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# Horizontal and vertical gene transfer drive sediment antibiotic resistome in an urban lagoon system

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## ABSTRACT

Rapid urbanization has resulted in pervasive occurrence of antibiotic resistance genes (ARGs) in urban aquatic ecosystems. However, limited information is available concerning the ARG profiles and the forces responsible for their assembly in urban landscape lagoon systems. Here, we employed high-throughput quantitative PCR (HT-qPCR) to characterize the spatial variations of ARGs in surface and core sediments of Yundang Lagoon, China. The results indicated that the average richness and absolute abundance of ARGs were 11 and 53 times higher in the lagoon sediments as compared to pristine reference Tibetan lake sediments, highlighting the role of anthropogenic activities in ARG pollution. Co-occurrence network analysis indicated that various anaerobic prokaryotic genera belonging to Alpha-, Deltaproteobacteria, Bacteroidetes, Euryarchaeota, Firmicutes and Synergistetes were the potential hosts of ARGs. The partial least squares-path modeling (PLS-PM) analysis revealed positive and negative indirect effects of physicochemical factors and heavy metals on the lagoon ARG profiles, via biotic factors, respectively. The horizontal (mediated by mobile genetic elements) and vertical (mediated by prokaryotic communities) gene transfer may directly contribute the most to drive the abundance and composition of ARGs, respectively. Furthermore, the neutral community model demonstrated that the assembly of sediment ARG communities was jointly governed by deterministic and stochastic processes. Overall,

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this study provides novel insights into the diversity and distribution of ARGs in the benthic habitat of urban lagoon systems and underlying mechanisms for the spread and proliferation of ARGs.

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## Introduction

Antibiotic resistance genes (ARGs), either induced or acquired by the bacterial cells to transform into antibiotic resistant bacteria (ARB), are among the emerging environmental concerns (Roope et al., 2019). ARGs disseminate by the moving ARBs and transfer into other bacteria via horizontal gene transfer (HGT) through mobile genetic elements (MGEs) (i.e., integrons, plasmids and transposons) (Pruden et al., 2013; Qiao et al., 2018; Zhu et al., 2018). The enrichment of ARGs in the urban ecosystems by various human activities (e.g., widespread antibiotic use, organic fertilization and sewage discharge) and high environmental persistence of ARGs are threatening for human health (Collignon et al., 2018; Zhu et al., 2018). Consequently, the antibiotic resistance has become a priority area among the public health concerns (Pruden et al., 2013; Roope et al., 2019). Hence, a comprehensive understanding of the assembly processes involved in the ARG communities in the urban ecosystems is direly needed for devising strategies to manage the dissemination and environmental accumulation of ARGs.

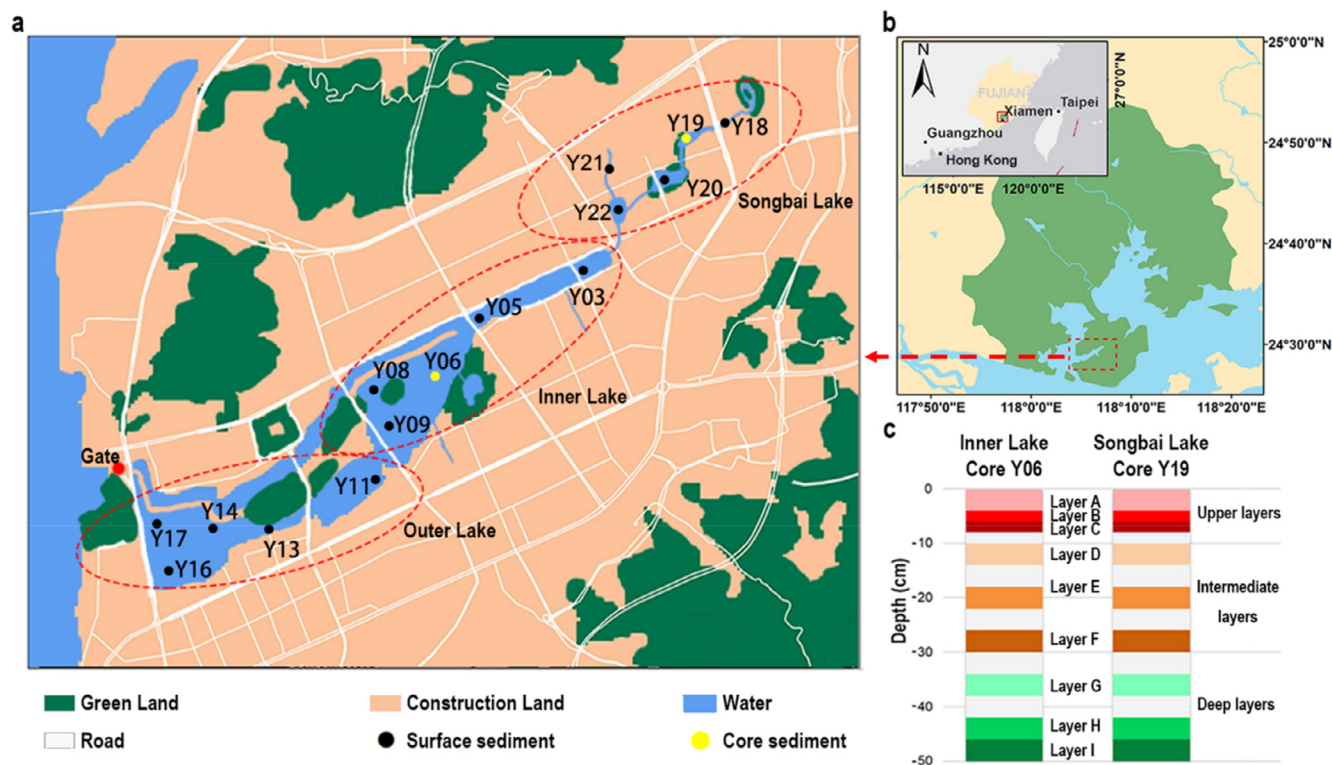
Urban aquatic ecosystems are hotspots for the spread of ARGs (Peng et al., 2020; Rodriguez-Mozaz et al., 2015). In the urban aquatic ecosystem, sediments not only act as sink but also become secondary sources of ARG contamination (Mao et al., 2014). For instance, recent studies have reported that treated and untreated wastewater and urban runoff introduced ARBs and ARGs to the urban aquatic sediments and eventually to the marine sediments (Guo et al., 2018b; Rodriguez-Mozaz et al., 2015; Zhang et al., 2016; Zhao et al., 2020; Zhu et al., 2017). Meanwhile, it is highly possible that ARGs from the sediment phase may enter into the water phase by physical (e.g., resuspension and diffusion) and biological (e.g., HGT and food chains) processes, thereby causing secondary pollution of the water bodies (Di Cesare et al., 2020; Han et al., 2017; Hes et al., 2018; Mao et al., 2014; Yang et al., 2016). However, there is a knowledge gap about the assembly of ARGs in the coastal lagoon sediments and about the responsible driving forces (Audouit et al., 2017). The coastal lagoon sediments have 13% share of the coastlines around the world and play important role in supporting biological productivity, biodiversity conservation, and economic and sociocultural activities (Audouit et al., 2017). Since the urban lagoons have been under the influence of intensive anthropogenic disturbances (Audouit et al., 2017; Wang et al., 2020a), the spread and accumulation of ARGs and ARBs may occur in urban lagoons and pose a great threat to urban dwellers.

It has been suggested that chemical pollutants, such as antibiotics and heavy metals were mainly responsible for the dissemination and assembly of ARGs in sediments of the

aquatic ecosystems (Dickinson et al., 2019; Ohore et al., 2020; Zhang et al., 2016; Zhu et al., 2018). However, other abiotic factors (e.g., temperature, pH, salinity and nutrients) might also pose selective stresses in driving the evolution, proliferation and spread of ARGs (Guo et al., 2018b; Ohore et al., 2020; Zhang et al., 2019). Biotic factors including prokaryotic communities and MGEs can also influence the sediment ARG profiles. Some earlier studies suggest that the abundance and composition of ARGs were greatly altered by prokaryotic communities through vertical gene transfer (VGT) in the estuarine sediments (Zhao et al., 2020; Zhou et al., 2017; Zhu et al., 2017), while in others, MGEs mediated HGT processes were proposed as one of the major mechanisms responsible for determining the ARG dynamics in urban aquatic ecosystems (Fang et al., 2019; Peng et al., 2020). However, little information is available concerning the relative importance of such abiotic and biotic factors that influence the ARG profiles in urban lagoon sediments.

In order to predict the ARG dissemination and to mitigate their contamination, exploration of the ecological processes underlying the ARG community assembly is of great importance (Hu et al., 2020). Empirical evidence suggests that deterministic and stochastic processes drive the microbial community assembly, simultaneously (Zhou and Ning, 2017). A case study on a drinking water reservoir showed that stochastic processes played a dominant role in the assembly of ARG communities (Guo et al., 2018c). In contrast, the deterministic processes predominated in shaping the ARG community composition in downstream area of an urbanized river (Fang et al., 2019; Peng et al., 2020). Moreover, Hu et al. (2020) found that the spread of class 1 integron *intI1* could motivate the deterministic processes (i.e., homogenizing selection) for stable ARG communities in two disconnected sub-watersheds. However, there is still a knowledge gap on the relative importance of deterministic and stochastic processes in driving the ARG community assembly in sediments, especially in urban aquatic ecosystems.

In the present study, we employed high-throughput quantitative PCR (HT-qPCR) to profile the abundance and composition of ARGs in surface and core sediments from an urban lagoon (Yundang Lagoon) located in southeast China. We aimed to: (1) evaluate the ARG contamination levels in surface and core sediments of the lagoon; (2) distinguish the relative importance of abiotic (i.e., sediment physicochemical variables and heavy metals) and biotic factors (i.e., MGEs, prokaryotic biomass and community composition) in influencing the ARG profiles; and (3) uncover the ecological processes underlying the ARG community assembly. We hypothesized that the abiotic and biotic factors could indirectly and directly affect the ARG profiles, respectively. Where, the major effects of abiotic factors may be mediated via biotic factors, thereby making the



**Fig. 1 – (a) Map of sampling sites in Yundang Lagoon. The circles in black and yellow represent the sites. Sites Y03, Y05, Y06, Y08 and Y09 were in Inner Lake; Sites Y11, Y13, Y14, Y16 and Y17 were in Outer Lake; Sites Y18, Y19, Y20, Y21 and Y22 were in Songbai Lake. (b) Location of Yundang Lagoon in Xiamen, China. (c) Overview of core sediments from Site Y06 (Inner Lake) and Site Y19 (Songbai Lake). Upper layers include segments A, B and C; Intermediate layers include segments D, E and F; and deep layers include segments G, H and I of the sediment core.**

deterministic processes predominantly responsible for shaping the assembly of ARG communities.

## 1. Material and methods

### 1.1. Site description

Yundang Lagoon, constructed by altering the natural Yundang Harbor is located in a highly urbanized city of Xiamen south-east China (Fig. 1a and b). The urbanization rate of the city was estimated as 85.2% in 2017 (Xiamen Municipal Bureau of Statistics, 2018; <http://www.stats-xm.gov.cn/>). The opening of the Yundang Harbor was closed by land reclamation to transform the harbor into a semi-closed coastal lagoon. Nowadays, the Yundang Lagoon has a catchment area of ~37 km<sup>2</sup> (accounting up 1/3 of Xiamen Island) with surface water area and average depth of ~1.6 km<sup>2</sup> and ~2.5 m, respectively (Wang et al., 2020a). Yundang Lagoon is composed of Outer, Inner, and Songbai lakes extending from west to east (Fig. 1a). As part of an urban water body, Yundang Lagoon is in a serious eutrophic state and has been contaminated with organic and inorganic contaminants originating from the domestic wastewater and sewage discharge, especially due to rainy season runoff (Chen et al., 2010; Uddin et al., 2020; Wang et al., 2020a).

### 1.2. Field sampling and physicochemical analysis

Sediment samples were collected from Yundang Lagoon during 27–28th June 2017. Two sediment sampling strategies were applied for this study. Firstly, a total of 15 surface sediments (0–5 cm sampling depth) were collected from five sites each of Outer, Inner, and Songbai lakes of Yundang Lagoon (Fig. 1a). For comparison/reference purpose, surface sediment samples from three salt lakes in Tibetan Plateau (i.e., Awong Co, Bieruoze Co and Tuosu Hu lakes, Appendix A Fig. S1) (Guo et al., 2018a; Xiong et al., 2012). These lakes had similar salinity levels but experienced relatively few anthropogenic disturbances as compared to the Yundang lagoon.

In the second strategy, core sediment samples (6 cm diameter, 50 cm depth) were collected from Inner (Site Y06, 24°28'52"N, 118°6'18"E) and Songbai lakes (Site Y19, 24°29'38"N, 118°7'34"E), respectively (Fig. 1a). Based on the average sedimentation rate (4 cm/year) in the investigated coastal area (Yu et al., 2016), each core sediment sample was divided into 9 sub-sections A (0–4 cm), B (4–6 cm), C (6–8 cm), D (10–14 cm), E (18–22 cm), F (26–30 cm), G (34–38 cm), H (42–46 cm) and I (46–50 cm). The sub-sections A, B and C represented the upper layer; D, E and F represented the intermediate layer; and G, H and I represented the deep layer sediments (Fig. 1c).

The sediment samples were transferred into pre-sterilized 50 mL Falcon tubes and stored at –80°C until further analysis. The water content of sediment sample was measured ac-

according to a previous study (Magni et al., 2019). Sediment samples were freeze-dried, ground and passed through a 2 mm mesh sieve. Subsequently, carbon, nitrogen and sulfur contents (TC%, TN% and TS%) were analyzed by using a Vario Max CNS elemental analyzer (Elementar, Germany). The particle size of the sediments (D50) was determined by a Mastersizer 3000 laser particle size analyzer (Malvern, UK) (Magni et al., 2019). Eight heavy metal elements (Cd, Pb, Cr, Mn, As, Ni, Cu and Zn) were measured in the sediment samples by inductively coupled plasma mass spectroscopy (ICP-MS) (Agilent 7500CX, Agilent Technologies, USA) as described in our previous study (Nkinahamira et al., 2019). The pH and electronic conductivity (EC) of the sediment samples were determined by a multi-parameter meter (HACH, USA) in a 1:2.5 (W/V) suspension of dried sediments and distilled water. The sediment samples were extracted with 2 mol/L KCl and dissolved inorganic nitrogen (DIN),  $\text{NH}_4\text{-N}$ ,  $\text{NO}_x\text{-N}$  ( $\text{NO}_2\text{-N} + \text{NO}_3\text{-N}$ ) and soluble reactive phosphorus (SRP) were determined by using Lachat QC8500 Flow Injection Auto-analyzer (Lachat Instruments, USA) (Hu et al., 2014).

### 1.3. DNA extraction, 16S rRNA gene amplicon sequencing and sequence analysis

An aliquot (0.3 g) of sediment sample was used for the extraction of environmental DNA by using a modified protocol established previously (Hu et al., 2014). The universal primer pair 515F (5'-GTG YCA GCM GCC GCG GTA-3') and 907R (5'-CCG YCA ATT YMT TTR AGT TT-3') were used to amplify V4 and V5 regions of 16S rRNA genes. The PCR product was purified and mixed at equal concentrations and then sequenced on an Illumina MiSeq platform (Majorbio Bio-Pharm Technology Co. Ltd., China) with paired-end strategy ( $2 \times 300$  bp). The sequence data generated in this study is publicly available at the NCBI Short Reads Archive (SRA) database under accession number PRJNA578248.

The raw sequences were denoised and assembled by using the DADA2 v1.13 (Callahan et al., 2016; Hu et al., 2019). Subsequently, the high-quality sequences were clustered into amplicon sequence variants (ASVs) with 100% identity. The taxonomic assignment was conducted by using the RDP classifier with the SILVA database v132 (Quast et al., 2013) at 80% confidence threshold.

### 1.4. HT-qPCR and real-time qPCR

The HT-qPCR was performed via the SmartChip Real-time PCR (Warfergen Inc., Fremont, USA) based on previous studies (Wang et al., 2020a; Zhu et al., 2017). A total of 296 primer sets including 285 primer sets for all major classes of ARGs, 10 primer sets for MGEs (8 transposase genes and 2 integrase genes) and one primer set for 16S rRNA genes were applied (Guo et al., 2018c; Zhu et al., 2017). The qPCR reaction system and amplification conditions were performed as described previously (Guo et al., 2018c; Zhu et al., 2017). All qPCR reactions were conducted in three technical replicates, and each chip included the 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., 2018c; Looft et al., 2012). Relative abundance (copy number of target

gene/copy number of 16S rRNA genes) and normalized abundance (copy number of target gene/bacteria cell) of ARGs and MGEs were calculated according to previous studies (Guo et al., 2018c; Wang et al., 2020b; Zhu et al., 2017).

The absolute abundance of ARGs and MGEs was calculated by using the normalized abundance to the absolute copy number of 16S rRNA genes (Wang et al., 2020b), which was quantified by real-time qPCR. All real-time qPCR reactions were performed in triplicate for each sample with negative controls on a LightCycler 480 instrument (Roche, Switzerland) by using a SYBR® Green I based approach (Guo et al., 2018c; Su et al., 2015).

### 1.5. Network analysis

The co-occurrence pattern among ARGs, MGEs and prokaryotic genera was identified by employing network analysis by using an online tool-Molecular Ecological Network Analyses Pipeline (<http://ieg4.rccsu.edu/MENA/>). Briefly, only the variables (i.e., ARGs, MGEs and prokaryotic genera), with > 50% occurrence in all samples (> 16 of 33 samples) were used for building the network (Wang et al., 2020a). Prior to the construction of a Pearson correlation-based network, the variables were log-transformed and missing values were filled with a very small number (0.01) if paired valid values were available (Deng et al., 2012). This step can ensure a more statistically reliable correlation coefficient between two variables. A co-occurrence association was considered statistically robust with a Pearson correlation coefficient ( $r$ ) > 0.77 and  $P$  value < 0.05 (Deng et al., 2012). The network was visualized by Gephi v0.9.2 (<https://gephi.github.io/>).

### 1.6. Statistical analyses

Principal component analysis (PCA) was used to identify the variation in sediment physicochemical properties by using PAST v2.03 (Hammer et al., 2001). The Kruskal-Wallis test was performed to test the statistical significance of sediment physicochemical properties among different groups (i.e., Outer, Inner and Songbai lakes) by using the R package *pgirmess* (Giraudeau et al., 2018). The Mantel test was used to explore the relationship between the composition of ARGs and prokaryotic communities by using the R package *vegan* (Oksanen et al., 2019). Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distance matrices was used to explore the variation in the composition of ARG communities by using PAST v2.03 (Hammer et al., 2001). ANOSIM test was conducted to test the statistically significant differences in the composition of ARGs from different lagoon areas (e.g., Outer, Inner and Songbai lakes). Furthermore, the correlation between the absolute abundance of ARGs and MGEs was determined by using Pearson correlation and Spearman rank correlation tests.

Partial least squares-path modeling (PLS-PM) was carried out to evaluate the direct and indirect effects of biotic (e.g., prokaryotic communities and MGEs) and abiotic (e.g., physicochemical variables and heavy metals) factors on the abundance and composition of ARG profiles by using the R package *plsppm* (Sanchez et al., 2015). Initially, the permutation test (999

simulations) was used to identify the variables with significant effect on ARG profiles. The significant variables were then divided into five groups: physicochemical variables (i.e., water content, TN% and TC%), heavy metals (i.e., Cd, Pb, Cr, Mn, As, Ni, Cu and Zn), prokaryotic communities (dominant prokaryotic phyla), prokaryotic biomass (i.e., the absolute abundance of 16S rRNA genes) and MGEs. The overall performance of the PLS-PM model was evaluated by using the goodness of fit index (GoF).

The neutral community model (NCM) was employed to evaluate the relative importance of stochastic processes (e.g., random dispersal and ecological drift) on the assembly of prokaryotic taxonomic (i.e., ASVs) and functional (i.e., ARGs) communities (Hu et al., 2019; Peng et al., 2020; Sloan et al., 2006). The parameter  $R^2$  was used to estimate the percentage of the community explained by the NCM, since, it could predict the overall fit to the model (Sloan et al., 2006). The parameter  $Nm$ , which is used to estimate the dispersal between communities, was calculated as the community size ( $N$ ) times the immigration rate ( $m$ ) (Peng et al., 2020).

## 2. Results

### 2.1. Physicochemical properties of sediment in Yundang Lagoon

PCA ordination indicated that the physicochemical properties of the surface sediments from different areas of Yundang Lagoon (i.e., Outer, Inner, and Songbai lakes) tended to form different clusters (Appendix A Fig. S2). The results of Kruskal-Wallis test demonstrated that the grain size (D50), SRP and DIN in surface sediments from Songbai Lake were significantly higher ( $P < 0.05$ ) than those in the Inner Lake (Appendix A Fig. S3). Moreover, the core sediments from Songbai Lake had significantly higher grain size (D50), water content, TN%, TC%, and heavy metal (Cd, Pb, As, Ni, Cu and Zn) contents than those in Inner Lake sediments (Kruskal-Wallis test,  $P < 0.05$ ), while the DIN and  $\text{NH}_4\text{-N}$  of the core sediments showed a reverse pattern (Kruskal-Wallis test,  $P < 0.05$ ) (Appendix A Fig. S4). At vertical scale, EC, DIN and  $\text{NH}_4\text{-N}$  showed a decreasing trend from upper to deep layers of the core sediment samples (Appendix A Figs. S5 and S6).

### 2.2. Occurrence and abundance of sediment antibiotic resistome

A total of 84 ARGs and 3 MGEs were detected in Yundang Lagoon sediments, while only 4 ARGs and 1 MGEs were detected in Tibetan lake sediments. The detected ARGs mainly belonged to aminoglycoside,  $\beta$ -lactam, multidrug and macrolide-lincosamide-streptogramin B (MLSB) types (Fig. 2c, d and Appendix A Fig. S7). Songbai Lake had the highest average numbers of ARGs (33), while Tibetan lakes had lowest (2) number of ARGs in the surface sediments (Appendix A Fig. S7a). Similarly, Songbai Lake had highest absolute and normalized abundances of ARGs and MGEs (average  $1.1 \times 10^{10}$  copies/g and 0.94 copies per bacterial cell for ARGs,  $3.2 \times 10^8$  copies/g and 0.02 copies per bacterial cell for MGEs), followed

by Outer (average  $1.8 \times 10^9$  copies/g and 0.20 copies per bacterial cell for ARGs,  $6.2 \times 10^7$  copies/g and 0.01 copies per bacterial cell for MGEs) and Inner lakes (average  $4.4 \times 10^8$  copies/g and 0.13 copies per bacterial cell for ARGs,  $1.8 \times 10^7$  copies/g and 0.01 copies per bacterial cell for MGEs) (Fig. 2a and c). Not surprisingly, Tibetan lake sediments harbored the lowest absolute and normalized abundances of ARGs (average  $8.6 \times 10^7$  copies/g and 0.01 copies per bacterial cell) (Fig. 2a and c).

The core sediments showed a decrease in the average detected ARGs with increase in the depth with the exception of the upper layers of the Core Y19, which harbored the lowest average number of ARGs (Appendix A Fig. S7b). A similar decreasing trend was also found for the absolute abundance of ARGs (Fig. 2b). However, different patterns were observed for the normalized abundance of ARGs (Fig. 2d). The intermediate layers exhibited the highest ( $2.43 \pm 0.54$ ), while the upper layer had the lowest ( $1.36 \pm 0.27$ ) normalized abundance of ARGs in the Core Y19 of Songbai Lake. Meanwhile, the upper layers of Core Y06 from Inner Lake exhibited the highest normalized abundance of ARGs ( $0.17 \pm 0.06$ ), followed by the deep ( $0.15 \pm 0.12$ ) and intermediate ( $0.10 \pm 0.06$ ) layers.

A significantly positive correlation ( $r = 0.732$ ,  $P < 0.001$ ) was found between the absolute abundance of total ARGs and MGEs in Yundang Lagoon sediments (Appendix A Fig. S8a). Similar results were also found in different sampling groups, including surface sediments ( $r = 0.716$ ,  $P < 0.01$ ), core sediments ( $r = 0.844$ ,  $P < 0.01$ ) and sediments from Songbai Lake ( $r = 0.879$ ,  $P < 0.001$ ) (Appendix A Fig. S8b and c).

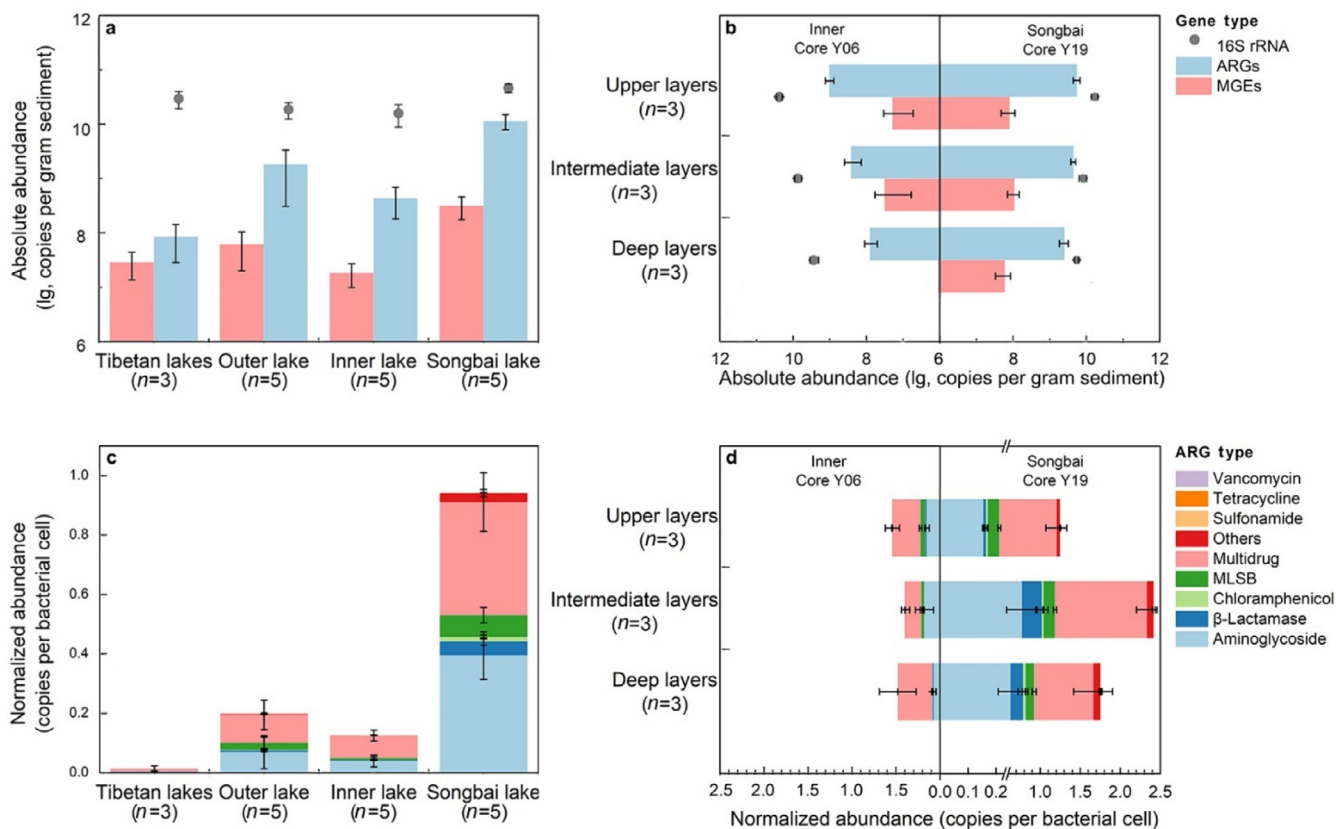
### 2.3. Patterns and assembly processes of sediment antibiotic resistome

NMDS ordination analysis showed that ARG communities from the surface sediments of Songbai and Tibetan lakes had unique ARG community compositions, while sediment samples from Outer and Inner lakes tended to cluster together (Fig. 3a). This pattern was also supported by the results of ANOSIM test ( $P < 0.05$ ). The core sediment samples from Songbai and Inner lakes formed distinct clusters, while the different sediment layers tended to cluster separately (Fig. 3b). Moreover, the Mantel test indicated that the ARG community composition was closely associated with that of prokaryotic communities ( $r = 0.801$ ,  $P = 0.001$ ).

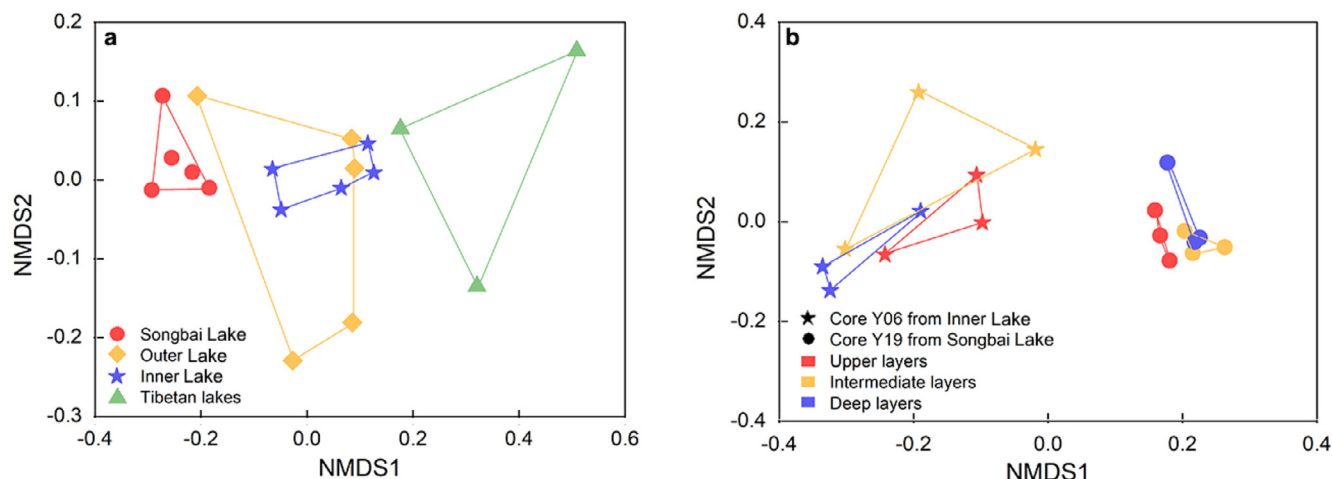
The NCM models explained a medium proportion in the variations of occurrence of prokaryotic ASVs ( $R^2 = 0.450$ ) and ARGs ( $R^2 = 0.466$ ) (Fig. 4). These results suggest that stochastic processes played certain role in shaping the assembly of both prokaryotic and ARG communities.

### 2.4. Characterization of prokaryotic biomass and community composition

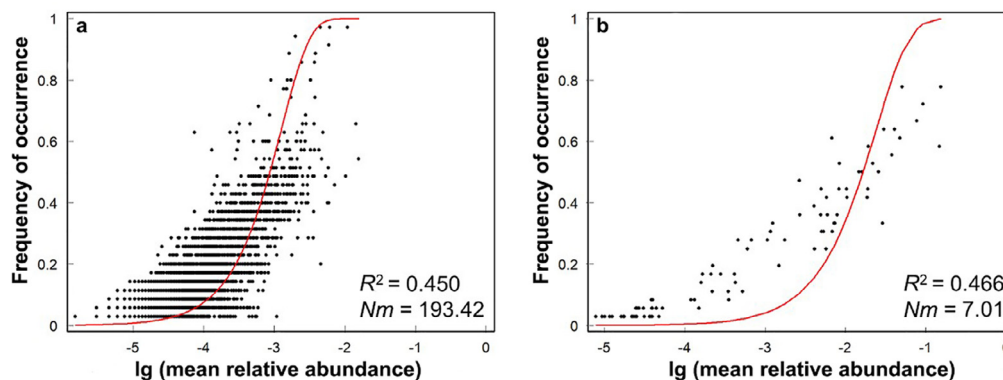
The results of the real-time PCR analysis demonstrated that Songbai Lake had the highest absolute abundance of prokaryotic 16S rRNA genes (average  $4.6 \times 10^{10}$  copies/g), followed by Outer (average  $1.9 \times 10^{10}$  copies/g) and Inner lakes (average  $1.6 \times 10^{10}$  copies/g) (Fig. 2a). For the vertical scale, the average absolute abundance of prokaryotic 16S rRNA genes decreased with increasing depth of the core sediments (Fig. 2b).



**Fig. 2 – Absolute abundance of 16S rRNA genes, ARGs and MGEs, expressed as copies per gram of the sediments in different sampling groups (a: surface sediments; b: core sediments). The normalized abundance of ARGs, expressed as copies per cell, in different sampling groups (c: surface sediments; d: core sediments). ARGs are further classified into aminoglycosides,  $\beta$ -lactams, chloramphenicol, macrolide-lincosamide-streptogramin B (MLSB), multidrug, sulfonamides, tetracycline, vancomycin and others. Gene copy numbers per cell are calculated on the basis of 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 ( $n = 3$  or 5) in each group.**



**Fig. 3 – Non-metric multidimensional scaling (NMDS) plots for the distribution patterns of ARG communities from different sampling groups. (a) Surface sediments of Yundang Lagoon and Tibetan lakes. (b) Core sediments from site Y06 (Inner Lake), and site Y19 (Songbai Lake). Each core sediment was divided into upper, intermediate and deep layers.**



**Fig. 4 – Neutral community model applied to assess the relative importance of stochastic processes on the assembly of prokaryotic (a) and ARG communities (b).  $R^2$  indicates the goodness-of-fit for the neutral model.  $Nm$  indicates the community size ( $N$ ) multiplies immigration ( $m$ ). The solid red lines indicate the best-fit to the neutral model.**

A total of 1,275,511 high-quality sequences were generated from all 33 samples, which were clustered into 12,017 ASVs. The results of the taxonomic assignment indicated that Bacteroidetes, Deltaproteobacteria, Gammaproteobacteria, Fimicutes, Chloroflexi, Euryarchaeota, Acidobacteria, Planctomycetes, Synergistetes and Actinobacteria were the most 10 abundant phylum/class in the lagoon sediments, accounting for average 14.6%, 13.1%, 10.2%, 8.7%, 7.9%, 7.2%, 4.7%, 4.7%, 3.5% and 3.3% of the total 16S rRNA gene sequences, respectively (Appendix A Fig. S10). Furthermore, SC103, DMER64, *Lutimonas*, *Woeseia*, *Methanobacterium*, *Mesotoga*, *Romboutsia*, *Methanosaeta*, *Clostridium\_sensu\_stricto\_1* and *Actibacter* were the top 10 dominant genus in the lagoon sediments that accounted for 1.8%, 1.7%, 1.6%, 1.5%, 1.4%, 1.2%, 1.2%, 1.0%, 1.0% and 0.9% of the total communities, respectively.

### 2.5. Associations among ARGs, MGEs and prokaryotic genera

The network analysis indicated that all ARG subtypes, except for *mexF* (multidrug), fell into one of the three network modules (module 2), and had significantly positive associations with various anaerobic prokaryotic genera (Fig. 5). For example, *Desulfomicrobium* (Deltaproteobacteria), DMER64 (Bacteroidetes), *Paludibacter* (Bacteroidetes), *Clostridium\_sensu\_stricto\_1* (Firmicutes), *Methanosaeta* (Euryarchaeota), *Methanospirillum* (Euryarchaeota) and *Thermovirga* (Synergistetes) were closely associated with aminoglycoside (e.g., *aadA*, *aadA2* and *aac(6′)-Ib*), multidrug (e.g., *qacEdelta1* and *ereA*) and MLSB (e.g., *ermF*) resistance subtypes. Moreover, several fecal- (i.e., *Gallicola*, *Intestinibacter* and *Methanobacterium*) (Ditchfield et al., 2012; Naphtali et al., 2019; Ohashi and Fujisawa, 2019) and sewage-prokaryotic genera (i.e., *Defluviococcus* and *Trichococcus*) (Burow et al., 2007; McLellan and Roguet, 2019) from module 0 also showed significant associations with several ARG subtypes (e.g. *aadA*, *aadA1*, *aadA2*, *aac(6′)-Ib*, *qacEdelta1*, *ereA* and *catB3*). In addition, the *int11* was found to be associated with four aminoglycoside (*aadA\_01*, *aadA2\_01*, *aac(6′)-Ib\_01* and *aac(6′)-Ib\_03*), one mul-

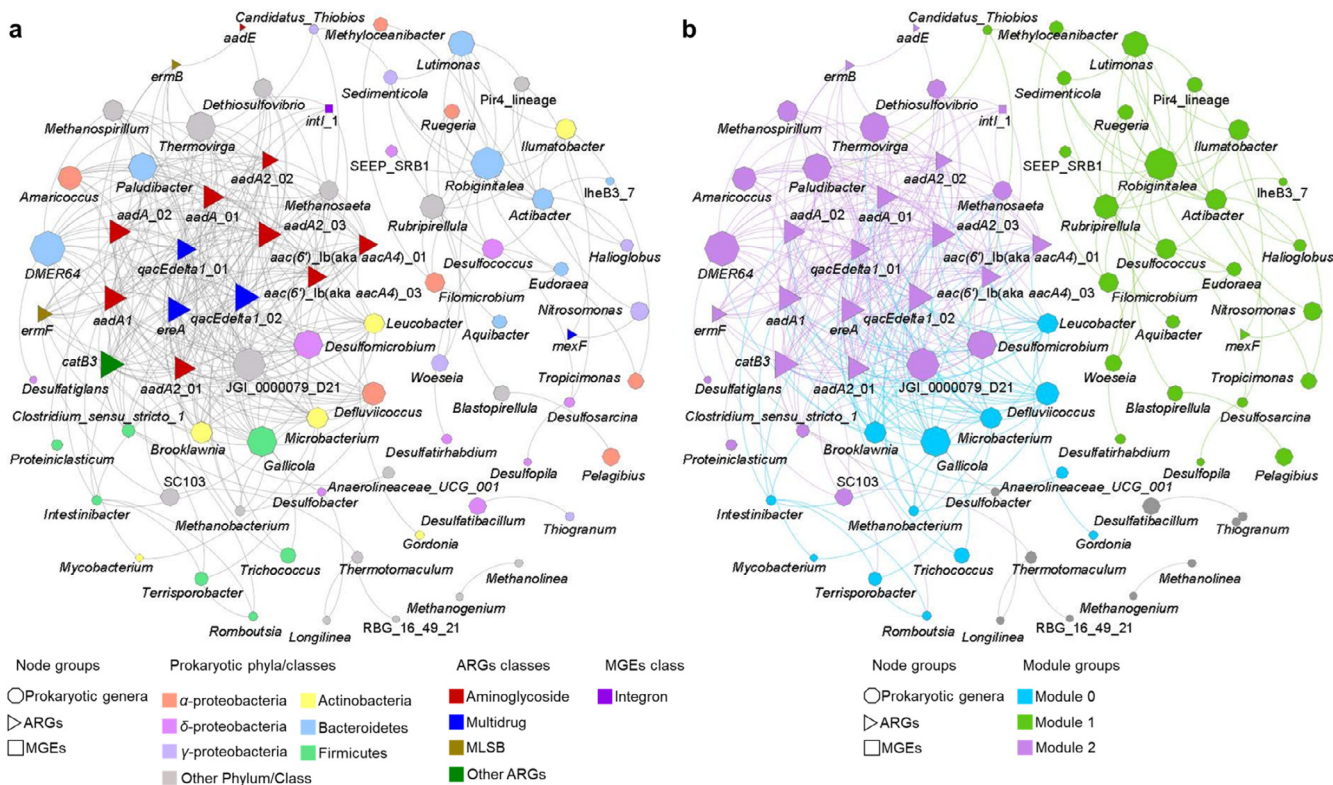
tidrug (*qacEdelta1\_02*) resistance subtypes and genus *Thermovirga*.

### 2.6. Effects of abiotic and biotic factors on the ARG profiles

The PLS-PM analysis was used to assess the direct and indirect effects of abiotic and biotic factors on the ARG profiles in Yundang Lagoon sediments (Fig. 6a and b). It was found that MGEs and prokaryotic community compositions (Taxa) had the largest positive total standardized effects (sum of direct and indirect effects) on the abundance (Fig. 6c) and composition (Fig. 6d) of ARGs, respectively. Furthermore, the PLS-PM analysis also suggested that the sediment physicochemical variables (Env) were positively associated with the abundance of ARGs indirectly via the prokaryotic biomass (Biom) and community compositions. While the main effect of heavy metals (Metals) on the ARG profiles was mediated by the prokaryotic biomass. It is also notable that the main effect of prokaryotic biomass on the abundance of ARGs was indirectly driven via MGEs.

## 3. Discussion

The characteristic pattern of ARGs occurrence has been extensively explored in the urban aquatic ecosystems, including sewage treatment plants (STPs), drinking water reservoirs, polluted rivers and estuarine areas (Liu et al., 2018; Qiao et al., 2018; Zhu et al., 2017). However, the urban landscape lagoon systems that represent the human-environment interfaces in the modern coastal cities received little attention for determining ARG pollution levels. To our knowledge, our study provided the most comprehensive insights of the ARG profiles and responsible driving forces in lagoon sediments by using the HT-qPCR for the first time. We found that (1) the abundance and composition of ARGs were mainly shaped by horizontal (via MGEs) and vertical (via prokaryotic communities) gene transfer, respectively; and (2) deterministic and stochastic processes may remodel the assembly of ARG communities



**Fig. 5 – Network of co-occurring prokaryotic genera, ARGs and MGEs in Yundang Lagoon sediments. (a) Colors of the nodes indicate different phyla/classes of prokaryotes, or types of ARGs and MGEs. (b) Colors of the nodes indicate different modules. Each node represents prokaryotes genus (octagon), ARG (triangle) or MGE subtypes (quadrangle). The size of each node is proportional to the number of connections (i.e., degree). The edges indicate strong (Pearson Correlation Coefficient ( $r$ ) > 0.77) and significant ( $P$  < 0.05) positive correlations between nodes.**

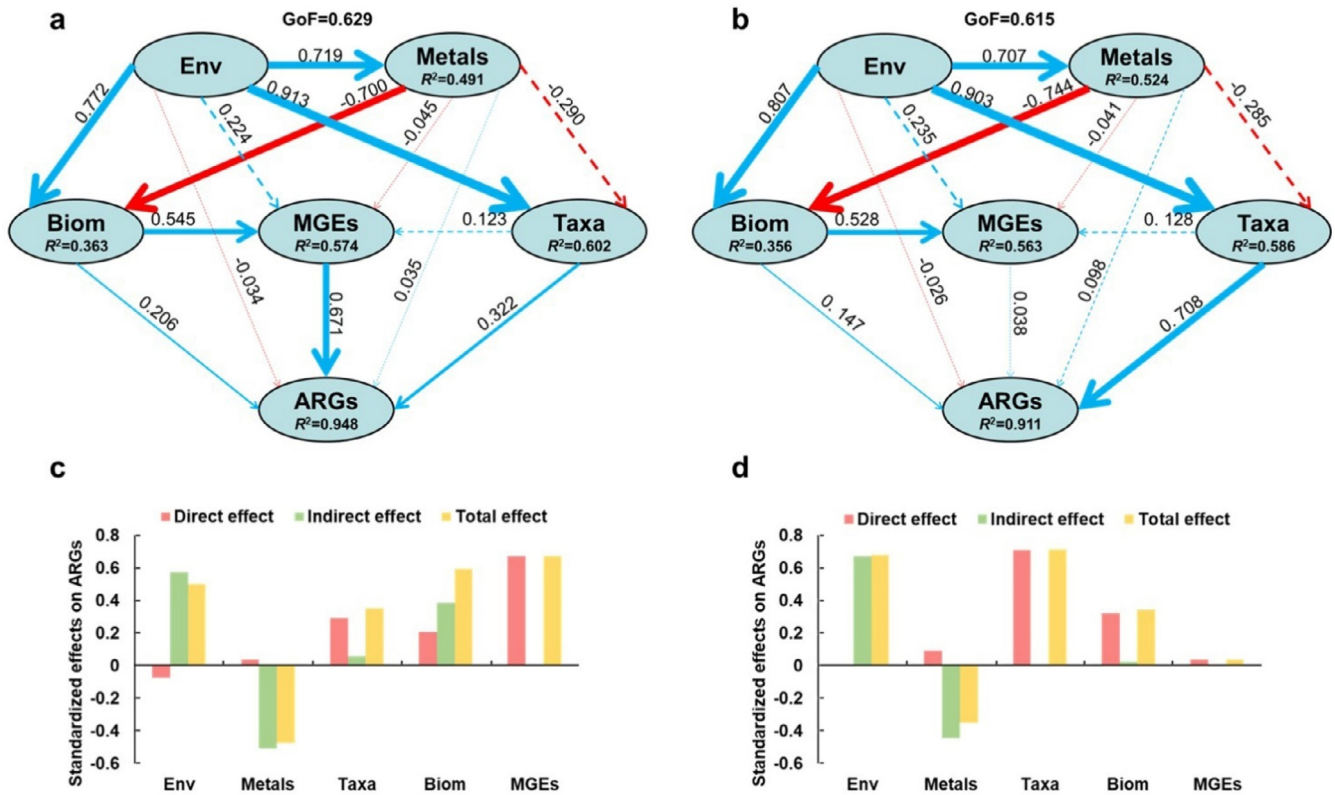
simultaneously, via influencing their host (prokaryotic) communities.

### 3.1. Contribution of anthropogenic activities in ARG pollution levels

Our study revealed that the average richness (i.e., the detected number) and absolute abundance of ARGs in the surface sediments of Yundang Lagoon were around 11 and 53 times higher than those of Tibetan lake sediments (Fig. 2 and Appendix A Fig. S2). These results suggest that the anthropogenic activities (e.g., urbanization) were responsible for enhancing the ARG pollution levels in the urban lagoon sediments. These patterns are in line with the previous findings that showed that human activities accelerated the spread and dissemination of ARGs (Chen et al., 2013, 2016). Moreover, antibiotic deactivation and efflux pump were identified as the dominant antibiotic resistance mechanisms in the sediments from Yundang Lagoon and Tibetan lakes, respectively (Appendix A Fig. S9). This provided a further support for the above speculation. Since the antibiotic deactivation may closely associate with the resistance to common anthropogenic antibiotics (Chen et al., 2013), while the efflux pump was used by the microorganisms to fight against selective environmental stresses in the pristine environments (McCann et al., 2019; Van Goethem et al., 2018).

The richness and abundance of ARGs (studied by HT-qPCR) in the lagoon sediments were comparable with those in the sediments from other urban water systems such as drinking water river-reservoir systems (91–124 ARGs and  $10^8$ – $10^9$  copies/g) (Jiang et al., 2018; Wu et al., 2020) and estuarine areas (70–158 ARGs and  $10^7$ – $10^8$  copies/g) (Zhao et al., 2020; Zhu et al., 2017). However, the richness and abundance of ARGs in the lagoon sediments were relatively lower than those in the polluted riverine sediments (122–139 ARGs and  $10^9$ – $10^{10}$  copies/g) (Cheng et al., 2020; Quintela-Baluja et al., 2019), influents ( $118 \pm 41$  ARGs and  $10^{11}$ – $10^{12}$  copies/L) and activated sludge ( $95 \pm 46$  ARGs and  $10^{12}$ – $10^{13}$  copies/L) of STPs (An et al., 2018). These comparative results indicate that the Yundang Lagoon had moderate levels of ARG pollution in its sediment phase. However, the spatial differences in the richness and abundance of ARGs between different areas of Yundang Lagoon suggest that the ARG pollution levels varied among the Outer, Inner and Songbai lakes. This may be attributed to two following reasons: (1) the Outer and Songbai lakes were reported to be influenced by highly intensive human activities, such as receiving greater volume of domestic and hospital sewages, than the Inner Lake (Chen et al., 2010; Wang et al., 2020a); and (2) the greater water circulation in the Inner Lake compared to the other two lakes (Uddin et al., 2020) may result in the accumulation of ARGs through sedimentation processes. In fact, the different sediment layers from the Inner





**Fig. 6** – Partial least squares path model (PLS-PM) showing the effects of abiotic and biotic factors on ARG abundance (a) and composition (b) in Yundang Lagoon sediments. Standardized direct and indirect effects on the ARG abundance (c) and composition (d) calculated by PLS-PM. Sediment physicochemical variables (Env) include water content, TN% and TC%. Heavy metals (Metals) included Cd, Pb, Cr, As, Ni, Cu and Zn. Prokaryotic community (Taxa) included dominant prokaryotic phyla. Prokaryotic biomass (Biom) and MGEs was based on the total abundance of 16S rRNA genes and MGEs, respectively. Model was assessed by using goodness of fit (GoF) statistic. Blue and red arrows indicate positive and negative effects, respectively. The higher path coefficients are shown as wider arrows. Solid and dashed lines indicate significant ( $P < 0.05$ ) and non-significant ( $P > 0.05$ ) relationships, respectively. Path coefficients and coefficients of determination ( $R^2$ ) are calculated after 999 permutations.

Lake had lower richness and abundance of ARGs as compared to the corresponding sediment layers from the Songbai Lake (Fig. 2b and d). Such patterns may be attributed to both historical and contemporary anthropogenic disturbances. Further studies with more comprehensive data at a large spatio-temporal scale are needed to fully understand the ARG dynamics in the urban lagoons.

### 3.2. Role of sediment physicochemical factors and heavy metals in the proliferation of ARGs

As expected, our results reveal that the sediment physicochemical factors (e.g., water content, TN% and TC%) and heavy metals (e.g., Cd, Pb, Cr, Mn, As, Ni, Cu and Zn) mainly pose positive and negative indirect effects on the lagoon ARG profiles, via prokaryotic community composition and biomass, respectively (Fig. 6). These findings are partially consistent with prior investigations showing that carbon- and nitrogen-related organic/nutrient matters played a critical role in influencing the abundance and composition of ARGs by structuring the composition of their respective host (microbial) communities in aquatic environments (Chen et al., 2019c; Guo et al.,

2018b). In the lagoon sediments, the ARG-carrying prokaryotic species may not only take advantage of such nutrient-rich environments (Zhao et al., 2017), but also outcompete the non ARG-carrying species under certain harsh conditions, such as chemical (e.g., antibiotics and biocides) pollution in the sediment phase (Deng et al., 2020; Jutkina et al., 2018). Previous studies have shown that the water bodies and sediments of Yundang Lagoon were contaminated with pharmaceuticals and personal care products (Rashid et al., 2019; Zhang et al., 2011), which may have facilitated the proliferation of ARG-carrying prokaryotes (Hu et al., 2020).

Empirical evidence suggests that heavy metals could promote the spread and dissemination of ARGs due to the co-selection phenomenon of antibiotic and metal resistance genes (Baker-Austin et al., 2006; Dickinson et al., 2019; Ohore et al., 2020). However, this may not be the case in our study, because heavy metals were found to cause a decrease in the ARGs abundance in the lagoon sediments. One possible explanation for such negative effect of heavy metals on ARGs could be the inhibition in the growth of prokaryotes by high levels of heavy metal contamination. Previous studies have demonstrated that heavy metals may not only inhibit

microbial enzyme activities, but also lead to the formation of reactive oxygen species (Huang and Shindo, 2000; Li et al., 2014). These effects may result in the poor cell growth or cell death of microorganisms (Giller et al., 2009; Song et al., 2018), thereby causing negative response of the prokaryotic abundance to the heavy metals (Fig. 6a and b). Since the high microbial abundance would favor the occurrence of both HGT and VGT (Bengtsson-Palme and Larsson, 2015; Chen et al., 2019b), such limitations in the proliferation of prokaryotes might mitigate ARG contamination in the lagoon sediments, to some degree. An earlier study suggested that anthropogenic disturbances from the surrounding areas was responsible for causing high contamination of several heavy metals in Yundang Lagoon to pose high ecological risks for sediment biota (Uddin et al., 2020). Nevertheless, the concentrations of heavy metals in Yundang Lagoon might not be too high to trigger the co-selection of antibiotic and metal resistance genes (Baker-Austin et al., 2006; Dickinson et al., 2019). However, given the essential roles of some unmeasured pollutants (e.g., polycyclic aromatic hydrocarbons (PAHs) and antibiotics) in promoting the propagation and spread of ARGs (Chen et al., 2017a; Zhu et al., 2018), further studies with a more comprehensive metadata collection are required to quantify and distinguish the effects of heavy metals, PAHs and antibiotics on the ARG profile of the lagoon sediments.

### 3.3. Horizontal and vertical gene transfer determined the abundance and composition of ARGs, respectively

It was not surprising that MGEs had strong positive direct effect on the ARG abundance in the lagoon sediments (Fig. 6c and Appendix A Fig. S8). This is consistent with prior findings, where the HGT mediated by MGEs was one of the major mechanisms involved in promoting the dissemination of ARGs across different aquatic ecosystems (Chen et al., 2017b; Ju et al., 2019; Liao et al., 2018). For example, Hu et al. (2020) revealed that the *intI1* played the most crucial role in influencing the abundance and composition of ARGs in a subtropical watershed. The findings of Zhu et al. (2017) showed that the *intI1* caused serious allochthonous pollution of ARGs in estuarine sediments across the coastline of China. Chen et al. (2019c) found that MGEs were the key factors responsible for shaping the ARG profiles in water and sediment in reservoir system but not in soil. The same study suggested that HGT and VGT were responsible for the proliferation and spread of ARGs in non-soil (i.e., water and sediment) and soil phases, respectively, while the ARG profiles in water phase were also influenced by VGT to some extent (Chen et al., 2019b). However, these findings were not consistent with the ARG community composition in our study. The PLS-PM models suggested that the VGT played a dominant role in shaping the composition of ARGs in the lagoon sediments (Fig. 6d). This pattern was also confirmed by the results of Mantel test, where the composition of ARG communities was found strongly correlated with sediment prokaryotic communities. Our network analysis further endorsed by the results of PLS-PM models. For instance, several typical benthic prokaryotic genera, such as *Clostridium\_sensu\_stricto\_1* (Sasi Jyothsna et al., 2016), *Desulfomicrobium* (Miralles et al., 2007), *Methanosaeta* (Antony et al., 2012),

*Methanospirillum* (Hu et al., 2015), *Paludibacter* (Fang et al., 2017) and *Thermovirga* (Goker et al., 2012) showed multiple associations with various ARG subtypes, and altogether formed an independent module. However, limited associations were found between *intI1*, ARGs and prokaryotic genus (i.e., *Thermovirga*) (Fig. 5). These phenomena further highlight that the sediment prokaryotic taxa may play a more important role in shaping the composition of ARGs as compared to MGEs. In addition, strong co-occurrence of fecal/sewage indicator prokaryotes and ARGs suggests that sewage leakage and overflows may be one of the main causes of ARG contamination in Yundang Lagoon sediments (Wang et al., 2020a). It is possible that the ARG carrying fecal/sewage prokaryotes may have settled down in the sediment phase of the lagoon, and contributed to the *in situ* dissemination of ARGs (Chen et al., 2019a).

### 3.4. Stochastic and deterministic processes remodeled the assembly of ARG communities

Mounting evidences suggest that anthropogenic disturbances can enhance the relative role of deterministic processes in the assembly of ARG communities (Hu et al., 2020; Peng et al., 2020). For instance, Peng et al. (2020) found that the changes in land use may pose strong deterministic effects on the assembly of antibiotic resistome in the urbanized area of Houxi River. Moreover, Corno et al. (2019) reported that the discharge of STP effluents may result in a deterministic assembly of ARG communities in receiving freshwater bodies. As discussed earlier, the crucial roles of both abiotic (i.e., physicochemical variables and heavy metals) and biotic (i.e., prokaryotic biomass and communities) factors in influencing the composition of ARG communities in this study (Fig. 6) imply that the ARG dynamics in lagoon sediments was mainly driven by deterministic processes. However, beyond our expectations, the NCM analysis revealed that stochastic processes may also play a certain role in the assembly of the sediment ARGs (Fig. 4). This is also in agreement with previous findings, where the deterministic and stochastic processes were reported to determine the assembly of taxonomic and functional communities of prokaryotes, simultaneously (Liu et al., 2018; Zhou and Ning, 2017). However, in contrary to the previous observations regarding the decoupling between taxonomic and functional (i.e., ARGs) prokaryotic communities (Fang et al., 2019; Peng et al., 2020), the composition of ARGs in Yundang Lagoon was closely associated with their respective taxonomic counterparts (i.e., ASVs). Hence, similar goodness of fits for neutral community models for taxonomic and functional communities (Fig. 4) suggest that the stochastic processes were responsible for remodeling ARG community assembly, via shaping their host (prokaryotic) communities. These results further support our conclusion that VGT played a more important role in affecting the composition of ARGs in the lagoon sediments. The ARG-carrying prokaryotic cells in the suspended sediment particles flow along with water to cause their dispersal between different sampling locations, likewise, ARG-carrying prokaryotes migrate from surface to deep layer sediments to cause random assembly of prokaryotic taxonomic and functional communities (Deng et al., 2020; Di Cesare et al., 2020; Wang et al., 2020a).

#### 4. Conclusions

This study uncovered the comprehensive profiles of the benthic ARGs in an urban landscape lagoon system. Comparison with sediment samples from Tibetan saline lakes indicated that urbanization-associated anthropogenic activities appreciably enhanced the richness and abundance of ARGs in the lagoon sediments. The fecal- and sewage-indicators co-existed with various ARGs of aminoglycoside, multidrug and MLSB types to indicate domestic sewage sources of ARG contamination. The sediment environmental properties (i.e., physicochemical variables and heavy metals) had indirect effects on the ARG profiles, while MGEs and prokaryotic communities were directly responsible for shaping the abundance and composition of ARGs, respectively. However, the stochastic processes may play a certain role in influencing the ARG profiles in parallel with their hosts (prokaryotic communities). Hence, we propose that both HGT and VGT were the major pathways for the spread and proliferation of ARGs in the benthic habitat of Yundang Lagoon, and underscore the need for making specific management strategies for reducing the potential threats to public health from ARGs in urban lagoons.

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#### Appendix A. Supplementary data

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jes.2020.09.004](https://doi.org/10.1016/j.jes.2020.09.004).

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