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研究报告

台风利奇马对城市淡水系统中微生物群落和抗生素耐药性基因的影响

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Feng Xinqian, Liu Shuqi, Han Axiang, Guan Wanchun. Effects of typhoon Lekima on microbial communities and antibiotic resistance genes in urban freshwater systems[J]. Microbiology China, 2022, 49(7): 2428-2441

摘　要：【背景】极端天气事件(如台风)带来的强风和降水，会给水生生态系统造成短暂和持久的影响。然而，很少有研究关注台风对水生微生物群落和抗生素耐药性基因(antibiotic resistance genes，ARGs)的影响。【目的】对台风前后城市淡水水域的微生物群落和抗性基因组成进行研究分析，更好地认识极端天气对淡水生态系统的干扰。【方法】在台风前后从 4 个地点采集了水样，通过宏基因组分析，检测了台风利奇马对温州休闲水域微生物群落和抗性基因的影响。除水生微生物群落和抗性基因外，还分析了每个采样点的物理、化学参数，包括温度、pH、溶解氧、叶绿素 a、可溶性活性磷、硝酸盐、亚硝酸盐和铵。【结果】台风登陆后，大多数地点的 pH、溶解氧和叶绿素 a 都有所增加。然而，台风对九山湖的影响要弱于对三垟湿地的影响。台风登陆后，变形菌门、蓝菌门和拟杆菌门的相对丰度增加，而放线菌门的相对丰度下降。在属水平上，栖湖菌的微生物多样性和相对丰度显著增加。在所有的环境因子中，铵是影响微生物群落结构的最重要的环境因子。另外，在所有样本中均检测到 35 个机会性致病菌类群。台风后，铜绿假单胞菌的相对丰度增加。ARGs 显示了空间(采样点间)和时间(台风前后)的变化。冗余分析表明，水总无机氮是影响抗性基因分布的主要环境因子。【结论】这些发现为极端天气(如台风)如何影响淡水系统中的微生物群落和抗性基因提供了新的见解。台风登陆增加了城市淡水系统的公共安全风险，因此，检验检疫方法和手段应该前移，加

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强对环境健康安全的评价和分析，这将有助于减轻抗生素耐药性和致病菌扩散的风险。

关键词：抗生素耐药基因；宏基因组分析；微生物群落；台风；淡水系统

Effects of typhoon Lekima on microbial communities and antibiotic resistance genes in urban freshwater systems

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Abstract: [Background] Extreme weather events, such as typhoons, bring transient and lasting effects on aquatic ecosystems due to strong winds and precipitation. However, very few studies have focused on the effects of typhoons on aquatic microbial communities and antibiotic resistance genes (ARGs). **[Objective]** To understand the effect of extreme weather on freshwater ecosystem, we analyzed the microbial communities and ARGs in urban freshwater before and after typhoon. **[Methods]** Water samples were collected from four sites before and after the typhoon. The effects of Lekima on the microbial communities and ARGs in recreational waters in Wenzhou were examined by metagenomic analysis in summer, 2019. In addition to the aquatic microbial communities and ARGs, the physical and chemical parameters of water samples from each sampling site were analyzed, including temperature, pH, dissolved oxygen, chlorophyll a, soluble active phosphorus, nitrate, nitrite, and ammonium. **[Results]** The pH, dissolved oxygen, and chlorophyll a at most sites increased after the typhoon, and the typhoon had a weaker effect on Jiushan Lake than on Sanyang Wetland. The relative abundances of *Proteobacteria*, *Cyanobacteria*, and *Bacteroidetes* increased, while that of *Actinobacteria* decreased after the typhoon. At the genus level, *Limnohabitans* showcased significantly increased diversity and relative abundance after the typhoon. Among all the environmental factors, ammonium was the key environmental factor affecting microbial community structure. In addition, 35 opportunistic pathogen taxa were detected in all samples. The relative abundance of *Pseudomonas aeruginosa*, an important opportunistic pathogen, increased after the typhoon. The ARGs showed spatial (among sampling sites) and temporal (before and after the typhoon) variation. A redundancy analysis showed that water total inorganic nitrogen was the main environmental factor affecting the distribution of ARGs. **[Conclusion]** These findings provide new insights into how extreme weather (e.g., typhoons) influences microbial communities and ARGs in freshwater system. Typhoon landing increases the public safety risk of urban freshwater system. Therefore, the testing and quarantine should be carried out in advance to strengthen the evaluation and analysis of environmental health and safety, which will help to reduce the risk of antibiotic resistance and pathogen diffusion.

Keywords: antibiotic resistance genes; metagenomic analysis; microbial community; typhoon; freshwater system

Extreme weather events, such as typhoons and heatwaves, are widely known to have transient and lasting effects on ecosystems[1-3]. Among them, strong winds and precipitation associated with typhoons can affect aquatic environments via short-term runoff events from watersheds and physical mixing of the water column[4]. Water mixing increases after typhoon landing, with a lower temperature, increasing nutrient (nitrate and carbon) and chlorophyll a (Chl-a) concentrations in the surface water[5-7]. Tseng et al. found that typhoons and rainfall were the main environmental factors influencing the community structure in subtropical freshwater reservoirs[8]. Further, it is expected that the risk of typhoon landing in coastal areas will increase, and the intensity will be strengthened[9]. Therefore, it is more and more important to pay attention to the impact of typhoon, the extreme weather on water parameters.

Aquatic systems are a potential reservoir for various biopollutants, including antibiotic resistance genes (ARGs) and human pathogens[10-11]. As emerging contaminants, ARGs are attracting increasing public attentions[12]. More and more evidence shows that clinical resistance is closely related to environmental ARGs and bacteria[13]. ARGs may be transmitted from the environment to human pathogens through direct or indirect contact with antibiotic resistant bacteria[14], which will pose a threat to clinical medication, weaken the effect of antibiotic treatment and endanger public health. ARGs have been detected in various aquatic environments, including sediment[15], drinking water[16-17], and oceans[18]. The occurrence and distribution of ARGs are influenced by many factors, such as geographical location, seasonal variations, and human activities[19-21]. In terms of seasonal variation, for example, Zheng et al. found a higher abundance of ARGs in summer than in winter in a peri-urban river[21]. In aquatic ecosystems, bacteria play a crucial role in biogeochemical processes. The diversity and composition of a bacterial community is influenced by environmental factors[22-23], while bacterial communities significantly affect the shifts of ARG structure[24]. The impact of environmental changes on phytoplankton in water bodies, which were brought about by typhoons, has been documented. For example, a study in the northeastern of South China Sea showed a higher abundance of *Dinophyta* and a lower abundance of *Bacillariophyta* and *Cyanophyta* after the typhoons Roke and Haitang[25]. However, few studies have investigated the impact of extreme weather on plankton bacterial communities and ARG composition in water bodies, especially urban water, which is significantly affected by human activities.

On August 10th, 2019, typhoon Lekima landed in southeastern Zhejiang Province and hit areas along the eastern coast of China with heavy rainfall. In order to explore the effects of the typhoon on the microbial community structure and ARGs in the lakes, the typhoon was traced and eight samples were collected from four different sampling sites, two in Jiushan Lake (JS) and two in Sanyang Wetland (SY), before and after the typhoon Lekima landed. Jiushan Lake, which is a shallow water in the city center, is a very popular natural swimming pool for local people. Sanyang Wetland carries a large number of citizens for leisure and entertainment activities. Thus, this study will improve our understanding of how typhoons influence microbial communities and ARG composition in urban waters.

1 Materials and Methods

1.1 Sampling and environmental measurements

Four sampling sites (JS.1 and JS.2 in Jiushan Lake, SY.1 and SY.2 in Sanyang Wetland) in Wenzhou, a coastal city in southeast Zhejiang Province, were selected, and eight water samples from 0.5 m depth were collected before and after typhoon Lekima landed (6–7 August and 12–13 August 2019, respectively). All water samples were stored in sterilized Nalgene 1-L polycarbonate bottles and transported to the laboratory in an icebox within 2 h of collection.

Environmental parameters including water temperature (T), pH, and dissolved oxygen (DO) were measured *in situ* by a YSI 550 portable dissolved oxygen meter. In addition, chlorophyll a (Chl-a), soluble active phosphorus (PO_4^{3-}), nitrate (NO_3^-), nitrite (NO_2^-), total inorganic nitrogen (TIN), soluble reactive phosphorus (SRP) and ammonium (NH_4^+) were measured using standard methods as described previously[26-27].

1.2 DNA extraction and shotgun metagenomic sequencing

Approximately 300 mL of each water sample was pre-filtered through 5 μm pore-size filters (Millipore, Billerica, MA) to remove impurities. Then, each filtrate was filtered again using a 0.22 μm membrane filter to capture and concentrate microbes. The filters were cut into strips using sterile scissors, placed into 5 mL sterile tubes, and stored at –20 °C until DNA extraction. Water samples were labeled as JS.1, JS.2, SY.1, SY.2, JS.1T, JS.2T, SY.1T, and SY.2T (JS: Jiushan Lake; SY: Sanyang Wetland; T: samples collected after the typhoon). DNA was extracted using a PowerWater DNA Isolation Kit (QIAGEN, Hilden) according to the manufacturer's instructions. DNA quality was assessed using a DeNovix DS-11 microspectrophotometer (DeNovix, Wilmington, DE). The degradation and contamination of DNA samples was electrophoretically examined on 1% agarose gels. Qualified DNA samples were sent to Novogene (Beijing, China) for Illumina shotgun high-throughput sequencing using the PE 150 (paired-end sequencing, 150 bp reads) sequencing strategy. A total amount of 1 μg DNA per sample was used as input material for library construction with an insert size of 350 bp, followed by high-throughput sequencing on an Illumina HiSeq 4000 platform (Illumina, San Diego, CA).

1.3 Metagenomic sequence analysis

The raw sequencing data were pre-processed for quality control using Readfq V8 (public domain). The raw reads with low-quality bases, >10 N bases, or>15 bp of overlap with adapters were filtered out. The sequencing output of each water sample was approximately 12 Gb. The filtered high-quality sequences were used for subsequent analysis. High-quality short reads of DNA were assembled using MEGAHIT and Scaftigs>500 bp were used for downstream analyses. The assembled Scaftigs were used to predict open reading frames (ORFs) using MetaGeneMark. A non-redundant gene catalog was constructed after removing redundancy using CD-HIT (V 4.5.8, parameters: -c 0.95, -G 0, -aS 0.9, -g 1, -d 0). The high-quality reads from the sample were aligned against the gene catalog using Bowtie2 to determine the relative gene abundance for each gene.

To investigate the microbial composition, the generated quality-filtered genes were aligned to the NR database (version: 2018-01-02) of NCBI using DIAMOND V0.9.9 software (free domain)[28] with an E-value$\leq 10^{-5}$. Human pathogenic bacteria were identified by referring to a list of 51 species provided in a previous study[29]. The abundance of a taxonomic group was calculated by summing the abundance of genes annotated to a feature.

To explore the diversity and abundance of ARGs, the quality-filtered genes were aligned to the CARD database using Resistance Gene Identifier (RGI) software[30-32] for annotation and BLASTp comparison (E-value$\leq 10^{-30}$). The alignment results were used to calculate both the total and the relative abundance of ARGs. The former was calculated by dividing the number of all ARG-annotated genes by the number of total mapped genes and expressed in genes per million mapped genes, while the latter was calculated by dividing the number of annotated genes for each ARG by the total number of genes for all ARGs and expressed as a percentage. Data are available at the NCBI Sequence Read Archive under the project with the accession No. of PRJNA638205.

1.4 Statistical analysis

Histograms were created using OriginPro 8.5 and R V2.15.3 was used to create boxplots, plots of the diversity index (Inverse Simpson), and the bubble graph. Redundancy analysis (RDA) was performed to investigate the correlation between the top 10 abundant genera, ARGs, and environmental factors. Principal component analysis (PCA) was performed to analyze microbial community structure similarity among all samples. RDA and PCA were conducted in CANOCO V5 (Biometrics, Wageningen, the Netherlands) for windows. Statistical analysis was performed with IBM SPSS Statistics V22 (IBM, Armonk, NY, USA) using one-way ANOVA (LSD test) and Wilcoxon signed-rank test.

2 Results

2.1 Environmental parameters

Physical and chemical parameters from each sampling site are shown in Table 1. The pH, DO, and Chl-a contents in most of the sampling sites increased after the typhoon. There were spatial and temporal differences in nutrients. In SY, the concentrations of NO_3^-, NO_2^-, TIN, and the ratio of N to P (N/P) increased after the typhoon, except for SRP and NH_4^+. However, the typhoon had a weaker effect on JS; only SRP and NH_4^+ decreased after the typhoon. Whether the typhoon landed or not, TIN was higher and SRP was lower in SY than in JS.

2.2 Microbial abundance and composition

Microbial communities were predominantly comprised of five phyla: *Proteobacteria* (23.59%–32.93%), *Actinobacteria* (8.67%–25.37%), *Cyanobacteria* (3.70%–10.10%), *Bacteroidetes* (4.00%–9.27%), and *Verrucomicrobia* (1.20–2.35%) (Figure 1). At the genus level, *Synechococcus* and *Limnohabitans* were dominant in all samples (Figure 2). The relative abundance of *Limnohabitans* increased significantly after the typhoon (P<0.05, one-way ANOVA test) (Figure 2). The Simpson index was used to evaluate microbial diversity. The microbial diversity in each sample increased

表 1 水体理化参数(n=3)

Table 1 Physical and chemical properties of the water samples (n=3)

Site	T (°C)	pH	DO (mg/L)	Chl-a (μg/L)	NO_2^- (μmol/L)	NH_4^+ (μmol/L)	NO_3^- (μmol/L)	SRP (μmol/L)	TIN (μmol/L)	N/P
JS.1	33.6	8.2	9.3	72.28	2.24	0.48	33.63	6.24	35.45	5.69
	(0)	(0)	(0)	(1.62)d	(0.02)f	(0.07)e	(3.39)d	(0.04)a	(4.28)d	(0.72)e
JS.2	33.1	7.59	6.3	41.88	1.89	1.25	31.41	6.15	34.55	5.62
	(0)	(0)	(0)	(1.34)e	(0.02)g	(0.24)e	(0.63)d	(0.04)a	(0.88)d	(0.15)e
SY.1	32.9	7.49	5.02	80.19	7.08	13.44	66.40	3.07	86.92	28.29
	(0)	(0)	(0)	(2.34)c	(0.03)c	(0.83)b	(2.97)c	(0.04)d	(3.64)c	(1.41)c
SY.2	31	6.92	2.07	26.47	6.78	15.79	65.15	4.39	87.72	20.00
	(0)	(0)	(0)	(0.67)h	(0.03)d	(0.54)a	(0.73)c	(0.06)c	(1.20)c	(0.1)d
JS.1T	33.3	8.54	9.89	117.95	2.75	0.47	26.95	5.61	29.10	5.18
	(0)	(0)	(0)	(1.59)a	(0.02)e	(0.04)e	(3.76)d	(0.09)b	(1.50)d	(0.60)e
JS.2T	32.7	7.72	7.6	36.06	1.83	2.06	19.37	4.48	23.26	5.20
	(0)	(0)	(0)	(0)f	(0)h	(0.08)e	(0.63)e	(0)c	(0.56)d	(0.12)e
SY.1T	25	7.64	6.5	94.33	26.64	4.14	127.31	1.55	158.09	101.87
	(0)	(0)	(0)	(5.6)b	(0.06)a	(0.48)d	(2.44)a	(0.02)f	(2.22)a	(0.23)a
SY.2T	25	7.29	6.2	35.00	22.39	6.58	122.57	2.06	151.53	73.70
	(0)	(0)	(0)	(0.40)g	(0)b	(0.85)c	(3.93)b	(0.06)e	(3.21)b	(3.73)b

Note: T: Temperature; DO: Dissolved oxygen; Chl-a: Chlorophyll a; SRP: Soluble reactive phosphorus; TIN: Total inorganic nitrogen; N/P: Ratio of total inorganic nitrogen and SRP. The numbers in brackets represent the standard deviation. P<0.05, One-way ANOVA (LSD test) results among the different samples are indicated by superscript letters. The data with identical superscript letters indicate that the mean values are not significantly different.

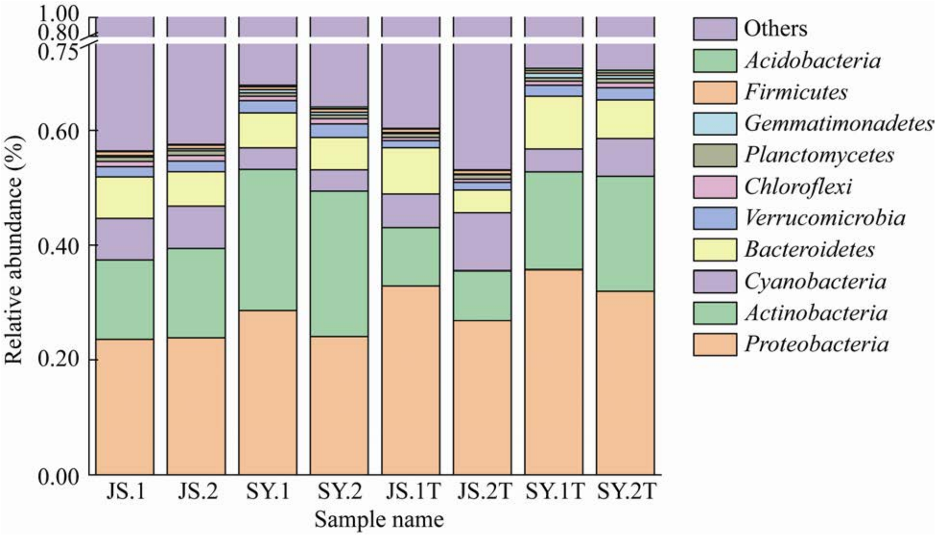

图 1 九山湖和三垟湿地的水样在门分类水平上的微生物群落结构

Figure 1 Microbial community structure in the water samples in Jiushan Lake (JS) and Sanyang Wetland (SY) at the phylum level.

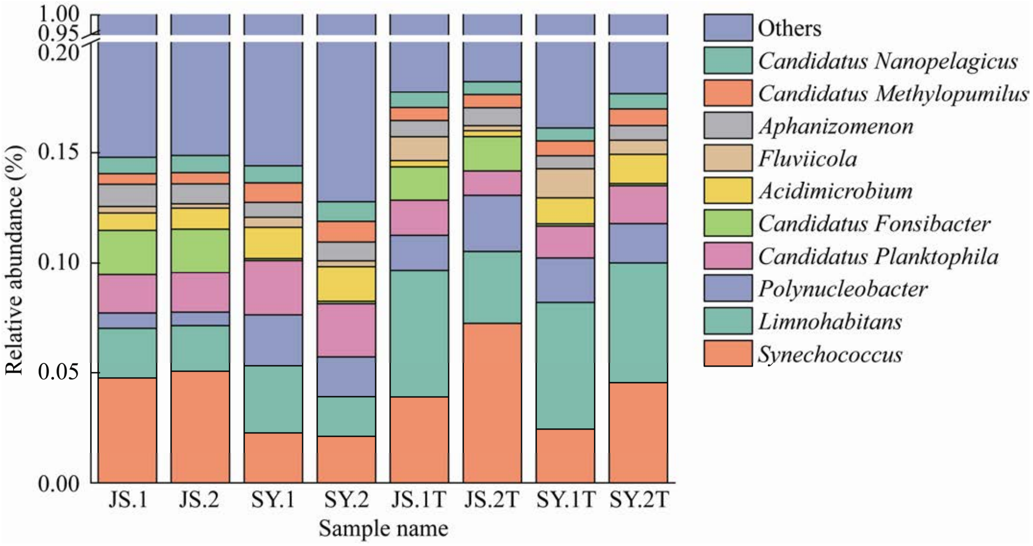

图 2 九山湖和三垟湿地的水样在属分类水平上的微生物群落结构

Figure 2 Microbial community structure in water samples in Jiushan Lake (JS) and Sanyang Wetland (SY) at the genus level.

significantly after the typhoon (P=0.029, Wilcoxon test) (Figure 3). The microbial community in SY had a higher diversity than that in JS (Figure 3).

PCA analysis was performed to evaluate the similarity in microbial community structure of all samples. The results suggested that all samples collected before the typhoon were significantly different from those collected after the typhoon (Figure 4). In order to explore the main environmental factors that affect the distribution of planktonic bacteria, we performed an RDA analysis of the water environmental factors and the

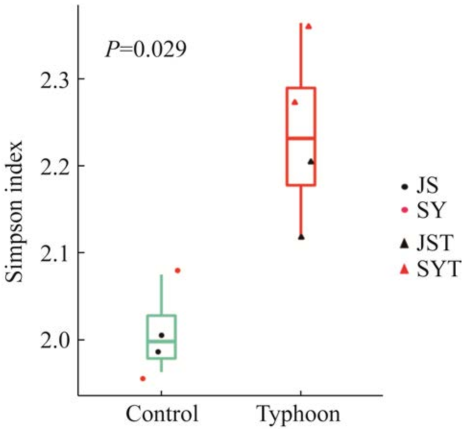

图 3　属水平两组的 α 多样性

Figure 3　Alpha diversity index of two groups at the genus level.

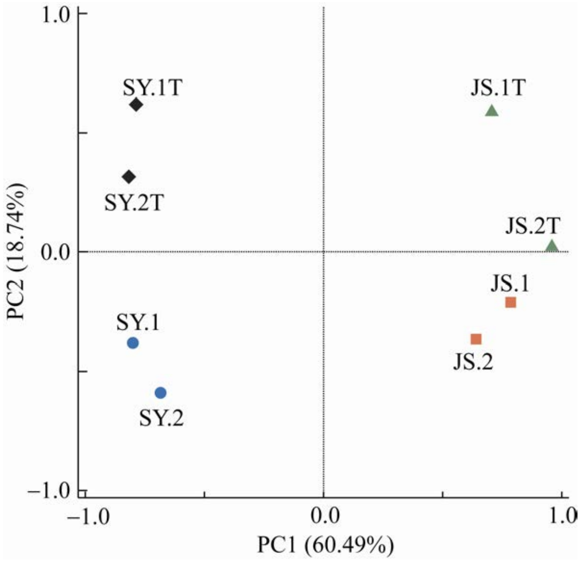

图 4　在属分类水平上的微生物群落的主成分分析

Figure 4　Principal component analysis (PCA) of the microbial community at the genus level.

dominant bacteria at the genus level. The main environmental factor affecting the distribution of microorganisms was NH_4^+ (P=0.038; Figure 5).

A total of 35 opportunistic pathogens were detected before and after the typhoon, and *Pseudomonas aeruginosa* accounted for the highest abundance in all samples. Other species with high abundances included *Escherichia coli*, *Chlamydia trachomatis*, and *Clostridium botulinum* (Figure 6). After the typhoon, the abundance of *Pseudomonas aeruginosa* in each sample tended to increase.

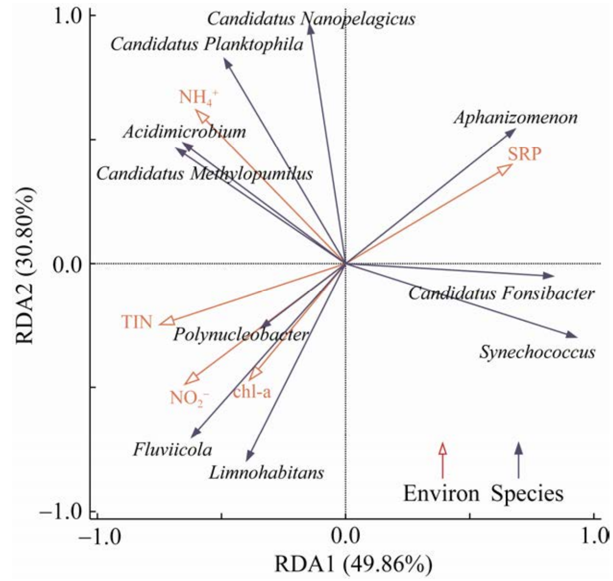

图 5　环境因子和 top 10 的属的冗余分析

Figure 5　Redundancy analysis (RDA) of environmental factors and the top 10 genera.

2.3　Diversity and abundance of ARGs

In JS, 396 and 383 ARGs were detected before and after the typhoon, respectively, of which 348 ARGs were shared whether the typhoon landed or not, 48 were unique before typhoon and 35 after typhoon. In SY, 420 and 421 ARGs were detected before and after the typhoon, respectively, of which 374 ARGs were common whether the typhoon landed or not, 46 were peculiar before typhoon and 47 after typhoon (Figure 7). The top 20 resistance genes accounted for 30.11%–36.45% of all resistance genes in each sample (Figure 8), and they mainly expressed resistance to aminoglycoside drugs. The typhoon affected the ARG composition in both JS and SY significantly, indicating spatial and temporal variation. In JS, the main ARGs were *ade*F, *cat*, and *APH*3-Vb before the typhoon and *cat*, *aad*6, and *Bacillus subtilis mpr*F after the typhoon. However, the main resistance genes in SY were

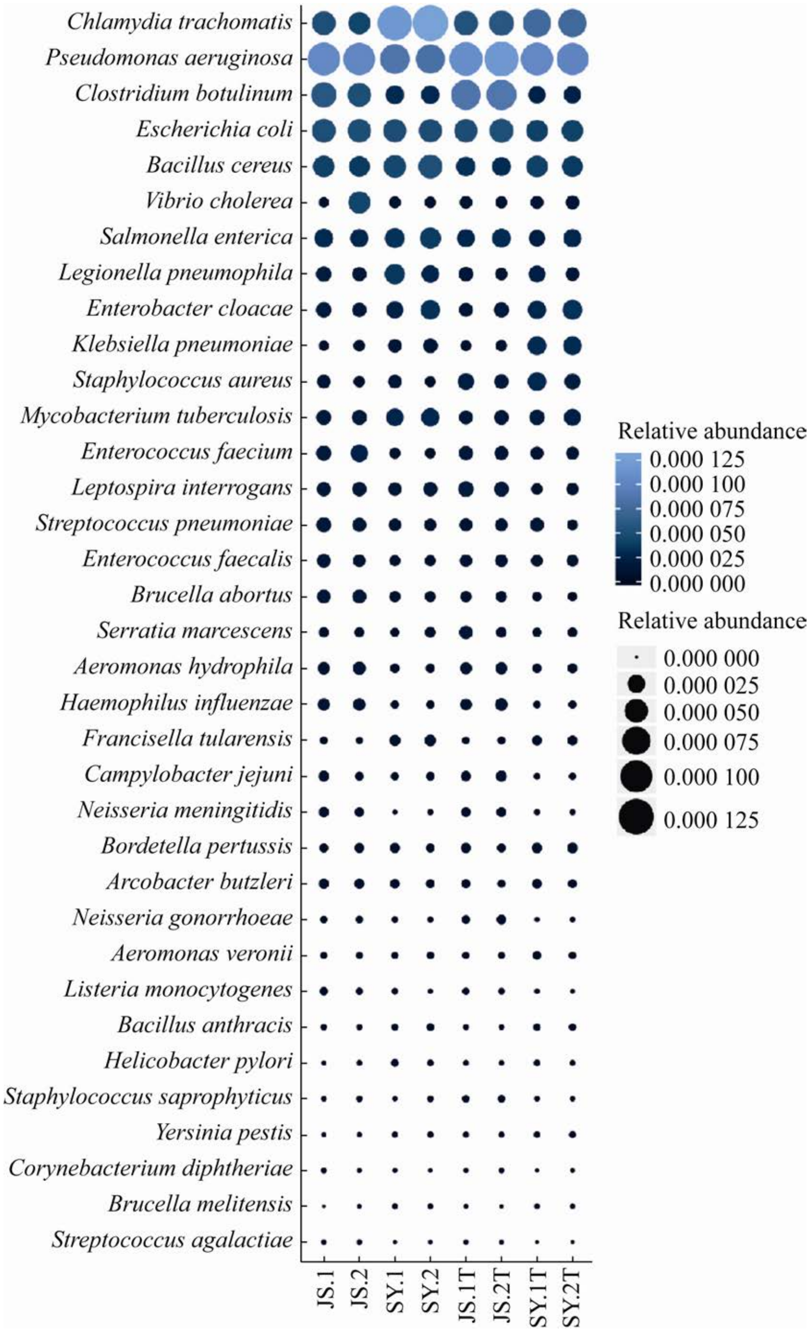

图 6 条件致病菌相对丰度的气泡图

Figure 6 Bubble graph showing the relative abundance of potentially pathogenic bacteria. Relative abundance was defined as the ratio of the number of each potentially pathogenic bacteria sequences to the total number of potentially pathogenic bacteria sequences. The sum was 1.

*Mycobacterium tuberculosis rps*L, *abc*A, and *ade*F before the typhoon and *Mycobacterium tuberculosis rps*L, *ade*F, and *cmr*A after the typhoon. The resistance genes *ade*F, *APH*6-Ia, *van*SM, and *sul*1 had a high proportion (>1%) in all eight water samples, at 2.61%–4.77%, 1.36%–3.27%, 1.00%–2.77%, and 1.00%–2.27%, respectively.

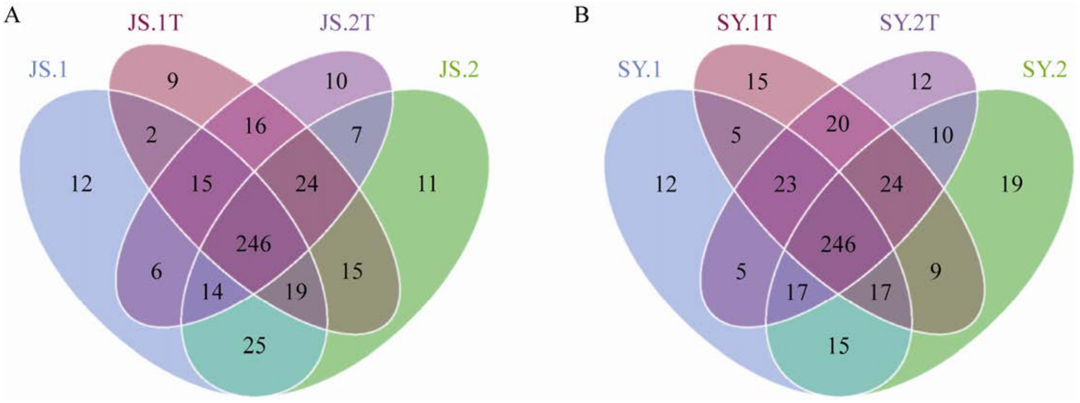

图 7 水样中共有和特有的抗性基因 A：九山湖；B：三垟湿地

Figure 7 Shared and unique ARGs in water samples. A: Jiushan Lake; B: Sanyang Wetland.

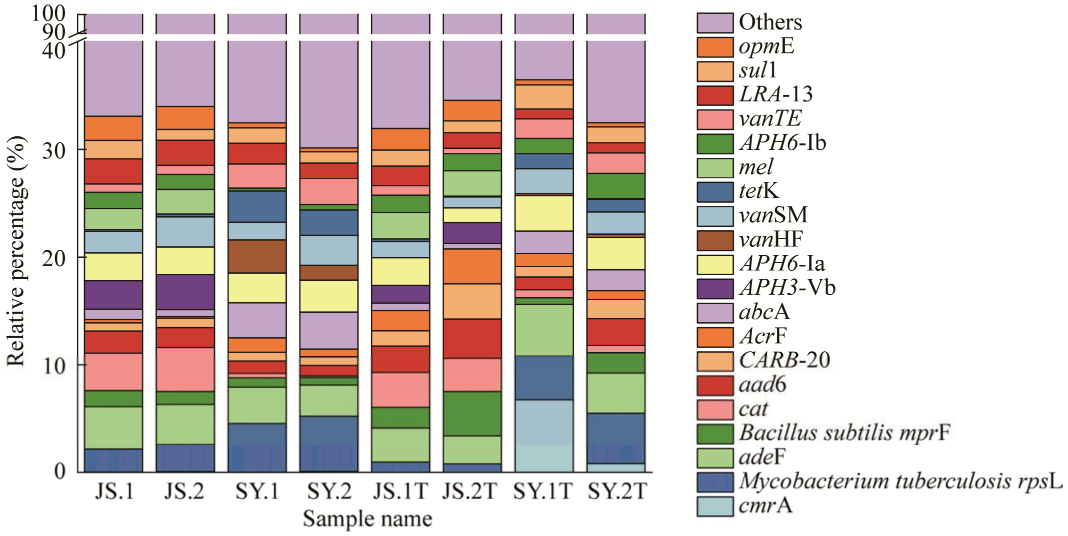

图 8 抗性基因在各个样本中的相对百分比

Figure 8 Relative percentage of antibiotic resistance genes (ARGs) in each sample.

Differences in ARG compositions of each sample were further analyzed by PCA (Figure 9). Generally, JS samples were different from SY samples, irrespective of the typhoon, which indicated spatial differences in ARG composition between the sampling sites. After the typhoon, there were clear differences between the two samples in each sampling site, that is, SY.1T was different from SY.2T and JS.1T was different from JS.2T. The distance between SY.1T and SY.2T was more significant than that between JS.1T and JS.2T (Figure 9).

Water environmental factors and the top 10 ARGs with the largest relative percentages among the eight samples were used in a redundancy analysis (RDA) to explore the correlation between water environmental factors and the distribution of resistance genes. The first two axes explained 79.1% of the ARG variations, and the environmental factors SRP, T, TIN, NH_4^+, NO_2^-, and NO_3^- were all significantly correlated with the distribution of ARGs ($P<0.05$) (Figure 10). TIN was the most important factor driving the distribution of ARGs, explaining 44.8% of the variation.

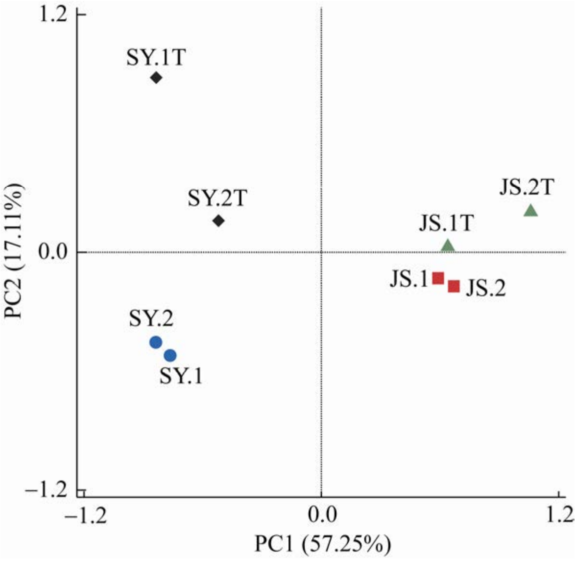

图 9 所有抗性基因的主成分分析

Figure 9 Principal components analysis (PCA) of all antibiotic resistance genes (ARGs).

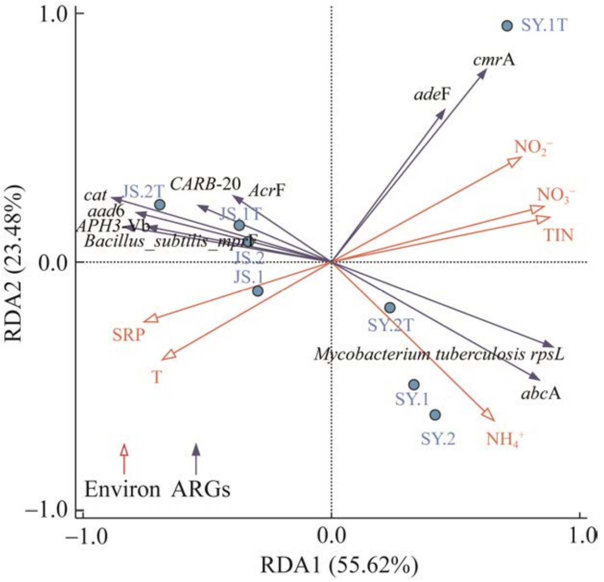

图 10 环境因子和 **top 10** 抗性基因的冗余分析

Figure 10 Redundancy analysis (RDA) of environmental factors and the top 10 antibiotic resistance genes (ARGs).

3 Discussion

Freshwater microbial communities have strong adaptability to natural and human disturbances. Based on the intensity of disturbances, the community structure usually returns to its original state within a few weeks or months[33-34]. Therefore, we completed sampling before and one week after typhoon Lekima landed to explore the impact of typhoon weather on the microbial community structure and ARGs in the water environment.

The microbial community structure analysis showed that *Proteobacteria* were dominant in all water samples, consistent with the results in other environmental samples such as soil, aquaculture water, and sewage[35-37]. In addition, the microbial community structure underwent a dynamic change before and after the typhoon. *Proteobacteria*, *Cyanobacteria*, *Bacteroides*, and *Actinobacteria* were the dominant phyla in water before the typhoon. After typhoon landing, the relative abundance of the first three phyla were increased, while that of *Actinobacteria* was decreased. At the genus level, the alpha diversity of microbes increased significantly after the typhoon. These results may be related to the typhoon's strong winds and heavy rains when it landed. High winds stir the water body, and the microorganisms in the bottom mud may mix into the upper water[4]. In addition, an increase in surface runoff could have carried nutrients and microorganisms from surrounding water bodies to the sampling sites, increasing microbial diversity[6,38]. The microbial community composition of the samples in Jiushan Lake and Sanyang Wetland was significantly different before and after the typhoon. In this study, increased Chl-a and nutrient concentrations were detected in the water after the typhoon, which was consistent with the previous results[5,39]. Several studies have shown that differences in bacterial community composition may be related to changes in nitrogen, temperature, pH, and total phosphorus available in water[22,40]. In this study, there was a significant correlation between the NH_4^+ and bacterial community composition. The sensitivity of bacteria to environmental factors may be related to the ecological environment in which they live. Bacteria living in different water bodies have different tolerances to environmental factors, leading to different results when analyzing different samples.

Typhoons accompanied by heavy rain may bring pathogenic bacteria into urban water systems. In 2013, after typhoon Haiyan, there was an outbreak of gastroenteritis in communities in Leyte, the Philippines. Researches showed that *Aeromonas hydrophila* probably caused the outbreak of acute gastroenteritis. The typhoon damaged part of the water supply pipeline, causing the spread of bacteria through contaminated water[41]. In this study, *P. aeruginosa*, *E. coli*, *C. trachomatis*, *C. botulinum*, *Enterobacteriaceae*, and other human pathogens had relatively high abundances in Jiushan Lake and Sanyang Wetland after the typhoon. *Pseudomonas aeruginosa*, the most important human pathogenic bacteria in *Pseudomonas*, had the highest relative abundance among all water samples. A previous study found that the number of *P. aeruginosa* extracted from lake water correlated with the number of swimmers[42]. In addition, recreational waters may also be colonized by *P. aeruginosa*. In Wales, an outbreak of *Pseudomonas folliculitis* following an outdoor water skiing activity affected 26% of the participating children[43]. This illustrates the impact of typhoons on pathogenic bacteria in water bodies and the importance of monitoring of water bodies after typhoons.

Water mixing caused by strong winds and surface scour caused by heavy rain during a typhoon can increase nutrients and other components in water[6,38]. Such changes in the water environment may further affect the distribution of ARGs by changing the composition of the microbial community and the content of antibiotics. The RDA showed that the distribution of ARGs was significantly correlated with various environmental factors, with TIN being the main factor. A recent study showed that the load of five target ARGs (*sul*1, *sul*2, *tet*O, *tet*W, and *erm*F) in urban rivers during storms was significantly higher than that in the background period under the same conditions, highlighting the role of storms in driving ARG transportation in urban water environments[44]. This indicates that a change in the environment affects the distribution of ARGs to a certain extent. In this study, there was no significant difference in the types of ARGs in each sampling site before and after typhoon, but there were significant changes in the composition of ARGs in Jiushan Lake and Sanyang Wetland before and after the typhoon. The composition of ARGs in Sanyang Wetland fluctuated more significantly after the typhoon than those in Jiushan Lake (Figure 9), which may be because Sanyang Wetland is connected with the Wenzhou River and its water exchange is more frequent than that in Jiushan Lake, which is a closed water body. The proportion of the ARGs *sul*1, *van*SM and *ade*F were high in each sample before and after the typhoon, indicating that the presence of these resistance genes was relatively stable and that the typhoon had little impact on them. Sulfonamides were the first synthetic antibiotics and have been widely used since the 1930s. Widespread resistance to sulfonamides has emerged in clinically isolated bacteria[45]. Various studies have detected the presence of s*ul*1 widely in urban sewage[46]. *van*SM is a subtype of the *van*S gene, which expresses resistance to vancomycin. Vancomycin resistance is also a common resistance gene in water. *ade*F is the membrane fusion protein gene of the active efferent pump AdeFGH, which expresses resistance to multiple antibacterial drugs[47]. Just as ARGs may be transferred or acquired by native aquatic bacteria, increasing drug resistance may subsequently be transferred to pathogenic bacteria[48]. Therefore, the stable presence of these resistance genes in samples and the expression of resistance to common antibiotics should be of concern.

Existing studies have shown that the frequency and intensity of typhoon will increase in the future[9], so the impact of typhoon on microbial community and antibiotic resistance genes in aquatic ecosystem will be more and more significant. Metagenomics provides a powerful tool for detecting unculturable microorganisms and mutating ARGs. Although the duration of typhoons is relatively short, the damage and impact they bring to the environment, species composition and community structure are enormous, and may have a lasting impact on surface water quality. The effect of typhoons on the metabolic characteristics of the urban water microbial community needs further analysis to understand functional changes, such as more sampling water areas and long-term observations.

4 Conclusion

The data in this study represent a baseline metagenomic characterization of the environmental waters of Wenzhou during typhoon Lekima. It will be a useful guide for future systematic studies aiming to evaluate pathogens and ARGs in different urban water environments. In addition, this information can be later incorporated to improve public health surveillance for ARGs and antibiotic-resistant pathogens.

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