Spatiotemporal distributions of sulfonamide and tetracycline resistance genes and microbial communities in the coastal areas of the Yangtze River Estuary

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ARTICLE INFO

ABSTRACT

In this paper, water and sediments were sampled at eight monitoring stations in the coastal areas of the Yangtze River Estuary in summer and autumn 2021. Two sulfonamide resistance genes (sul1 and sul2), six tetracycline resistance genes (tetM, tetC, tetX, tetA, tetO, and tetQ), one integrase gene (intI1), 16 S rRNA genes, and microbial communities were examined and analyzed. Most resistance genes showed relatively higher abundance in summer and lower abundance in autumn. One-way analysis of variance (ANOVA) showed significant seasonal variation of some ARGs (7 ARGs in water and 6 ARGs in sediment). River runoff and WWTPs are proven to be the major sources of resistance genes along the Yangtze River Estuary. Significant and positive correlations between intI1 and other ARGs were found in water samples ($P < 0.05$), implying that intI1 may influence the spread and propagation of resistance genes in aquatic environments. Proteobacteria was the dominant phylum along the Yangtze River Estuary, with an average proportion of 41.7%. Redundancy analysis indicated that the ARGs were greatly affected by temperature, dissolved oxygen, and pH in estuarine environments. Network analysis showed that Proteobacteria and Cyanobacteria were the potential host phyla for ARGs in the coastal areas of the Yangtze River Estuary.

1. Introduction

Environmental pollution is a global problem that affects the quality of life of humans and other living organisms. Nanotechnology and nanostructures have shown great promise in remediating contaminated environments, as evidenced by recent studies (Sahar et al., 2020). The unique physicochemical properties, high reactivity, and large surface area of nanomaterials make them particularly suitable for environmental remediation (Sahar et al., 2019). Various types of nanoparticles, such as metal nanoparticles (MNPs) (Sahar and Masoud, 2016), metal oxide nanoparticles (MONPs) (Sahar et al., 2021), and novel nanomaterials (Maryam et al., 2023), have been investigated for this purpose. This paper's findings can provide valuable data and references for researchers working on new technologies, such as nanomaterials and nanostructures, for pollutant removal and environmental remediation.

A new class of emerging pollutants, known as antibiotic resistance genes (ARGs), has been identified (Pruden et al., 2006). The increasing resistance of bacteria (both pathogenic and non-pathogenic) to antibiotics is a significant concern, as it poses risks to the environment and human health (Sharma et al., 2016; Wellington et al., 2013).

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Antibiotic-resistant bacteria (ARB) constitute one of the greatest risks to human health in the 21st century, proposed by the World Health Organization (WHO). (Zheng et al., 2017). Bacteria can acquire ARGs through gene mutation and generational inheritance and obtain exogenous resistance genes via transduction, conjugation and transformation (Alekshun and Levy, 2007). Integrons, an important mobile genetic element (MGE), can capture and carry antibiotic resistance genes through the site-specific recombination system of gene cassettes, leading to the dissemination and horizontal transfer of ARGs (Stalder et al., 2012; Stokes and Hall, 1989; Zhu et al., 2013). Class I integron (*int*I1) is a crucial integron gene that frequently shows a strong correlation with ARGs (Zhang et al., 2018; Li et al., 2020). Transposons can capture *int*I1, thereby increasing the horizontal transfer potential of ARGs (Gillings et al., 2014). Antibiotics at low concentrations in the environment can act as interspecific or intraspecific signaling molecules for microbial species, mediating the selective adaptation of microbial communities to antibiotics (Aminov, 2009), and affecting the spread of ARGs.

Microbial communities, as an activated carrier of ARGs, are essential for the occurrence and dissemination of ARGs (Qiu et al., 2019; Ke et al., 2023). However, the presence of bacteria in the environment, particularly pathogenic bacteria, can also carry ARGs through horizontal transfer of genes, which poses a significant threat to human health and socio-economic development (Kheiri and Akhtari, 2016). Moreover, many studies have shown that the aquatic environment is a significant reservoir for the occurrence of ARGs (Marti et al., 2014; Zhu et al., 2017; Guo et al., 2018). The physicochemical properties of ARGs further promote their interaction with bacteria, thus increasing the likelihood of bacteria carrying ARGs. Microbial communities can stimulate the enrichment of ARGs under selective pressure (Knapp et al., 2010). In 2017, China accounted for 45% of global consumption of antimicrobial veterinary medications, and it is expected to remain the leading user (43%) by 2030 (Tiseo et al., 2020). The occurrence and spread of ARGs in estuarine environments are affected by both human activities and hydrometeorological factors during combined physical–chemical-biological processes (Zhang et al., 1999; Jiao et al., 2007). Antibiotics, heavy metals, microbial communities and environmental factors such as temperature, pH, organic carbon (TOC), are closely related to the prevalence and dissemination of ARGs. (Wang et al., 2020; Xu et al., 2015; Zheng et al., 2017). A variety of resistance genes, such as tetracyclines, sulfonamides, and aminoglycosides, have been detected in most estuaries in China (Zhu et al., 2017; J.Y. Chen et al., 2019; J. Chen et al., 2019; Lu et al., 2019). However, the seasonal and spatial distributions of ARGs and microbial communities in the coastal areas of the Yangtze River Estuary are still unclear, and the main influencing factors and seasonal response mechanisms underlying the shift of ARGs have not been fully explored.

Previous researches have demonstrated that the two typical classes of ARGs that predominate in estuarine environments are sulfonamide and tetracycline resistance genes (J.Y. Chen et al., 2019; J. Chen et al., 2019; Lu et al., 2019). The most commonly detected ARGs in the aquatic environment were the tetracycline and sulfonamide ARGs. (Jiang et al., 2013; Yan et al., 2018). The class I integron (*int*I1) is an important integron gene that is usually highly correlated with ARGs (Zhang et al., 2018; Li et al., 2020). Fluorescence quantitative PCR (qPCR) was used to identify the spatiotemporal distributions of 10 target genes such as two sulfonamide resistance genes (*sul*1 and *sul*2), six tetracycline resistance genes (*tet*M, *tet*C, *tet*X, *tet*A, *tet*O, and *tet*Q) and the *int*I1 and 16 S rRNA genes obtained from water and sediment samples. The 16 S amplicon sequencing technique was utilized to analyze the characteristics of microbial communities. The correlation between ARG and environmental factors such as temperature, dissolved oxygen, pH value, electrical conductivity, turbidity and salinity and the co-occurrence mechanisms between microflora and ARGs were explored, and the response mechanisms between ARGs and microbial communities in the coastal area of the Yangtze River Estuary were revealed. The results can provide a scientific basis for pollution control and eco-risk evaluation of ARGs in estuarine environments.

2. Materials and methods

2.1. Sampling collections

Surface water and paired sediment samples were collected at 8 sampling sites on 14th July (summer) and 16th October (autumn), 2021, in the coastal areas of the Yangtze River Estuary (Fig. 1). 8 sampling sites are named Station No.1 Liuhekou (LHK), Station No.2 Shidongkou (SDK), Station No.3 Wusongkou (WSK), Station No.4 Zhuyuan (ZY), Station No.5 Sanjiagang (SJG), Station No.6 Chaoyangnongchang (CYNC), Station No.7 Dazhihe (DZH) and Station No.8 Nanhui (NH). Station No.1 (LHK), Station No.3 (WSK), and Station No.7 (DZH) are located at the confluence of the Yangtze River and urban inland rivers (Liuhe River, Huangpu River and Dazhihe River), which are influenced by both river runoff and estuarine tidal effects. Station No.2 (SDK) and Station No.4 (ZY) are located downstream of wastewater treatment plants (WWTPs), influenced by the discharge of domestic sewage and sludge. Station No.6 (CYNC) is located on an intertidal flat, Station No.5 (SJG) is located downstream of the Jiuduansha Wetland National Nature Reserve, and Station No. 8 (NH) is located in the Nanhui dongtan wetlands.

Surface water (2 L) was collected at each sampling site and placed in ultrapure water-washed polyethylene bottles, while 100 g of surface layer sediment (0–5 cm) was quantified using an electronic balance (GL124–1SCN, Sartorius, Germany) and stored in sterilized plastic bags. Three parallel samples were taken for each sample to eliminate manual errors. All samples were transported on ice (4 °C). The physical and chemical properties of water were measured onsite by a portable multiparameter water quality analyzer (AP-5000, AQUAREAD, UK). Station No. 3 (WSK) has been selected as representative sampling site to detect the physic-chemical properties of sediment such as pH, organic matter (OM), cation exchange capacity (CEC), moisture content (MC) and soil mechanical composition (Table S2).

2.2. DNA extraction and qPCR for quantifying ARGs

The water sample (1 L) was filtered through a glass fiber membrane with a pore size of 0.22 μm, and 5 mg of the filtered suspended matter was weighed for DNA extraction. Total DNA in water and sediment samples was extracted using a DNA Extraction Kit (MP Biomedicals, USA). The integrity of the extracted DNA was verified by 1.5% agarose gel electrophoresis, and the DNA concentration was further quantified by a spectrophotometer (NanoDrop2000, USA). To detect target genes, polymerase chain reaction (PCR) and quantitative PCR (qPCR) were carried out on all DNA samples after they had been kept in a − 80 °C refrigerator.

In this study, two classes of eight target genes, *int*I1 and 16S rRNA gene were quantified by fluorescent quantitative PCR (qPCR instrument: Bio-Rad CFX96 touch, CA, USA). Primer sequences, fragment sizes, annealing temperatures, and references corresponding to the fragments of ARGs are shown in Table S2. The qPCR system included (20 μl): 2 ×Taq Pro Universal SYBR qPCR Master Mix 10 μl (Vazyme, Nanjing, China), upstream and downstream primers 0.4 μl, DNA template 2 μl, and ddH2O 7.2 μl. The thermal cycling reaction program for gene amplification included a predenaturation at 95 °C for 30 s and 40 cycles (each cycle was 95 °C for 10 s, annealing for 30 s, and the annealing temperature is shown in Table S3), and the melting curve temperature was set to increase from 60 °C to 95 °C. The standard curve equations and R^2 values for the target genes are shown in Table S3. Target genes were normalized by 16 SrRNA gene, which reduces the error caused by the interference of DNA extraction efficiency and background value of microorganisms. (Lu et al., 2015). The relative abundance formula of the target gene is:
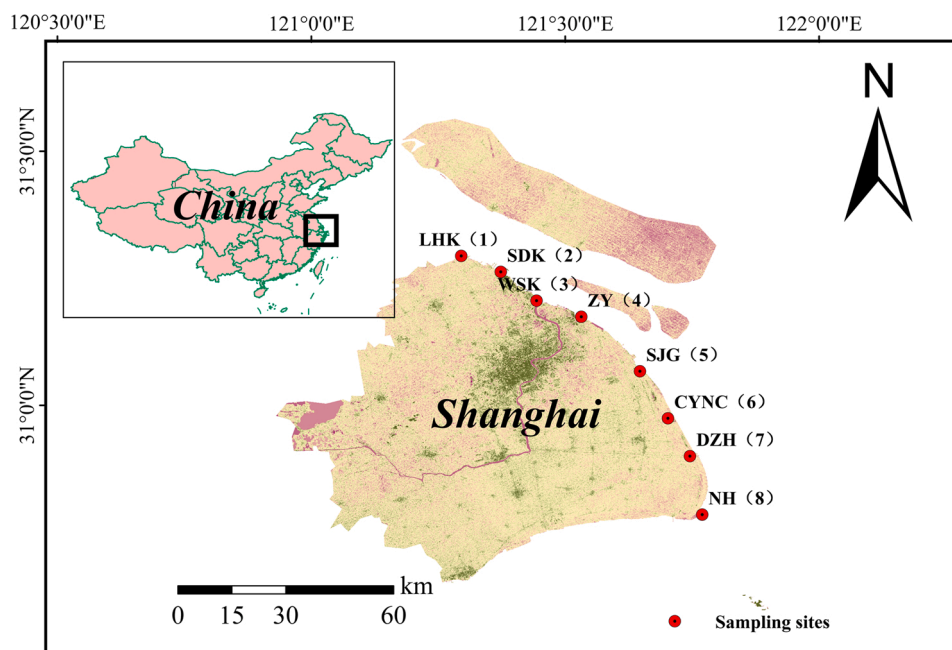
Fig. 1. Location of sampling sites.

Relative abundance=Copies of target gene/Copies of 16 S rRNA gene　(1)

The ARG distribution in water and sediment samples was studied using the partition coefficient (Kd) (Lu et al., 2019).

Kd=As/Aw　(2)

where As is the absolute abundance of ARGs in the sediment, and Aw represents the absolute abundance of ARGs in the surface water.

2.3. 16 S amplicon sequencing

All samples were delivered to APExBIO Technology, LLC (Shanghai, China) for 16 S amplicon sequencing. Our target was the V3–V4 hypervariable region of the bacterial 16 S rRNA gene. PCR was started immediately after the DNA was extracted. The 16 S rRNA V3–V4 amplicon was amplified using 2 ×Hieff® Robust PCR Master Mix (Yeasen,10105ES03, China). Two universal bacterial 16 S rRNA gene amplicon PCR primers (PAGE purified) were used: the amplicon PCR forward primer 341 F (ACTCCTACGGGAGCAGCA) and amplicon PCR reverse primer 805 R (GGACTACHVGGGTWTCTAAT). The reaction was set up as follows: microbial DNA (10 ng/μl) 2 μl; amplicon PCR forward primer (10 μM) 1 μl; amplicon PCR reverse primer (10 μM) 1 μl; 2 ×Hieff® Robust PCR Master Mix (Yeasen, 10105ES03, China) (total 30 μl). The plate was sealed and PCR performed in a thermal instrument (Applied Biosystems 9700, USA) using the following program: 1 cycle of denaturing at 95 °C for 3 min, first 5 cycles of denaturing at 95 °C for 30 s, annealing at 45 °C for 30 s, elongation at 72 °C for 30 s, then 20 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 30 s and a final extension at 72 °C for 5 min. The PCR products were checked using electrophoresis in 2% (w/v) agarose gels in TBE buffer (Tris, boricacid, EDTA) stained with ethidium bromide (EB) and visualized under UV light.

Raw sequencing data were obtained using the Illumina NovaSeq PE250 platform. Potential chimeric sequences were removed by Trimmomatic PE (Bolger et al., 2014). Raw data were filtered, deduplicated and base corrected to obtain valid sequences that could be used for subsequent analysis (Clean Data). ASVs (amplicon sequence variants) with 100% similarity were clustered using Divisive Amplicon Denoising

Algorithm 2 (DADA2) (Callahan et al., 2016). According to the Silva database, each ASV sequence was noted and analyzed by QIIME 2 (Quast et al., 2012; Pelin et al., 2014).

Alpha diversity indices such as Chao1, Shannon, and Simpson indices are important indicators for the assessment of microbial community diversity (Francesca et al., 2018). The Chao1 index is used to estimate the species richness. The Shannon index is used to describe the homogeneity of species and communities and the Simpson index is used to estimate the diversity of bacteria.

2.4. Statistical analysis

Statistical analysis of ARG abundances was performed using Origin software (Version 2021, Origin Lab Corporation, USA). One-way analysis of variance (ANOVA) was used to compare gene abundances across seasons and points, and $P < 0.05$ represented substantial divergence. The Tutools platform (https://www.cloudtutu.com), a free online data analysis website, was used to carry out the principal component analysis (PCA) (Guo et al., 2020), redundancy analysis (RDA), microbial diversity index box line plots and clustering stacked histograms. Network analysis has been widely used in biological and ecological sciences to explore potential associations within/between microbial taxa, ARGs and proteins in complex microbial communities (Ju and Zhang, 2015; Yu et al., 2022). In this study, network analysis between ARGs and microbial communities were based on Spearman's correlation coefficients ($r > 0.6$) and significance levels ($P < 0.05$) and visualized by Gephi platform (Li et al., 2023) to explore the co-occurrence patterns of ARGs and microbial communities.

3. Results

3.1. Absolute abundances of ARGs

The absolute abundances of ARGs in all samples from coastal areas of the Yangtze River Estuary in July and October are shown in Fig. S1. W1-W8 and S1-S8 represent the water samples and sediment samples collected at Station Nos.1–8, respectively. The detection rate of all target genes was 100% at the 8 sampling sites. In general, the absolute abundances of most ARGs in water samples were higher in July than in
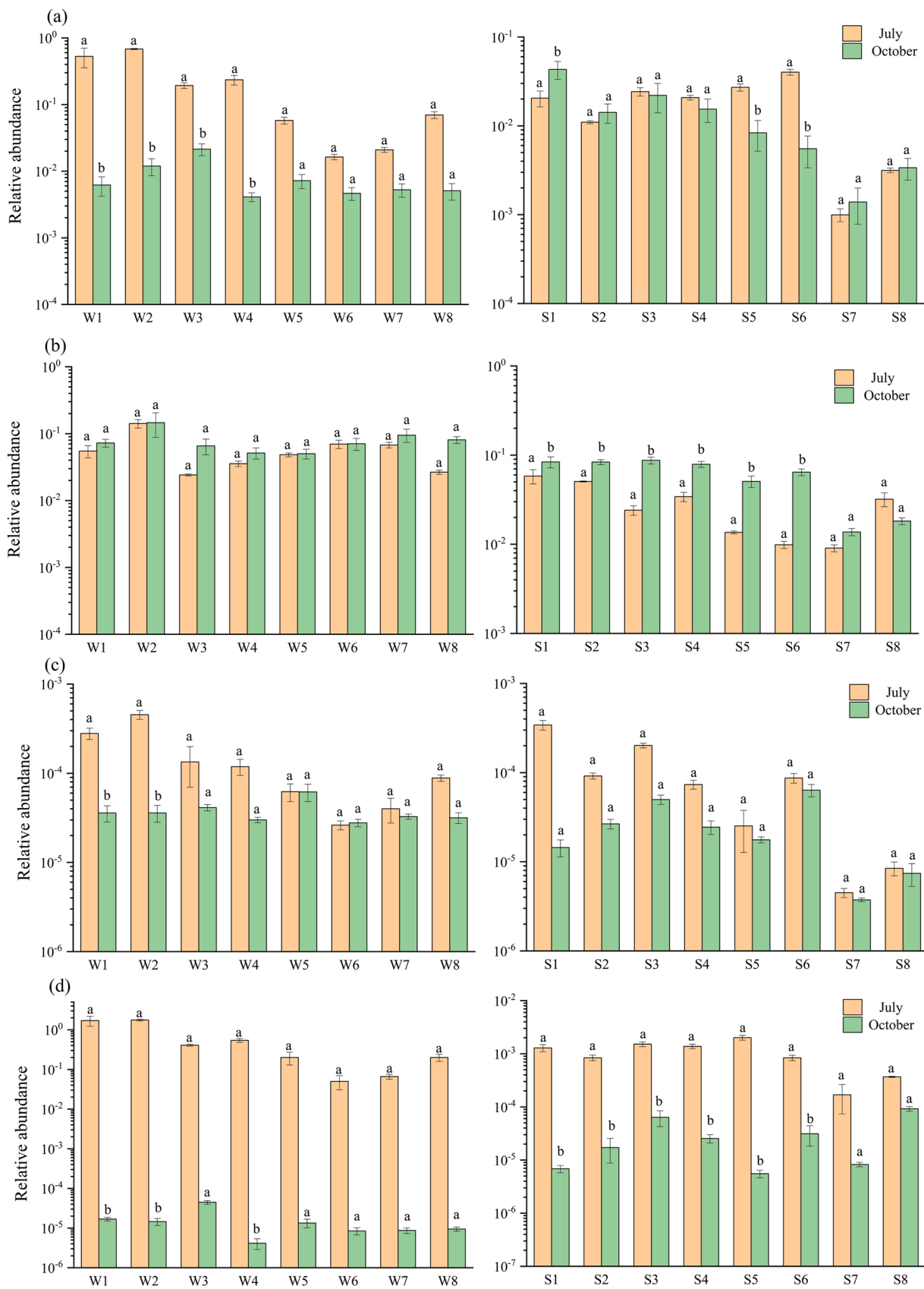
Fig. 2. The relative abundance of ARGs in the coastal areas of the Yangtze River Estuary in July and October. (a): *sul*1, (b): *sul*2, (c): *tet*M, (d): *tet*C, (e): *tet*X, (f): *tet*A, (g): *tet*O, (h): *tet*Q, and (i): *int*I1. Significant differences among different sampling sites are indicated by the different letters above the bars in the two different seasons ($P < 0.05$).
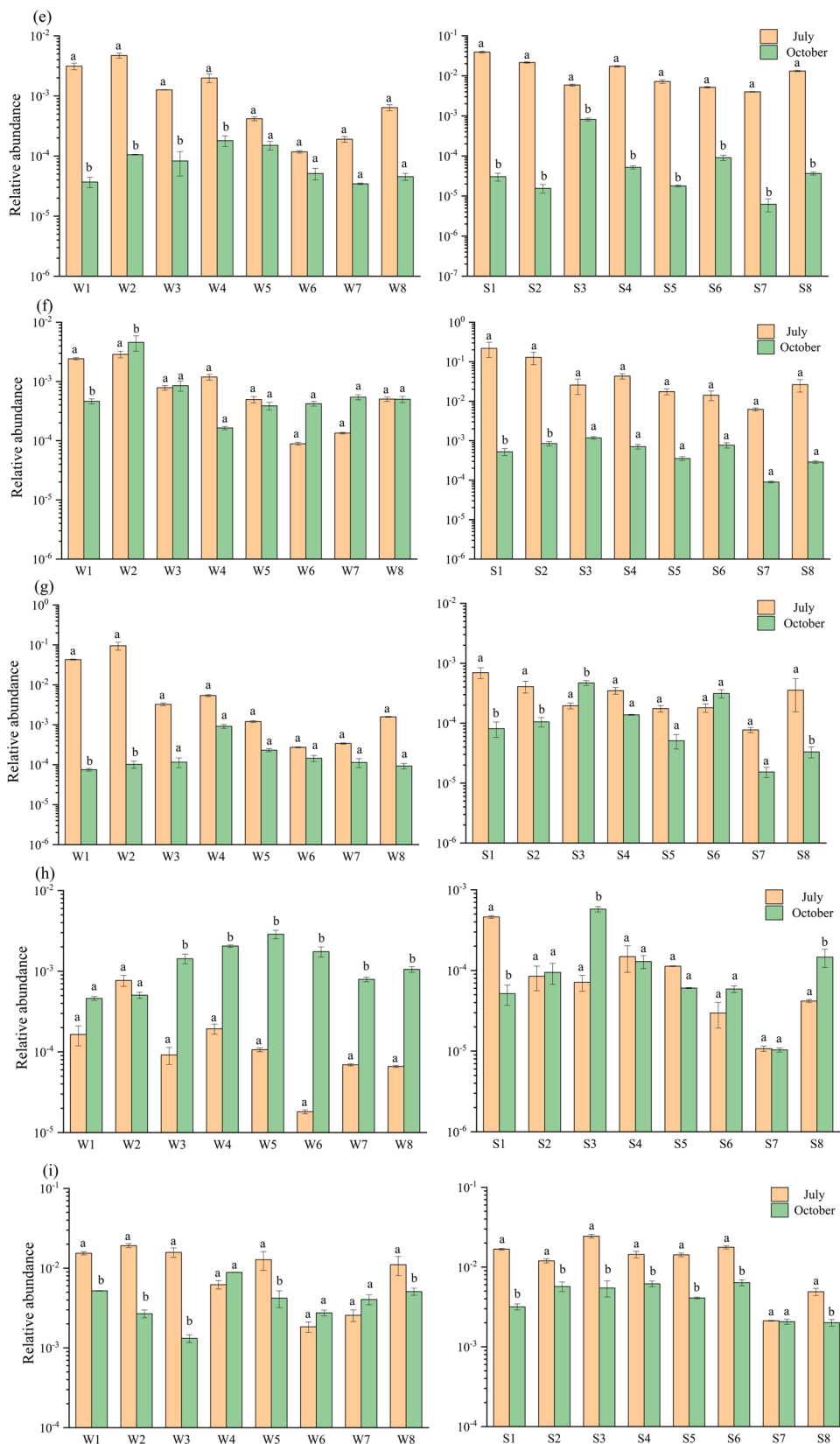
Fig. 2. (*continued*).

October, while in sediment samples, the absolute abundances were similar during the two surveys. Similar conclusions have been obtained from previous studies (Guo et al., 2018; Wang et al., 2022). For sulfonamide resistance genes, the absolute abundance of *sul*1 in surface water ranged from 5.44×10^4 to 9.75×10^4 copies/mL in July and 1.08×10^3 to 2.54×10^4 copies/mL in October. Conversely, the absolute abundance of *sul*2 was slightly higher in October (1.34×10^4 to 2.56×10^5 copies/mL) than in July (7.36×10^3 to 3.28×10^5

copies/mL). A negative correlation with temperature ($r = -0.42$, $P = 0.1$) and class I integron (intI1) ($r = -0.31$, $P = 0.2$) may cause such phenomenon. ARGs in sediment samples showed a different trend from those in surface water. The total absolute abundances of tetracycline resistance genes in all samples were 2.26×10^5 copies/mL and 2.34×10^8 copies/g in July, respectively, which were higher than those in October (4.13×10^3 copies/mL and 3.80×10^6 copies/g, respectively). The two tetracycline resistance genes (tetA and tetM) were widely abundant in the sediment with average abundances of 4.60×10^7 and 5.70×10^7 copies/g. The result of tetA was approximately 3 magnitudes higher than those of sediment samples in the Yangtze Estuary (Guo et al., 2018; Lin et al., 2015), which may suggest that tetA contamination has been increasingly severe in recent years. Spearman correlation analysis showed a positive correlation between temperature and most ARGs (sul1, tetM, tetC, tetX, tetO and intI1) and temperature had a significant positive correlation with total ARGs ($P < 0.05$). Besides, the ARG abundance in July (1.95×10^5 copies/mL on average) was higher than that in October (1.86×10^5 copies/mL on average). In the Zhangxi River, Zheng et al. (2018) discovered that the absolute abundance of ARGs peaked in the summer (2.81×10^9 copies/L on average). It turns out that water temperature is an important factor that promotes the transformation of ARGs (Su et al., 2017). Higher temperature promotes the growth of bacteria, leading to the increasing frequency of conjugation and transformation between ARGs and bacteria, which accelerates the diffusion and migration of ARGs (Guo et al., 2018; Son et al., 2018). These results indicated that temperature has a significant effect on ARGs.

3.2. Relative abundances of ARGs

Fig. 2 shows the relative abundances of the 9 genes in all samples. The total relative abundances of ARGs in water and sediment samples ranged from 1.39×10^{-1} to 4.05 and 2.41×10^{-2} to 4.75×10^{-1} in July and 6.53×10^{-2} to 1.66×10^{-1} and 1.73×10^{-2} to 1.31×10^{-1} in October, respectively. In both water and sediment samples, sul1 was the dominant gene at most sampling sites along the coastal areas of the Yangtze River Estuary, with relative abundances ranging from 1.64×10^{-2} to 1.02 (July) and 4.11×10^{-3} to 2.14×10^{-2} (October) in water samples and from 9.97×10^{-4} to 4.02×10^{-2} (July) and 1.39×10^{-3} to 4.32×10^{-2} (October) in sediment samples (Fig. 2a). In this study, sul genes accounted for 56% of all target genes, which indicates sul genes dominate in the coastal areas of the Yangtze River Estuary (Yang et al., 2017). For tetracycline resistance genes, the relative abundances of tetM (Fig. 2c), tetC (Fig. 2d), tetX (Fig. 3e), tetA (Fig. 2f) and tetO (Fig. 2g) were all higher in July than in October, while tetQ (Fig. 3h) was opposite, with greater values in October. According to the present study, tetC, tetM, tetX, and tetA are sensitive to temperature. The relative abundance of ARGs in river and lake waters was significantly negatively correlated with runoff (Jiang et al., 2013). In the coastal areas of the Yangtze River Estuary, rainfall in summer increases the discharge of runoff, which has a dilutional effect on the water column of the estuarine environment, yet low water discharges in autumn may promote the enrichment of ARGs. One-way analysis of variance (ANOVA) on the variation for ARGs was conducted and visualized by Origin software (Fig. S2). The result shows that significant seasonal variation for sul1, tetM, tetC, tetX, tetO, tetQ and intI1 in water samples and sul2, tetC, tetX, tetA, tetO, intI1 in sediment samples.

3.3. Microbial diversity and community structure

The alpha diversity indices of July and October are compared in Fig. S3, and the seasonal differences were analyzed using the Wilcoxon rank sum test, where $P < 0.05$ indicates a significant variation. The mean values of the Chao1 index, Shannon index, and Simpson index for sediment samples were 2297, 6.98, and 0.996, respectively, which were higher than those for water samples, indicating higher bacterial richness and biodiversity in sediments than in water. The highest average value of the Shannon index (6.54) appeared at Station No.4 (ZY) for water samples, while Station No.2 (SDK) had the highest average value of the Shannon index (7.19) for sediment samples, which were both attributed
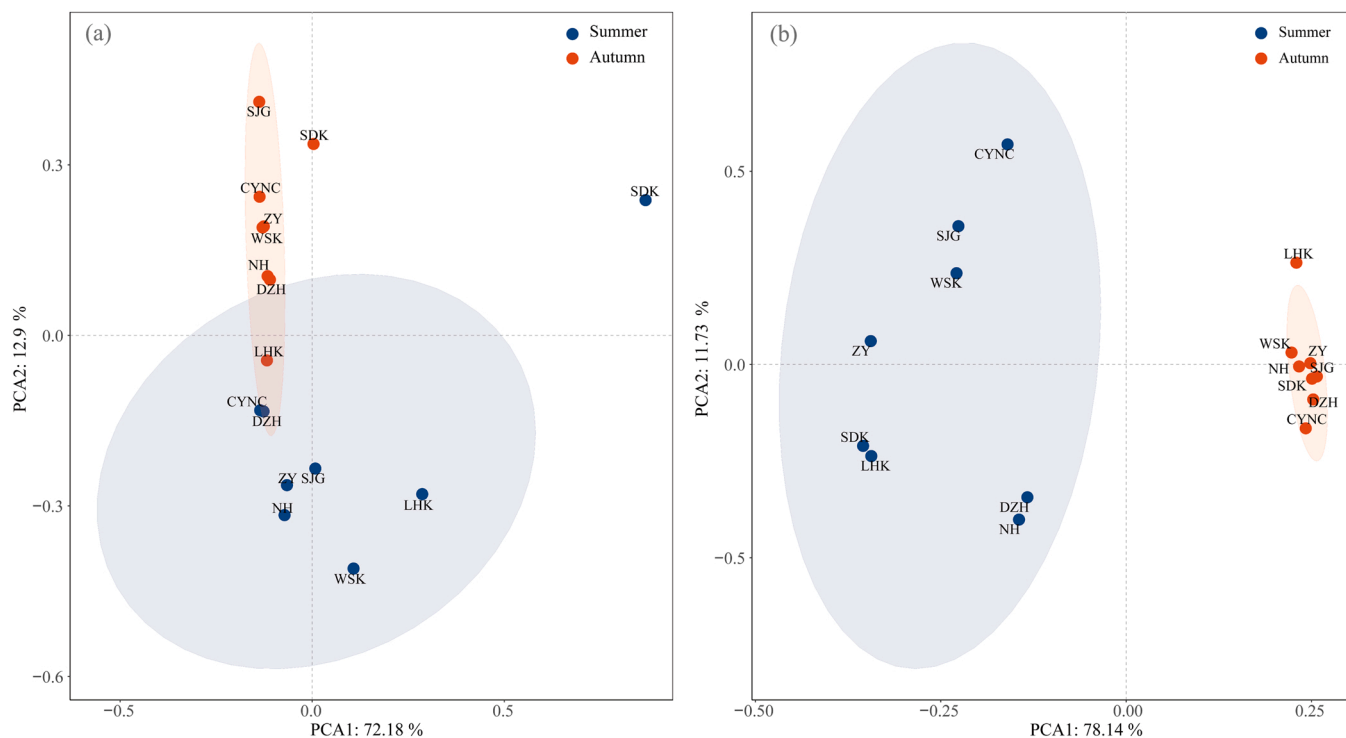

Fig. 3. Clustering stacked histogram of the top 20 most abundant bacterial genera in water and sediment phases among different seasons. (a) Water sample; (b) Sediment sample.

to the effluent discharge from WWTPs that promoted bacterial colonization and increased the microbial community diversity in surrounding aquatic environments (Luo et al., 2019; Yang et al., 2017). The alpha diversity index showed a significant difference between July and October ($P < 0.05$) in the water samples, while the opposite was observed in the sediment samples ($P > 0.05$). This was attributed to the fact that the biological environment in sediments is relatively stable (Wang et al., 2021), and temporal and spatial variations in the microbial diversity were more apparent in water samples than in sediments.

The microbial community composition at phylum-level in July and October is shown in Fig. S4. The top 20 phyla in terms of relative abundance were selected for analysis. Proteobacteria was the dominant phylum along the Yangtze River Estuary, with an average proportion of 41.7%. This result is in line with the results of other studies investigating the bacterial composition of the Yangtze River Estuary (Guo et al., 2018; Liu et al., 2015; Sun et al., 2014). Luo et al. (2019) found that discharge from WWTPs can significantly increase the proportion of Proteobacteria in the aqueous environment. In the present study, Proteobacteria increased 20.3% after sewage treatment from WWTPs. In autumn, the relative abundance of Actinobacteriota in water samples (25.2%) was significantly higher than that in summer (13.0%), and its abundance was second only to Proteobacteria. The relative abundance of Cyanobacteria (13.8%) in the water samples was considerably higher in summer than in autumn. Studies have shown that Proteobacteria and Bacteroidota were the dominant phyla in the aquatic environment along the Yangtze River Estuary, may be involved in the biochemical cycling processes of numerous substances (Guo et al., 2018). Proteobacteria and Bacteroidota were the dominant bacteria in sediment samples, which may have a significant impact on the biochemical cycle of the Yangtze River Estuary.

Clustering stacked histograms of microbial community genera at 8 sampling sites are shown in Fig. 3. In general, *Chloroplast* was the dominant genus in the aquatic environment of the study region, with average relative abundances of 13.1% in water samples and 10.6% in sediment samples. The results were comparable to those reported in a previous study in this area (Xu et al., 2023). Significant differences in the distribution of microbial generic levels in all samples. In the water samples, the dominant genera *Hydrogenophaga*, *Chloroplast*, and *Rheinheimera* accounted for 15.7%, 17.5%, and 15.9% in July, respectively, which were significantly higher than those in October. The dominant genera in sediments were *Chloroplast* and *Luteolibacter*, which were 15.6% and 10.2% in July and 5.7% and 6.2% in October, respectively. The spatial differences in microbial genera at Station No. 2 (SDK) and Station No. 4 (ZY) were mainly influenced by the effluent discharge from WWTPs (Kim and Aga, 2007; Yang et al., 2017; Yu et al., 2020). Microbial community of nitrifying-enriched activated sludge (NAS) plays an important role in improving wastewater treatment technology (Sepehri and Sarrafzadeh, 2018). In sediment samples, microbial genera were similar at Station No.2 (SDK) and Station No.4 (ZY) with the dominant genus *Nitrospira*, which is usually the dominant homogeneous genus in the activated sludge system of WWTPs (Harms et al., 2003). The anaerobic biological treatment processes increased the risk of sludge enrichment of *Nitrospira* in the aqueous environment (Yao and Peng, 2017).

4. Discussions

4.1. Distributions of ARGs

The 8 sampling sites along the coastal areas of the Yangtze River Estuary suffered ARG pollution. The absolute abundances of all ARGs in water samples ranged from 2.77×10^5 to 5.35×10^6 copies/mL, with an average of 1.90×10^6 copies/mL, 8.26-fold higher than in the Southern Ocean (2.5×10^5 copies/mL) (Jang et al., 2022). The average absolute abundances of ARGs in water samples (1.9×10^5 copies/mL) in the present study were approximately 2–3 magnitudes higher than those

in the Bohai Sea (2.05×10^2 to 7.25×10^3 copies/mL) and the Yellow Sea ($21.1–8 \times 10^3$ copies/mL) (Lu et al., 2019), suggesting that the maintenance and propagation of ARGs is more affected by human activities in the coastal area than in the sea. Compared with 18 other estuarine regions (Biliu Jiang, Liao He, Luan He, Yongdingxin He, Qiantang Jiang, Yong Jiang, Ou Jiang, Huotong Xi, Min Jiang, Jin Jiang, Jiulong Jiang, Han Jiang, Long Jiang, Zhu Jiang, Nanliu Jiang, Qin Jiang and Fangcheng Jiang) in China (Zhu et al., 2017), the mean absolute abundances of ARGs in sediment samples were relatively higher in this study (3.34×10^5 to 3.56×10^9 copies/g). In general, the enrichment of ARGs in the coastal areas of the Yangtze River Estuary was at a high level among the domestic estuarine environments.

The average relative abundances of target genes in July were approximately 2–12 times higher than those in October. The average abundances of ARGs were significantly higher in Station No.1 (LHK), Station No.2 (SDK), Station No.3 (WSK), and Station No.4 (ZY) than in other sampling sites. This is because urban instream runoff (Liuhe River and Huangpu River) brings many pollutants (including antibiotics, heavy metals, PAHs, etc.) into the adjacent aquatic environment (Station No.1 (LHK) and Station No.3 (WSK)), which induce and synergistically select to produce ARGs. Meanwhile, external inputs induced by human activity also contribute to the spread and dissemination of ARGs near Station No. 2 (SDK) and Station No. 4 (ZY).

To further study the spatiotemporal distributions of ARG abundance, PCA was performed to assess the variation patterns of ARGs in different media at 8 sampling points (Fig. 4). The distances between scatters indicated the degree of variation. In both water and sediment samples, the two principal components explained 91.08% and 89.87% of the ARG variation, respectively, with PCA1 explaining 72.18% and 78.14% of the variation. The samples of summer and autumn clustered together respectively, and no correlation was observed between them. Only a few sampling sites were distributed outside of the 95% confidence ellipse, such as water samples at Station No.2 (SDK) and sediment samples at Station No.1 (LHK). The differences in ARG abundances in water samples might be attributed to the high nutrient content in the effluent discharge from WWTPs, which promoted the growth of bacteria (Luo et al., 2019; Jia et al., 2022). The variations in ARG abundances in sediment samples were mainly related to urban inland river runoff and pollutants (e.g., heavy metals, antibiotics, and resistant bacteria) caused by human activities (Guo et al., 2018). The discharge of treated water from WWTPs was also found to increase ARG abundances (Proia et al., 2018). In addition, the results of Guo et al. (2018) pointed out that SDK WWTPs and WSK Huangpu River intakes had a significant impact on the distributions of ARGs. Therefore, it is suggested that river runoff and WWTPs are major sources of ARGs in the Yangtze River Estuary.

4.2. Distribution coefficient of ARGs

The distribution coefficient (Kd), which is one of the most well-known parameters used to describe the distribution behavior of ARGs at the water–sediment interface (Lu et al., 2019), was calculated for 10 target genes by Eq. (2) and represented as box plots (Fig. S5). The distribution coefficient in July and October were generally above 1.0, indicating that ARGs easily accumulated in sediments. Previous studies have shown that sediments serve as sinks of antibiotic resistance genes (Guo et al., 2018; Lu et al., 2019). In aquatic environments, ARGs are readily adsorbed to particulate/suspended matter and settle. A stable biological environment such as the sedimentary phase is conducive to the reproduction of bacteria, thus promoting the migration and diffusion of ARGs (Wang et al., 2021). Overall, the mean Kd value of tetracycline resistance genes (2.77×10^4) was much higher than that of sulfonamide resistance genes (4.05×10^3). This indicates a higher adsorption capacity of tetracycline resistance genes in the sediment phase, which is consistent with the results in Chen and Zhou (2014) and Huang et al. (2019). Heavy use of tetracyclines has accumulated tetracycline-resistant genes in estuarine and coastal habitats. (Suzuki
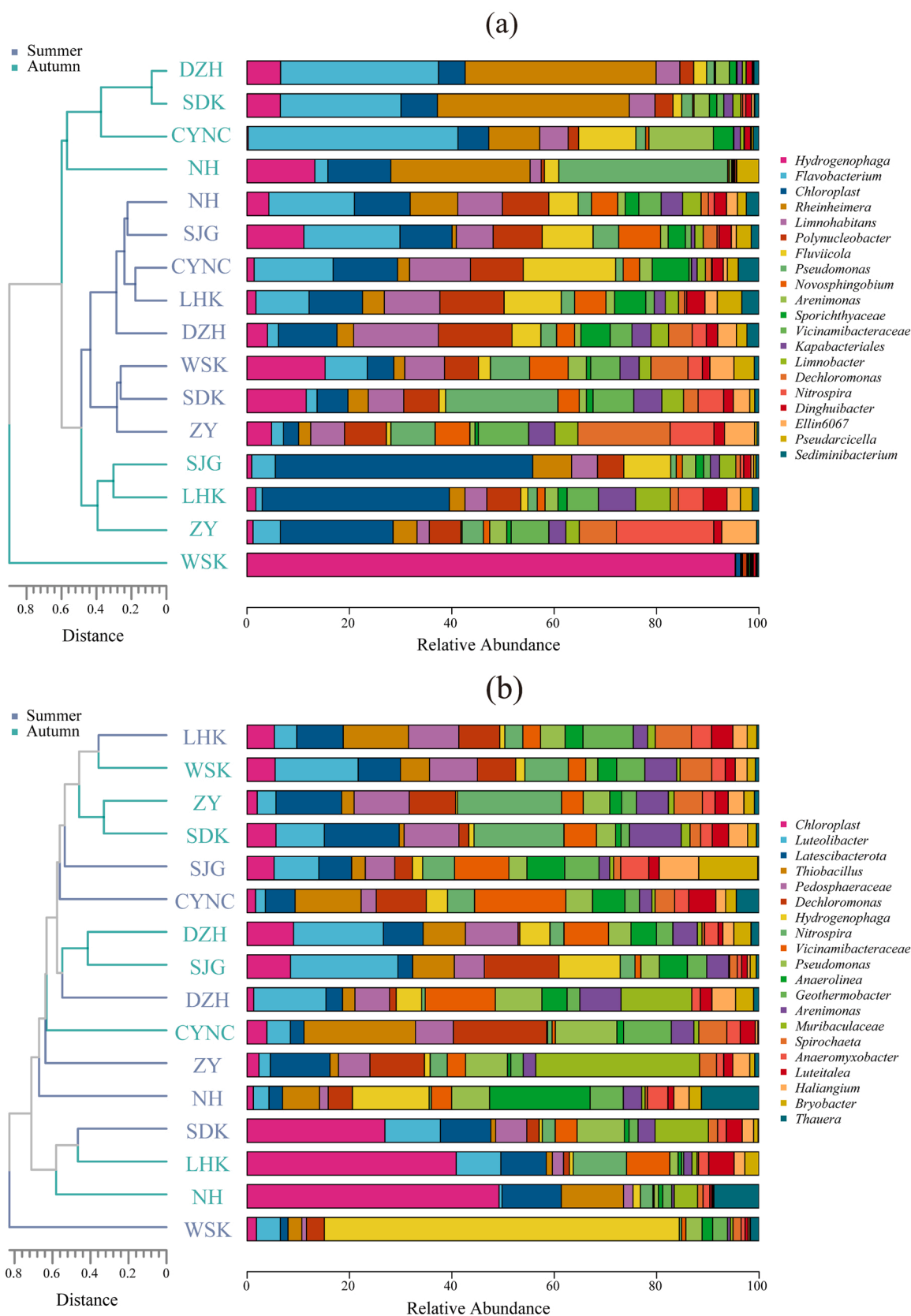
Fig. 4. PCA of ARG abundance in water and sediment samples taken along the nearshore area of the Yangtze Estuary with seasonal variations. ARG abundance at different sampling sites was clustered in blue and red ovals, representing summer and autumn, respectively. (a) Water sample; (b) Sediment sample.

et al., 2019; Zhang et al., 2018; Zheng et al., 2021). Among the 10 target genes, the average Kd value of *tet*A was the highest (1.22 × 10⁵), indicating that *tet*A was more easily enriched in sediments than other ARGs. The Kd value of the sulfonamide resistance genes had no seasonal variation, which was similar to the trend of *int*I1 because of the significant positive correlation between sulfonamide resistance genes and *int*I1 ($r = 0.881$, $P < 0.01$) (Guo et al., 2018; Lu et al., 2015; Zhang et al., 2018).

4.3. Correlations between intI1 and ARGs

In this paper, the correlation coefficients and *P* values between *int*I1 and other ARGs were calculated and analyzed using the Spearman rank correlation test (Cheng et al., 2021). As shown in Table 1, *int*I1 has a certain correlation with a variety of ARGs, especially in the water samples. A significant positive correlation between *sul*1 and *int*I1 was observed in all samples ($P < 0.05$), as *sul*1 often overlaps with the *int*I1 gene sequence (Chee-Sanford et al., 2009; Guo et al., 2018; Nigro et al., 2013) and is linked to *int*I1 with a broader host range (He et al., 2014). In the sediments, it was also found that the correlation between *int*I1 and most genes was not significant ($P > 0.05$), particularly the tetracycline resistance genes, which disagreed with previous studies. Eighteen ARGs were selected from 15 freshwater lakes along the Yangtze River as target genes, Yang et al. (2017) demonstrated a significant correlation between the tetracycline resistance gene *tet*G and *int*I1 ($P < 0.1$). Differences between the results in the present paper and those of Yang et al. (2017) were caused by the following: (1) The study area in Yang et al. (2017) was a freshwater lake along the Yangtze River, which was less affected by urbanization development; (2) Pollutants were retained longer in lakes than in estuarine areas (Wang et al., 2020); and (3) Although *int*I1 was a mobile genetic element that facilitates the horizontal transfer of ARGs in aquatic environments, other MGEs were widely available in nature, including integrons, transposons, phages, plasmids, and gene islands (Elsas and Bailey, 2002; Marti et al., 2014; Zhang et al., 2020). In addition, the occurrence of ARGs in sediments might also be affected by other environmental factors such as heavy metals and polycyclic aromatic hydrocarbons (PAHs) (Nguyen et al., 2019; Das et al., 2021).

4.4. Correlations between ARGs and environmental factors

The relationship between the relative abundance of resistance genes and environmental factors in water samples was examined using the Spearman rank test and shown by origin (Fig. 5a). There were 72 correspondences between 9 target genes and 8 environmental factors. *int*I1 was significantly positively correlated with most genes. *Sul*1 and *tet*M genes were also significantly positively correlated with 5 ARGs (*tet*C, *tet*X, *tet*A, *tet*O and *int*I1), indicating that *sul*1 and *tet*M can act as indicator genes to regulate the dissemination of ARGs in the coastal area of the Yangtze Estuary. *Sul*2 is negatively associated with *int*I1, which may be due to that *sul*2 was not found in the *int*I1 gene sequence (Lin et al., 2015) and that the diffusion of *sul*2 was mediated by other mechanisms, such as plasmids and pBP1 (Skold, 2000). Environmental factors have a significant influence on the distributions of ARGs in estuarine and coastal habitats (Yang et al., 2018; Zheng et al., 2021; Wu et al., 2022). According to Fig. 5a, water temperature and conductivity were significantly positively correlated with most ARGs ($P < 0.05$), and the opposite correlation was observed for pH. This indicates that water temperature and conductivity are key influencing parameters governing the spread of ARGs. Zheng et al. (2018) found that the abundance of ARGs was higher in summer than other seasons (2.81 × 10⁶ copies·mL⁻¹), while that in spring was the lowest in the whole year. Loftin et al. (2008) also pointed out that temperature conditions can influence the number of tetracycline residues, which affected the concentration of tetracycline resistance genes.

The relationship between target genes and environmental factors were estimated using RDA (Li et al., 2020; Wang et al., 2022). As shown in Fig. 5b, RDA1 and RDA2 explained 76.48% and 15.33%, respectively, and the total explanation rate reached 91.81%, suggesting that the RDA model is feasible. In Fig. 5, salinity was negatively correlated with most ARGs (*sul*1, *tet*M, *tet*C, *tet*X, *tet*A and *tet*O), with only *tet*C showing a significant negative correlation with salinity ($P < 0.05$). However, RDA analysis showed that salinity was not the key factor influencing the ARG distributions in the coastal areas of the Yangtze River Estuary, despite being a vital environmental factor in estuary areas (Lu et al., 2015). Temp, DO, and pH were the most important environmental factors influencing ARG distributions, as indicated by their longer projections on the RDA1 axis. pH affects the number of tetracycline-resistant colonies, thereby promoting their production (Zhang et al., 2014).

### 4.5. Correlations between ARGs and microbial communities

Microbial communities can have a direct impact on the occurrence of ARGs by encoding resistance genes (Ju et al., 2016; Wang et al., 2020). In this paper, the cooccurrence patterns of ARGs and microbial communities in the coastal areas of the Yangtze River Estuary were explored using a network analysis method based on Spearman correlation (Fig. 6). The top 20 microbial phyla including Proteobacteria, Actinobacteriota, Bacteroidota, Cyanobacteria, Verrucomicrobiota, Planctomycetota, Acidobacteriota, Chloroflexi, Firmicutes, Nitrospirota, Patescibacteria, Gemmatimonadota, Bdellovibrionota, Myxococcota, Desulfobacterota, Bacteria, Armatimonadota, Latescibacterota, MBNT15 and NB1.j and target genes including *sul*1, *sul*2, *tet*M, *tet*C, *tet*X, *tet*A, *tet*O, *tet*Q, *int*I1 and 16 S rRNA genes were selected for network cooccurrence analysis. There were 200 correspondences found between microbial phyla and ARGs in both water and sediment samples, respectively. In water samples, a significant correlation ($P < 0.05$) was found between 16 microbial phyla (including Actinobacteriota, Bacteroidota, Verrucomicrobiota, Acidobacteriota, Cyanobacteria, Chloroflexi, Nitrospirota, Patescibacteria, Gemmatimonadota, Myxococcota, Desulfobacterota, Bacteria, Armatimonadota, Latescibacterota, MBNT15 and NB1.j) and all taget genes including *sul*1, *sul*2, *tet*M, *tet*C, *tet*X, *tet*A, *tet*O, *tet*Q, *int*I1 and 16 S rRNA genes. In detail, 3 phyla (Actinobacteriota, Bacteroidota and Cyanobacteria) showed a significantly positively correlation with a wide range of ARGs including *sul*2, *tet*M, *tet*X, *tet*O, *tet*Q, *int*I1 and 16 S rRNA genes in water samples ($r > 0.6$, $P < 0.05$), while most of the remaining phyla were significantly

Table 1
Correlation analysis between intI1 and other ARGs in water and sediment samples.

Seasons	Types		*sul*1	*sul*2	*tet*M	*tet*C	*tet*X	*tet*A	*tet*O	*tet*Q
Summer	Water	*r*	0.881 * *	0.048	0.881 * *	0.881 * *	0.786 *	0.786 *	0.786 *	0.690
(2021.7.15)		*P*	< 0.01	0.911	< 0.01	< 0.01	0.021	0.021	0.021	0.058
	Sediment	*r*	0.786 *	0.143	0.405	0.667	0.048	0.143	0.143	0.310
		P	0.021	0.736	0.320	0.071	0.911	0.736	0.736	0.456
Autumn	Water	*r*	0.794 *	0.904 * *	0.952 * *	0.690	0.714 *	0.619	0.905 * *	0.619
(2021.10.15)		*P*	0.019	< 0.01	< 0.01	0.058	0.047	0.102	< 0.01	0.102
	Sediment	*r*	0.755 *	0.429	0.405	0.095	0.452	0.738 *	0.810 *	0.119
		P	0.030	0.289	0.320	0.823	0.260	0.037	0.015	0.779

Notes: *r*: Spearman's rank order correlation analysis, *P*: *P value*, * : significant at $P < 0.05$, and * *: significant at $P < 0.01$.
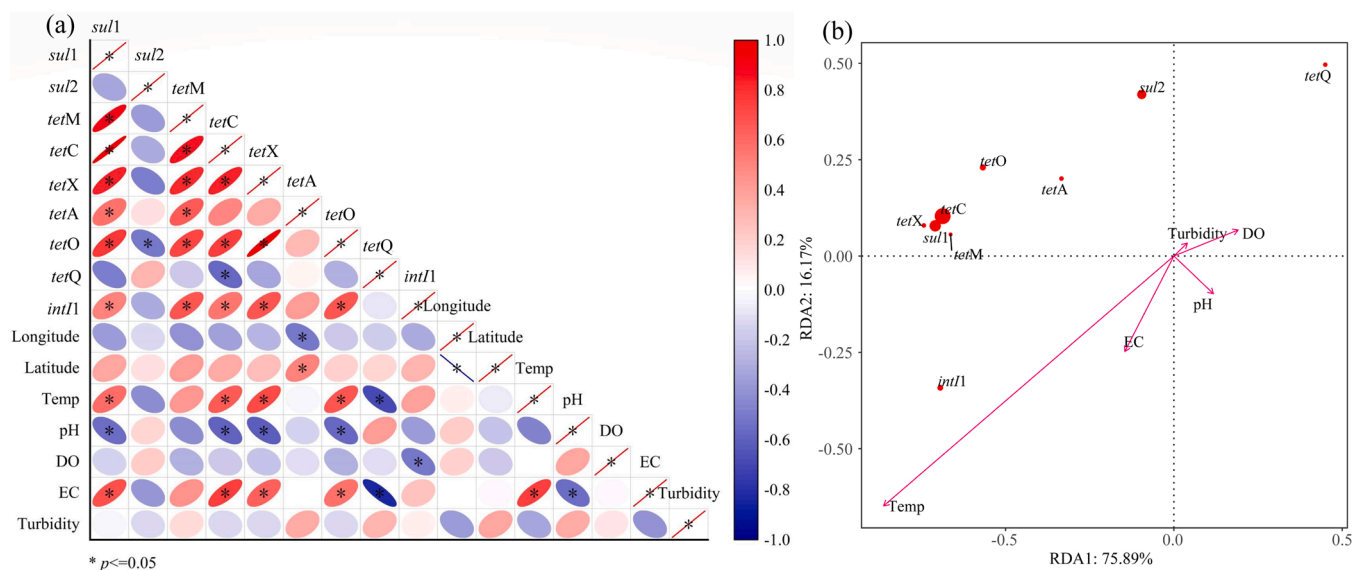
Fig. 5. (a) Correlation analysis between ARGs and environmental factors in different water samples. The red ellipse represents a positive correlation, whereas the blue ellipse represents a negative correlation. The flatter the ellipse is, the larger the correlation coefficient, and * represents $P \leq 0.05$. (b) RDA based on the relative abundances of the ARGs and environmental factors in different water samples. The length of the arrow indicates the degree of correlation, and the size of the red circle indicates the relative abundances of the ARGs.
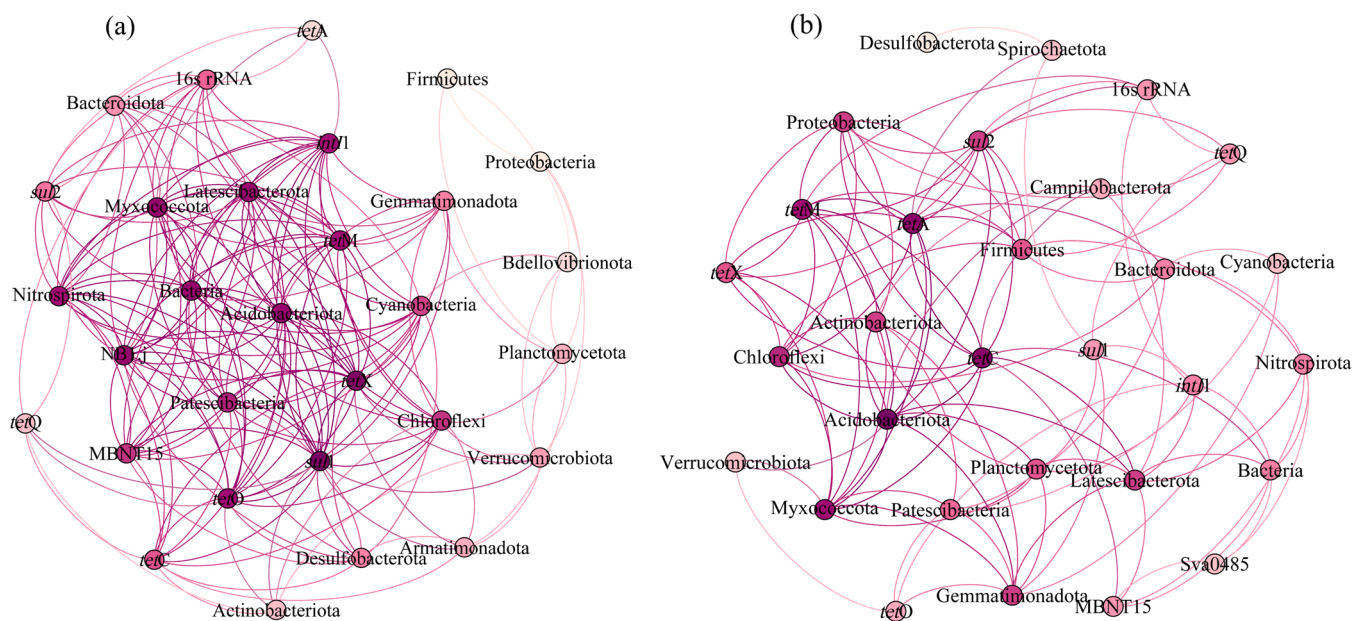
Fig. 6. Cooccurrence network analysis between the top 20 phyla and ARGs. Spearman correlation coefficient threshold ($|r| > 0.6$, $P < 0.05$). (a) Water sample;(b) Sediment sample.

negatively correlated with ARGs ($r < -0.6$, $P < 0.05$). When microbial phyla are significantly and positively correlated with target ARGs, these phyla may be potential hosts for ARGs (Xu et al., 2023). Three potential hosts (Bacteroidota, Cyanobacteria and Actinobacteriota) were detected in the water samples, as shown in Fig. 6a. For the sulfonamide resistance genes, no host information was detected for *sul*1, and one potential host (Bacteroidota) was present for *sul*2. Cyanobacteria were the common potential hosts for tetracycline resistance genes (*tet*X, *tet*O and *tet*M) because Cyanobacteria was significantly correlated with *int*I1. In sediment samples, a significant correlation ($P < 0.05$) was found between 16 microbial phyla including Proteobacteria, Bacteroidota, Acidobacteriota, Verrucomicrobiota, Chloroflexi, Planctomycetota, Firmicutes, Actinobacteriota, Myxococcota, Campilobacterota,

Patescibacteria, Gemmatimonadota, Latescibacterota, Spirochaetota, Bacteria and MBNT15 and 9 taget genes including *sul*1, *sul*2, *tet*M, *tet*C, *tet*X, *tet*A, *tet*O, *tet*Q and *int*I1. Five microbial phyla (Proteobacteria, Acidobacteriota, Firmicutes, Campilobacterota and Spirochaetota) showed a significantly positively correlation with 4 target genes (*sul*1, *sul*2, *tet*C and *tet*Q), and the remaining 11 phyla showed a negatively correlation with ARGs. Five potential host phyla (Proteobacteria, Acidobacteriota, Firmicutes, Campilobacterota and Spirochaetota) were detected in sediment samples (Fig. 6b). Only *sul*1, *sul*2, *tet*C, and *tet*Q had potential host phyla. Specifically, there were many potential host bacteria of *sul*2 (Proteobacteria, Acidobacteriota, and Firmicutes), indicating that *sul*2 was greatly affected by the microbial community in sediments. Spirochaetota was identified as potential host for *sul*1,

Campilobacterota was identified as potential host for *tet*C, and Proteobacteria and Firmicutes were identified as potential host for *tet*Q. Proteobacteria and Firmicutes were the common potential host for *sul*2 and *tet*Q. Wang et al. (2020) investigated the correlation between microbial communities and six target genes in Hong Lake and found that the abundance of Gemmatimonadetes, Proteobacteria, Firmicutes, and Verrucomicrobia showed a positive relationship with ARG abundance, suggesting that bacteria belonging to these phyla may be hosts of ARGs. A negative correlation between the microbial phylum and the target genes implied that the enrichment of the phylum may restrain the abundance and propagation of ARGs. Chen et al. (2019) showed that *Thiobacillus* has some biodegradation effect on oxytetracycline, which may indirectly inhibit the diffusion and transfer of ARGs and reduce the concentration of ARGs. In this study, we found a significant correlation between Proteobacteria and Firmicutes and antibiotic resistance genes (ARGs) in sediment samples. These two phyla were identified as common potential hosts for *sul*2 and *tet*Q, indicating that they may carry a wide range of ARGs, while other phyla play different roles in the co-occurrence pattern. Previous research has shown that ARGs can easily transfer between potential hosts, especially in Proteobacteria (Li et al., 2010). Human pathogenic bacteria, such as *Salmonella*, *Aspergillus*, *Campylobacter*, *Shigella*, and *E. coli*, can acquire these ARGs, leading to the formation of superbugs (Fischbach and Walsh, 2009). The spread of bacterial resistance renders many antibiotics ineffective, and induce people to misuse antibiotics, exacerbating the spread of resistance and posing a serious threat to public health and the environment.

5. Conclusions

The study carried out water and sediment samples at 8 sampling points along the Yangtze River Estuary in July and October, respectively. The spatiotemporal distributions of 10 ARGs and microbial communities were analyzed. The correlations between ARGs and microbial communities were explored. The main findings of this paper are as follows:

(1) Most resistance genes showed relatively higher abundance in summer and lower abundance in autumn. One-way analysis of variance (ANOVA) showed significant seasonal variation of some ARGs (7 ARGs in water and 6 ARGs in sediment). PCA and abundance analysis of ARGs showed that inland river runoff and effluent discharge from WWTPs had a large impact on the distributions of ARGs. The distribution coefficient in July and October were generally above 1.0, indicating that ARGs easily accumulated in sediments. In water samples, *int*I1 showed a positive correlation with most ARGs, while the opposite was true in sediment samples.
(2) In terms of microbial community diversity analysis, the richness and multiplicity of bacteria in sediment samples were much higher than those in water samples. Temporal and spatial differences in water samples had a significant effect on microbial community diversity. Proteobacteria was the dominant phylum along the Yangtze River Estuary. Significant seasonal variations were observed at the microbial genus level, and the relative abundances of the dominant genera in July were higher than those in October. Microbial community diversity and community structure were associated with effluent discharge from WWTPs.
(3) Redundancy analysis demonstrated that temperature, DO and pH were important environmental factors influencing the ARG distributions in the coastal areas of the Yangtze River Estuary. A significant correlation ($P < 0.05$) was found between 16 microbial phyla and most target genes in water and sediment samples. Network analysis showed that *sul*2 had one potential host (Bacteroidota), and three tetracycline resistance genes (*tet*X, *tet*O and *tet*M) had one common potential host (Cyanobacteria) in water samples. In sediments, Proteobacteria was a shared potential host

for *sul*2 and *tet*Q. Environmental factors and the microbial community had a great impact on the migration and transformation of ARGs in the coastal areas of the Yangtze River Estuary.

While our method does not yield a large dataset for in-depth analysis of antibiotic resistance genes (ARGs) and microbial community structure in the coastal areas of the Yangtze River Estuary, our findings on the co-occurrence of ARGs and their likelihood of occurrence in bacteria can aid decision-making on technical and management strategies to mitigate pollution problems caused by ARGs and multidrug resistance in bacteria along the coastal areas of the Yangtze River Estuary.

CRediT authorship contribution statement

Shuguang Liu: Conceptualization, Methodology, Software. **Qiuhong Xu**: Data curation, Writing – original draft. **Sha Lou**: Methodology, Writing – review & editing. **Junbiao Tu**: Investigation. **Wenjun Yin**: Investigation. **Xin Li**: Investigation. **Yuchen Jin**: Investigation. **Larisa Dorzhievna Radnaeva**: Investigation. **Elena Nikitina**: Investigation. **Aleksei Nikolavich Makhinov**: Methodology, Writing – review & editing. **José Tavares Araruna**: Writing – review & editing. **Irina Viktorovna Fedorova**: Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The authors are unable or have chosen not to specify which data has been used.

Acknowledgment

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115025.

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