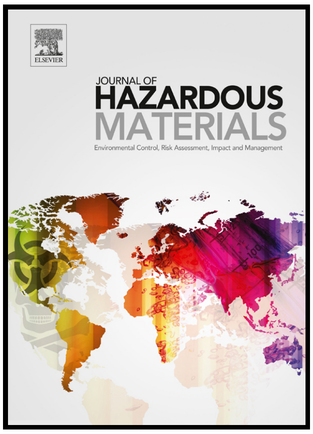

Fecal pollution mediates the dominance of stochastic assembly of antibiotic resistome in an urban lagoon (Yundang lagoon), China*Stochastic assembly of lagoon ARGs*

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Fecal pollution mediates the dominance of stochastic assembly of antibiotic resistome in an urban lagoon (Yundang lagoon), China

Running title: *Stochastic assembly of lagoon ARGs*

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Abstract

Sewage and fecal pollution cause antibiotic resistance genes (ARGs) pollution in urban lagoons. Seasonality also affects ARG dynamics. However, knowledge of factors controlling ARG community assembly across seasons is still limited. Here, we revealed the seasonal variation of ARGs and depict the underlying assembly processes and drivers via high-throughput quantitative PCR in an urban lagoon, China. A higher richness and abundance of ARGs were observed in summer and winter compared to spring and fall (Kruskal-Wallis test, $P < 0.05$). Both ARG and prokaryotic communities were mainly governed by stochastic processes, however these processes drove ARGs and prokaryotes differently across seasons. Stochastic processes played a more dominant role in shaping ARG communities in summer (average stochasticity: 83%) and winter (75%), resulting in a stable antibiotic resistome. However, no such seasonal pattern of stochastic processes was determined for prokaryotes, indicating a decoupling of the assembly process of ARGs and prokaryotes. Moreover, fecal microorganisms (e.g., *Bacteroidetes* and *Faecalibacterium*) mediated the stochastic processes of ARG profiles, via enhancement of prokaryotic biomass and mobile genetic elements abundances. The *tnp*A-07 transposase was the key for the horizontal gene transfer. These findings will enhance our understanding of how fecal pollution shapes ARG community assembly in urban lagoons.

Keywords:

Urban lagoon; Fecal pollution; Antibiotic resistance genes; Prokaryotic community; Stochastic processes

1. Introduction

The spread of antibiotic resistance is a global threat to humans, and is estimated will be the cause 10 million deaths each year by 2050 (Meredith, et al., 2015). Antibiotic resistance genes (ARGs) could persist in the natural environments even after the antibiotic resistant bacteria (ARB) have died since ARGs can transfer to new hosts, and lead to the failure of antibiotic therapy (Han, et al., 2020). Urban landscape water that has been modified by human activity, engineering, and design to serve as an important environment–human interface, where city dwellers have a great possibility to come in direct or indirect contact with ARGs (Hou, et al., 2020, Morse, 2015). Consequently, the spread of ARB and ARGs in various urban landscape water bodies, including urban lakes (Yang, et al., 2017), ponds (Hou, et al., 2020), and rivers (Berglund, et al., 2015) may pose a serious threat to human health. Widespread antibiotic use, organic fertilization with manure, and discharge of antibiotic residues from hospitals, livestock farms, pharmaceutical industries, and wastewater treatment plants (WWTPs) all contribute to the accumulation of ARGs in urban landscape water (Proia, et al., 2018, Zhu, et al., 2013). More importantly, due to horizontal gene transfer (HGT), ARGs could be exchanged and transferred among environmental microbes and pathogens through mobile genetic elements (MGEs) such as integrons, plasmids, and transposons (Zheng, et al., 2020). Therefore, recently the public calls for a comprehensive understanding of the pollution sources, and variation, of the antibiotic resistome in urban landscape water to manage and reduce the dissemination and accumulation of ARGs (Hou, et al., 2020, Lorenzo, et al., 2018).

It has been shown that deterministic and stochastic processes can simultaneously drive the spatio-temporal variation of the antibiotic resistome (Hou, et al., 2019a, Hu, et al., 2020, Peng, et al., 2020). Previous studies have investigated the patterns and processes of ARG and microbial community assembly in great depth via qualitative assessments (Fang, et al., 2019). Mounting evidences suggests that abiotic (e.g., antibiotics, biocides, heavy metals, and nutrients) and biotic factors (e.g., MGEs, metal resistance genes, and microbial communities) are responsible for the dissemination and assembly of ARGs in the urban water environments (Hu, et al., 2020, Peng, et al., 2020, Zheng, et al., 2017). For instance, Corno et al. (2019) found that deterministic factors (i.e., higher proportions of WWTP effluents) played a dominant role in shaping the antibiotic resistome in freshwater bodies receiving WWTP effluents (Corno, et al., 2019). In contrast, stochastic processes dominated the ARG community assembly in urban ponds due to the random horizontal gene transfer mediated by MGEs (Hou, et al., 2020). Peng et al. (2020) demonstrated that the ecological processes of ARG profiles shifted from stochastic to deterministic processes as the level of urbanization surrounding a river increased, where land use and MGEs may be the key drivers (Peng, et al., 2020). However, revealing the key abiotic and biotic factors regulating the balance of deterministic and stochastic processes in ARG community assembly is still challenging.

It has been suggested that fecal pollution rather than environmental selection can explain ARG abundances in anthropogenically impacted environments (Karkman, et al., 2019). Thornton et al. found that higher abundances and diversity of ARGs are associated with human fecal contamination in river surface water (Thornton, et al., 2020). Raw wastewater and WWTP effluents themselves contain a wide array of ARBs and ARGs in fecal materials from humans and animals, which could facilitate the accumulation of ARGs and/or the HGT processes in downstream river networks (Hu, et al., 2020). In addition, a previous study revealed significant seasonal variations of ARGs in the wild fish gut and relevant peri-urban water environments, and the bacterial community was the main contributor to ARG changes (Zhou, et al., 2021). Other factors such as urban stormwater runoff (Lee, et al., 2020b) and residual antibiotics (Huang, et al., 2019) also can shape ARG profiles in aquatic ecosystems. For instance, wastewater with residual antibiotics could form a selection pressure for ARGs (Karkman, et al., 2018). However, little information is available concerning the relative importance of fecal pollution and other factors that influence the assembly processes of ARG communities in urban landscape water bodies, especially for urban coastal lagoons.

Coastal lagoons present nearly 15% of the world’s shorelines, and are favorable habitats for primary producers and are highly productive ecosystems (Sivaperuman, et al., 2018). Despite their relevance for conservation, coastal lagoons suffer multiple pressures from eutrophication, sewage discharge, and diverse forms of modification in their watersheds, caused by urbanization (Conde, et al., 2015). Urban coastal lagoons are highly dynamic in nature as they exhibit seasonal variability in their physical and chemical characteristics (Anthony, et al., 2009, Wang, et al., 2020). Anthropogenic stressors, such as changes in land use, freshwater withdrawal from ground and surface water sources, sewage discharge with fecal materials, and nonpoint source pollution also promote the variation of urban lagoon ecosystems (Anthony, et al., 2009). These factors could further affect the dynamics of ARG and prokaryotic communities. Although some studies have investigated the seasonal variation of the structure within ARG and prokaryotic communities of urban aquatic systems (Caucci, et al., 2016, Peng, et al., 2019, Zheng, et al., 2018), very few studies have focused on the temporal dynamics in assembly mechanisms for ARG profiles and their potential hosts (i.e., prokaryotic communities) in urban lagoons. It was observed that seasonal variations of antibiotic resistance levels in WWTPs effluents might lead to seasonal changes of

ARGs in receiving water bodies (Caucci, et al., 2016). These variations may provide the ideal environment for bacteria to acquire and retain ARGs by HGT, which may maintain/increase antibiotic resistance development and transference of ARGs in the indigenous communities (Thomas and Nielsen, 2005). For instance, the bacterial community was closely associated with ARG communities and impacted the transfer of ARGs in urban waters (Hou, et al., 2020, Xiang, et al., 2018, Zhou, et al., 2017). Recent studies also reported that the dynamics of ARG and bacterial communities showed a decoupled behavior in urban landscape waters (Fang, et al., 2019, Peng, et al., 2020). However, more knowledge of the ARG community assembly across seasons in urban lagoons is needed, as this would be crucial for modeling, predicting, and controlling ARG in the urban landscape water in coastal cities.

Here, we employed a high-throughput quantitative PCR (HT-qPCR) approach to characterize the distribution pattern of ARGs in the surface waters of an urban lagoon over four different seasons. To identify the potential fecal pollution sources of the lagoon, the occurrence and abundance of 23 fecal indicator genes were also examined. We aimed to (i) characterize the seasonal variation in the abundance and composition of the antibiotic resistome; (ii) investigate the assembly dynamics of ARG and prokaryotic communities as well as their regulating factors; (iii) identify the major pollution sources of ARGs in the urban lagoon; and (iv) uncover the co-occurrence pattern of ARGs, MGEs, and fecal markers in the lagoon waters. Additionally, we hypothesized that since fecal pollution introduced by sewage may contribute to the random HGT of ARGs in the lagoon, a decoupling in assembly processes in prokaryotic taxonomic (i.e., OTUs) and functional (i.e., ARGs) communities across seasons could be observed. Taken together, this study will advance our knowledge about the temporal characteristics of ARG community assembly in urban lagoons and help to develop effective management strategies for the prevention of ARG dissemination.

2. Materials and Methods

2.1 Field sampling and physicochemical analysis

Coastal Yundang lagoon consists of Outer, Inner, and Songbai lakes, with a surface water area of ~1.6 km^2 and an average depth of ~2.5 m, respectively (Fig. 1) (Wang, et al., 2020). Being an important part of the urban landscape water bodies in Xiamen city, China, Yundang lagoon has been heavily contaminated by domestic and industrial wastewater discharge before 1990 (Uddin, et al., 2020). Although continuous efforts were made for restoring the environmental degradation of coastal Yundang lagoon, heavy rain can cause raw sewage to overflow into Yundang lagoon since combine sewer systems were adopted before 2020, where the stormwater runoff was dumped into the same pipes that are used for domestic wastewater (The Standing Committee of Xiamen People's Congress, 2020). To investigate the seasonal pattern of ARGs in Yundang lagoon, we collected a total of 56 surface water samples at a depth of 0.5 m for all four seasons [i.e., fall (Late September, 2014), winter (December, 2014), spring (April, 2015), and summer (Early August, 2015)]. These samples were collected from a total of 14 sites (five sites in the Outer lake, four sites in the Inner lake, and five sites in the Songbai lake) covering the main water channel of Yundang lagoon. The climate information of these seasons was shown in Table S1. We also collected four influent and four effluent samples from a nearby WWTP as a comparison (Fig. 1).

Water temperature, salinity, pH, and dissolved oxygen (DO) were determined *in-situ* using a HACH portable meter (HACH, CO, USA). Transparency was measured using a Secchi disk. Three subsamples (500-600 mL for each subsample) were collected from at 5-m intervals from each site. Aliquots of water sample (400 mL) was filtered through 0.45 μm pore size cellulose filters (Millipore, MA, USA) and stored at 4℃ for nutrient analysis. Ammonia (NH_4-N), nitrite (NO_2-N), nitrate (NO_3-N), and soluble reactive phosphorus (SRP) were determined following the methods described in our previous study (Hu, et al., 2017). Dissolved inorganic nitrogen (DIN) was defined as the sum of NH_4-N, NO_2-N, and NO_3-N. Other physicochemical analyses including suspended solids (SS), chemical oxygen demand (COD_{Mn}), and biological oxygen demand (BOD_5) were analyzed using standard methods (China's State Environment Protection Agency, 2002). In addition, 400-500 mL subsamples of each water sample were pre-filtered through 20 μm mesh (Millipore, MA, USA) and then filtered by 0.22 μm Sterivex-GP filters (Millipore, MA, USA). The filters were stored at -80°C until molecular analysis.

2.2 DNA extraction, 16S rRNA gene amplicon sequencing and sequence analysis

DNA was extracted from the Sterivex-GP filters according to our previous modifications (Hu, et al., 2018). 16S rRNA gene amplicon sequencing was used to explore the lagoon prokaryotic community composition (Wang, et al., 2020). Briefly, the V4-V5 region of 16S rRNA genes was amplified by using 515F (5'-GTG YCA GCM GCC GCG GTA-3') and 907R (5'-CCG YCA ATT YMT TTR AGT TT-3') (Hu, et al., 2017). PCR reactions were performed in 25 μL in triplicate containing 12.5 μL of AmpliTaq™ Gold PCR Master Mix (2 ×) (Applied Bio-systems, CA, USA) and 0.4 μM of forward and reverse primers, and 20 ng of template DNA. PCR conditions were comprised of an initial denaturation at 95°C for 5 min, 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 90 s, and a final extension at 72°C for 10 min.

Triplicate PCR products were combined and purified using the Quant-iT^{TM} PicoGreen^{TM} dsDNA Assay Kit (Invitrogen, Waltham, MA, USA) (Hou, et al., 2019b). The purified PCR products were sequenced on an Illumina MiSeq platform (Shanghai Majorbio Bio-Pharm Technology Co. Ltd., Shanghai, China) with 2 × 300 bp paired-end strategy. The sequences generated in this study were deposited into the NCBI Short Reads Archive (SRA) database under accession number PRJNA328583.

The 16S rRNA gene sequences were analyzed by using a pipeline combined with LotuS (Hildebrand, et al., 2014) and QIIME v1.9.1 (Caporaso, et al., 2010) by following the procedure described in Wang et al. (2020). Briefly, the paired-end sequence reads were quality trimmed, merged, and clustered into operational taxonomic units (OTUs) at a cutoff of 97% identity by using LotuS tool with default parameters (Hildebrand, et al., 2014). The representative sequence of each OTU was classified by employing the RDP classifier with the SILVA database v132 (Quast, et al., 2013) at a confidence threshold of 80%. The potential fecal microorganisms were identified at the genus level as our previous study (Hu, et al., 2020).

2.3 HT-qPCR analysis of ARGs and fecal indicator genes

HT-qPCR was performed via the SmartChip Real-time PCR (Warfergen Inc., Fremont, CA, USA) based on previous studies (Wang, et al., 2020, Zhu, et al., 2017a). The distribution pattern of ARGs and MGEs were analyzed by using a total of 296 primer sets including 285 primer sets targeting all major classes of ARGs, 10 primer sets for MGEs (eight transposase genes and two integrase genes), and one primer set for 16S rRNA genes (Guo, et al., 2018, Zhu, et al., 2017a). Meanwhile, to identify potential sources of fecal pollution in Yundang lagoon, we applied 23 primer sets targeting the fecal indicator genes of human, ruminants (cows and sheep), pigs, poultry, horses, and pets (dogs and cats), respectively (Harwood, et al., 2014, Wang, et al., 2020). The sensitivity and specificity of the fecal marker primers were tested and evaluated by analyzing 155 fecal samples of multiple hosts (i.e., humans, pigs, chickens, ducks, dogs, cattle, sheep, deer, horses, cats, geese, and rabbits) as showed in our previous work (An, et al., 2020). The sensitivity of the fecal marker primers was > 80% (except duck and horse) and the specificity was > 99% (An, et al., 2020).

Amplification was conducted in a 100 nL reaction system and the thermal cycle was set as follow: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 30 s, 60°C for 30 s (Guo, et al., 2018, Wang, et al., 2020). All qPCR reactions were conducted in three technical replicates, and each chip included a non-template control for each primer set. The results of HT-qPCR were quality trimmed and analyzed by using SmartChip qPCR Software (Guo, et al., 2018, Looft, et al., 2012, Wang, et al., 2020). The relative abundance (copy number of the target gene/copy number of 16S rRNA gene) and normalized abundance (copy number of the target gene/bacteria cell) of ARGs and MGEs were calculated according to previous studies (Guo, et al., 2018, Wang, et al., 2020). Specifically, the average copy number of 16S rRNA genes per bacterial cell is estimated at four based on the Ribosomal RNA Operon Copy Number Database (Zhu, et al., 2017b). The normalized abundances of ARGs and MGEs were estimated by dividing the concentration of each sample by its corresponding number of bacterial cells, which can be calculated by dividing the copy number of the 16S rRNA genes by four (Hou, et al., 2020).

To quantify the absolute abundance of ARGs and fecal indicator genes, we quantified the absolute copy number of 16S rRNA genes using on a LightCycler 480 instrument (Roche, Switzerland) with an SYBR® Green approach as previously described (Wang, et al., 2020). All real-time qPCR reactions were performed in triplicate for each sample with negative controls.

2.4 Network analysis

To reveal the co-occurrence of ARGs, MGEs, fecal indicator genes and prokaryotic genera, a network analysis was performed using the random matrix theory-based network approach (http://ieg4.rccc.ou.edu/MENA/). Briefly, only the variables (i.e., ARGs, MGEs, fecal indicator genes and prokaryotic genera), with > 50% occurrence in all samples (> 28 of 56 samples) were used for building the network (Wang, et al., 2020). Prior to the construction of the network, the missing values were filled with 0.01 if paired valid values were available to ensure a more statistically reliable correlation coefficient between two variables (Deng et al., 2012). Only correlations with significance ($P < 0.05$) that exhibited a strong value (Spearman correlation $\rho > 0.69$) were displayed by using Gephi v0.9.2 (https:/gephi.github.io/).

2.5 Neutral community model and null model analyses

The neutral community model (NCM) was employed to evaluate the effects of random dispersal and ecological drift on the assembly of the prokaryotic taxonomic (i.e., OTUs) and functional (i.e., ARGs) communities (Hu, et al., 2019, Peng, et al., 2020, Sloan, et al., 2006). The parameter R^2, which indicates the overall fit to the NCM, was used to quantify the variation in community assembly explained by the NCM (Sloan, et al., 2006). The parameter *Nm* could be used to estimate the dispersal between communities (Peng, et al., 2020). Moreover, we further applied an ecological null model (Zhang, et al., 2019b) to evaluate the relative importance of deterministic and stochastic processes on the community assembly (Hou, et al., 2020). Briefly, the compositional stochasticity (ST_{Ratio}) was calculated by comparing the observed similarity (S_{obs}) of the actual communities and the expected similarity (E_{exp}) of null expected communities (1000 random shuffles of the original community data) (Zhang, et al., 2019b).

$$ST_{Ratio} = 1 - (S_{obs} - E_{exp})/S_{obs}$$

2.6 Statistical analysis

Principal component analysis (PCA) was performed using PRIMER v7.0 (Clarke and Gorley, 2015) and used to identify the variation in physicochemical variables of the lagoon waters. Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis distance was employed to determine the β-diversity pattern of prokaryotic taxonomic (i.e., OTUs) and functional (i.e., ARGs) communities. In addition, the analysis of similarity (ANOSIM) and permutational multivariate analysis of variance (Adonis) were used to test the significance of differences of the ARG community compositions among different seasons. Mantel test was used to explore the associations between the physicochemical variables and prokaryotic taxonomic (i.e., OTUs) or functional (i.e., ARGs) communities with 999 permutations by using the R package vegan (Oksanen, et al., 2019). In addition, the relationship between the absolute abundance of ARGs and MGEs or fecal indicator genes was determined by using both Pearson and Spearman correlation tests.

Partial least squares-path model (PLS-PM) was used to evaluate the direct and indirect effects of biotic (e.g., prokaryotic communities, MGEs and fecal indicator genes) and abiotic (e.g., physicochemical variables and nutrients) factors on the abundance and assembly processes (i.e., compositional stochasticity) of lagoon ARG communities by using the R package plspm (Sanchez, et al., 2015). Initially, the permutation test (999 simulations) was used to identify the significant variables affecting the ARG profiles. The significant variables were then divided into six groups: physicochemical variables (i.e., DO and pH), nutrients (i.e., DIN and SRP), fecal indicator genes (i.e., human HF183-BacR287 and dog BacCan-UCD), prokaryotic community (i.e., dominant prokaryotic phyla), prokaryotic biomass (i.e., the absolute abundance of 16S rRNA genes) and MGEs (i.e., the absolute abundance of MGEs). The overall performance of the PLS-PM model was evaluated by using the goodness of fit index (GoF) as described elsewhere (Wang, et al., 2021).

3. Results

3.1 Occurrence and relationship of lagoon ARGs and fecal indicator genes

PCA analysis indicated that Yundang lagoon waters had distinct physicochemical parameters during each season (Fig. S1 and S2). Results of the HT-qPCR analysis showed that both the average numbers and normalized abundances of ARGs detected in lagoon waters in summer and winter were significantly higher (93 and 86 per sample, respectively; 1.07 and 1.08 copies per cell, respectively) than in spring and fall (58 and 57 per sample, respectively; 0.27 and 0.25 copies per cell, respectively) (Kruskal-Wallis test, $P < 0.05$) (Fig. 2). By comparison, the average numbers of ARGs in the influents of WWTPs (115) were much higher than in the lagoon waters during different seasons, with the exception of summer (Kruskal-Wallis test, $P < 0.05$) (Fig. 2A). Despite decreasing after WWTP treatment, the average number of ARGs in the effluents (82) was similar to those in lagoon waters (Kruskal-Wallis test, $P > 0.05$). However, unlike the average numbers of ARGs, the normalized abundances of ARGs in both influents and effluents of WWTPs displayed significantly higher values than those of the lagoon waters except for summer (Kruskal-Wallis test, $P < 0.05$) (Fig. 2B).

Interestingly, being similar to the normalized abundances of ARGs, the absolute abundance of ARGs (3.5×10^6 copies/mL) and MGEs (1.4×10^6 copies/mL) in lagoon waters in summer were also significantly higher than the values for lagoon waters in spring (5.9×10^5 and 2.2×10^5 copies/mL for ARGs and MGEs, respectively) and fall (3.5×10^5 and 1.1×10^5 copies/mL for ARGs and MGEs, respectively) (Kruskal-Wallis test, $P < 0.05$) (Fig. S3). Correlation analysis indicated that the absolute abundance of ARGs positively associated with the absolute abundance of MGEs in lagoon waters, independent of whether influent and effluent samples were included (Fig. 3C and D). These ARGs covered eight major ARG types (i.e., aminoglycoside, β-lactamase, chloramphenicol, multidrug, Macrolide-Lincosamie-Streptogamin B (MLSB), sulfonamide, tetracycline, and vancomycin). Yundang lagoon waters shared a large proportion of ARGs with the WWTP influents (78% of the total number of ARGs in Yundang lagoon) and effluents (64%) (Fig. S4A). Moreover, Yundang lagoon waters in winter shared a higher number of ARGs with summer than with other seasons (Fig. S4B).

We also quantified the abundance of fecal indicator genes, and found that human fecal markers, including HumM2, *B. fragilis gyrB*, HF183-BFDrev, HF183-BacR287, and Human mtCytb, were detected throughout the sampling sites in summer and winter (Fig. S5A and B). Dog fecal indicator genes (mainly BacCan-UCD) were detected at most of the sites in summer and all sites in winter (Fig. S5C and D). The Spearman correlation analysis indicated that there were strong positive correlations among different fecal indicator genes, especially between HF183-BFDrev and HF183-BacR287 ($\rho = 0.939$, $P < 0.001$, Fig. S6). HF183-BacR287 was used as the representative human fecal marker for further analysis considering its higher occurrence (87.5%). Notably, both the absolute and relative abundance of HF183-BacR287 detected in summer and winter were significantly higher than in lagoon waters in spring and fall (Kruskal-Wallis test, $P < 0.05$) (Fig. S5A and B). It was also noticed that the potential fecal microorganisms at the genus level shared a similar seasonal variation trend as the fecal markers ($\rho = 0.804$, $P < 0.001$) (Fig. S6). For instance, the potential fecal microorganisms at the genus level were detected at all sampling sites in winter and the relative abundance of them was lower compared to other seasons (Fig. S6). Interestingly, there were strong positive relationships between the absolute abundances of fecal markers and total ARGs (Pearson and Spearman correlation, $P < 0.001$ for HF183-BacR287 or BacCan-UCD and ARGs) (Fig. 3A and B).

3.2 Seasonal variation in the composition and assembly processes of lagoon antibiotic resistome

NMDS ordination analysis showed that the antibiotic resistome of the lagoon waters for each season (i.e., spring, summer, fall, and winter) was distinct (ANOSIM and Adonis tests, $P < 0.001$), except for those of spring and fall (ANOSIM test, $P > 0.05$) (Fig. 4A and Table S2). Moreover, the lagoon antibiotic resistome in summer and winter tended to cluster together with the resistomes from the influents and effluents of WWTPs. Similarly, the prokaryotic taxonomic communities of the lagoon waters in different seasons were highly distinct from each other (ANOSIM and Adonis tests, $P < 0.001$) (Fig. 4B and Table S2). However, the association between the compositions of ARG and prokaryotic taxonomic communities was relatively weak (Mantel test, r = 0.313, $P < 0.001$).

It was observed that stochastic processes predominated the assembly of both the ARG (average compositional stochasticity for all seasons: 73.61%) and prokaryotic (67.81%) communities in Yundang lagoon (Fig. 5). However, the seasonal variation in the relative importance of the stochastic processes was inconsistent between the two communities. For example, the stochastic processes played a more dominant role in shaping the ARG communities in summer (average compositional stochasticity: 83%) and winter (75%) compared to spring (68%) and fall (68%) (Kruskal-Wallis test, $P < 0.05$) (Fig. 5A). Regarding prokaryotic communities, however, the compositional stochasticity in fall was significantly higher (83%) than for other seasons (Kruskal-Wallis test, $P < 0.05$) (Fig. 5B). It is worth noting that the compositions of ARG communities in summer and winter were more similar than those from spring and fall (Kruskal-Wallis test, $P < 0.05$) (Fig. S8A), indicating that the lagoon antibiotic resistome tended to be more stable in its composition when the relative importance of stochasticity was higher. No such pattern was observed for prokaryotic taxonomic communities (Fig. S8B).

Consistent with this, the frequency of ARG occurrence in summer ($R^2 = 0.521$) and winter ($R^2 = 0.563$) showed a better fit to the NCM than the frequency in spring ($R^2 = 0.359$) and fall ($R^2 = 0.301$) (Fig. S9), suggesting that stochastic processes could explain a higher proportion of the ARG communities in summer and winter as compared to other seasons. We also tested the fit to the NCM for the prokaryotic taxonomic profiles and found the profile of all seasons combined as one fitted the NCM poorly ($R^2 = 0.385$), while there was a medium fit to the NCM for the prokaryotic taxonomic profiles of each individual season ($R^2 = 0.664$ for spring; $R^2 = 0.688$ for summer; $R^2 = 0.503$ for fall; $R^2 = 0.678$ for winter) (Fig. S10).

3.3 Direct and indirect effects of abiotic and biotic factors on the lagoon antibiotic resistome

The Mantel tests indicated that pH, MGEs, human fecal marker HF183-BacR287, and dog fecal marker BacCan-UCD were significantly correlated with the composition of ARG communities ($r > 0.25$, $P < 0.05$), while the compositional stochasticity of ARGs had significantly correlation with pH and MGEs ($r > 0.25$, $P < 0.05$) (Fig. S11). It was shown that temperature, pH and salinity of Yundang lagoon surface water samples ranged from 17.6 to 31.9°C, 7.3 to 8.9, and 11 to 30‰ in different seasons, respectively (Fig. S2). As for the prokaryotic taxonomic communities, the key factors were temperature, DIN, and SRP, while the compositional stochasticity of prokaryotic communities was correlated by temperature and DO (Fig. S11). In Yundang lagoon water, prokaryotic communities were dominated by Bacteroidetes (average 32.3%), Alphaproteobacteria (31.8%), Gammaproteobacteria (13.9%), and Cyanobacteria (4.5%) among four seasons (Fig. S12). Some of those dominant taxa, such as Cyanobacteria, Firmicutes and Actinobacteria, showed clear seasonal variation. Specifically, Cyanobacteria may play an important role in fall, accounting for 16.1% of the fall prokaryotic communities, while Firmicutes and Actinobacteria at some sampling sites had relatively higher relative abundances in spring and summer than the other seasons, respectively (Fig. S12).

The results of the PLS-PM analysis indicated that physicochemical variables (i.e., DO and pH), nutrients (i.e., DIN and SRP), and prokaryotic taxonomic communities negatively affected the absolute abundance and compositional stochasticity of the lagoon antibiotic resistome in an indirect manner (Fig. 6A and B). However, compared to prokaryotic taxonomic communities, the physicochemical variables and nutrients had higher negative total standardized effects on the absolute abundance and compositional stochasticity of the lagoon antibiotic resistome (Fig. 6C and D). MGEs directly affected the ARG absolute abundance and the compositional stochasticity of ARG communities, and had the largest positive total standardized effects (0.636 and 0.456 for the absolute abundance and compositional stochasticity of ARGs, respectively) (Fig. 6 C and D). Moreover, the prokaryotic biomass (i.e., the abundance of 16S rRNA genes) and fecal markers (i.e., HF183-BacR287 and BacCan-UCD) positively affected the absolute abundance and compositional stochasticity of ARGs. Notably, fecal markers were a major indirect influence for the absolute abundance and compositional stochasticity of ARGs, which was mediated by the prokaryotic biomass and MGEs (Fig. 6A and B).

3.4 Co-occurrence patterns among ARGs, fecal markers and prokaryotic genera

The co-occurrence network analysis indicated that the human fecal marker HF183_BacR287 was strongly associated with four ARG subtypes (i.e., *cml*A1_01, *cml*A1_02, *erm*B, and *tet*E), two transposases (i.e., IS613 and *tnpA*_07) and phyla Bacteroidetes (e.g., genus *Bacteroides*) and Firmicutes (e.g., genus *Phascolarctobacterium*) (Fig. 7). Except for *tnpA*_07 and *erm*B (which belonged to Module 1), they fell into Module 3, clustering with multiple fecal prokaryotic genera (i.e., *Eubacterium*, *Megamonas*, *Paludibacter*, *Parabacteroides*, *Phascolarctobacterium*, *Prevotella*, *Roseburia*, and *Tolumonas*) (Fig. 7). Moreover, it was observed that IS613 and *tnpA*_07, which exhibited strong connections with particular prokaryotic genera and ARGs, belonged to Modules 3 and 1, respectively. *TnpA*_07 showed the highest node degree (12) among all MGEs (Table S3). Among the 12 connections, *tnpA*_07 was significantly associated with HF183_BacR287, *Prevotella*, *Bacteroides, Parabacteroides*, and *Paludibacter* in Module 3, while *tnpA*_07 was found to connect with seven ARGs (i.e., *aadA*1, *aph*_2_Id_02, *bla*$_{\mathrm{OXA10\_01}}$, *bla*$_{\mathrm{VEB}}$, *erm*B, *lnu*B_02, and *sat*4) in Module 1 (Table S4). This may indicate the importance of *tnp*A_07 as the keystones in HGT.

Among the prokaryotes, *Paludibacter* (40), *Prevotella* (38), *Faecalibacterium* (32), and *Bacteroides* (24) were the top four genera with the highest node degrees. *Paludibacter* and *Prevotella* were closely associated with many ARG subtypes, including aminoglycoside (e.g., *aac_6__Ib_aka_aac*A4_02 and *aac_6__Ib_aka_aac*A4_03), β-lactamase (e.g., *bla*$_{\mathrm{VEB}}$), chloramphenicol (e.g., *cml*A1_01), tetracycline (e.g., *tetE* and *tetR*_02) in Module 3, and MLSB resistance genes (e.g., *lnu*B_02 and *ermB*) in Module 1, resulting in a close relationship between these modules. *Faecalibacterium* in Module 2 was clustered with multiple fecal and sewage prokaryotic genera (e.g., *Proteiniclasticum*, *Clostridium*, *Cloacibacterium*, *Eubacterium*, and *Ruminococcus*). *Faecalibacterium* as well as fecal and sewage prokaryotic genera all had close associations with *Paludibacter* in Module 3.

4. Discussion

Seasonal changes can exacerbate the environmental fluctuations of urban aquatic ecosystems, resulting in difficulty modeling and predicting the source(s) and dissemination of ARGs (Fang, et al., 2019). Human activities could further influence the accumulation and transmission of ARGs and make it more complicated to control them in urban aquatic ecosystems. Until now, only a few existing studies have described the spatial and temporal distribution of ARGs in urban rivers/streams subjected to anthropogenic activities (Hu, et al., 2020, Peng, et al., 2020, Peng, et al., 2019, Xiang, et al., 2018). Some of them also have studied the variation of the assembly processes of the ARG communities among seasons (Peng, et al., 2019, Xiang, et al., 2018). However, information about the key factors that mediate the balance between deterministic and stochastic assembly for urban aquatic ARG communities at the temporal scale is still missing. Here we investigated the seasonal variation in ARG profiles of an urban lagoon over four seasons using HT-qPCR. We also went one step further, elucidating the community assembly mechanisms and the associated driving forces affecting ARG profiles to provide basic guidance for predicting and controlling ARGs.

4.1 Seasonal variation of the diversity and abundance of ARGs in lagoon waters

A previous study showed that the bacterial communities of an urban reservoir displayed a seasonal pattern, while ARG community composition did not exhibit seasonality (Fang, et al., 2019). However, the distribution pattern and composition of ARGs varied seasonally in Yundang lagoon (Figs. 2 and 3). The richness of ARGs (average numbers of ARGs per sample ranging from 57 to 93) in different seasons was within the range of the richness observed in an urban river (Peng, et al., 2019), whereas the normalized abundance of ARGs in different seasons (0.25 ~ 1.08) fluctuated more than those of the same river (0.35 ~ 0.75) (Peng, et al., 2019). Interestingly, the lagoon waters in summer and winter had higher ARG richness and abundances than in spring and fall (Fig. 2). The NMDS ordination also showed that the antibiotic resistome of lagoon waters in summer and winter had a closer link to the antibiotic resistome of WWTP influents and effluents (Fig. 2). This indicated that raw or treated sewage might have a higher input of ARGs to Yundang lagoon waters in summer and winter. A previous study showed that the influents of 32 WWTPs from 17 Chinese cities exhibited a higher abundance of ARGs in summer than other seasons (Su, et al., 2017). Moreover, the seasonality of antibiotic prescriptions for outpatients may contribute to the higher level of urban sewage ARGs in winter (Caucci, et al., 2016) as well as the ARGs in receiving water bodies. However, more data for wastewater discharge from local WWTPs are needed for making incontestable conclusions.

We previously found that the dominant ARGs in Yundang sediments were aminoglycoside, multidrug, β-lactamase, and MLSB resistance genes (Wang, et al., 2021), which had a total of 84 ARGs and the normalized abundance of ARGs ranging from 0.13 to 0.94. These sediment ARGs might be further released to lagoon waters through sediment resuspension due to disturbance from wind-wave action, heavy rainfall and benthic organisms (Luo, et al., 2010). However, besides these genes, more diverse ARGs existed in lagoon waters including tetracycline, sulfonamide, and chloramphenicol resistance genes (Fig. 2), suggesting other sources, such as urban runoff may contribute to the dissemination of ARGs in lagoon waters (Dong, et al., 2019). In urban watershed of Singapore with non-point source pollutions, *tet*X and β-lactamase B genes were found to be more abundant (Low, et al., 2016). In fact, some precipitation occurred five days prior to our sampling events in both summer and winter (Table S1). Given the high ratio of watershed area to water area (23:1) in Yundang lagoon, the urban runoff could have been contaminated by an array of point and non-point sources, including land applied manure, septic tanks, and sewer overflow, may contribute to the input of ARGs (Garner, et al., 2017, Wang, et al., 2020). Although pretreatment of manure was usually conducted before it is applied organic fertilizers, standard composting was insufficient to control ARGs (Sardar, et al., 2021). Our previous study targeting the same area (Yundang lagoon) showed that extreme precipitation events could lead to an increase in the abundance of human fecal markers in lagoon waters (Wang, et al., 2020). The exogenous input of fecal microorganisms may enhance the antibiotic resistance levels in receiving water (Lee, et al., 2020a, Thornton, et al., 2020).

Our results of fecal markers further validated the aforementioned assumption. The absolute abundances of total ARGs and human (HF183-BacR287) or dog (BacCan-UCD) fecal markers had strong positive correlations (Figs. 4 and S7). However, the occurrence and absolute abundances of human fecal markers were about 2 times and 15-50 times higher than those of dog fecal markers, respectively. The relatively higher abundances of human fecal markers were found in most sites from summer and winter than other seasons (Fig. S5), suggesting that human fecal pollution may also contribute to the higher diversity and abundance of lagoon ARGs in summer and winter. Likewise, increases in ARG diversity and abundance in the Ganges river was accompanied by the increases in human gut microbiome-associated sequences (Zhang, et al., 2019a).

4.2 Distinct seasonal assembly dynamics for ARG and prokaryotic communities

Many studies have shown that ARG and prokaryotic communities are shaped by different assembly processes (Guo, et al., 2018, Hou, et al., 2019a, Hou, et al., 2020, Peng, et al., 2020). In urban ponds, the ARG and prokaryotic taxonomic communities were mainly shaped by stochastic and deterministic processes, respectively (Hou, et al., 2020). On the contrary, deterministic and stochastic processes, respectively, affected ARG and prokaryotic community assembly in the urbanizing watershed (Peng, et al., 2020). Inconsistent with these previous results, we found that stochastic processes played a dominant role in the assembly of both the ARG and prokaryotic communities of Yundang lagoon. Although continuous actions and management have led to the improvement of the water quality and decreasing influence of human since 2000 (Uddin, et al., 2020), eutrophication was still one of the main environmental issues of Yundang lagoon (Zheng, et al., 2020). The eutrophication-induced high productivity may be one of the factors promoting the relative importance of stochastic processes in local community assembly (Zhou and Ning, 2017). Remarkably, our results showed that the seasonal variation in the relative importance of the stochastic processes was inconsistent between ARG and prokaryotic communities (Figs. 5 and S7). This indicated a decoupling of ARG and prokaryotic community assembly across seasons.

The high stochastic assembly from ARG communities in summer and winter resulted in a more stable antibiotic resistome than those from other seasons (Figs. 5A and S7). A similar phenomenon, that an increase in the proportion of WWTP effluents into the receiving water bodies might promote the stabilization of the ARG communities, has been observed previously (Corno, et al., 2019). Such stabilization of ARGs can occur even if the bacterial community composition is not stable (Corno, et al., 2019), which is consistent with our results (Fig. S8). Gao et al. (2020) have suggested that ecological processes underlying microbial taxonomy assembly may differ from those of regulating microbial gene structure along the dissemination chain of antibiotics (Gao, et al., 2020).

Our results revealed that the seasonal variation of the abundance of ARGs in Yundang lagoon was strongly associated with the variation of MGE abundances (Figs. 3C and D). MGEs played an important effect on the stochastic ratio of ARG communities (Figs. 6C and D), which may cause the variation of the assembly processes of ARG communities. The abundant MGEs could enhance the probability of HGT of ARGs (Xiang, et al., 2018), promoting stochastic processes (Hou, et al.,

2020) and differences of ARG and prokaryotic community assembly across seasons. Moreover, other factors including the water quality variables, human fecal markers and prokaryotic biomass also influenced the assembly dynamics of the ARG communities (Fig. 6). These results are consistent with the findings of Thornton et al. (2020) who observed that multiple environmental factors shaped ARG communities in a WWTP effluents affected urban watershed. Given the significant influence of fecal pollution on the diversity and abundance of ARGs (see discussion above), we proposed that fecal pollution may play a certain role in leading to the uncoupled relationship between the prokaryotic and ARG community assemblies. However, only a few environmental factors (i.e., temperature and pH) were found to associate with the stochasticity of the prokaryotic communities (Fig. S11), further investigations are needed to decipher the mechanisms underlying the assembly dynamics of lagoon prokaryotes.

4.3 Fecal pollution favoring the dominance of stochastic assembly for ARG communities

Our network analysis suggested that human fecal marker HF183-BacR287 strongly associated with multiple fecal prokaryotic genera, including *Bacteroides*, *Paludibacter*, *Prevotella*, *Tolumonas*, *Parabacteroides*, and *Phascolarctobacterium* (Fig. 7) (Deusch, et al., 2014, Liao, et al., 2018, Xu, et al., 2017). Moreover, as the keystone genera, *Bacteroidetes*, *Faecalibacterium*, *Paludibacter*, and *Prevotella*, all showed close relationships with many ARGs and other fecal genera, which have been found to be dominant and typical in the fecal microbiota of pigs (Kubasova, et al., 2018). This may indicate that ARGs have spread extensively across different strains, species or even mammal hosts, since no swine fecal markers were detected. It was also found that the relative abundance of *Bacteroides* and *Paludibacter* were significantly correlated with the aminoglycoside, β-lactamase, MLSB, sulfonamide, and tetracycline resistance genes, and the abundance of transposases in an urban river (Zhou, et al., 2017). Similarly, our previous study found that MLSB and tetracycline resistance genes had non-random co-occurrence with *Bacteroides*, *Blautia*, *Clostridium*, *Faecalibacterium*, and *Ruminococcus* in a subtropical watershed (Hu, et al., 2020). These phenomena provide further support that fecal pollution emerged as one of the major determinants for ARG profiles in urban lagoons.

Remarkably, one of the MGEs, *tnp*A-07, showed close associations with both multiple ARGs and fecal genera, indicating that *tnp*A-07 may play a central role in HGT of ARGs from fecal microorganisms to environmental microorganisms in Yundang lagoon. Considering the dominant role of MGEs in influencing ARG abundance and the stochastic ratio of ARG communities in Yundang lagoon (Fig. 6), the stability of ARGs in summer and winter mainly resulting from stochastic processes meditated by *tnp*A-07 (Figs. 5 and 7) via HGT, can be proposed. Fang et al. (2019) reported transposase genes IS613, *tnp*A-03, *tnp*A-04, and *tnp*A-07 showed a significant correlation with some types of ARGs (e.g., aminoglycoside, beta-lactam, and tetracycline) in an urban reservoir (Fang, et al., 2019). *Tnp*A-07 also co-occurred with β-lactamase, chloramphenicol, and MLSB resistance genes in rural environments (Cheng, et al., 2020).

Unlike the previous study where the resistome had a deterministic trajectory in the effluents contaminated freshwater bodies (Corno, et al., 2019), we observed a stochastic proliferation of the lagoon antibiotic resistome. In our study, fecal indicator genes were identified as one of the important drivers for regulating the stochastic assembly of ARG communities (Figs. 6B and D). Although, as mentioned earlier, MGEs played a distinguishing role in shaping ARG profiles, the input of fecal microorganisms can promote the compositional stochasticity of ARGs, via enhancement the prokaryotic biomass and MGE abundances. Lee et al. demonstrated that the mobility and HGT of human-related ARGs between taxa caused the ARG bloom in the downstream river (Lee, et al., 2020a). Therefore, fecal microorganisms may promote the HGT of ARGs among them and indigenous lagoon microorganisms, indirectly resulting in the dominance of stochastic assembly for the ARG communities. In addition, relatively high stochasticity levels (Fig. 5) might have resulted in the high abundance, richness and stable composition of ARG communities, which increases the risk of ARBs and ARGs spreading across the urban lagoons as well as human populations. Similarly, in Lake Baiyang sediment of China, the stochastic processes made a higher contribution than the deterministic processes on the ARG profile, where the total coverages and alpha-diversity of ARGs were significantly higher comparing to Lake Tai sediment (Chen, et al., 2021). Collectively, our findings improve the understanding of how fecal pollution affects the ARG community assembly in urban lagoons. Addressing how other factors affect the fecal microorganisms in transferring ARGs among themselves and environmental microorganisms is the next step for better management of the dissemination of antibiotic resistance in urban lagoons.

5. Conclusions

This study characterized the distribution pattern of ARGs in Yundang lagoon, China at different seasons, and clearly showed that human fecal pollution is one of the major causes of ARGs. Although stochastic processes play a dominant role in shaping both ARG and prokaryotic profiles, the seasonal assembly dynamics of ARG communities was inconsistent with that of prokaryotic communities. The stochastic processes of ARG profiles were mainly affected by fecal bacteria via promoting the prokaryotic biomass and MGE abundances. *Tnp*A-07, one of the MGEs, is the keystone of the HGT. These results enhanced our understanding of drivers and mechanisms of ARG assembly and dynamics in urban landscape lagoons. Our findings could also provide a reference for future work targeting microbial contaminants in urban coastal lagoon worldwide. Further studies combined with the high-frequency sampling and microcosm experiments should be undertaken to enhance our conclusions and establish the causal relationships between fecal pollution and ARG community assembly.

CRediT author statement

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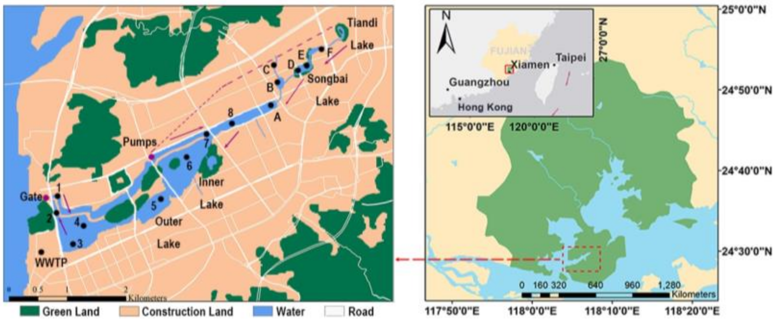


Fig. 1. Location and sampling map of the Yundang Lagoon, Xiamen, China. Purple arrows indicate the water flow direction in Yundang Lagoon. WWTP, wasterwater treatment plant. This map is revised based on our previous study (Wang et al., 2020).

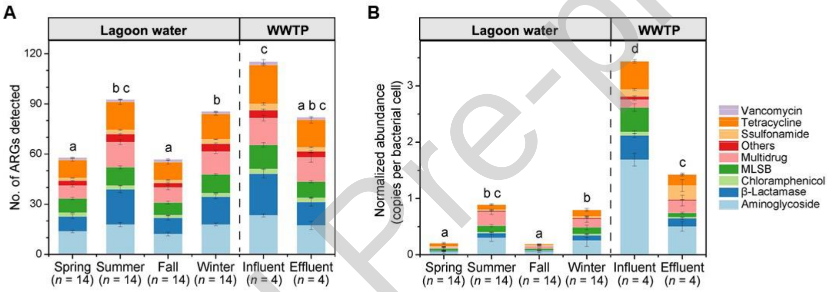


Fig. 2. (A) Number of ARGs detected in Yundang lagoon surface water in different sampling groups. (B) The normalized abundance of ARGs, expressed as copies per cell, in different sampling groups. ARGs are further classified into aminoglycosides, β-lactams, chloramphenicol, macrolide-lincosamide-streptogramin B (MLSB), multidrug, sulfonamides, tetracycline, vancomycin, or others. Gene copy numbers per cell were calculated based on the 16S rRNA gene copy numbers by assuming that the average number of 16S rRNA genes in each genome is 4.1 according to the Ribosomal RNA Operon Copy Number Database. Error bars represent standard error (s.e.) of sampling replicates (n= 14 or 4) in each group. Different lowercase letters (a, b, c and d) indicate significant differences (Kruskal-Wallis test, $P < 0.05$) between different groups (e.g., spring, summer, fall, winter, influent, and effluent). Marked with the same lowercase letters indicate no significant difference ($P > 0.05$).

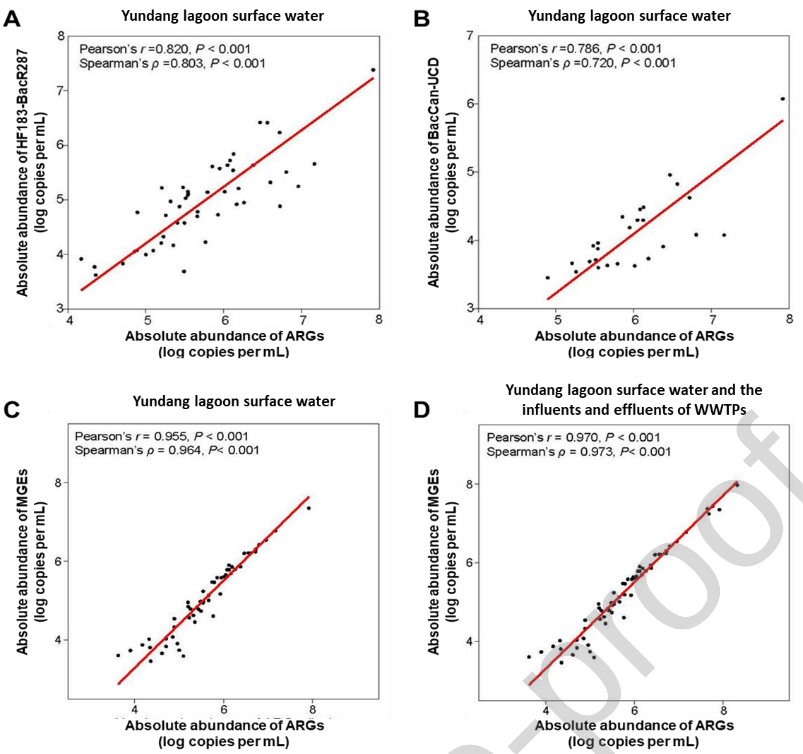


Fig. 3. Correlation between the absolute abundance of total ARGs and human (A) and dog (B) fecal indicator genes in Yundang lagoon surface water. Correlation between the absolute abundance of ARGs and MGEs within Yundang lagoon surface water (C) and the surface water of Yundang lagoon and the influents and effluents of WWTPs (D).

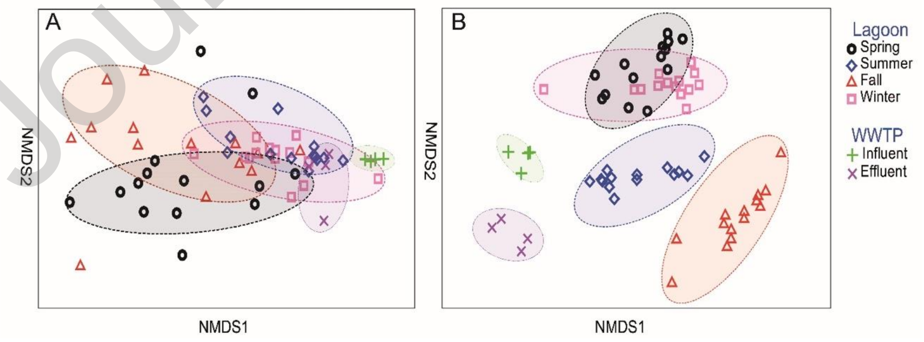


Fig. 4. Non-metric multidimensional scaling (NMDS) plots of the distribution patterns of the antibiotic resistome (A) and the prokaryotic community (B) in Yundang lagoon surface water in different seasons and the influents and effluents of WWTPs.

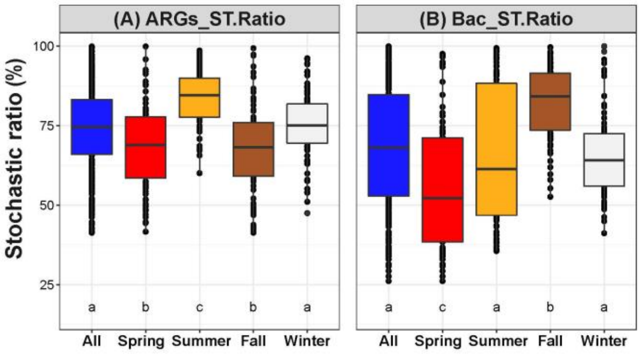


Fig. 5. Changes of stochastic ratio of the antibiotic resistome (A) and prokaryotic communites (B) in Yudang lagoon surface water in different seasons. The column labeled with "All" inidcated the average compositional stochasticity for all seasons. Different lowercase letters indicate significant differences (Kruskal-Wallis test, $P < 0.05$) between different groups.

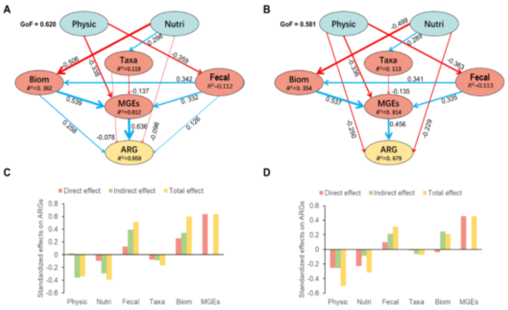


Fig. 6. Partial least squares path models (PLS-PM) showing the effects of different factors on ARG absolute abundance (A) and stochastic ratio of ARG communities (B) in lagoon water. Standardized direct and indirect effects on the ARG absolute abundance (C) and stochastic ratio of ARG communities (D) are calculated from the PLS-PM. Water physicochemical variables (Physic) include DO and pH. Nutrients (Nutri) included DIN and SRP. Fecal indictor genes (Fecal) includes human fecal indicator gene (HF183-BacR287) and dog fecal indicator gene (BacCan-UCD). Taxonomic community (Taxa) was based on dimension reduced (PCoA with Bray-Curtis similarity distance) relative abundance of dominant prokaryotic phyla (≥ 0.5%). Prokaryotic biomass (Biom) and MGEs was based on the total abundance of 16S rRNA genes and MGEs, respectively. Models were assessed using goodness of fit (GoF) statistic. Blue and red arrows indicate positive and negative effects, respectively. Abiotic factors, biotic factors and ARGs are marked by blue, red, and yellow, repsectively. The higher path coefficients are shown as wider arrows. Solid lines indicate significant ($P < 0.05$) relationships. Path coefficients and coefficients of determination (R^2) are calculated after 999 bootstraps.

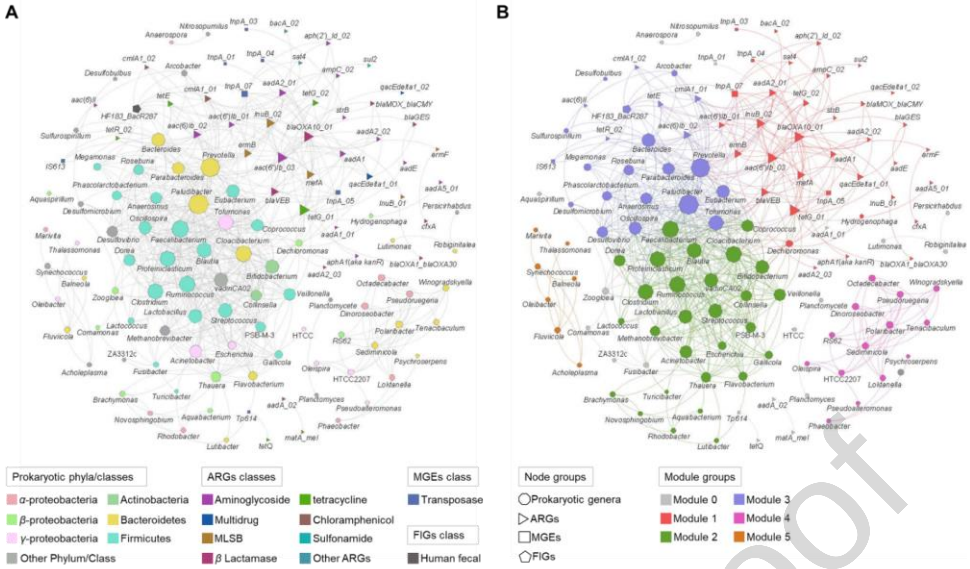
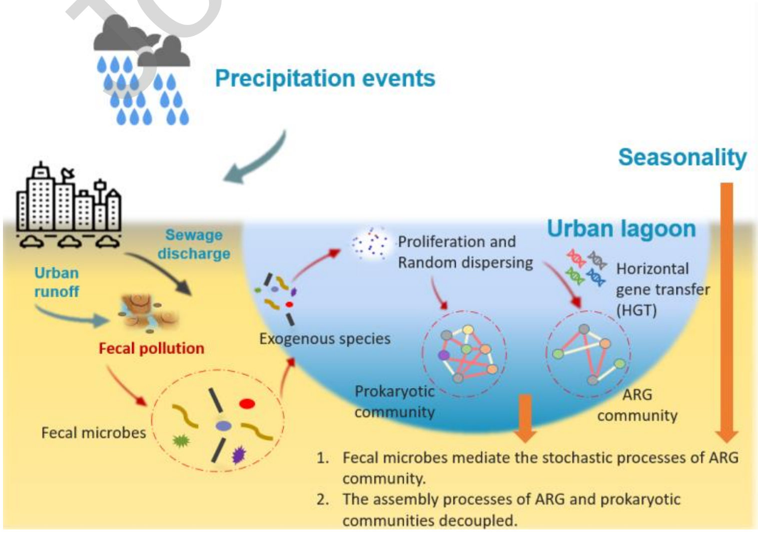


Fig. 7. Network of co-occurring prokaryotic genera, fecal indicator genes (FIGs), ARGs, and MGEs in lagoon water. (A) Colors of the nodes indicate different phyla/classes of prokaryotes, fecal sources, or classes of ARGs and MGEs. (B) Colors of the nodes indicate different modules. Each node represents a genus of prokaryotes (octagon), FIG (pentagon), ARG (triangle) or MGE (quadrangle). The size of each node is proportional to the number of connections (i.e., degree). The edges indicate strong (Pearson correlation coefficient r > 0.69) and significant positive correlations ($P < 0.05$) between nodes.

Graphical abstract



1. Fecal microbes mediate the stochastic processes of ARG community.
2. The assembly processes of ARG and prokaryotic communities decoupled.

CRediT author statement

Liyuan Hou: Conceptualization, Formal analysis, Validation, Writing – original draft, Visualization. **Hongjie Wang**: Investigation, Methodology, Formal analysis, Data curation, Software, Visualization. **Qinfu Chen**: Investigation, Methodology, Resources. **Jianqiang Su**: Methodology, Resources. **Mahmoud Gad:** Methodology, Resources, Visualization. **Jiangwei Li**: Investigation, Methodology. **Sikandar I. Mulla**: Investigation, Methodology. **Chang-Ping Yu**: Resources, Supervision, Funding acquisition. **Anyi Hu**: Conceptualization, Data curation, Software, Validation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Fecal pollution is the main pollution source of ARGs in Yundang lagoon.
- Stochastic processes play a major role in shaping ARG and prokaryotic profiles.
- ARG but not prokaryotes profiles had higher stochasticity in summer and winter.
- Fecal microbes mediate the stochastic processes of ARG profiles via HGT.
- The transposase gene *tnp*A-07 was one of the key MGEs for HGT.