Mass-immigration shapes the antibiotic resistome of wastewater treatment plants

Lanping Zhang[1,2,3], Bob Adyari[1,2,3,4], Liyuan Hou[5,6], Xiaoyong Yang[7], Mahmoud Gad[8], Yuwen Wang[1,9], Cong Ma[10], Qian Sun[1,2,3], Chang-Ping Yu[1], Anyi Hu[1,2,3*]

1. CAS Key Laboratory of Urban pollutant Conversion, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China
2. University of Chinese Academy of Sciences, Beijing 100049, China
3. Fujian Key Laboratory of Watershed Ecology, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China
4. Department of Environmental Engineering, Universitas Pertamina, Jakarta 12220, Indonesia
5. Department of Civil and Environmental Engineering, Utah state university, Utah UT 84322, USA
6. Utah Water Research Laboratory, Utah State University, Logan, Utah 84322, USA
7. School of Environmental and Material Engineering, Yantai University, 30 Qingquan Road, Yantai 264005, China
8. Water Pollution Research Department, National Research Centre, Giza 12622, Egypt
9. Marine biology Institute, Shantou University, Shantou 515063, China
10. Xiamen Municipal Environmental Technology Co., Ltd., Xiamen 361001, China

***Correspondence to:**

Dr. Anyi Hu; Email: ayhu@iue.ac.cn; Tel: 86-592-6190582.

Abstract

Wastewater treatment plants (WWTPs) are the hotspots for the spread of antibiotic resistance genes (ARGs) into the environment. Understanding the behavior and assembly processes of ARG communities in each treatment unit in WWTPs can promote ARG surveillance and control. Despite this, current ARG surveillance in WWTPs lacks comprehensive assessments of the city-level and short-term daily variations. Thus, in this study, 285 ARGs and ten mobile gene elements (MGEs) were monitored in seven WWTPs in Xiamen via high-throughput qPCR for seven days. The results showed that the average daily load of ARG to WWTPs was about 1.21×10^{20} copies/d, and a total of 1.44×10^{18} copies/d was discharged to the environment across the entire city. Genes conferring resistance to beta-lactam, multidrug, aminoglycoside, and macrolide-lincosamide-streptogramin B (MLSB) were dominant. Interestingly, there was no daily difference in the richness and abundance of ARGs as well as ARG community composition. Stochastic processes were the main force determining the assembly of ARG communities in WWTPs. The relative importance of stochastic processes in each treatment unit ranked in the order of influent (INF) > effluent (EFF) > active sludge (AS). The results of SourceTracker, variation partitioning analysis (VPA) and hierarchical partitioning (HP) analysis indicated that bacterial and ARG communities from upstream treatment units played an increasing dominant role in shaping ARG communities in AS and EFF, respectively, suggesting the importance of mass-immigration of bacteria and ARGs from the source on ARG transport in wastewater treatment units. This emphasizes the need to revise the way we mitigate ARG contamination but focus on the source of ARGs in urban wastewater.

Keywords: Wastewater treatment plant; Antibiotic resistance genes; Mass immigration; Assembly mechanisms; Daily variation; Mass loads

1. Introduction

Extensive consumption of antibiotics in humans and livestock exerts a major selective pressure on the environmental microbiome, contributing to the proliferation and spread of antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs) (Buchy et al., 2020). Therefore, ARB and ARGs have become major global health threats and concerns in environmental systems (Bengtsson-Palme and Larsson, 2015; Prestinaci et al., 2015). One Health framework, aiming to understand the mechanism of ARB movement across humans, animals, and the environment, can help guide antibiotic resistance management. Reliable surveillance data lays the foundation for the One Health framework (Lebov et al., 2017). Previous cross-regional investigations reveal that ARGs are widespread in both highly human-disturbed (e.g., urban city) and human-inaccessible areas (e.g., pristine plateau) (Chen et al., 2016; Hendriksen et al., 2019; J. Li et al., 2022; Liu et al., 2021; Yao et al., 2022). Human activities can promote ARG transfer between various environmental interfaces. For instance, wastewater discharge could cause the immigration of massive amounts of ARGs to the receiving environments (Harnisz et al., 2020; Schwermer and Uhl, 2021). Moreover, the immigration of ARGs not only increases their loads but also raises the risk of their proliferation in the receiving environments through vertical gene transfer (VGT) and horizontal gene transfer (HGT) (Li et al., 2019). HGT events happening within the genomes of bacteria or between bacterial cells from different taxonomic lineages are mediated by MGEs such as plasmids, transposons, and insertional sequences. On the contrary, VGT is the transmission of ARGs from parent to offspring via proliferation (Frost et al., 2005; Zhu et al., 2013). Bacteria with ARGs through HGT can further boost the spread of ARGs via secondary HGT and VGT (Li et al., 2019; Seoane et al., 2011). WWTPs are the key nodes in human-environment interfaces. Hence, comprehensive surveillance of ARG profiles and mass loads in WWTPs is pivotal to the development of suitable strategies for controlling the spread of ARGs in urban environments and assessing human health risks.

Nowadays, WWTPs are widely recognized as the hotspots for ARG proliferation and dissemination (Pruden et al., 2013). Previous studies showed that the biological treatment processes (e.g., activated sludge) of WWTPs contained a high density of bacteria and various kinds of contaminants (e.g., heavy metals, antibiotics, etc.). These contaminants not only facilitated the proliferation of ARB (Wang et al., 2020) but also promoted the exchange of ARGs among bacteria (Ju et al., 2019; Z.-H. Li et al., 2022; Lin et al., 2019; Su et al., 2020; Tong et al., 2019; Zhao et al., 2019; Zhu et al., 2013). Although WWTPs had a relatively high removal efficiency of ARGs for 1–2 log reduction, the effluent still harbored more than 10^7 copies/mL ARGs (An et al., 2018; Yin et al., 2022). The release or recycling of effluents could lead to increasing ARGs abundance and shifting in ARGs community composition in the receiving environments (Kampouris et al., 2021; Lee et al., 2021; Marano et al., 2019; Reichert et al., 2021; Schwermer and Uhl, 2021). In addition, numerous studies have demonstrated that ARG profiles in WWTP varied at different time scales. For example, at the seasonal scale, the diversity and composition of ARGs were significantly different between summer and winter in eleven WWTPs in China (An et al., 2018). And the temporal changes of ARG types in effluents were mainly related to the prevalent antibiotics (Yin et al., 2022). At the diurnal scale, the variations of ARGs were related to the dynamics of wastewater flow (Cai et al., 2022; Sun et al., 2021). However, the daily variation of ARGs in WWTPs at the city level is still limited. This is important for setting suitable sampling frequencies and monitoring targets to facilitate comparison within and among individual WWTPs. In addition, obtaining this information will help understand the assembly mechanisms underlying ARG spread within a short time, contributing to establishing suitable wastewater-based surveillance approaches in cities.

Stochastic (i.e., dispersal and drift) and deterministic processes (i.e., bio-interaction and environmental selection) collectively contribute to the community assembly (Daliang et al., 2019; Vellend, 2010; Zhou and Ning, 2017). However, after

decades of debate, the role of stochastic processes in structuring communities has been increasingly appreciated. For instance, the stochastic processes played a central role in structuring ARG communities in urban lakes (e.g., Baiyang Lake and Tai Lake). However, the difference in the relative importance of stochastic processes in Baiyang Lake and Tai Lake was correlated with environmental selection (Chen et al., 2021). In addition, Hou and colleagues showed that the stochastic processes played a dominant role in shaping ARG community assembly in urban water bodies under anthropogenic influences (Hou et al., 2021a, 2021b). And they suggested that the dominant role of stochastic processes may indicate the contribution of HGT to the spread of ARGs in different urban environments. Although many studies explored the influence of various factors (e.g., nutrients, chemical contaminants, operational parameters, etc.) on ARG profiles in WWTPs or bioreactors (Chen et al., 2020; Dai et al., 2022; Ju et al., 2019; Pallares-vega et al., 2019), the contribution of stochastic processes in ARG community assembly in the different treatment processes of WWTPs is still limited. Understanding the assembly mechanism of microbial communities including ARGs and their hosts is a promising way to provide information for predict their transport and spread.

Numerous studies reported that the combined effect of physiochemical parameters and biological interaction shapes the ARG profiles (Fang et al., 2019; Guo et al., 2018; Zhao et al., 2017). Notably, several recent studies have demonstrated that mass immigration via wastewater stream strongly determined the bacterial communities in active sludge (Ali et al., 2019; Dottorini et al., 2021; Mei and Liu, 2019). However, whether mass immigration (i.e., stochastic processes) plays a role in the ARG community assembly across the wastewater treatment processes is not known. Since influent wastewater flows across different WWTP treatment units, the mass immigration of microorganisms along the flow may shape the microbial and ARG communities in the downstream processes such as the activated sludge and effluents (Dottorini et al., 2021).

In this study, we employed high-throughput quantitative PCR (HT-qPCR) to investigate the daily variation of 285 ARGs and 10 MGEs in INF, AS and EFF of seven WWTPs in Xiamen, China for seven days. We aim to: □) characterize the daily variations of the abundance, diversity, and composition of ARGs in WWTPs across the whole city; □) explore the community assembly mechanisms of ARGs and identify the key biotic and abiotic factors shaping the ARG profiles in different wastewater treatment units; and □) assess the daily ARG mass loads to WWTPs and ARG mass loads in receiving environment at the city level, followed by potential health risk assessment. We assume that stochastic processes will have a higher contribution to the ARG community assembly in INF than in AS and EFF because the latter two will undergo specific environmental pressure (i.e., operation parameters). Among various factors (i.e., biotic and abiotic factors), the immigration of ARGs and microbial communities from upstream (e.g., INF) will affect the ARG profiles in the downstream processes (i.e., AS and EFF). The result of this study will extend our understanding of ARG dynamics as well as the underlying assembly mechanisms in WWTPs, providing fundamental information for surveillance and control of WWTP ARGs at a city-level perspective.

2. Methods and materials

2.1 Study area and sample collection

Xiamen is a typical subtropical monsoon city on the southeast coast of China (117°4′E-118°21′E, 24°24′N-24°54′N) with a small range of annual temperature variance (seasonal average temperature 13.7–27.9□). It covers an area of 1699 km^2 and has a population of 3.92 million in 2016 (Hu et al., 2018). Impressively, the urban land almost doubled to 334 km^2 and the total amount of municipal wastewater increased from around 210 million tons to 320 million tons during the past 10 years (Xiamen Environmental Protection Bureau, 2007–2016). 92% of the municipal wastewater in Xiamen was treated by seven WWTPs. Among these WWTPs, six

WWTPs (listed as W2~W7) employ the activated sludge technology, while one (i.e., W1) uses a biological aerated filter treatment process. Detailed information on these WWTPs was shown in Table S1. More information about Xiamen city and residents representing the socioeconomic development such as the urbanization rate, per capita disposable income (PCDI), and per capita consumption expenditure was obtained from the Xiamen Bureau of Statistics (Xiamen Bureau of Statistics, 2017).

The 24-hr composite samples of INF, AS, and EFF were collected from seven WWTPs by using automatic samplers (Hachi, Sigma SD900) for 7 days (from Feb. 28th to March 6th, 2016). But there was no sampling event on March 5th, 2016 because the staff had the day off. From all WWTPs, six were equipped with one AS tank each and one (W5) was equipped with two tanks. For the first two days, we collected AS samples from the two AS tanks of W5 separately, and one AS composite sample for the subsequent days. Due to the limitations of sampling conditions, a total of 49 INF samples, 49 EFF samples, and 48 AS samples were finally collected. Among these samples, there were 46 pairs of INF-AS-EFF samples. All samples were kept on ice and shipped to the lab within 4 h. For microbial community analysis, 100~300 mL of INF and 800~1000 mL of EFF were filtered through the 0.2 μm pore size Sterivex-GP filter (Millipore, Billerica, MA, USA), while AS samples were concentrated by centrifuging at 8,000 rpm for 15 min. All microbial concentrated samples were stored at -80 □ before DNA extraction. The concentration of pharmaceuticals and personal care products (PPCPs) was measured following the protocol of EPA 1694 with slight modifications (Wang et al., 2018). Here, we classified PPCPs into antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), stimulants, and other PPCPs, and cited the mass loads of information on each PPCP type from our previous study (Wang et al., 2018). The water quality parameters, including chemical oxygen demand (COD), 5-day biological oxygen demand (BOD_5), total phosphorous (TP), total nitrogen (TN), ammonia nitrogen (NH_4^+-N), and suspended solids (SS) of INF and EFF, were provided by the wastewater treatment plants by using national standard methods

(China's State Environment Protection Agency, 2002). Activated sludge performance on specific removal rates of nutrients (TN, TP, and NH_4^+-N) and organic matters (indicated as chemical oxygen demand; COD) was calculated as the following equation (Han et al., 2021):

$$Removal\ rate = (C_{inf} - C_{eff})/C_{inf}$$

where C_{inf} and C_{eff} represent the concentration of each chemical in the INF and EFF (mg/L), respectively.

2.2 DNA extraction, 16S rRNA gene amplicon sequencing, and data processing

The genomic DNA of the 146 samples was extracted by using FastDNA SPIN Kit for Soil (Qbiogene-MP Biomedicals, Irvine, CA, USA) according to the manufacturer's protocol with modifications (Hu et al., 2018). The 16S rRNA amplicon sequencing was conducted to characterize the prokaryotic bacterial communities. The 16S rRNA genes in the hypervariable V4-V5 region were amplified with a universal primer set 515F and 907R (Hu et al., 2017). The PCR products were then purified and sequenced on Illumina HiSeq 2500 platform (Illumina Inc., San Diego, CA, USA) via a paired-end approach (2 × 250 bp). The raw sequence data can be downloaded from the NCBI short reads archive database under the BioProject number of PRJNA545043.

Raw 16S rRNA paired-end reads were trimmed and assembled using the LotuS pipeline (Hildebrand et al., 2014; Hu et al., 2018). Only high-quality reads that meet the following criteria were retained for subsequent analysis: (□) an average sequence quality > 27; (□) a sequence length > 170 bp; (□) no ambiguous bases; (iv) no mismatches to the barcodes or primers; and (v) a homopolymer run < 8 bp. The reads were then checked for chimera and clustered into operational taxonomic units (OTUs) with a threshold of 97% similarity by using UPARSE (Edgar, 2013).

2.3 HT-qPCR analysis of 285 ARGs and 10 MGEs

A total of 285 ARGs, 10 MGEs, and one 16S rRNA gene were determined on a Warfergen SmartChip real-time PCR system (Wafergen Bio Inc., USA) with the SYBR Green I® chemistry. The targeted ARGs involved the genes that were resistant to all major classes of antibiotics, while those MGEs included 8 transposase and 2 integron genes. HT-qPCR was carried out in a volume of 100 nL reaction system and was under the following thermal cycle condition: initial denaturing at 95°C for 10 min; followed by 40 cycles of denaturation at 95°C for 30 s and annealing at 60°C for 30 s (Hou et al., 2021b; Su et al., 2015; Zhu et al., 2017). The melting curve analyses were automatically generated by SmartChip qPCR software (2.7.0.1). Only the results that meet the following conditions were obtained for statistical analysis: (□) a threshold cycle (Ct) ≤ 31; (□) more than two replicates having been amplified were retained as positive; (□) reaction with one peak; (iv) amplification efficiency within 90–110%. The relative copy number of each gene can be calculated using:

$$\text{Relative gene copy number}_{gene} = 10^{(31-\text{Ct})/(10/3)}$$

Relative abundance (copies per 16S rRNA gene) and absolute abundance (copies per mL) of ARGs and MGEs was calculated by the following equation:

$$\text{Relative abundance}_{gene} = \frac{\text{Relative gene copy number}_{gene}}{\text{Relative gene copy number}_{16S\,rRNA}}$$

$$\text{Absolute abundance}_{gene} = \text{Relative abundance}_{gene} \times \text{absolute abundance of 16S rRNA genes}$$

The absolute abundance of the 16S rRNA gene was measured on LightCycler® Roche 480 Real-time PCR instrument (Roche Inc., Basel, Switzerland). A total of 20 μL reaction system consisted of 10 μL LightCycler® 480 SYBR Green I Master Mix (2 ×) (Applied Bio-systems, CA, USA), 0.4 μM of 16S rRNA primer, 1 μg/L bovine serum albumin (Sigma, Steinheim, Germany) and 20 ng of template DNA. The standard curve was processed by 10-fold serially diluted 16S rRNA gene incorporated plasmids with insert length 61 bp bases. The PCR reactions were performed as follows: initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation

at 95°C for 15 s and annealing at 60°C for 1 min and extension at 72°C for 15 s. All qPCR reactions were conducted in triplicate for each sample and negative controls were included.

2.4 Daily ARG and MGE mass loads

Based on the total amount of wastewater and the served population, target gene (i.e., ARGs and MGEs) daily mass loads in different WWTPs across the whole city and target gene mass loads per inhabitant are calculated at the city level. ARG or MGE mass loads in different treatment units were following the equations:

$$\text{Daily target gene loads (copoies/d)} = \frac{\sum \text{absolute abundance of total target gene} \left(\frac{\text{copies}}{\text{mL}}\right) \text{in the target unit} \times \text{Q (mL/d)}}{7\ \text{(d)}}$$

$$\text{Daily target gene loads per capita (copies/(d} \cdot \text{capita))} = \frac{\text{Daily target gene loads (copoies/d)}}{\text{population size}}$$

Where Q is the flow rate (mL/d) of the corresponding WWTP. Daily mass loads of ARGs and MGEs per capita in INF and EFF represent the citizen's daily discharge of ARGs and MGEs to WWTP and potential discharge loads to the environment or release to humans, respectively.

2.5 Null model analysis

The null model was utilized to determine the relative contribution of stochastic and deterministic processes to the assembly of ARG and bacterial communities (Hou et al., 2021b). The principle of the null model can be recognized as follows: the expected similarity of null expected communities was calculated by 1000 random shuffles based on the raw community data. The richness and proportion of species occupancy of the expected community were consistent with the observed community

in this step. Then, we calculated the value of the relative importance of the stochastic processes by:

$$\text{Stochasticity } (\%) = 1 - (S_{obs} - E_{exp})/S_{obs}$$

where S_{obs} means the observed similarity of the original communities, and E_{exp} means the average similarity of null expected communities.

2.6 SourceTracker analysis

The relative contribution of communities (ARGs or OTUs) from different source habitats (i.e., INF, AS, EFF and unknown source) to sink habitats was assessed by using SourceTracker v2.0 with default parameter settings based on previous studies (Gad et al., 2022; Knights et al., 2011; Li et al., 2018). For the sink of AS, the source includes INF and the unknown source, while the sources became INF, AS and the unknown source for the sink of EFF. The unknown source represents the indigenous communities or those from uninvestigated environments. For each sample, the predicted proportion for each of the potential sources was run 5 times to calculate the average proportion. The accuracy of SourceTracker prediction and corresponding standard deviation (SD) within each habitat were evaluated by the consistency between the predicted source and the original habitat (Gad et al. 2022). The ARGs and OTUs with an occurrence of less than 1% were discharged during this analysis.

2.7 Statistical analysis

Daily variations of ARG richness and abundance for seven days were assessed by the coefficient of variance (CV) and were calculated as the following formula: CV = SD/the mean value of ARG richness or abundance. The time-dependent ARG and bacterial community structures were analyzed by using a time decay model. The time decay model takes the time interval matrices between samples as the independent variable, and the β-diversity matrices based on the Bray-Curtis distance between

samples as the dependent variable. The variables were fitted with a linear model and the obtained slope and R^2 were used to evaluate the collinear relationship.

The β-diversity patterns of ARG and bacterial communities were revealed by nonmetric multidimensional scaling (NMDS) analysis based on Bray-Curtis distance matrices. The significance of the differences within ARG and bacterial communities among wastewater treatment units or WWTPs were performed by permutational multivariate analysis of variance (Adonis) and analysis of similarity (ANOSIM).

VPA was used to distinguish the relative effects of biotic, abiotic factors, and MGEs on the variance of β-diversity of ARG communities in each treatment process (Hu et al., 2017). The biotic factors included the first two NMDS axes of the bacterial communities and the corresponding source proportions of ARG and bacterial communities predicted by SourceTracker. Here, we only included the source proportions in the analysis of ARG communities from AS and EFF. The abiotic factors included physicochemical parameters of INF and EFF and the nutrient removal rate (i.e., TN, TP, NH_4^+-N, and COD) of WWTPs. Here, MGEs were included as an independent factor because they were commonly recognized as an important driver causing the HGT of ARGs. Prior to VPA, the multicollinearity between variables in each factor group was assessed by the variation inflation factor (VIF), using “vifstep” function from the R package usdm (Naimi et al., 2014). The variables with VIF < 10 were retained. To quantify the relative importance of an individual element of factors, HP analysis was performed via the “rdacca.hp” function from the R package *rdacca.hp* (Lai et al., 2022).

Statistical analysis and visualization were conducted using R 4.1.0 with the R package phyloseq (McMurdie and Holmes, 2013), vegan (Oksanen et al., 2012), and ggplot2 (Wickham, 2010).

3. Results

3.1 A full profile of ARGs in WWTPs across the whole city

A total of 208 ARG subtypes were detected in INF, AS, and EFF of seven WWTPs of Xiamen, corresponding to 8 major ARG types (i.e., aminoglycoside, beta-lactam, chloramphenicol, MLSB, multidrug, sulfonamide, tetracycline, and vancomycin resistance genes). The number of ARG subtypes in different treatment units decreased as follows: INF (94~141) > EFF (31~115) > AS (44~98) (**Fig. S1A**). 156 of 208 ARG subtypes (75%) were shared by three habitats (i.e., INF, EFF, and AS) (**Fig. S1B**). The shared ARGs mainly belonged to beta-lactam (43 ARG subtypes), multidrug (33), aminoglycoside (31), and MLSB (23) resistance genes.

The results indicated that the relative abundance of ARGs in INF was the highest (average 0.56 copies/16S rRNA gene), and then decreased more than 50% in AS to 0.20 copies/16S rRNA gene. But in EFF this value increased again to 0.33 copies/16S rRNA gene (**Fig. 1A**). Being inconsistent with this trend, AS had the highest absolute abundance of ARGs (average 4.14×10^9 copies/mL), followed by INF (1.34×10^8 copies/mL) and then EFF (2.40×10^6 copies/mL) (**Fig. 1B**). According to the absolute abundance of ARGs, aminoglycoside (ranging from 2.70×10^4 to 5.36×10^9 copies/mL), multidrug (3.49×10^4~4.82×10^9 copies/mL), MLSB (5.81×10^3~9.03×10^8 copies/mL) and beta-lactam (9.90×10^3~3.06×10^9 copies/mL) resistance genes were the top 4 dominant ARG types.

As for MGEs, the relative abundance in INF was the highest value (average 0.092 copies/16S), followed by EFF (0.055 copies/16S) and AS (0.016 copies/16S) (**Fig. 1A**), while the absolute abundance is in the following order: AS (average 3.81×10^8 copies/mL) > INF (2.26×10^7 copies/mL) > EFF (4.10×10^5 copies/mL) (**Fig. 1B**). Notably, among MGE subtypes, the relative abundance of integrons in EFF increased to a level comparable to that in INF. Spearman correlation analysis indicated that the relative abundances of MGEs and ARGs were significantly correlated with each other in both INF and EFF ($r \geq 0.50$, $P < 0.001$), but not in AS (**Fig. S2**). In addition, the relative abundance of transposases correlated significantly with the majority of ARG

types in both INF and EFF ($P < 0.05$). Interestingly, the relationship between integrons and ARG types was stronger in INF than in EFF (**Fig. S3**).

3.2 Daily variations of ARG abundance, diversity, and composition in different treatment units

NMDS ordination analysis indicated that both ARGs and bacterial communities tended to cluster based on the sample habitats (i.e., treatment units) (**Fig. 2A & C**). The treatment unit ($R^2 = 0.21$, $P < 0.001$) and WWTPs ($R^2 = 0.25$, $P < 0.001$) showed similar effects on bacterial community compositions of WWTPs based on Adonis tests. However, the overall variance of the ARG communities driven by the treatment unit ($R^2 = 0.35$, $P < 0.001$) was much higher than that of WWTPs ($R^2 = 0.14$, $P < 0.001$). Significance differences have been observed in both ARG and bacterial communities between EFF and INF (ARGs: $R^2 = 0.19$, $P < 0.01$; bacteria: $R^2 = 0.14$, $P < 0.01$), EFF and AS (ARGs: $R^2 = 0.20$, $P < 0.01$; bacteria: $R^2 = 0.11$, $P < 0.01$), as well as INF and AS (ARGs: $R^2 = 0.43$, $P < 0.01$; bacteria: $R^2 = 0.26$, $P < 0.01$). However, the differences in both ARG and bacterial communities between EFF and INF, and EFF and AS were much smaller than those between INF and AS. This indicated the significant shifts of ARG and bacterial communities from INF to AS. In addition, bacteria and ARG communities showed pronounced spatial differences across the treatment units, especially in AS (ARGs: $R^2 = 0.66$, $P < 0.001$; bacteria: $R^2 = 0.78$, $P < 0.001$) (**Fig. S4**).

The daily variation of the ARG communities among different treatment units during the sampling period was presented in **Fig. 3**. The daily variation of ARG richness showed statistically significant differences between INF and AS, and INF and EFF (Wilcoxon test; $P < 0.05$). The relative abundance of ARGs exhibited much smaller daily fluctuations compared to the absolute abundance based on the CV analysis (**Fig. 3A**). Among different treatment units, AS had the greatest variation in ARG richness over time but the smallest variation in ARG relative abundance

and OTU richness. Based on the time decay model, we found how the dissimilarity of ARG community composition changes with time. No linear association between the dissimilarity of ARG community composition and time has been observed. However, the dissimilarity of the bacterial community composition showed a strong positive correlation with time (**Fig. 3B**). This indicated that the daily variation of the ARG community within the same treatment unit was stable.

3.3 Immigration of ARGs across different treatment units

SourceTracker analysis conducted the prediction of the source habitats (i.e., the treatment units) for ARG communities in 78% (108/138) samples and for bacterial communities in 91% (126/138) samples successfully (**Figs. S5A and S6A**). Specifically, as for ARG communities, 89% samples (41/46), 89% samples (41/46), and 57% samples (26/46) were predicted to be from INF, AS, and EFF with a probability of 73%±15%, 79%±14%, and 62%±16%, respectively. This supported the findings of NMDS ordination that the samples tended to cluster by their habitats (**Fig. 2A & C**), suggesting that each habitat had unique characteristics of ARG communities. However, as the wastewater flows between different treatment units, the immigration process of the upstream communities (i.e., INF) contributed to downstream communities (e.g., AS and EFF). For instance, the contribution of INF to the ARG community downstream (i.e., AS and EFF) increased greatly along with treatment units from 25.5% in AS to 49.6% in EFF. AS also contributed to 24.3% of the ARG community in EFF (**Fig. S5B**). As a comparison, the contribution of INF to the bacterial community in AS was 56.1%, while INF and AS had similar contributions (~33%) to the bacterial community in EFF (**Fig. S6B**).

3.4 Assembly mechanisms and controlling factors underlying ARGs in WWTPs

The null model analysis showed that stochastic processes played a dominant role in shaping the ARG communities in WWTPs (the average stochastic ratio > 50%), and the relative importance of stochastic processes ranked as follows: INF (average 83.7% ± 9.3%) > EFF (64.6% ± 13.9%) > AS (58.8% ± 13.9%) (**Fig. 2B**).

VPA results showed that biotic (i.e., local bacterial communities and immigration of bacteria and ARGs), abiotic (i.e., physicochemical parameters) factors and MGEs could explain 36.5%~40.5% of the total variance of ARG communities in different treatment units (**Fig. 4A, C, & E**). Although the pure effects of abiotic factors and MGEs are relatively low in three treatment units, the interaction effect of these factors was high in INF. Notably, the pure effects of biotic factors on the total variance of ARG communities increased greatly as wastewater flowed to the downstream treatment units (5.5% in INF, 13.2% AS and 23.5% in EFF). The relative importance of an individual factor to the variance of ARG communities was assessed by HP analysis (**Fig. 4B, D, & F**). The results indicated that bacterial immigration from INF is the most important to the total variance of the ARG communities in AS. And ARG immigrations from INF and AS were the top two important factors affecting ARG communities in EFF. Besides, the pure effects of MGEs on ARG communities decreased along with treatment units.

3.5 Daily mass loads of ARGs to the urban environment at the city level

Daily mass loads received by WWTPs across the whole city were 1.21×10^{20} copies/d ARGs and 2.01×10^{19} copies/d MGEs. And WWTPs discharged 1.44×10^{18} copies/d ARGs and 2.43×10^{17} copies/d MGEs to the receiving environment. The citizen's daily ARG and MGE mass loads to WWTPs can be calculated accordingly, which are up to 3.13×10^{13} copies/d/capita and 5.20×10^{12} copies/d/capita, respectively. And potential discharge loads to the environment or releasing to humans from WWTPs were 3.72×10^{11} copies/d/capita, 6.31×10^{10} copies/d/capita, respectively (**Fig. 5A & B**).

Spearman correlation analysis indicated that total PPCPs mass loads, other PPCPs mass loads, and socioeconomic factors (i.e., urbanization rate and PCDI) were significantly correlated with six major types of ARG mass loads and the MGE mass loads, while antibiotics and NSAIDs had no significant correlation with any types of ARGs (**Fig. 5C**). In addition, MGE loads had the highest correlation coefficient with the total PPCPs mass loads.

4. Discussion

To facilitate the monitoring and control of ARG dissemination, setting up a wastewater-based surveillance approach as well as understanding the ecological processes and driving forces of the ARG communities along the wastewater treatment processes are required. Unlike the previous studies that mainly focused on the fate of ARGs in WWTPs, we targeted the variation and transport of ARGs in different wastewater treatment units. Here, we found the stable daily variance of ARGs at the city level through large-scale and continuous daily sampling. Furthermore, stochastic processes were the dominant assembly mechanism of ARG communities in WWTPs, although the importance in each treatment unit varied. Among the driving factors, the immigration of bacteria and ARG in the upstream treatment unit significantly shaped the downstream ARG community. At last, we estimated the daily mass loads of ARGs to the WWTPs and the ones released from WWTPs at the city level.

4.1 Stable daily ARG profile and loads throughout WWTPs inspired ARG surveillance strategies

WWTPs are recognized as the breeding reservoirs for ARG dissemination, which harbor ARGs with high diversity and abundance. However, there is limited information regarding the continuous ARG pollution at various WWTP units during wastewater treatment operations. A comprehensive understanding of the daily

dynamics and profile of ARGs throughout WWTP units is highly needed to build wastewater-based surveillance approaches for One Health. Here, we found that the composition of the daily ARG communities tended to be stable throughout seven days from all WWTPs in the same city (**Fig. 3B**). Interestingly, the ARG richness and total ARG relative abundance in INF showed low daily variations (**Fig. 3A**). This indicated that the ARGs profiles introduced by sewage formed a relatively stable pattern across entire city, compared to the EFF, which is affected by daily treatment process variability (Majeed et al., 2021). Another reason for such abundant and stable ARGs is the plateau effect of microbes. That is, microbes have reached the highest carrying capacity of ARGs in special environment. The plateau effect was frequently observed in field irrigation experiments (Dalkmann et al., 2012; Kampouris et al., 2021). For instance, the relative abundance of *sul1* in fields irrigated with untreated wastewater for 1.5 years was similar to that in fields irrigated with wastewater for 10 years (Dalkmann et al., 2012). This phenomenon requires continuous stimulation of high ARGs to stabilize (Kampouris et al., 2021). Furthermore, the high stability of ARG profiles in INF indicates the locational reproducibility, suggesting that applying a low sampling frequency of sewage would be useful for ARG surveillance in global WWTPs. Similar phenomenon, such as shifts and increases in variability of ARGs from INF to EFF, have been observed in other studies (Lira et al., 2020; Majeed et al., 2021). The relatively higher daily variation of ARGs in EFF also presents advantage in assessing WWTP performance and providing a measure of loads and potential associated exposures to the receiving environment. Additionally, it may be better than by INF surveillance alone for detecting and dealing with anomalies of concern (Majeed et al., 2021). Another landmark study in Hong Kong WWTPs with similar climate and geographical location showed no distinct seasonal variations over 9 years span. However, the ARG composition in AS shifted every 2-3 years (Yin et al., 2019). In this study, no such turnover period was captured, and it is unclear to what extent the observed patterns are generalizable across all WWTPs. Future research would

focus long period sampling for explore the ARG turnover pattern and dynamics across each treatment processes.

Our study revealed that the relative abundance of ARGs showed higher dynamic robustness than the absolute abundances of ARGs, which is consistent with a previous study (Sun et al., 2021). This study suggested that applying the ARG relative abundance as the index can avoid minor variations in sample processing. In addition, our hourly composite sampling over a 24-hour period reduced the impact of accidental residential water use and provided a closer representation of daily discharge of ARGs into WWTPs (Cai et al., 2022; Sun et al., 2021). Overall, the above results provided novel insights into sampling and monitoring strategies for antibiotic resistance surveillance, particularly for monitoring anomalous genes of clinical concern in WWTPs, that is, short-term sampling for ARGs profiles can provide representative information for the distribution of ARGs in WWTPs (at least a week).

4.2 Immigration process determined the assembly of the ARG communities

The ecological mechanisms of ARG community assembly have been investigated mainly in urban semi-natural environments, such as urban ponds and reservoirs (Fang et al., 2019; Guo et al., 2018; Hou et al., 2021b; Peng et al., 2020). However, little is known about the ARG community assembly processes in engineered environments. Here, the central role of stochastic processes in the ecological assembly processes of ARG communities in WWTPs was revealed. It is consistent with our previous study with a small sample size (Hou et al., 2021b). The prevalence of such high stochasticity in ARG communities throughout WWTPs in the current study may be attributed to other factors beyond the effects of small-scale habitats and homogeneous physicochemical conditions (Peng et al., 2020; Sloan et al., 2006). The high correlation between the abundance of ARGs and MGEs in INF suggested that HGT may play an important role in the spread of ARGs (**Fig. S3**). HP analysis found that

MGEs were the second most important factor in determining the ARG communities in INF (**Fig. 4B**). Indeed, large amounts of nutrients provided beneficial conditions for nourishing microbes as well as pathogens, which could facilitate cell contact and their HGT of ARGs there (He et al., 2022; Zhao et al., 2019).

In AS, we found that the immigration of bacteria from INF and the local bacterial communities was more vital compared to the other two factors (i.e., MGEs and abiotic factors) (**Fig. 4C & D**). In a previous study, the bacterial community was considered to be the key factor that drove the ARG profiles in the high anthropogenic environment (Su et al., 2015). The variation in the microbial community composition was concurrent with stable metabolic functional structure and performance (Louca and Doebeli, 2017). In the AS bioreactor, to maintain high efficiency in nutrients removal, the growth of species with desired function or high adaptability is facilitated via operation parameters such as solids retention time and recycle ratio, which is a deterministic process that may affect the proliferation of ARGs (Dai et al., 2022; Dottorini et al., 2021; Wang et al., 2022). This indicated the importance of VGT for ARG reproduction in AS. Recently, researchers confirmed that the VGT is significantly involved in the ARG community of AS via single-cell microfluidics (Li et al., 2019; Wei et al., 2021). Thus, the existing understanding suggested deterministic factors, such as the design and operation of AS bioreactors, are suggested to contribute to the AS microbial communities. However, we showed that microbial immigration from upstream (stochastic processes) mainly controls the ARG communities in AS. Similarly, Dottorini et al. found that the influent microbial community immigration determined the assembly of the AS microbial communities (Dottorini et al., 2021). Previously, INF microbial communities were often considered as the seeding for the AS communities. However, here, mass immigration is responsible for both the presence and abundance of ARGs in the AS, especially for those species with ARGs that are not fit for the function of AS.

In EFF, the low bacterial density and diversity, as well as the low concentration of nutrients and antibiotics limited the frequency of VGT occurrence, while the high potential for natural transformation of ARG via HGT was confirmed (Dong et al., 2019; Jin et al., 2020). Interestingly, our analysis revealed that the immigration of upstream ARGs had the highest effects on shaping the ARG communities, followed by the upstream and local bacterial communities and then MGEs (**Fig. 4F**). Tong et al. think bacteria host with ARGs may survive better compared with the bacteria without ARGs during the treatment processes (Tong et al., 2019). This explained why the immigration of ARGs played a more important role in controlling the ARG community than the bacterial community. This also tells us the source ARG community is essential for the ARG discharges from WWTPs to the environment, challenging us to revise our understanding to control ARGs in sewer systems before WWTPs. Besides, the contribution of the unknown source for the ARG community in AS and EFF decreased along with the treatment units, indicating the limitation of the general source library of ARGs in WWTPs. Future studies focusing on providing more source information into the analysis and therefore increasing the range of identifiable sources in WWTPs are needed.

Although researchers found that EFF largely reflected AS and INF by microbes escaping settling, it is hard to quantify the influence from upstream (Majeed et al., 2021). We reported the first result highlighting the importance of the immigration of bacteria and ARGs from the source/upstream in shaping the downstream ARGs communities in WWTPs. Our results revealed that the shift of the ARG community structure is mainly affected by the immigration from upstream treatment units, but the immigration of the bacterial and ARG communities contributed differently. WWTPs were continuously fed by upstream sewage sources and were open ecosystems (Dottorini et al., 2021; Lee et al., 2015). The INF including bacteria was considered to be the main source of biomass in AS and may influence the diversity, abundance, and assembly of local microbial communities microbes via microbial immigration (Ali et

al., 2019; Dottorini et al., 2021; Mei and Liu, 2019; Saunders et al., 2016; Zhang and Meng, 2021). Although microbial immigration constantly contributes to community assemblage in natural and engineered microbial ecosystems, the impact of microbial immigration varied due to different immigration intensities (Corno et al., 2019; Mei et al., 2019). ARGs are prone to persist in the receiving environment once introduced (Kampouris et al., 2021). For example, effluents of WWTPs rapidly stabilized the antibiotic resistome in receiving freshwater, even if bacterial community composition was not yet stable, implying the occurrence of ARGs and pathogens downstream was affected by the exchange of ARGs from EFF (Corno et al., 2019; Harnisz et al., 2020; Reichert et al., 2021). However, no evidence was found of taxa that could drive the stabilization of ARGs in receiving environment (Corno et al., 2019). Recently, coupling a mass balance model with qPCR allows the calculation of the effects of treatment performance and hydrologic characteristics on ARG communities in receiving environment (Lee et al., 2021; Schwermer and Uhl, 2021). However, the relative importance of the immigration of bacterial and ARG communities in shaping ARG communities remains veiled in engineered ecosystems. Here, we found that the immigration of bacteria and ARG community determined the ARG communities in AS and EFF, respectively. The results fill up the gap by providing basic information on the dissemination of ARGs throughout WWTP treatment units and novel insights for policy maker to consider the role of mass immigration when developing strategies to control ARGs.

4.3 WWTPs as ARG dissemination source under socioeconomic development at the city level

With the rapid development of industry, antibiotics are widely used in animal husbandry and disease treatment, thus making the resistance gradually become a serious problem. In our study, we found that socioeconomic factors are closely related to ARG pollution across the whole city from the perspective of ARG loads.

Population size is the key factor driving fluoroquinolones, macrolide antibiotics, sulfamethoxazole, chloramphenicol, and daily loads of the corresponding ARGs in INF (Elder et al., 2021). Xiamen is a coastal city with rapid industrialization and economic development. Two WWTPs with the highest ratio of domestic wastewater in INF contained the highest relative abundance of total ARGs. This indicated that domestic wastewater which is closely associated with population size is the main source of ARGs. Our previous study found that socioeconomic factors (i.e., urbanization rates) are significantly correlated with PPCP daily mass load per capita (Wang et al., 2018). Here, a positive correlation between two socioeconomic factors (i.e., urbanization rate and PCDI) and daily ARGs per capita was verified. This suggested that higher human activity facilitates ARG pollution in highly developed area.

A study found that two municipal WWTPs in South Korea covering < 40% of the population in Xiamen received average ARG daily loads of around 10^{20} copies/d and discharged 10^{16}-10^{18} copies/d to the environment (Lee et al., 2017). The total ARG daily mass loads in Xiamen WWTPs in China were in the same range, suggesting the burden of ARG pollution in highly developed cities. ARG and MGE mass loads are another vital indicators for ARG dissemination assessment (Kampouris et al., 2021). Thus, despite the fact that two orders of magnitude of ARGs were removed during the treatment processes of WWTPs, a massive amount of ARGs and MGEs were released into the environment across the whole city, leading to the subsequent wide-spread and high risk of exposure of ARGs to humans.

5. Conclusion

After characterizing the ARG dynamics throughout the wastewater treatment units in all WWTPs in a city for a week, the following conclusions were therefore drawn:

1. Short-term sampling for ARGs profiles can be representative enough for the distribution of ARGs in WWTPs in the longer term (at least a week), such as ARG

richness, relative abundance, and absolute abundance of total ARGs, as well as the compositional dissimilarities of the ARG communities in a week showed little daily variation, especially in INF.

2. Stochastic processes in assembly ARG communities were dominant in three treatment units, and the relative importance of stochastic processes in different treatment units ranked as follows: INF > EFF > AS. The immigration of the bacterial communities upstream and the local bacterial communities played an important role in shaping the ARG communities in AS. The immigration of ARG communities from upstream in WWTPs combined with the local bacterial communities contributed to the ARG dissemination in EFF. This emphasizes the need to revise the way we mitigate ARG contamination but focus on the source in the urban environment.

3. We assessed the total ARG daily mass load received by and discharged from WWTPs at the city level, filling the knowledge gaps on the total amount of ARGs in the urban water cycle. We have linked the socioeconomic factors at the city level with the ARG pollution from WWTPs, providing an assessment of human exposure to ARGs for highly developed cities.

Overall, this study highlighted the daily pattern of ARGs throughout the wastewater treatment units and the importance of mass immigration of ARGs and bacteria from upstream on the formation of ARG communities downstream. This provides the fundamental information for establishing wastewater-based surveillance strategies and ARG ecological behavior in WWTPs.

Author contributions

Lanping Zhang: Conceptualization, Investigation, Methodology, Data Analysis, Software, Visualization, Writing - original draft. **Bob Adyari**: Validation, Software, Writing - review & editing. **Liyuan Hou**: Validation, Writing - review & editing. **Xiaoyong Yang**: Investigation, Writing - review & editing. **Mahmoud Gad**: Writing - review & editing. **Yuwen Wang**: Investigation, Methodology. **Cong Ma**:

Investigation, Resources. **Qian Sun**: Methodology, Resources. **Chang-Ping Yu**: Resources, Funding acquisition. **Anyi Hu**: Conceptualization, Data curation, Validation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Acknowledgement

We thank Dr. Fuyi Huang and Miss. Xinyuan Zhou for their assistance with the methodology. This work was supported by the National Key R&D Program of China (2022YFE0120300) and the National Natural Science Foundation of China (U1805244 and 31870475). M. Gad was supported by the Science, Technology & Innovation Funding Authority, Egypt (44201).

References

Ali, M., Wang, Z., Salam, K.W., Hari, A.R., Pronk, M., Van Loosdrecht, M.C.M., Saikaly, P.E., 2019. Importance of species sorting and immigration on the bacterial assembly of different-sized aggregates in a full-scale Aerobic granular sludge plant. Environ. Sci. Technol. 53, 8291–8301. https://doi.org/10.1021/acs.est.8b07303

An, X.-L.L., Su, J.-Q.Q., Li, B., Ouyang, W.-Y.Y., Zhao, Y., Chen, Q.-L.L., Cui, L., Chen, H., Gillings, M.R., Zhang, T., Zhu, Y.-G.G., 2018. Tracking antibiotic resistome during wastewater treatment using high throughput quantitative PCR. Environ. Int. 117, 146–153. https://doi.org/10.1016/j.envint.2018.05.011

Bengtsson-Palme, J., Larsson, D.G.J.J., 2015. Antibiotic resistance genes in the environment: prioritizing risks. Nat. Rev. Microbiol. 13, 396–396. https://doi.org/10.1038/nrmicro3399-c1

Buchy, P., Ascioglu, S., Buisson, Y., Datta, S., Nissen, M., Tambyah, P.A., Vong, S., 2020. Impact of vaccines on antimicrobial resistance. Int. J. Infect. Dis. 90, 188–196. https://doi.org/10.1016/j.ijid.2019.10.005

Cai, M., Wang, Z., Gu, H., Dong, H., Zhang, X., Cui, N., Zhou, L., Chen, G., Zou, G., 2022. Occurrence and temporal variation of antibiotics and antibiotic resistance genes in hospital inpatient department wastewater: Impacts of daily schedule of inpatients and wastewater treatment process. Chemosphere 292, 133405. https://doi.org/10.1016/j.chemosphere.2021.133405

Chen, B., Yuan, K., Chen, X., Yang, Y., Zhang, T., Wang, Y., Luan, T., Zou, S., Li, X., 2016. Metagenomic Analysis Revealing Antibiotic Resistance Genes (ARGs) and Their Genetic Compartments in the Tibetan Environment. Environ. Sci. Technol. 50, 6670–6679. https://doi.org/10.1021/acs.est.6b00619

Chen, H., Liu, C., Teng, Y., Zhang, Z., Chen, Y., Yang, Y., 2021. Environmental risk characterization and ecological process determination of bacterial antibiotic resistome in lake sediments. Environ. Int. 147, 106345. https://doi.org/10.1016/j.envint.2020.106345

Chen, J., Zhang, Z., Lei, Z., Shimizu, K., Yao, P., Su, Z., Wen, D., 2020. Occurrence and distribution of antibiotic resistance genes in the coastal sediments of effluent-receiving areas of WWTPs, China. Bioresour. Technol. Reports 11, 100511. https://doi.org/10.1016/j.biteb.2020.100511

China’s State Environment Protection Agency, 2002. Water and Waste Water Monitoring and Analysis Methods. China Environ. Sci. Press.

Corno, G., Yang, Y., Eckert, E.M., Fontaneto, D., Fiorentino, A., Galafassi, S., Zhang, T., Di Cesare, A., 2019. Effluents of wastewater treatment plants promote the rapid stabilization of the antibiotic resistome in receiving freshwater bodies. Water Res. 158, 72–81. https://doi.org/10.1016/j.watres.2019.04.031

Dai, D., Brown, C., Bürgmann, H., Larsson, D.G.J.J., Nambi, I., Zhang, T., Flach, C.-F., Pruden, A., Vikesland, P.J., 2022. Long-read metagenomic sequencing

reveals shifts in associations of antibiotic resistance genes with mobile genetic elements from sewage to activated sludge. Microbiome 10, 20. https://doi.org/10.1186/s40168-021-01216-5

Daliang, N., Ye, D., M., T.J., Jizhong, Z., 2019. A general framework for quantitatively assessing ecological stochasticity. Proc. Natl. Acad. Sci. 116, 16892–16898. https://doi.org/10.1073/pnas.1904623116

Dalkmann, P., Broszat, M., Siebe, C., Willaschek, E., Sakinc, T., Huebner, J., Amelung, W., Grohmann, E., Siemens, J., 2012. Accumulation of pharmaceuticals, enterococcus, and resistance genes in soils irrigated with wastewater for zero to 100 years in central Mexico. PLoS One 7, e45397. https://doi.org/10.1371/journal.pone.0045397

Dong, P., Wang, H., Fang, T., Wang, Y., Ye, Q., 2019. Assessment of extracellular antibiotic resistance genes (eARGs) in typical environmental samples and the transforming ability of eARG. Environ. Int. 125, 90–96. https://doi.org/10.1016/j.envint.2019.01.050

Dottorini, G., Michaelsen, T.Y., Kucheryavskiy, S., Andersen, K.S., Kristensen, J.M., Peces, M., Wagner, D.S., Nierychlo, M., Nielsen, P.H., 2021. Mass-immigration determines the assembly of activated sludge microbial communities. Proc. Natl. Acad. Sci. U. S. A. 118, 1–11. https://doi.org/10.1073/pnas.2021589118

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

Elder, F.C.T., Proctor, K., Barden, R., Gaze, W.H., Snape, J., Feil, E.J., Kasprzyk-Hordern, B., 2021. Spatiotemporal profiling of antibiotics and resistance genes in a river catchment: Human population as the main driver of antibiotic and antibiotic resistance gene presence in the environment. Water Res. 203, 117533. https://doi.org/10.1016/j.watres.2021.117533

Fang, P., Peng, F., Gao, X., Xiao, P., Yang, J., 2019. Decoupling the dynamics of bacterial taxonomy and antibiotic resistance function in a subtropical urban

reservoir as revealed by high-frequency sampling. Front. Microbiol. 10, 1–12. https://doi.org/10.3389/fmicb.2019.01448

Frost, L.S., Leplae, R., Summers, A.O., Toussaint, A., 2005. Mobile genetic elements: the agents of open source evolution. Nat. Rev. Microbiol. 3, 722–732. https://doi.org/10.1038/nrmicro1235

Gad, M., Hou, L., Cao, M., Adyari, B., Zhang, L., Qin, D., Yu, C.P., Sun, Q., Hu, A., 2022. Tracking microeukaryotic footprint in a peri-urban watershed, China through machine-learning approaches. Sci. Total Environ. 806, 150401. https://doi.org/10.1016/j.scitotenv.2021.150401

Guo, Y., Liu, M., Liu, L., Liu, X., Chen, H., Yang, J., 2018. The antibiotic resistome of free-living and particle-attached bacteria under a reservoir cyanobacterial bloom. Environ. Int. 117, 107–115. https://doi.org/10.1016/j.envint.2018.04.045

Han, T., Zheng, J., Han, Y., Xu, X., Li, M., Schwarz, C., Zhu, L., 2021. Comprehensive insights into core microbial assemblages in activated sludge exposed to textile-dyeing wastewater stress. Sci. Total Environ. 791, 148145. https://doi.org/10.1016/j.scitotenv.2021.148145

Harnisz, M., Kiedrzyńska, E., Kiedrzyński, M., Korzeniewska, E., Czatzkowska, M., Koniuszewska, I., Jóźwik, A., Szklarek, S., Niestępski, S., Zalewski, M., 2020. The impact of WWTP size and sampling season on the prevalence of antibiotic resistance genes in wastewater and the river system. Sci. Total Environ. 741, 140466. https://doi.org/10.1016/j.scitotenv.2020.140466

He, Yike, Bai, M., He, Yaodong, Wang, S., Zhang, J., Jiang, S., Wang, G., 2022. Suspended particles are hotspots for pathogen-related bacteria and ARGs in coastal beach waters of northern China. Sci. Total Environ. 817, 153004. https://doi.org/10.1016/j.scitotenv.2022.153004

Hendriksen, R.S., Munk, P., Njage, P., van Bunnik, B., McNally, L., Lukjancenko, O., Röder, T., Nieuwenhuijse, D., Pedersen, S.K., Kjeldgaard, J., Kaas, R.S., Clausen, P.T.L.C., Vogt, J.K., Leekitcharoenphon, P., van de Schans, M.G.M.M.,

Zuidema, T., de Roda Husman, A.M., Rasmussen, S., Petersen, B., Bego, A., Rees, C., Cassar, S., Coventry, K., Collignon, P., Allerberger, F., Rahube, T.O., Oliveira, G., Ivanov, I., Vuthy, Y., Sopheak, T., Yost, C.K., Ke, C., Zheng, H., Baisheng, L., Jiao, X., Donado-Godoy, P., Coulibaly, K.J., Jergović, M., Hrenovic, J., Karpíšková, R., Villacis, J.E., Legesse, M., Eguale, T., Heikinheimo, A., Malania, L., Nitsche, A., Brinkmann, A., Saba, C.K.S., Kocsis, B., Solymosi, N., Thorsteinsdottir, T.R., Hatha, A.M., Alebouyeh, M., Morris, D., Cormican, M., O'Connor, L., Moran-Gilad, J., Alba, P., Battisti, A., Shakenova, Z., Kiiyukia, C., Ng'eno, E., Raka, L., Avsejenko, J., Bērziņš, A., Bartkevics, V., Penny, C., Rajandas, H., Parimannan, S., Haber, M.V., Pal, P., Jeunen, G.-J.J., Gemmell, N., Fashae, K., Holmstad, R., Hasan, R., Shakoor, S., Rojas, M.L.Z., Wasyl, D., Bosevska, G., Kochubovski, M., Radu, C., Gassama, A., Radosavljevic, V., Wuertz, S., Zuniga-Montanez, R., Tay, M.Y.F.F., Gavačová, D., Pastuchova, K., Truska, P., Trkov, M., Esterhuyse, K., Keddy, K., Cerdà-Cuéllar, M., Pathirage, S., Norrgren, L., Örn, S., Larsson, D.G.J.J., Heijden, T. Van der, Kumburu, H.H., Sanneh, B., Bidjada, P., Njanpop-Lafourcade, B.-M.M., Nikiema-Pessinaba, S.C., Levent, B., Meschke, J.S., Beck, N.K., Van, C.D., Phuc, N. Do, Tran, D.M.N., Kwenda, G., Tabo, D. adjim, Wester, A.L., Cuadros-Orellana, S., Amid, C., Cochrane, G., Sicheritz-Ponten, T., Schmitt, H., Alvarez, J.R.M., Aidara-Kane, A., Pamp, S.J., Lund, O., Hald, T., Woolhouse, M., Koopmans, M.P., Vigre, H., Petersen, T.N., Aarestrup, F.M., 2019. Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. Nat. Commun. 10, 1124. https://doi.org/10.1038/s41467-019-08853-3

Hildebrand, F., Tadeo, R., Voigt, A.Y., Bork, P., Raes, J., 2014. LotuS: an efficient and user-friendly OTU processing pipeline. Microbiome 2, 30. https://doi.org/10.1186/2049-2618-2-30

Hou, L., Wang, H., Chen, Q., Su, J.-Q.Q., Gad, M., Li, J., Mulla, S.I., Yu, C.-P.P., Hu, A., 2021a. Fecal pollution mediates the dominance of stochastic assembly of

antibiotic resistome in an urban lagoon (Yundang lagoon), China. J. Hazard. Mater. 417, 126083. https://doi.org/10.1016/j.jhazmat.2021.126083

Hou, L., Zhang, L., Li, F., Huang, S., Yang, J., Ma, C., Zhang, D., Yu, C.-P., Hu, A., 2021b. Urban ponds as hotspots of antibiotic resistome in the urban environment. J. Hazard. Mater. 403, 124008. https://doi.org/10.1016/j.jhazmat.2020.124008

Hu, A., Ju, F., Hou, L., Li, J., Yang, X., Wang, H., Mulla, S.I., Sun, Q., Bürgmann, H., Yu, C.-P., 2017. Strong impact of anthropogenic contamination on the co-occurrence patterns of a riverine microbial community. Environ. Microbiol. 19, 4993–5009. https://doi.org/10.1111/1462-2920.13942

Hu, A., Li, S., Zhang, L., Wang, H., Yang, J., Luo, Z., Rashid, A., Chen, S., Huang, W., Yu, C.-P., 2018. Prokaryotic footprints in urban water ecosystems: A case study of urban landscape ponds in a coastal city, China. Environ. Pollut. 242, 1729–1739. https://doi.org/10.1016/j.envpol.2018.07.097

Jin, M., Liu, L., Wang, D. ning, Yang, D., Liu, W. li, Yin, J., Yang, Z. wei, Wang, H. ran, Qiu, Z. gang, Shen, Z. qiang, Shi, D. yang, Li, H. bei, Guo, J. hua, Li, J. wen, 2020. Chlorine disinfection promotes the exchange of antibiotic resistance genes across bacterial genera by natural transformation. ISME J. 14, 1847–1856. https://doi.org/10.1038/s41396-020-0656-9

Ju, F., Beck, K., Yin, X., Maccagnan, A., McArdell, C.S., Singer, H.P., Johnson, D.R., Zhang, T., Bürgmann, H., 2019. Wastewater treatment plant resistomes are shaped by bacterial composition, genetic exchange, and upregulated expression in the effluent microbiomes. ISME J. 13, 346–360. https://doi.org/10.1038/s41396-018-0277-8

Kampouris, I.D., Agrawal, S., Orschler, L., Cacace, D., Kunze, S., Berendonk, T.U., Klümper, U., 2021. Antibiotic resistance gene load and irrigation intensity determine the impact of wastewater irrigation on antimicrobial resistance in the soil microbiome. Water Res. 193. https://doi.org/10.1016/j.watres.2021.116818

Knights, D., Kuczynski, J., Charlson, E.S., Zaneveld, J., Mozer, M.C., Collman, R.G., Bushman, F.D., Knight, R., Kelley, S.T., 2011. Bayesian community-wide culture-independent microbial source tracking. Nat. Methods 8, 761–765. https://doi.org/10.1038/nmeth.1650

Lai, J., Zou, Y., Zhang, J., Peres-Neto, P.R., 2022. Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rdacca.hp R package. Methods Ecol. Evol. 2022, 782–788. https://doi.org/10.1111/2041-210X.13800

Lebov, J., Grieger, K., Womack, D., Zaccaro, D., Whitehead, N., Kowalcyk, B., MacDonald, P.D.M., 2017. A framework for One Health research. One Heal. 3, 44–50. https://doi.org/10.1016/j.onehlt.2017.03.004

Lee, J., Jeon, J.H., Shin, J., Jang, H.M., Kim, S., Song, M.S., Kim, Y.M., 2017. Quantitative and qualitative changes in antibiotic resistance genes after passing through treatment processes in municipal wastewater treatment plants. Sci. Total Environ. 605–606, 906–914. https://doi.org/10.1016/j.scitotenv.2017.06.250

Lee, J., Ju, F., Maile-Moskowitz, A., Beck, K., Maccagnan, A., McArdell, C.S., Dal Molin, M., Fenicia, F., Vikesland, P.J., Pruden, A., Stamm, C., Bürgmann, H., 2021. Unraveling the riverine antibiotic resistome: The downstream fate of anthropogenic inputs. Water Res. 197, 117050. https://doi.org/10.1016/j.watres.2021.117050

Lee, S.H., Kang, H.J., Park, H.D., 2015. Influence of influent wastewater communities on temporal variation of activated sludge communities. Water Res. 73, 132–144. https://doi.org/10.1016/j.watres.2015.01.014

Li, B., Qiu, Y., Song, Y., Lin, H., Yin, H., 2019. Dissecting horizontal and vertical gene transfer of antibiotic resistance plasmid in bacterial community using microfluidics. Environ. Int. 131, 105007. https://doi.org/10.1016/j.envint.2019.105007

Li, J., Zhang, L., Li, Y., Liu, K., Liu, Y., Huang, S., Li, F., Chen, C.-T.A., Zhang, Y., Hu, A., 2022. A Comprehensive Profile of Antibiotic Resistance Genes in the Water Column of a Shallow-Sea Hydrothermal Vent Ecosystem. Sustainability 14, 1776. https://doi.org/10.3390/su14031776

Li, L.-G.G., Yin, X., Zhang, T., 2018. Tracking antibiotic resistance gene pollution from different sources using machine-learning classification. Microbiome 6, 93. https://doi.org/10.1186/s40168-018-0480-x

Li, Z.-H., Yuan, L., Wang, L., Liu, Q.-H., Sheng, G.-P., 2022. Coexistence of silver ion and tetracycline at environmentally relevant concentrations greatly enhanced antibiotic resistance gene development in activated sludge bioreactor. J. Hazard. Mater. 423, 127088. https://doi.org/10.1016/j.jhazmat.2021.127088

Lin, H., Jiang, L., Li, B., Dong, Y., He, Y., Qiu, Y., 2019. Screening and evaluation of heavy metals facilitating antibiotic resistance gene transfer in a sludge bacterial community. Sci. Total Environ. 695, 133862. https://doi.org/10.1016/j.scitotenv.2019.133862

Lira, F., Vaz-Moreira, I., Tamames, J., Manaia, C.M., Martínez, J.L., 2020. Metagenomic analysis of an urban resistome before and after wastewater treatment. Sci. Rep. 10, 8174. https://doi.org/10.1038/s41598-020-65031-y

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

Louca, S., Doebeli, M., 2017. Taxonomic variability and functional stability in microbial communities infected by phages. Environ. Microbiol. 19, 3863–3878. https://doi.org/10.1111/1462-2920.13743

Majeed, H.J., Riquelme, M. V., Davis, B.C., Gupta, S., Angeles, L., Aga, D.S., Garner, E., Pruden, A., Vikesland, P.J., 2021. Evaluation of Metagenomic-

Enabled Antibiotic Resistance Surveillance at a Conventional Wastewater Treatment Plant. Front. Microbiol. 12, 1–19. https://doi.org/10.3389/fmicb.2021.657954

Marano, R.B.M., Zolti, A., Jurkevitch, E., Cytryn, E., 2019. Antibiotic resistance and class 1 integron gene dynamics along effluent, reclaimed wastewater irrigated soil, crop continua: elucidating potential risks and ecological constraints. Water Res. 164, 114906. https://doi.org/10.1016/j.watres.2019.114906

McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS One 8, e61217.

Mei, R., Kim, J., Wilson, F.P., Bocher, B.T.W.W., Liu, W.-T., 2019. Coupling growth kinetics modeling with machine learning reveals microbial immigration impacts and identifies key environmental parameters in a biological wastewater treatment process. Microbiome 7, 65. https://doi.org/10.1186/s40168-019-0682-x

Mei, R., Liu, W.-T.T., 2019. Quantifying the contribution of microbial immigration in engineered water systems. Microbiome 7, 1–8. https://doi.org/10.1186/s40168-019-0760-0

Naimi, B., Hamm, N.A.S., Groen, T.A., Skidmore, A.K., Toxopeus, A.G., 2014. Where is positional uncertainty a problem for species distribution modelling? Ecography (Cop.). 37, 191–203. https://doi.org/10.1111/j.1600-0587.2013.00205.x

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Sólymos, P., Stevens, M.H.H., Wagner, H., 2012. Vegan: Community Ecology Package. R Packag. version 1.15-2. https://doi.org/http://cran.r-project.org/web/ packages/vegan/index.html

Pallares-vega, R., Blaak, H., Plaats, R. Van Der, Roda, A.M. De, Hernandez, L., Loosdrecht, M.C.M. Van, Weissbrodt, D.G., Schmitt, H., Gene, A., 2019. Determinants of presence and removal of antibiotic resistance genes during

WWTP treatment□: A cross-sectional study. Water Res. 161, 319–328. https://doi.org/10.1016/j.watres.2019.05.100

Peng, F., Guo, Y., Isabwe, A., Chen, H., Wang, Y., Zhang, Y., Zhu, Z., Yang, J., 2020. Urbanization drives riverine bacterial antibiotic resistome more than taxonomic community at watershed scale. Environ. Int. 137, 105524. https://doi.org/10.1016/j.envint.2020.105524

Prestinaci, F., Pezzotti, P., Pantosti, A., 2015. Antimicrobial resistance: a global multifaceted phenomenon. Pathog. Glob. Health 109, 309–318. https://doi.org/10.1179/2047773215Y.0000000030

Pruden, A., Larsson, D.G.J., Amézquita, A., Collignon, P., Brandt, K.K., Graham, D.W., Lazorchak, J.M., Suzuki, S., Silley, P., Snape, J.R., Topp, E., Zhang, T., Zhu, Y.-G., 2013. Management of Options for Reducing the Release of Antibiotics. Environ. Health Perspect. 121, 878–885.

Reichert, G., Hilgert, S., Alexander, J., Rodrigues de Azevedo, J.C., Morck, T., Fuchs, S., Schwartz, T., 2021. Determination of antibiotic resistance genes in a WWTP-impacted river in surface water, sediment, and biofilm: Influence of seasonality and water quality. Sci. Total Environ. 768, 144526. https://doi.org/10.1016/j.scitotenv.2020.144526

Saunders, A.M., Albertsen, M., Vollertsen, J., Nielsen, P.H., 2016. The activated sludge ecosystem contains a core community of abundant organisms. ISME J. 10, 11–20. https://doi.org/10.1038/ismej.2015.117

Schwermer, C.U., Uhl, W., 2021. Calculating expected effects of treatment effectivity and river flow rates on the contribution of WWTP effluent to the ARG load of a receiving river. J. Environ. Manage. 288, 112445. https://doi.org/10.1016/j.jenvman.2021.112445

Seoane, J., Yankelevich, T., Dechesne, A., Merkey, B., Sternberg, C., Smets, B.F., 2011. An individual-based approach to explain plasmid invasion in bacterial

populations. FEMS Microbiol. Ecol. 75, 17–27. https://doi.org/10.1111/j.1574-6941.2010.00994.x

Sloan, W.T., Lunn, M., Woodcock, S., Head, I.M., Nee, S., Curtis, T.P., 2006. Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environ. Microbiol. 8, 732–740. https://doi.org/10.1111/j.1462-2920.2005.00956.x

Su, J., Wei, B., Ou-Yang, W.-Y., Huang, F., Zhao, Y., Xu, H., Zhu, Y., 2015. Antibiotic Resistome and Its Association with Bacterial Communities during Sewage Sludge Composting. Environ. Sci. Technol. 49, 7356–7363. https://doi.org/10.1021/acs.est.5b01012

Su, Z., Li, A., Chen, J., Huang, B., Mu, Q., Chen, L., Wen, D., 2020. Wastewater discharge drives ARGs spread in the coastal area: A case study in Hangzhou Bay, China. Mar. Pollut. Bull. 151, 110856. https://doi.org/10.1016/j.marpolbul.2019.110856

Sun, S., Geng, J., Li, B., Ma, L., Sun, X., Meng, F., Qi, H., Shen, J., 2021. Temporal variations of antibiotic resistance genes in influents and effluents of a WWTP in cold regions. J. Clean. Prod. 328, 129632. https://doi.org/10.1016/j.jclepro.2021.129632

Tong, J., Tang, A., Wang, H., Liu, X., Huang, Z., Wang, Z., Zhang, J., Wei, Y., Su, Y., Zhang, Y., 2019. Microbial community evolution and fate of antibiotic resistance genes along six different full-scale municipal wastewater treatment processes. Bioresour. Technol. 272, 489–500. https://doi.org/10.1016/j.biortech.2018.10.079

Vellend, M., 2010. Conceptual Synthesis in Community Ecology. Q. Rev. Biol. 85, 183–206. https://doi.org/10.1086/652373

Wang, C., Yang, Y., Wang, Y.Y., Wang, D., Xu, X., Wang, Y.Y., Li, L., Yang, C., Zhang, T., 2022. Absolute quantification and genome-centric analyses elucidate

the dynamics of microbial populations in anaerobic digesters. Water Res. 224, 119049. https://doi.org/10.1016/j.watres.2022.119049

Wang, J., Chu, L., Wojnárovits, L., Takács, E., 2020. Occurrence and fate of antibiotics, antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARB) in municipal wastewater treatment plant: An overview. Sci. Total Environ. 744, 140997. https://doi.org/10.1016/j.scitotenv.2020.140997

Wang, Y.Y., Li, Y., Hu, A., Rashid, A., Ashfaq, M., Wang, Y.Y., Wang, H., Luo, H., Yu, C.P., Sun, Q., 2018. Monitoring, mass balance and fate of pharmaceuticals and personal care products in seven wastewater treatment plants in Xiamen City, China. J. Hazard. Mater. 354, 81–90. https://doi.org/10.1016/j.jhazmat.2018.04.064

Wei, Z., Feng, K., Wang, Z., Zhang, Y., Yang, M., Zhu, Y.-G.G., Virta, M.P.J.J., Deng, Y., 2021. High-Throughput Single-Cell Technology Reveals the Contribution of Horizontal Gene Transfer to Typical Antibiotic Resistance Gene Dissemination in Wastewater Treatment Plants. Environ. Sci. Technol. 55, 11824–11834. https://doi.org/10.1021/acs.est.1c01250

Wickham, H., 2010. A Layered Grammar of Graphics. J. Comput. Graph. Stat. 19, 3–28. https://doi.org/10.1198/jcgs.2009.07098

Xiamen Bureau of Statistics, 2017. 2017 Yearbook of Xiamen Special Economic Zone. China Statisitcs Press. https://doi.org/http://www.stats-xm.gov.cn/2017/main0.htm

Yao, Y., Liu, Z., Yip, K.K., Pu, Y., Cheng, W., Li, M., Habimana, O., 2022. Cross-regional scale pollution of freshwater biofilms unveiled by antibiotic resistance genes. Sci. Total Environ. 818, 151835. https://doi.org/10.1016/j.scitotenv.2021.151835

Yin, X., Deng, Y., Ma, L., Wang, Y., Chan, L.Y.L., Zhang, T., 2019. Exploration of the antibiotic resistome in a wastewater treatment plant by a nine-year

longitudinal metagenomic study. Environ. Int. 133, 105270. https://doi.org/10.1016/j.envint.2019.105270

Yin, X., Yang, Y., Deng, Y., Huang, Y., Li, L., Chan, L.Y.L., Zhang, T., 2022. An assessment of resistome and mobilome in wastewater treatment plants through temporal and spatial metagenomic analysis. Water Res. 209, 117885. https://doi.org/10.1016/j.watres.2021.117885

Zhang, S., Meng, F., 2021. Core activated sludge communities are influenced little by immigration: Case study of a membrane bioreactor plant. J. Environ. Sci. 102, 244–255. https://doi.org/10.1016/j.jes.2020.09.024

Zhao, Y., Cocerva, T., Cox, S., Tardif, S., Su, J.-Q., Zhu, Y.-G., Brandt, K.K., 2019. Evidence for co-selection of antibiotic resistance genes and mobile genetic elements in metal polluted urban soils. Sci. Total Environ. 656, 512–520. https://doi.org/10.1016/j.scitotenv.2018.11.372

Zhao, Z., Wang, J., Han, Y., Chen, J., Liu, G., Lu, H., Yan, B., Chen, S., 2017. Nutrients, heavy metals and microbial communities co-driven distribution of antibiotic resistance genes in adjacent environment of mariculture. Environ. Pollut. 220, 909–918. https://doi.org/10.1016/j.envpol.2016.10.075

Zhou, J., Ning, D., 2017. Stochastic Community Assembly: Does It Matter in Microbial Ecology? Microbiol. Mol. Biol. Rev. 81. https://doi.org/10.1128/MMBR.00002-17

Zhu, Y.-G.G., Johnson, T.A., Su, J.-Q.Q., Qiao, M., Guo, G.-X.X., Stedtfeld, R.D., Hashsham, S.A., Tiedje, J.M., 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proc. Natl. Acad. Sci. 110, 3435–3440. https://doi.org/10.1073/pnas.1222743110

Zhu, Y.-G.G., Zhao, Y., Li, B., Huang, C.-L.L., Zhang, S.-Y.Y., Yu, S., Chen, Y.-S.S., Zhang, T., Gillings, M.R., Su, J.-Q.Q., 2017. Continental-scale pollution of estuaries with antibiotic resistance genes. Nat. Microbiol. 2, 16270. https://doi.org/10.1038/nmicrobiol.2016.270

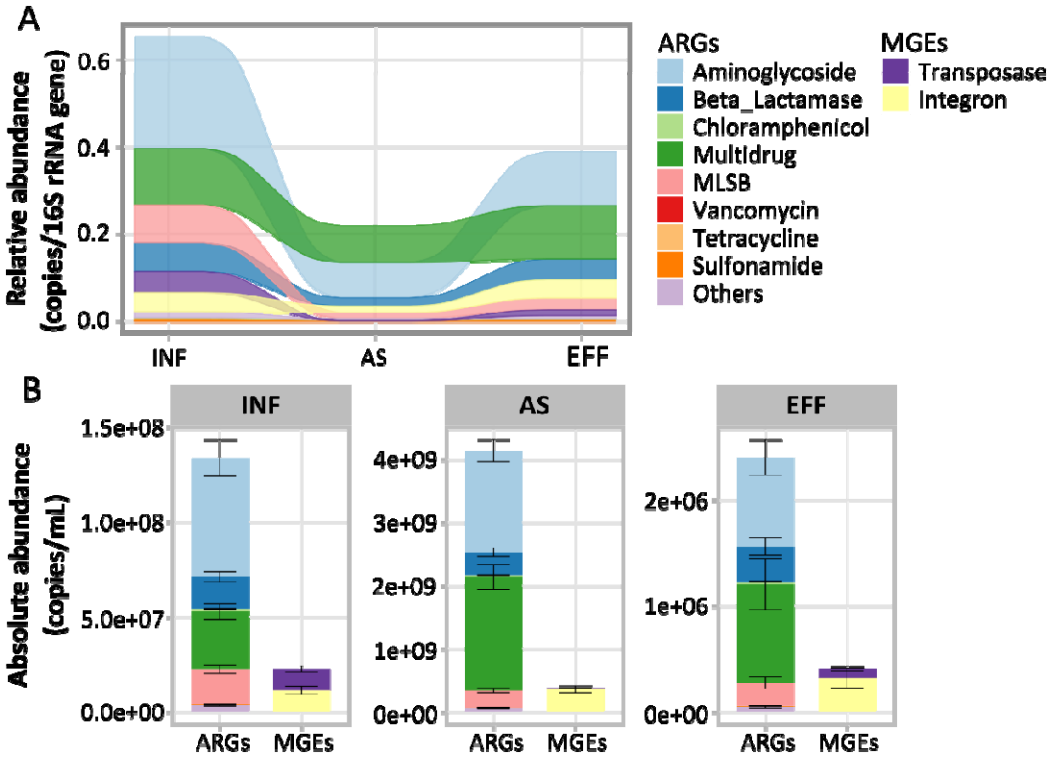

Figure 1. Relative abundance (A) and absolute abundance (B) of ARGs and MGEs in different wastewater treatment units. MLSB stands for Macrolide-Lincosamide-Streptogramin B.

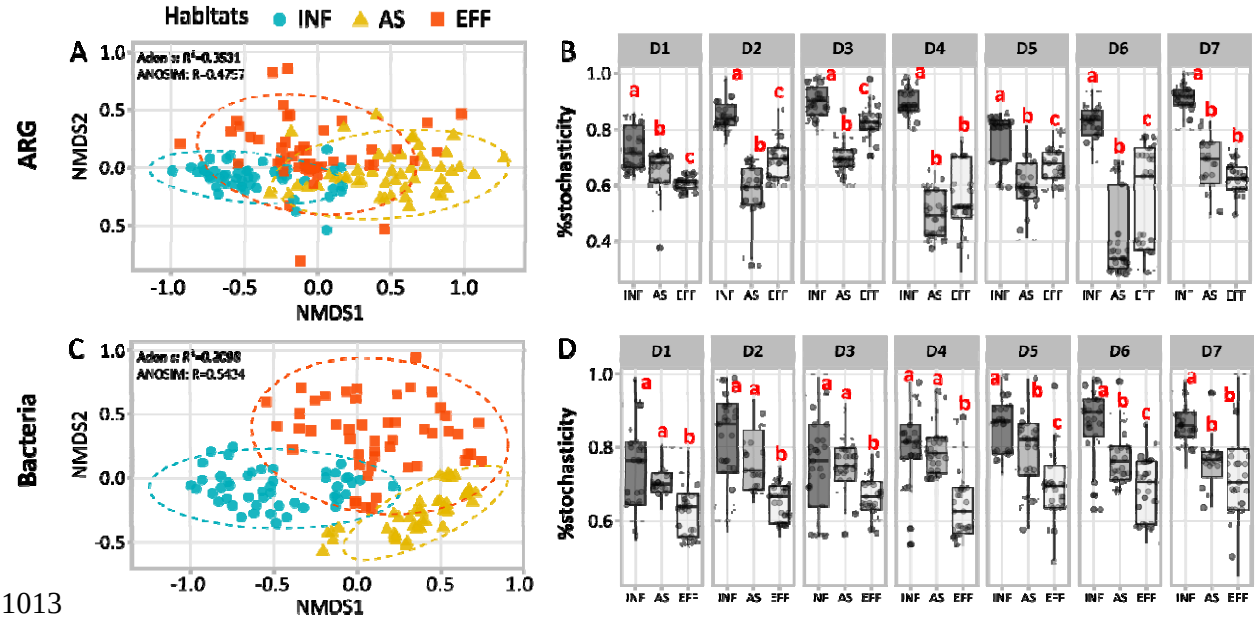

Figure 2. Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis distance showing the distribution pattern of ARG communities (A) and bacterial communities (C) in all WWTPs across the entire city. The ellipses were drawn at the 95% confidence level. Abundance-based β-null model was applied to assess the relative importance of the stochasticity process in governing the assembly of ARG (B) and bacterial communities (D) in each treatment unit during the sampling period (from day 1 to day 7). Significant differences were indicated by different letters among different units of the treatment process.

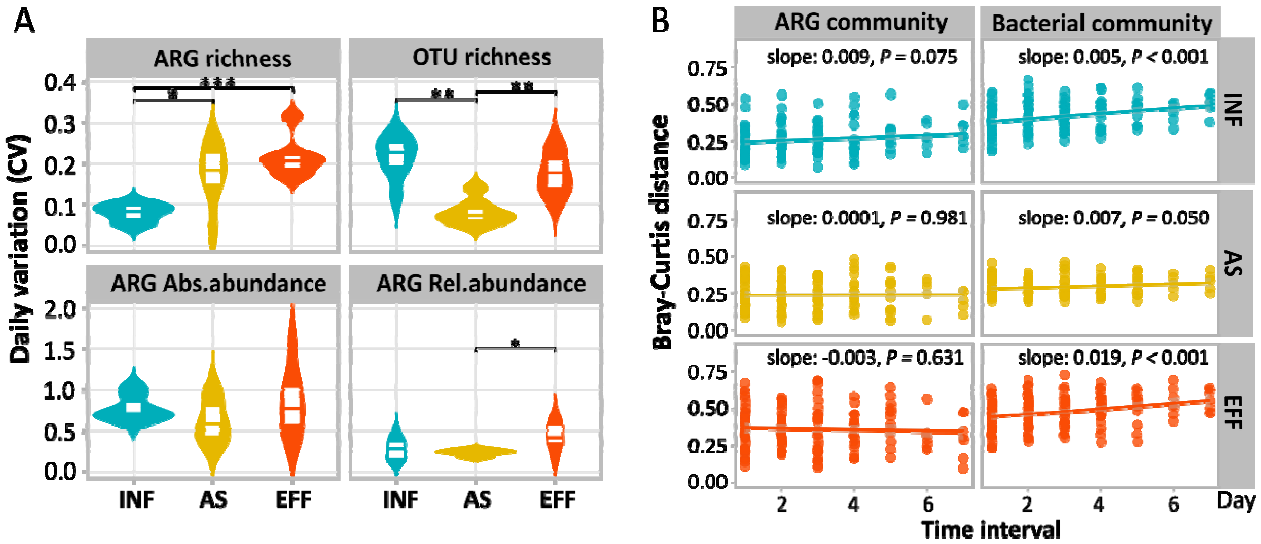

Figure 3. Daily variation of ARGs and bacteria at different wastewater treatment units. (A) Coefficient of variation (CV) of richness and abundance of overall ARG community and OTU richness, (B) time-decay relationships of the compositional dissimilarity of ARG and bacterial communities at different time intervals. Significant differences among different units of the wastewater treatment process were assessed by paired Wilcoxon test and indicated as follows: ***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$. Lines represent the fitted linear regression curve. The shaded areas represent the 95% confidential interval of the fitted linear regression curve.

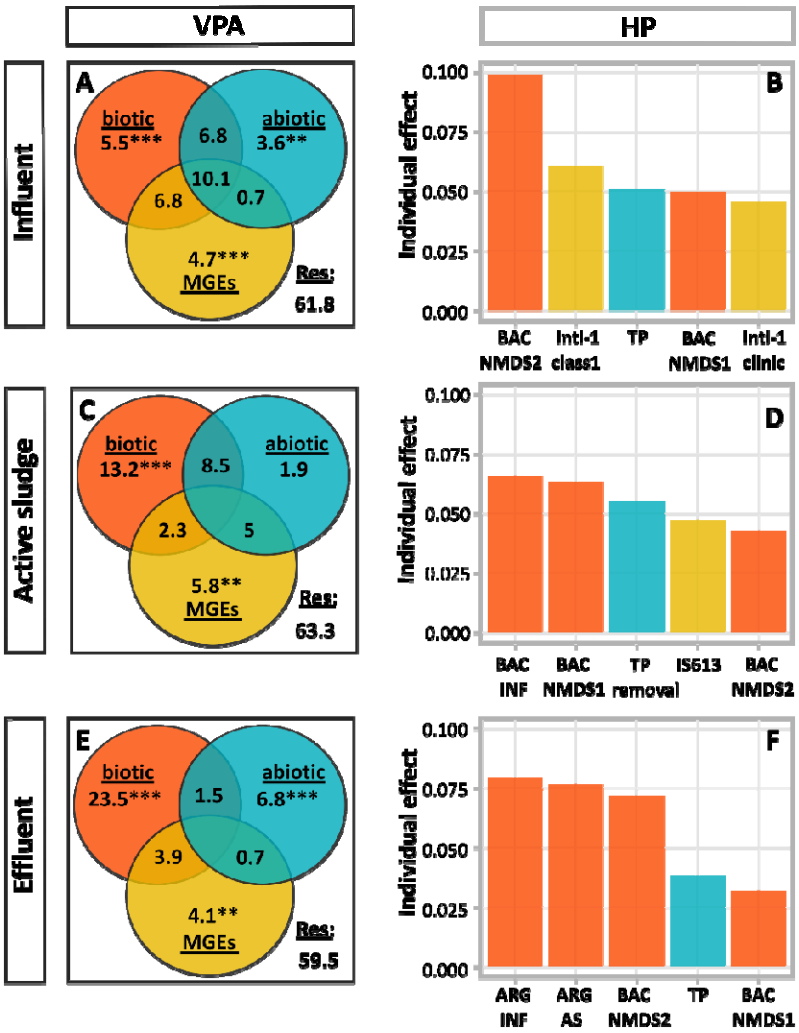

Figure 4. Variation partitioning analysis (VPA) of β-diversity variation of ARG communities among biotic, abiotic, and MGEs groups as well as their interactions (A, C, E). MGEs are considered to be a unique class of factors that contribute to the assembly of the ARG community, and therefore are separated from the biotic group as a single factor. The top five variables with the highest variance explained to β-diversity variation of ARG communities in each treatment unit are indicated by hierarchical partitioning (HP) analysis (B, D, F). The abbreviation of BAC NMDS1 and BAC NMDS2 represent the first two axes of NMDS results of the bacterial community in each treatment unit, and the abbreviation of BAC INF, BAC AS, ARG INF, and ARG AS represents the source of the bacterial community or ARG

community (upstream treatment units such as INF or AS). Significant differences were indicated as follows: ***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$.

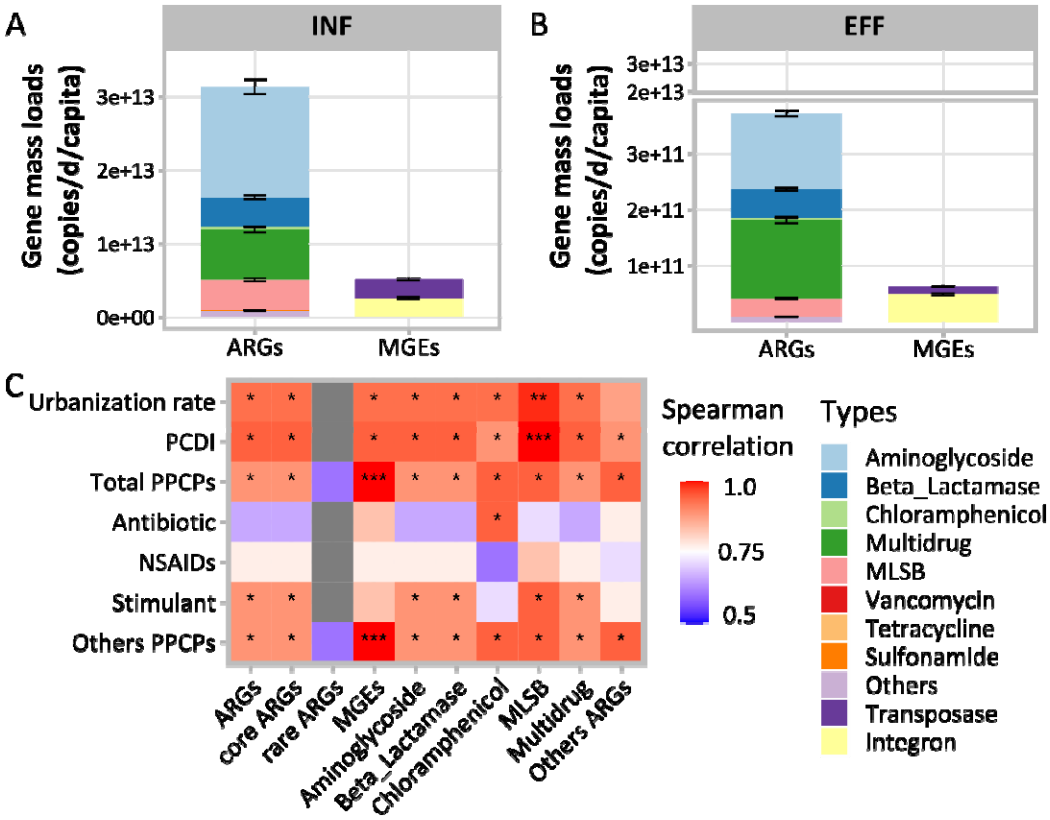

Figure 5. The daily ARG and MGE receiving loads per capita by WWTPs (A) and discharge loads from WWTPs (B) in Xiamen, China. Spearman correlation analysis was made between major types of the daily discharge ARG loads per capita and the daily PPCP loads in WWTPs and social development factors of the city (C). Significant differences were indicated as follows: ***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$.