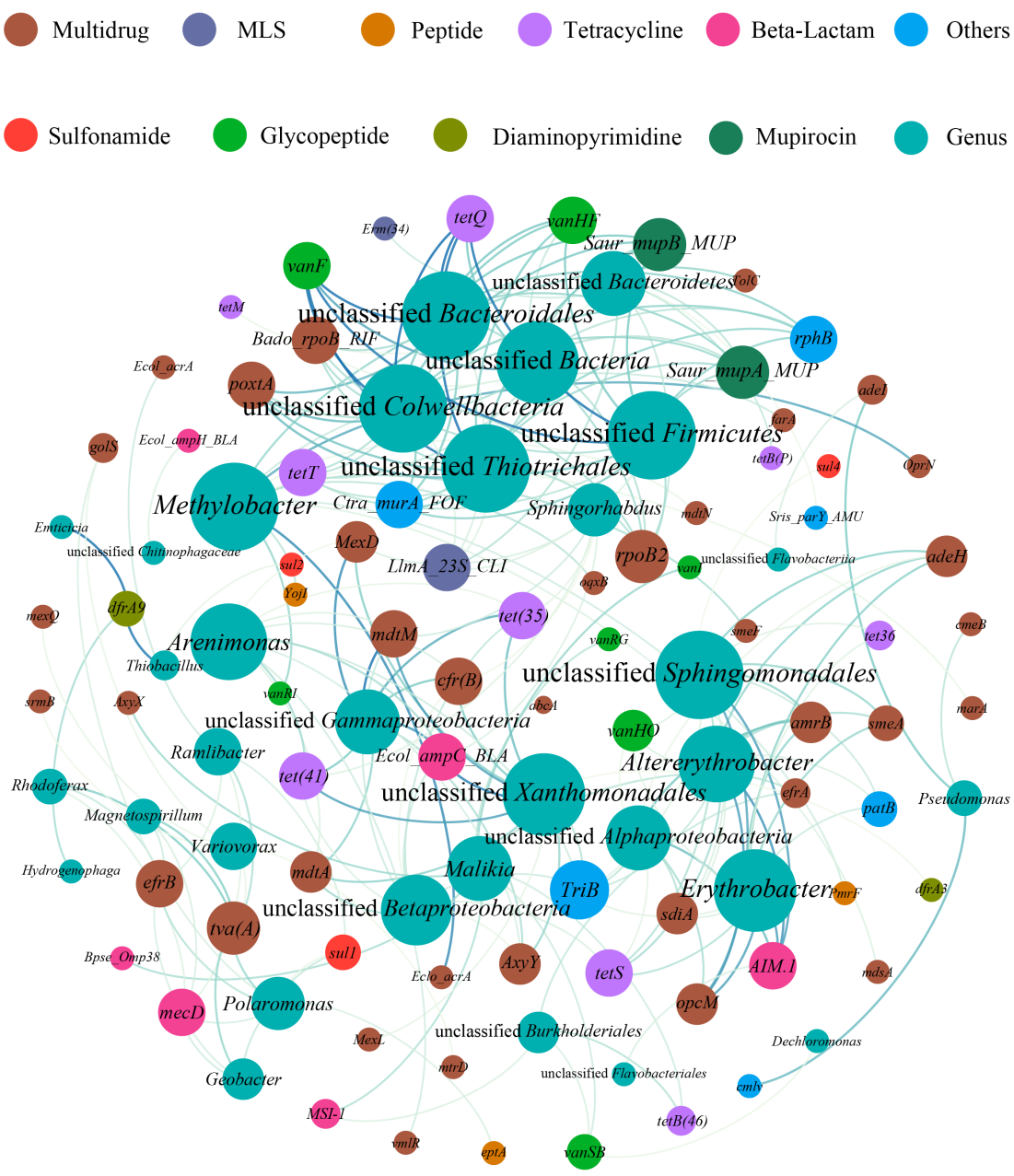



Fig. 11 Network analysis among ARG subtypes and bacteria communities at genus level in groundwater samples.

## 3.5 Factors influencing the spatial variation of ARGs in soil-groundwater systems

Numerous studies have indicated that ARGs in surface soil can migrate to deeper

soil layers via vertical transport, potentially contaminating groundwater (Huang et al., 2013; Li et al., 2022a). In this study, previously obtained metagenomic data from soil cores in the industrial park were integrated with groundwater data to investigate the distribution and migration patterns of ARGs and ARBs within the soil-groundwater system. The results are presented in Fig. 12 and Fig. 13 (Miao et al., 2025).

Through metagenomic analysis of four paired soil-groundwater samples, 1229 and 998 ARG subtypes were detected in the soil cores and groundwater, respectively. Among these, 855 subtypes (accounting for 91.0% of the total detected in groundwater) were co-present in both media. The overall abundance levels of ARGs in the two media were similar (averaging 15,814.8 ppm in soil cores and 14,587.2 ppm in groundwater), and their composition and abundance distribution exhibited high similarity. Multidrug, macrolide, glycopeptide, tetracycline, and peptide resistance genes showed high abundance in both compartments and were relatively enriched in the deep soil layer (T5). These results indicate that ARGs from deep soil layers can migrate into groundwater and persist stably over time, thereby influencing the composition of the groundwater resistome.

The study further identified the primary drivers influencing the distribution of ARGs within the soil-groundwater system, including antibiotics, heavy metals, PAHs, mobile genetic elements (MGEs), microbial communities, and environmental physicochemical properties. Among these, microbial communities are widely recognized as a key factor shaping the spatial distribution of ARGs. ARBs can facilitate long-distance transport via carriers such as soil particles and aerosols, thereby

participating in the global circulation of resistance genes. To clarify the specific composition of ARB in the study area, a co-occurrence network was constructed, revealing potential host relationships between 56 microbial genera and 260 ARG subtypes. This approach enabled the systematic identification of key potential ARB in the industrial park's soil-groundwater system.

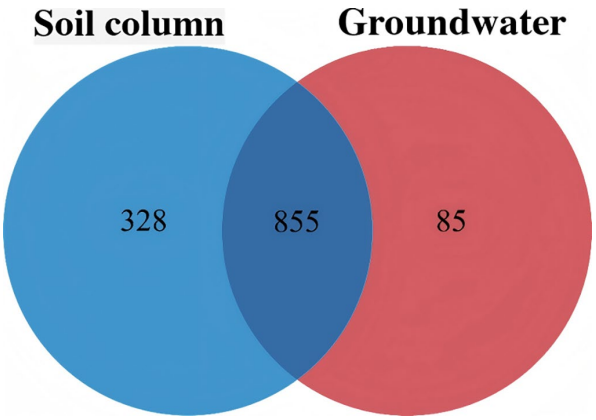



Fig. 12 Distribution of ARG subtypes species in soil columns and groundwater samples.

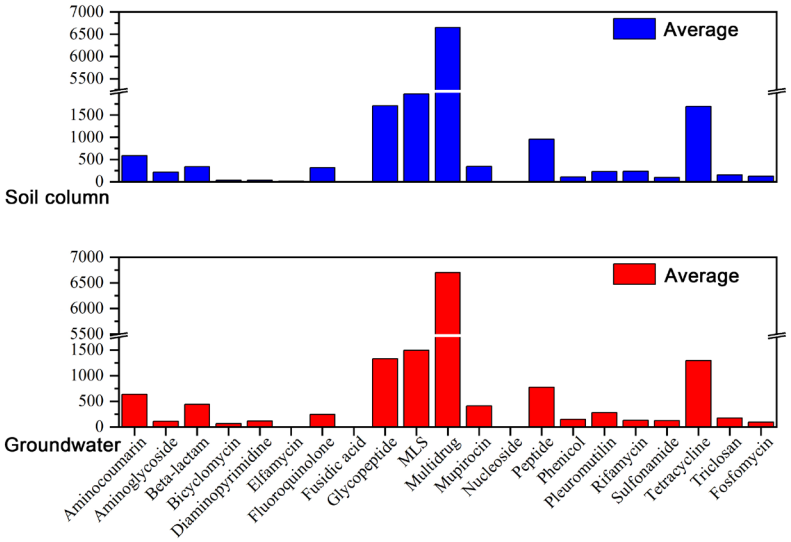



Fig. 13 Comparison of normalized abundance of 21 ARGs in soil columns and groundwater samples.

### 3.6 Environmental drivers of microbial taxa abundance

The relationships between the microbial community in groundwater and antibiotics, PAHs, and physicochemical properties were explored using redundancy analysis (Fig. 14, Fig. 15, Fig. 16). The results indicate that 13 environmental factors, including multiple antibiotics (OFC, ETM, NFC, MLs, FQs, TC), PAHs (Ace, Chr, Pyr, Flu), and physicochemical parameters (pH, DOC, DO), were significantly correlated with the bacterial community structure in groundwater. Among these, contaminant factors (antibiotics and PAHs) played a predominant role, further confirming that contaminants are the key factors influencing the microbial community structure in the groundwater of this region. This finding is consistent with previous studies reporting that antibiotics and PAHs are important factors affecting the microbial community structure in groundwater (Guo et al., 2022; Sun et al., 2022).

As shown in Fig. 14, the influence of different antibiotics on the microbial community varies. For instance, Proteobacteria exhibited a significant positive correlation solely with tetracycline, while showing significant negative correlations with norfloxacin, ofloxacin, erythromycin, fluoroquinolones, and macrolide antibiotics. PAHs alter the microbial community structure to adapt to the contaminated environment by promoting the growth of microorganisms with degrdation capabilities, and the effects of different categories of PAHs on various microbial communities also differ (Sun et al., 2022). In the groundwater of the study area, Ace demonstrated the highest correlation among all PAH factors significantly associated with the microbial community, whereas Pyr and Flu showed significant positive correlations with a

broader range of bacterial phyla (Fig. 15).

Water quality parameters also influenced the microbial composition. pH and dissolved organic carbon showed significant positive correlations with major bacterial phyla, except for Proteobacteria, while dissolved oxygen was significantly positively correlated only with Proteobacteria and negatively correlated with other major phyla. These findings differ from some prior research results; variations in geographical location, groundwater physicochemical parameters, and contaminant concentration levels may account for the discrepancies in the influence of environmental factors (Xiong et al., 2022) (Fig. 16).

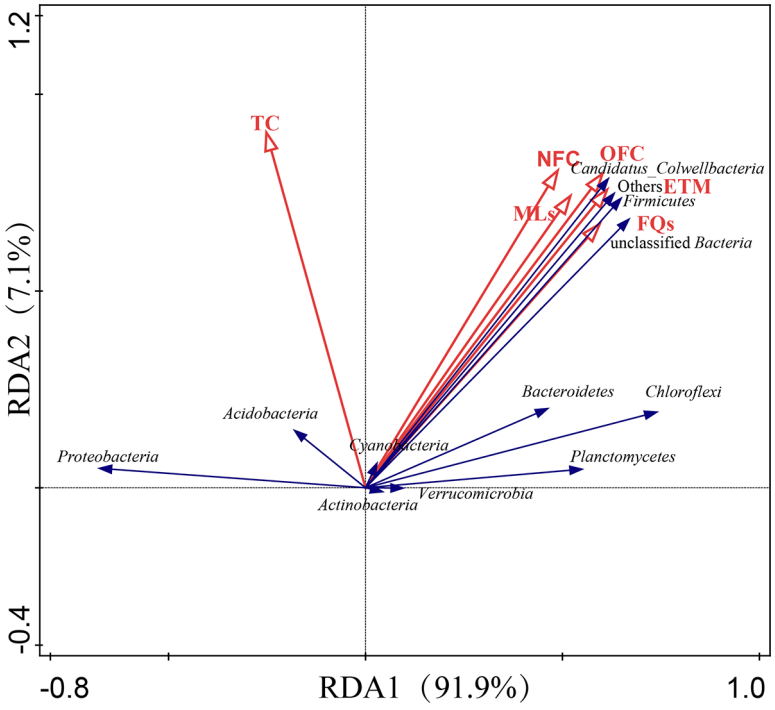
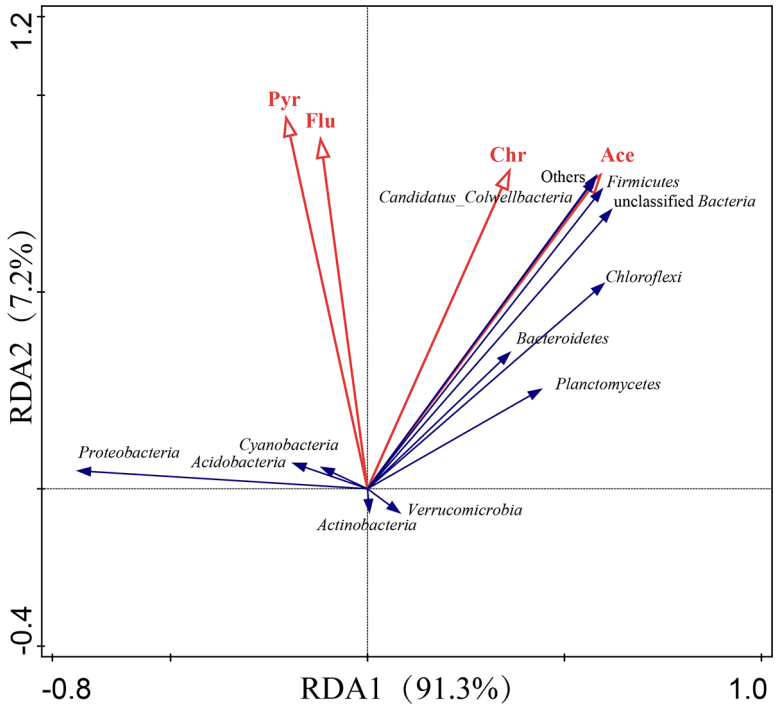
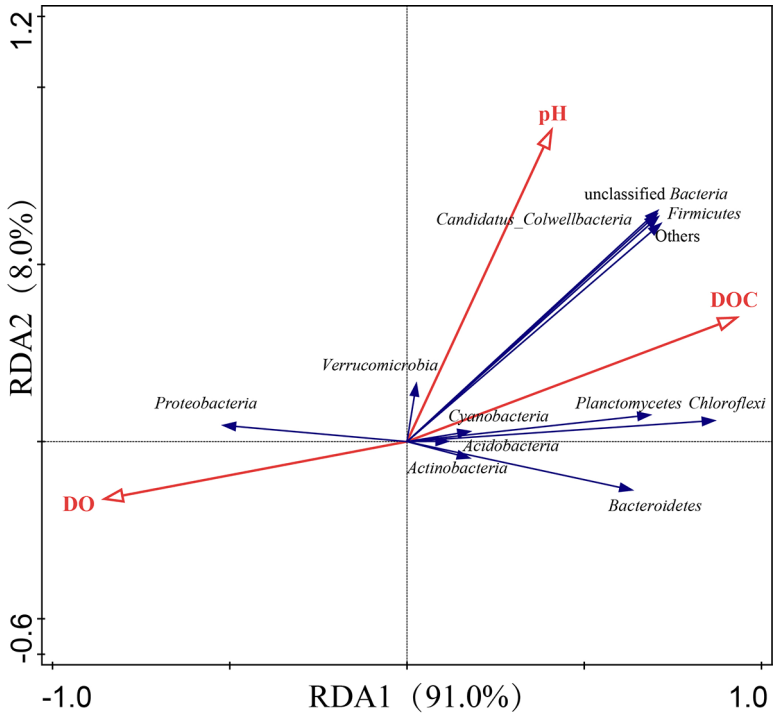



Fig. 14 RDA showing the influence from antibiotics on microbial communities in groundwater.




581

582 Fig. 15 RDA showing the influence from PAHs on bacteria communities in groundwater.




583

584 Fig. 16 RDA showing the influence from physicochemical properties on bacteria communities in

585 groundwater.

586

# 4 Discussion

## 4.1 Distribution and transport of antibiotics and potential controlling factors

### 4.1.1 Distribution and Migration of Antibiotics in Groundwater

The concentration of sulfonamide antibiotics (SAs) in the groundwater of this study area is significantly higher than those reported in Hubei Province, China (Tong et al., 2020), Barcelona, Spain (Lopez-Serna et al., 2013), Guizhou Province, the Ganges Basin, India (Sharma et al., 2019), Beijing, China (Chen et al., 2017d), and Tianjin, China (Hu et al., 2010). However, it is lower than that observed in Heilongjiang Province, China (Ma et al., 2021) and similar to levels documented in North Carolina, USA (Gray et al., 2020).

The concentration of fluoroquinolone antibiotics (FQs) is notably higher than those reported in Beijing, China (Chen et al., 2017d), the Ganges Basin, India (Sharma et al., 2019), and Heilongjiang Province, China (Ma et al., 2021). It is lower than the level observed in Barcelona, Spain (Lopez-Serna et al., 2013) and comparable to those in Hubei Province, China (Yao et al., 2017a) and Tianjin, China (Hu et al., 2010).

The concentration of tetracycline antibiotics (TCs) is markedly higher than those reported in Beijing, China (Chen et al., 2017d) and Tianjin, China (Hu et al., 2010), but lower than that in Hubei Province, China (Yao et al., 2017a). It is similar to levels recorded in Barcelona, Spain (Lopez-Serna et al., 2013) and Guizhou Province.

Macrolide antibiotic (MLs) concentrations in the groundwater are relatively low, significantly lower than those reported in North Carolina, USA (Gray et al., 2020) and

608 Hubei Province, China (Yao et al., 2017a), and comparable to levels in Heilongjiang
609 Province, China (Ma et al., 2021). Chloramphenicol antibiotic (CPs) concentrations are
610 modest and similar to those reported in Tianjin, China (Hu et al., 2010).

611 Compared with surface water in other regions, the concentrations of SAs and TCs
612 in this study area are significantly lower than those in surface water from Hubei
613 Province (Tong et al., 2020), the Huangpu River in Shanghai (Chen and Zhou, 2014),
614 and lake water from Hubei Province (Tong et al., 2020). The concentration of FQs is
615 broadly similar to that in the Huangpu River, Shanghai (Chen and Zhou, 2014), but
616 notably lower than those in surface water and lake water from Hubei Province (Tong et
617 al., 2020). MLs concentrations are comparable to those reported in the Huangpu River,
618 Shanghai (Chen and Zhou, 2014), as well as in surface water and lake water from Hubei
619 Province (Tong et al., 2020). CPs concentrations are significantly lower than those
620 reported in the Huangpu River, Shanghai (Chen and Zhou, 2014).

621 In summary, the overall antibiotic contamination level in the groundwater of the
622 study area is moderate. Compared to surface water, the concentrations of SAs and TCs
623 in the groundwater of this area are significantly lower than those in surface waters of
624 Hubei (China), the Huangpu River (Shanghai), and lake waters in Hubei (China). The
625 concentration of FQs is comparable to that in the Huangpu River (Shanghai) but notably
626 lower than those in surface waters and lake waters of Hubei (China). The concentration
627 of MLs is similar to those in the Huangpu River (Shanghai), surface waters of Hubei
628 (China), and lake waters of Hubei (China), while the concentration of CPs is
629 significantly lower than that in the Huangpu River (Shanghai). Overall, the antibiotic

630 contamination level in the groundwater of the study area is considered moderate.

631 The distribution and transport of antibiotics in groundwater are jointly regulated
632 by multiple processes, including adsorption, biodegradation, and climatic conditions
633 (Zainab et al., 2020). Their adsorption behavior is influenced by factors such as their
634 physicochemical properties, initial concentration, ionic strength, temperature, and pH
635 (Loftin et al., 2008). In groundwater, lower microbial abundance and diversity, as well
636 as insufficient nutrients and dissolved oxygen, can inhibit the biodegradation rate of
637 antibiotics and may even promote their transformation into more stable and harmful
638 metabolites, leading to persistent accumulation in aquifers (Gartiser et al., 2007; Sui et
639 al., 2015; Lapworth et al., 2012). Furthermore, climatic conditions—such as rainfall
640 frequency and intensity—and groundwater recharge characteristics also affect the
641 mobility of antibiotics in aquatic systems (Zainab et al., 2020). Simultaneously, the
642 combined effects of multiple coexisting contaminants may significantly influence the
643 transport and transformation processes of antibiotics.

644

645 **4.1.2 Potential controlling factors**

646 To investigate the key factors influencing the distribution of antibiotics in
647 groundwater, redundancy analysis was conducted between groundwater
648 physicochemical parameters and the concentrations of five antibiotic classes (as well
649 as total antibiotics). The results showed that dissolved organic carbon (DOC), salinity,
650 and dissolved oxygen (DO) were significantly correlated with the concentrations of all

five antibiotic classes and total antibiotics. Specifically, total antibiotic concentration, along with sulfonamides (SAs), fluoroquinolones (FQs), tetracyclines (TCs), and macrolides (MLs), exhibited significant positive correlations with DOC and salinity, and negative correlations with DO. In contrast, chloramphenicols (CPs) showed a positive correlation with DO and negative correlations with DOC and salinity. These findings align with previous research, which indicates that most antibiotics degrade more readily under aerobic conditions, while chloramphenicol (a member of the CPs class) is more efficiently removed in anaerobic environments (Lai et al., 1995). This suggests that the molecular structure of antibiotics is a key determinant of their migration behavior under similar oxygen conditions. Among all significantly correlated physicochemical parameters, DOC ($r^2$ = 0.83, p = 0.003) emerged as the most influential environmental factor affecting antibiotic concentrations. DOC likely facilitates the transport and enrichment of antibiotics in groundwater by acting as a carrier for organic pollutants and providing adsorption sites (Fig. 5).

Furthermore, analysis of polycyclic aromatic hydrocarbon (PAH) contamination indicates that, compared to groundwater from other regions both domestically and internationally, the total concentration of 16 PAHs in the groundwater of this study area is significantly higher than those reported in Taiyuan, China (135 ng $L^{-1}$) (Li et al., 2015c) and Jharkhand, India (22 ng $L^{-1}$) (Ambade et al., 2021), and is similar to the level reported in Rawalpindi, Pakistan (763 ng $L^{-1}$) (Saba et al., 2012), reflecting a relatively high contamination level.

Redundancy analysis further revealed that multiple PAH congeners, including

673 total PAHs, showed significant positive correlations with four antibiotic classes
674 (excluding chloramphenicols) and total antibiotic concentrations, with total PAHs
675 exhibiting the strongest correlation. Additionally, the abundance of PAH-degrading
676 microorganisms—primarily Firmicutes and Actinobacteria—increased significantly
677 under high PAH exposure (Yu et al., 2017; Wang et al., 2017). Through the RND efflux
678 pump EmhABC, PAH-degrading bacteria can simultaneously expel both PAHs and
679 antibiotics (Yao et al., 2017b). Concurrently, the presence of antibiotics in the
680 environment can induce reactive oxygen species-mediated damage to PAH-degrading
681 bacterial cells and significantly inhibit their PAH degradation capacity (Fig. 6).

682 Therefore, we hypothesize that the combined microbial interactions between
683 PAHs and antibiotics in the groundwater environment may be the primary reason for
684 the observed significant positive correlation between PAHs and antibiotics in
685 groundwater.

686 **4.2 Pollution level, transfer of ARGs and inducing factors**

687 **4.2.1 Drivers of Groundwater Antibiotic Resistance Gene (ARG) Contamination**
688 **and Transfer**

689 Based on the results of redundancy analysis (RDA), the significantly correlated
690 influencing factors with high coefficients of determination ($r^2$) were categorized into
691 three groups: environmental factors (DOC); pollutants (Ace, Flu, Pyr, TPAH, NFC,
692 OFC, ETM, FQs, and MLs); and microbial communities (Proteobacteria, Firmicutes,
693 Chloroflexi, Candidatus_Colwellbacteria, TotalMic, and Shanno). Variance

partitioning analysis (VPA) revealed that these three categories of influencing factors collectively explained 70.9% of the variation in the distribution of ARGs in groundwater. Among them, pollutants — represented by antibiotics and PAHs — contributed the most (52.4%), serving as the primary drivers of ARG dissemination in the industrial park groundwater, followed by microbial communities (43.9%). These results indicate that pollutant inputs exert a more critical controlling role in the spread of ARGs in groundwater compared to microbial structure and environmental conditions (Fig. 17).

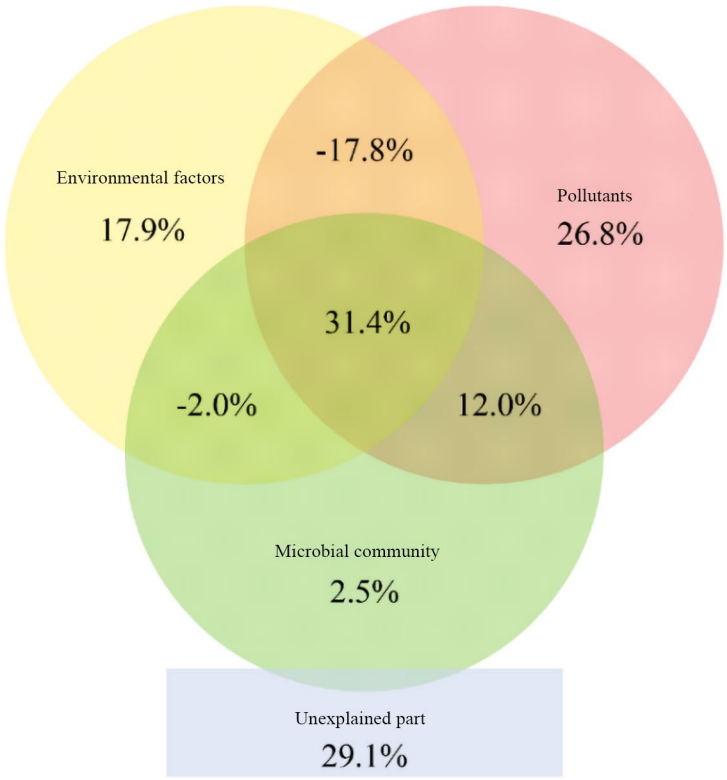



Fig. 17 Variation partitioning analysis (VPA) differentiating the effects of environmental factors, bacterial communities, and pollutants on the shift of ARGs in groundwater samples

Redundancy analysis (RDA) of the concentrations of 20 individual antibiotics, five major antibiotic classes, and total antibiotics in relation to 21 classes of ARGs in groundwater revealed significant positive correlations between tetracyclines (TCs),

fluoroquinolones (FQs), macrolides (MLs) and their corresponding ARG abundances, consistent with previous research (Liu et al., 2019c). On one hand, each class of antibiotics selectively inhibits the growth of susceptible bacteria, thereby providing greater survival advantages for the minority of resistant bacteria, which subsequently proliferate and accelerate the amplification and dissemination of their corresponding ARGs (Gullberg et al., 2011). On the other hand, certain bactericidal antibiotics (e.g., fluoroquinolones) can stimulate bacterial cells to produce reactive oxygen species, triggering the SOS response and inducing resistance mutations and ARG recombination, thereby further promoting the spread of ARGs (Hughes and Andersson, 2012; Kohanski et al., 2010). Additionally, MLs exhibited the highest correlation coefficient with ARGs, likely due to their relatively low detected concentrations in the groundwater of the study area (Hughes and Andersson, 2012). Under low-level antibiotic exposure, the continuous accumulation of multiple minor phenotypic mutations may be more conducive to the enrichment of ARBs (Miao et al., 2021) (Fig. 18).

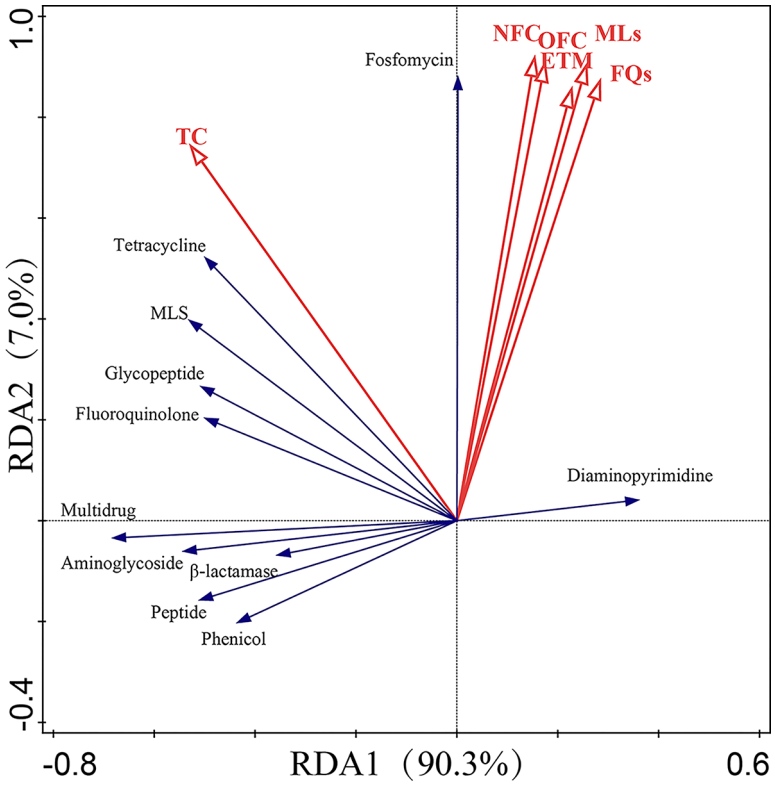



Fig. 18 RDA showing the influence from antibiotics on ARGs in groundwater samples.

Significant correlations between PAHs and ARGs have been confirmed in various types of soils (Azhogina et al., 2022). However, the influence of PAHs on ARG distribution in aquatic environments, particularly groundwater, remains poorly understood. In this study, redundancy analysis (RDA) was conducted on the concentrations of 16 individual PAHs and total PAHs (TPAH) in groundwater from the industrial park in relation to 21 classes of ARGs. The results showed that six PAH congeners (Ace, Phe, Flu, Pyr, BaA, Chr) and TPAH were significantly correlated with ARGs (Fig. 19). Consistent with previous studies (Wang et al., 2017), Phe, Flu, and Pyr exhibited significant positive correlations with most major ARG classes detected in groundwater, including multidrug, glycopeptide, macrolide, and tetracycline resistance genes, which may be related to the horizontal gene transfer of ARGs. Previous studies have found that 0.5 mg/L of Phe and 0.05 mg/L of Pyr significantly promoted RP4

plasmid-mediated horizontal transfer of ARGs by inducing elevated intracellular reactive oxygen species (ROS) levels, altering cell membrane permeability, and inhibiting mRNA expression of korA and korB genes.

Although TPAH showed the highest correlation coefficient with ARGs overall, it exhibited a significant negative correlation with the most dominant multidrug resistance genes in the study area. On one hand, the predominance of low-molecular-weight PAHs in the industrial park groundwater may form loosely aggregated complexes with adenine bases in plasmids via π-π interactions, thereby inhibiting horizontal transfer of ARGs (Kang et al., 2015). On the other hand, higher pollutant concentrations may inhibit the survival or even cause mortality of ARBs, thus slowing the dissemination of ARGs (Qiu et al., 2012). Therefore, we hypothesize that the higher proportion of low- to medium-ring PAHs and the elevated PAH contamination levels in the groundwater of this study area may have suppressed the proliferation and spread of certain ARG classes.

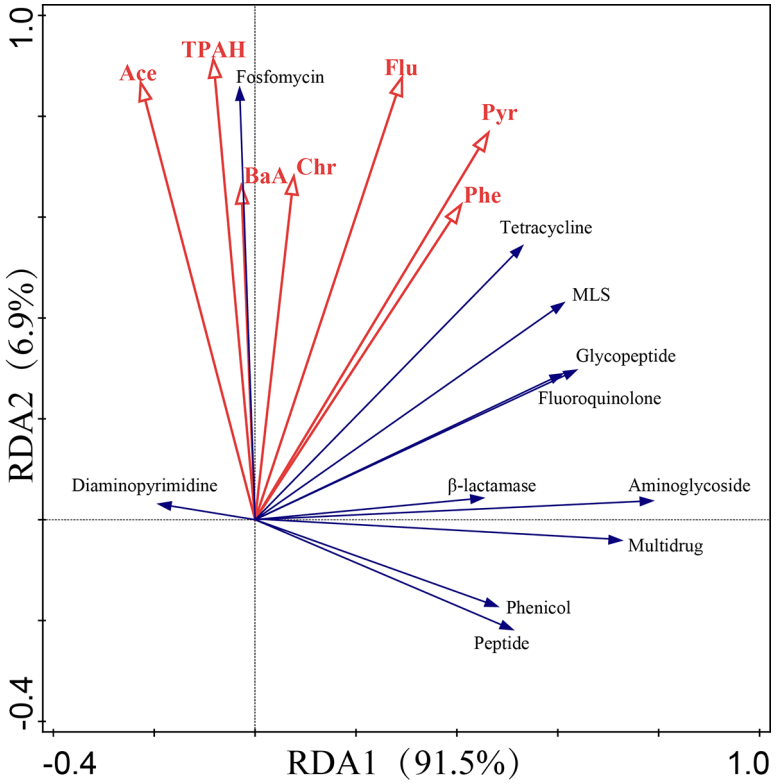



Fig. 19 RDA showing the influence from PAHs on ARGs in groundwater samples.

Redundancy analysis (RDA) indicated that environmental factors such as dissolved organic carbon (DOC), pH, and salinity in groundwater showed significant associations with the distribution of ARGs. Among these, DOC exhibited the most significant correlation, with its concentration negatively correlated with most ARGs. This finding aligns with previous studies emphasizing the notable influence of nutrient-related factors on ARGs (Gao et al., 2020). However, it contrasts with some research reporting positive correlations between ARGs and total organic carbon (TOC) or similar parameters. This discrepancy may be attributed to the unique environmental characteristics of the industrial park groundwater examined in this study: elevated levels of antibiotic and polycyclic aromatic hydrocarbon (PAH) contamination, combined with stressors such as hypoxia and oligotrophic conditions, may collectively enhance microbial stress tolerance, thereby altering the abundance and distribution

patterns of ARGs (Danielopol et al., 2000; Hemme et al., 2010; Yun et al., 2022). This likely explains the observed differences between groundwater in Shanghai's industrial parks and surface water bodies in Shanghai (Fig. 20).

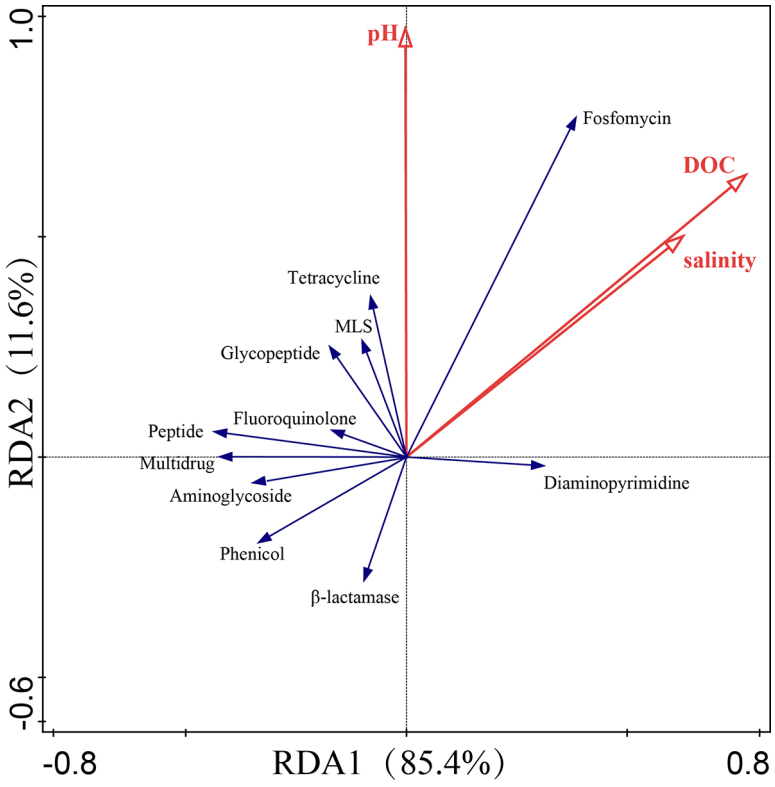



Fig. 20 RDA showing the influence from physicochemical properties on ARGs in groundwater samples.

Mobile genetic elements (MGEs) are key drivers in the distribution and transport of ARGs in groundwater. Redundancy analysis was performed on four categories of mobile genetic elements (integrons, transposons, insertion sequences, plasmids) and total MGEs, in relation to 21 classes of antibiotic resistance genes, based on their standardized abundance. This study also conducted a correlation network analysis (Spearman's $r > 0.6$, $p < 0.01$) between ARG subtypes and MGEs, revealing co-occurrence patterns between ARG subtypes and MGEs in groundwater, with results shown in Fig. 22. The co-occurrence network consisted of 159 nodes (including 74

ARG subtypes and 85 MGEs) and 586 edges. Among these, the 85 MGEs comprised 55 transposons, 16 insertion sequences, 13 integrons, and 1 plasmid. The integron-associated elements were the MGEs with the most connections to ARG subtypes, showing positive correlations with 28 and 27 ARG subtypes, respectively. This indicates that, besides transposons, integrons are also important factors in the distribution and transport of ARGs in groundwater, which is consistent with findings reported by Cerqueira et al (2019) (Fig. 21).

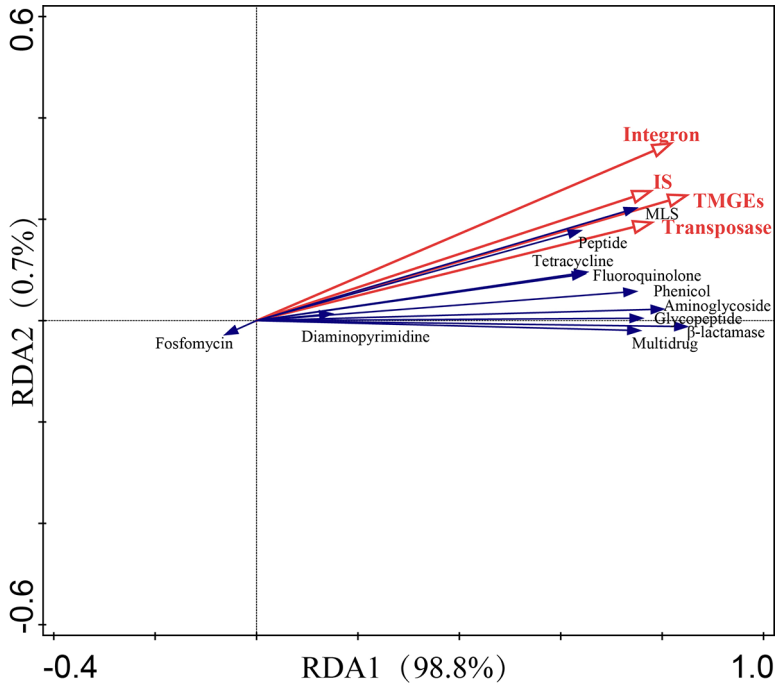



Fig. 21 RDA showing the influence from MGEs on ARGs in groundwater samples.

Redundancy analysis (RDA) demonstrated a significant association between the microbial community structure and the distribution of ARGs in the industrial park groundwater. Specifically, Proteobacteria and total microbial abundance (TotalMic) were positively correlated with most major ARG classes, whereas Chloroflexi, Planctomycetes, and the Shannon diversity index (Shanno) showed negative correlations with dominant ARGs. These results confirm that the microbial community

is another critical factor—alongside pollutants, environmental parameters, and MGEs—influencing the distribution and migration of ARGs in groundwater (Fig. 23).

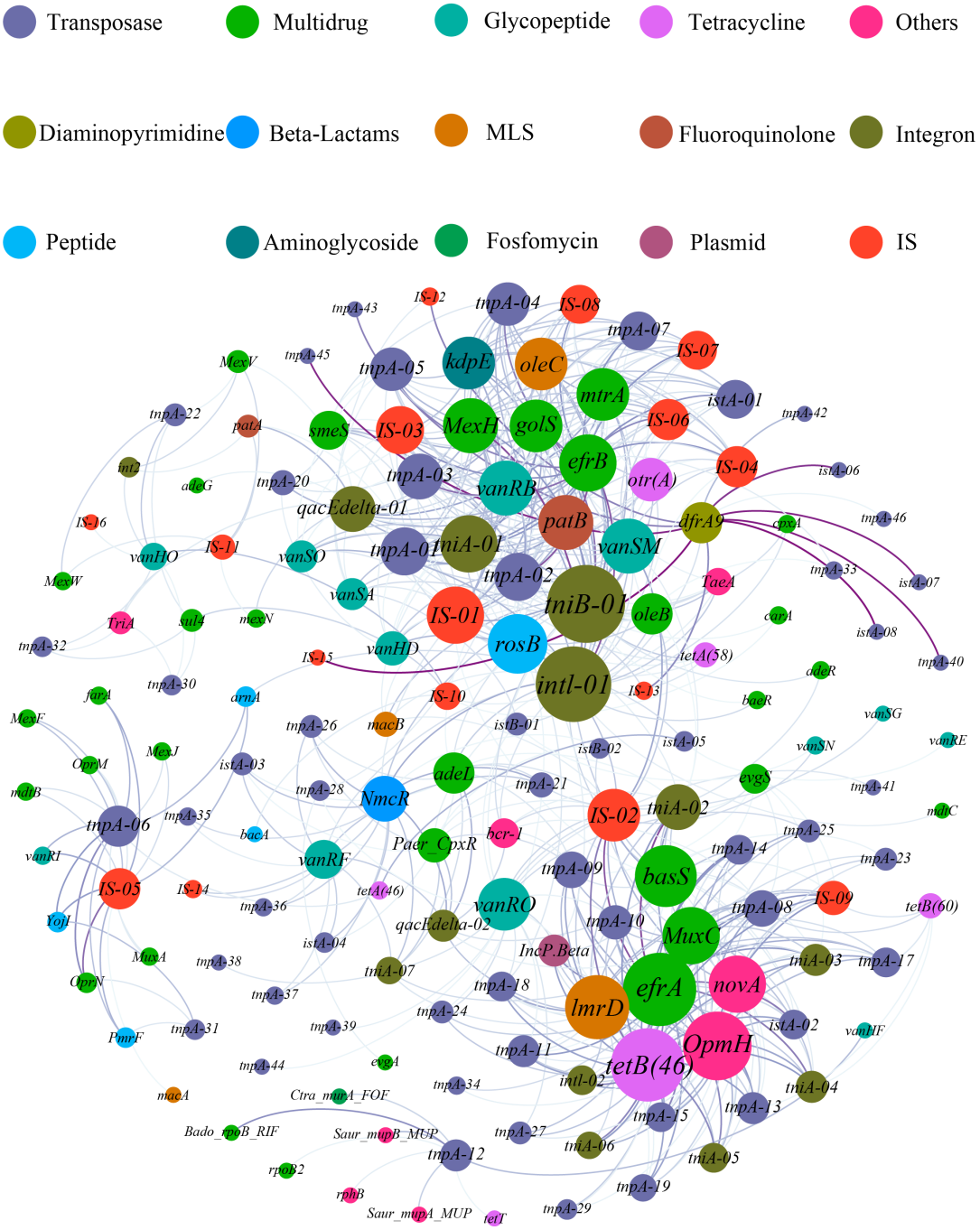



Fig. 22 Network analysis among ARG subtypes and MGEs in groundwater samples.

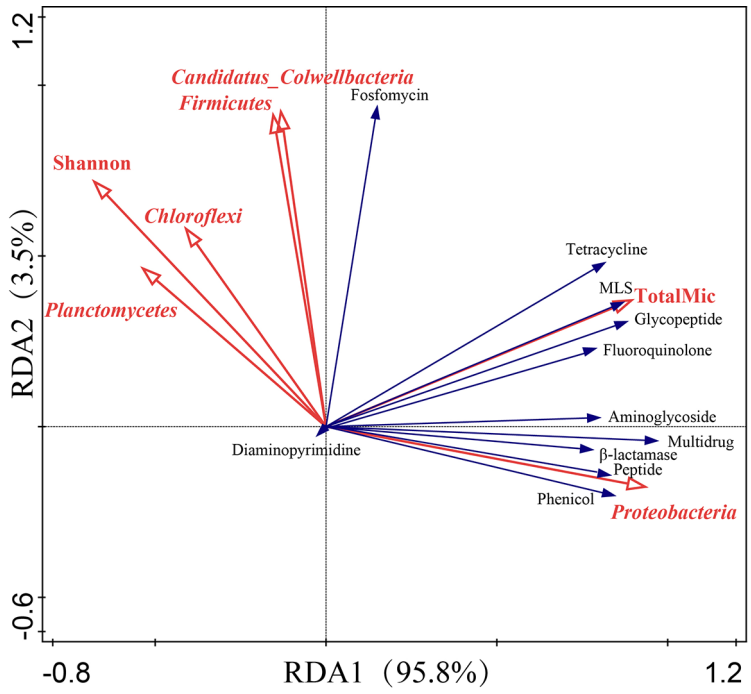



Fig. 23 RDA showing the influence from microbial community on ARGs in groundwater samples.

4.2.2 Efflux pump-mediated resistance and its role in the dissemination of antibiotic resistance genes

In the groundwater of the industrial park, efflux pumps constituted the dominant resistance mechanism for ARGs, accounting for an average of 65.4%, followed by antibiotic target modification (25.2%), antibiotic inactivation (4.0%), and target replacement (2.2%). This distribution aligns with findings from previous studies (Qiao et al., 2021; Yan et al., 2019). The compositional structure remained stable across most sampling sites. Among the efflux pump mechanisms, the RND type was predominant (41.1%), followed by ABC (31.5%) and MFS (14.3%) types. ARGs containing two or more efflux pump resistance mechanisms accounted for 9.5% of the total. This distribution pattern is similar to that observed in local soil cores (Miao et al., 2025).

The abundance and composition of ARGs were highly similar between soil cores

and groundwater in the industrial park. Multidrug, macrolide, glycopeptide, tetracycline, and peptide resistance genes were the dominant ARGs in the soil-groundwater system. The composition of ARG resistance mechanisms showed minimal variation between soil and groundwater, with efflux pump-based mechanisms and antibiotic target modification collectively exceeding 90% in both media. These results indicate that ARGs from soil can migrate into groundwater and persist stably over the long term, highlighting the need for focused attention on their potential risks to the groundwater environment (Fig. 24).

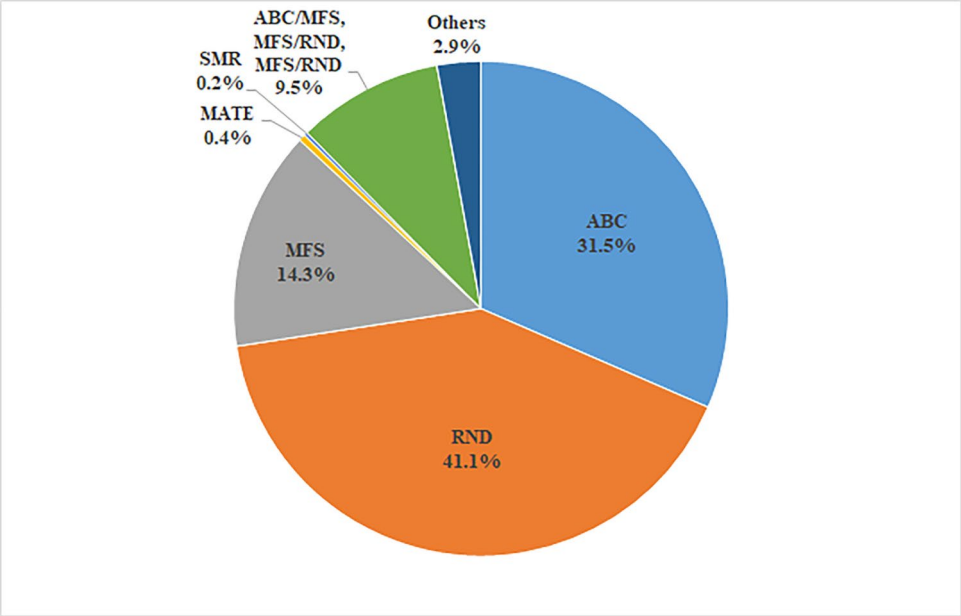



Fig. 24 Composition of efflux pump resistance mechanism of ARGs in groundwater samples.

### 4.3 Distribution and transfer of pathogens

Co-occurrence network analysis revealed extensive associations between ARGs and bacterial genera within the soil-groundwater system of the industrial park. The

834 network comprised 109 nodes (81 ARG subtypes and 28 bacterial genera) and 265
835 connecting edges. Taxonomically, Proteobacteria (17 genera) and Chloroflexi (4 genera)
836 dominated the bacterial composition, indicating their roles as key potential ARBs in the
837 distribution and migration of ARGs within the system. Among these, unclassified
838 Gammaproteobacteria exhibited the broadest connectivity, linking to 33 ARG subtypes,
839 while unclassified Alphaproteobacteria, unclassified Bacteroidota, Erythrobacter, and
840 other genera also showed significant correlations with more than 10 ARG subtypes.
841 Notably, by comparing potential hosts in soil and groundwater, seven shared bacterial
842 genera were identified, including unclassified Burkholderiales, Thiobacillus, and
843 Arenimonas. This suggests that these bacteria may carry ARGs and migrate vertically
844 along the soil profile into groundwater. Thus, the vertical migration of antibiotic-
845 resistant bacteria in the soil-groundwater system represents a critical pathway
846 contributing to ARG contamination in groundwater (Fig. 25).
847

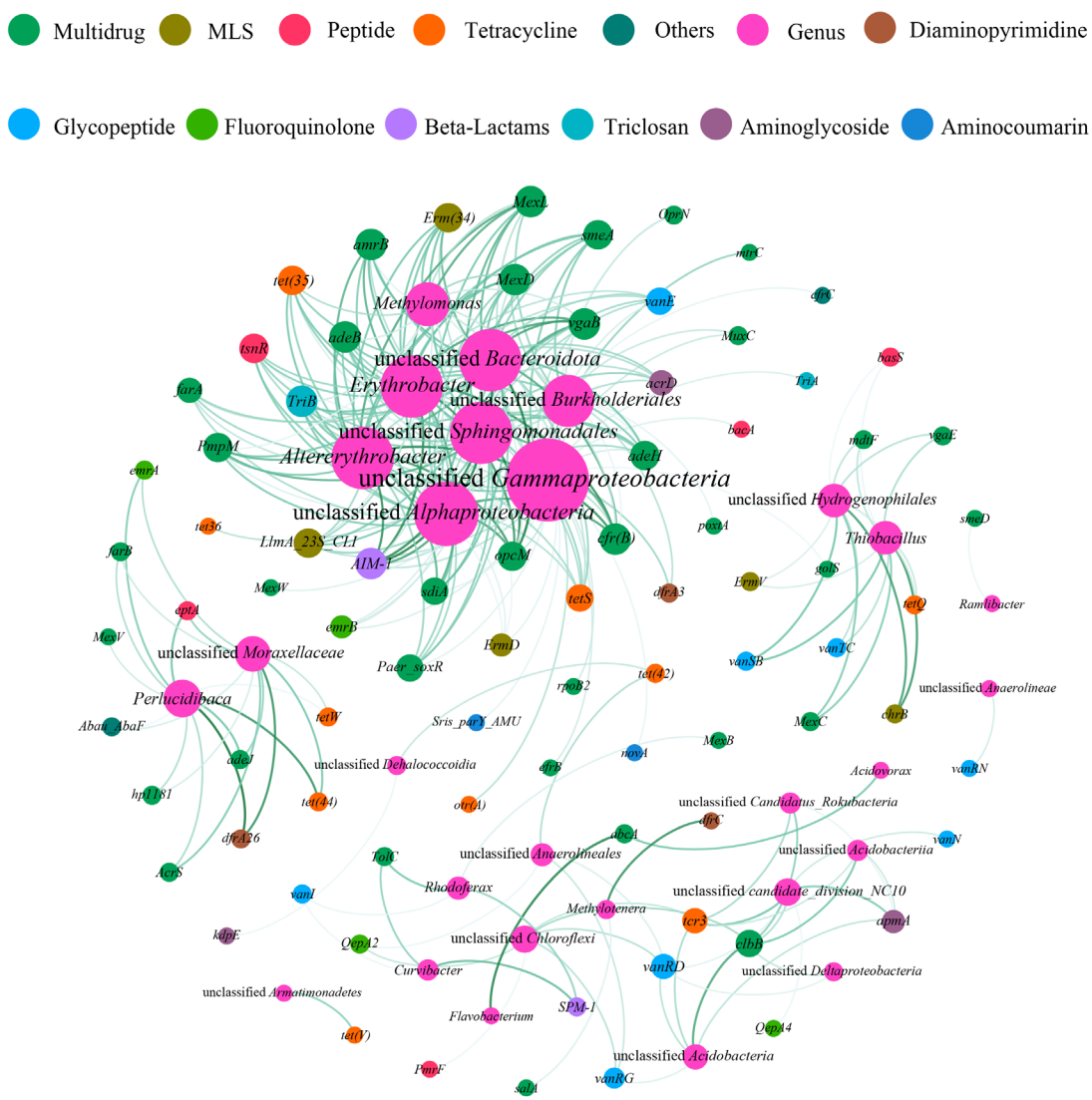



Fig. 25 Network analysis among ARG subtypes and bacteria communities at genus level in soil-groundwater system.

## 5 Conclusions

This study highlights the critical yet often overlooked risk posed by the vertical migration of antibiotics, ARGs, and potential pathogens in urban groundwater systems, which are simultaneously contaminated by multiple pollutants. Our findings demonstrate that the distribution and transport of antibiotics in groundwater are

858 governed by a combination of anthropogenic inputs, the physicochemical properties of
859 antibiotics, groundwater parameters, and interactions with co-occurring contaminants.
860 Sulfonamides were identified as the predominant antibiotic class in groundwater,
861 whereas fluoroquinolones exhibited higher enrichment in soil, likely due to their
862 polar/ionic functional groups and strong adsorption to soil matrices, which limit their
863 leaching into groundwater. Vertically, antibiotic concentrations decreased exponentially
864 with depth, with tetracyclines showing greater mobility than sulfonamides.

865 The composition and relative abundance of 21 ARG classes were highly similar
866 between soil cores and groundwater, indicating active exchange and migration of ARGs
867 across the soil-groundwater interface. In groundwater, contaminants—particularly
868 antibiotics and PAHs—were the primary drivers of ARG distribution, followed by the
869 structure of microbial communities. Mobile genetic elements (MGEs) and groundwater
870 physicochemical parameters also played significant roles in shaping the dissemination
871 of ARGs.

872 Integrating earlier research on ARG-hosting microorganisms in soil layers, a
873 comparison of potential hosts in soil and groundwater identified seven shared bacterial
874 genera, including unclassified Burkholderiales, Thiobacillus, and Arenimonas. This
875 suggests that these bacteria may act as vectors carrying ARGs along the soil profile into
876 groundwater. Consequently, the vertical migration of antibiotic-resistant bacteria (ARB)
877 through the soil-groundwater continuum represents a key pathway contributing to ARG
878 contamination in groundwater.

879 This work reveals interconnected pollution patterns in urban soil-groundwater

systems, where antibiotics, PAHs, and ARGs interact across deeper soil layers and groundwater, collectively threatening ecosystem security. Effective control of ARG dissemination will require integrated management strategies targeting co-pollutants such as PAHs and antibiotics to mitigate their selective pressure and reduce the spread of resistance determinants.

## 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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