

1 **Supporting Information for**

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3 **Salinity Gradients Determine the Resilience of Estuarine**

4 **Microbial Ecosystems to Extreme Weather Events**

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Supplementary Method:

Method S1: Meta data pre-process

A total of 129 16S rRNA raw sequencing datasets were obtained from seven publicly available studies, comprising five high-salinity environments (n = 113) and two riverine samples (n = 16). Detailed metadata for these samples are provided in Supplementary Dataset S1 (Additional File). To ensure comparability across datasets, sequences from different hypervariable regions (V4, V4-V5, V3-V4) were standardized by extracting the common V4 region. Quality control and primer trimming were performed using DADA2 and Cutadapt (v4.9) with an error rate threshold of 0.15. The forward primer 515F (5'-ATTAGATACCCTGGTAGTCC-3') was used as the reference position for truncation. Post-trimming, all sequences exhibited consistent 5' alignment, confirming uniform starting positions. Then, to minimize annotation bias due to variable sequence lengths, all reads were trimmed to a uniform length of 235 bp (the shortest high-quality sequence in the dataset). Downstream analyses were conducted at a standardized sequencing depth of 4,511 reads per sample to ensure equitable comparisons.

Method S2: Classification of bacteria

To investigate how microbial communities of varying abundance levels respond to typhoons, we classified all ASVs into six categories following recent studies[1]. The classification includes always abundant taxa (AAT), defined as ASVs maintaining a relative abundance $\geq 1\%$ across all samples, and conditionally abundant taxa (CAT), which show $\geq 0.01\%$ abundance in all samples while reaching $\geq 1\%$ in some samples. Always rare taxa (ART) were identified as ASVs consistently present at $< 0.01\%$ abundance in all samples, while conditionally rare taxa (CRT) exhibit $< 0.01\%$ abundance in some samples but never reach $\geq 1\%$ in any sample. We also identified moderate taxa (MT) that persistently maintain relative abundances between 0.01% and 1% across all samples, along with conditionally rare and abundant taxa (CRAT)

that demonstrate dynamic abundance ranges from rare ($<0.01\%$) to abundant ($\geq 1\%$). This comprehensive classification scheme enables detailed analysis of microbial community responses to environmental perturbations across different abundance thresholds.

Method S3: Estimate of the bacterial life history strategy

Based on the conventional classification of bacterial growth strategies and using rrn copy numbers from the rrnDB database as weighting factors, we referenced weighted rrn copy numbers for each ASV following Wang et al.[2]. Specifically, we used the rrn copy number of the subordinate taxon when available; otherwise, the parent taxon's rrn copy number was applied. The weighted rrn copy number for each sample was computed as the sum of each ASV's relative abundance multiplied by its corresponding rrn copy number. Bacterial communities with low weighted rrn copy numbers were classified as K-strategists, while those with high values were identified as r-strategists. This approach provides a refined assessment of bacterial functional strategies in response to environmental disturbance.

94 **Supplementary Table:**

95 **Table S1.** Significance analysis of environmental factors in four time periods
 96 from BT to 60DPT.

Kruskal-Wallis Test				
Variables	Chi-squared	df	p-value	Sig
Salinity	7.9215	3	0.04766	*
pH	3.1497	3	0.3691	n.s.
DO	7.352	3	0.06149	n.s.
Temperature	23.815	3	0.0000273	***
DOC	20.641	3	0.000125	***
TA	12.137	3	0.006928	***
Nitrite	23.796	3	0.00002755	***
Nitrate	6.552	3	0.08751	n.s.
Phosphate	7.2815	3	0.06345	n.s.
Silicate	5.3392	3	0.1486	n.s.

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99 **Table S2.** Comparison of multiple significant differences of environmental
100 factors in the BT-60DP4 time periods

Kruskal-Wallis-Dunn_test					
Variables	Comparisons	Chi-squared	P	P.adjusted	Sig
Salinity	3DPT- BT	7.921466	0.025544582	0.15326749	n.s.
	24DPT- BT	7.921466	0.324493344	1	n.s.
	60DPT - BT	7.921466	0.217609501	1	n.s.
	24DPT- 3DPT	7.921466	0.067384212	0.40430527	n.s.
	3DPT- 60DPT	7.921466	0.003156912	0.01894147	*
	24DPT- 60DPT	7.921466	0.108329234	0.6499754	n.s.
pH	3DPT- BT	3.149701	0.16888425	1	n.s.
	24DPT- BT	3.149701	0.4935186	1	n.s.
	60DPT - BT	3.149701	0.20829281	1	n.s.
	24DPT- 3DPT	3.149701	0.16482208	0.9889325	n.s.
	3DPT- 60DPT	3.149701	0.03828511	0.2297107	n.s.
	24DPT- 60DPT	3.149701	0.2129835	1	n.s.
DO	3DPT- BT	7.352018	0.030833559	0.18500135	n.s.
	24DPT- BT	7.352018	0.290309837	1	n.s.
	60DPT - BT	7.352018	0.111478009	0.66886805	n.s.
	24DPT- 3DPT	7.352018	0.007735502	0.04641301	*
	3DPT- 60DPT	7.352018	0.257853789	1	n.s.
	24DPT- 60DPT	7.352018	0.038264953	0.22958972	n.s.
Temperature	3DPT- BT	23.81542	0.002077284	0.0124637	*
	24DPT- BT	23.81542	0.1724477	1	n.s.
	60DPT - BT	23.81542	4.05759E-06	2.43455E-05	***
	24DPT- 3DPT	23.81542	0.02732528	0.1639517	n.s.
	3DPT- 60DPT	23.81542	0.05525091	0.3315055	n.s.
	24DPT- 60DPT	23.81542	0.000217746	0.001306475	**
DOC	3DPT- BT	20.6411	0.3130056	1	n.s.
	24DPT- BT	20.6411	0.02562414	0.153744825	n.s.
	60DPT - BT	20.6411	0.000199004	0.001194025	**
	24DPT- 3DPT	20.6411	0.007410089	0.044460537	*
	3DPT- 60DPT	20.6411	2.80372E-05	0.000168223	***
	24DPT- 60DPT	20.6411	0.05569169	0.334150142	n.s.
TA	3DPT- BT	12.13723	0.108486574	0.650919445	n.s.
	24DPT- BT	12.13723	0.247528354	1	n.s.
	60DPT - BT	12.13723	0.014746837	0.088481019	n.s.
	24DPT- 3DPT	12.13723	0.027624998	0.165749985	n.s.
	3DPT- 60DPT	12.13723	0.000323102	0.001938613	**
	24DPT- 60DPT	12.13723	0.067517771	0.405106627	n.s.
Nitrite	3DPT- BT	23.79593	0.04547562	0.2728537	n.s.
	24DPT- BT	23.79593	0.000575461	0.00345277	**

	60DPT - BT	23.79593	0.000001955	0.00001173	***
	24DPT- 3DPT	23.79593	0.05933620	0.3560172	n.s.
	3DPT- 60DPT	23.79593	0.00171844	0.01031066	*
	24DPT- 60DPT	23.79593	0.08607562	0.5164537	n.s.
Nitrate	3DPT- BT	6.555243	0.14182246	0.85093478	n.s.
	24DPT- BT	6.555243	0.00617952	0.03707712	*
	60DPT - BT	6.555243	0.21776667	1	n.s.
	24DPT- 3DPT	6.555243	0.07642220	0.45853321	n.s.
	3DPT- 60DPT	6.555243	0.38498687	1	n.s.
	24DPT- 60DPT	6.555243	0.04253788	0.25522727	n.s.
Phosphate	3DPT- BT	7.281492	0.02562414	0.15374482	n.s.
	24DPT- BT	7.281492	0.48704068	1	n.s.
	60DPT - BT	7.281492	0.29036163	1	n.s.
	24DPT- 3DPT	7.281492	0.02374606	0.14247638	n.s.
	3DPT- 60DPT	7.281492	0.00617952	0.03707712	*
	24DPT- 60DPT	7.281492	0.30158802	1	n.s.
Silicate	3DPT- BT	5.339198	0.05943715	0.35662292	n.s.
	24DPT- BT	5.339198	0.31300561	1	n.s.
	60DPT - BT	5.339198	0.24752835	1	n.s.
	24DPT- 3DPT	5.339198	0.14182246	0.85093478	n.s.
	3DPT- 60DPT	5.339198	0.01248701	0.07492209	n.s.
	24DPT- 60DPT	5.339198	0.12107354	0.72644127	n.s.

103 **Table S3.** Oligotrophs (K-strategists) and copiotrophs (r-strategists) defined in this
 104 study.

Classify	K/r- Strategists	Operon_copy
Phylum		
Actinobacteriota	Oligotroph	1.88
Bacteroidota	Copiotroph	3.67
Chloroflexi	Oligotroph	1.79
Firmicutes	Copiotroph	6.3
Gemmatimonadota	Oligotroph	1.85
α -proteobacteria	Oligotroph	1.95
β -proteobacteria	Copiotroph	3.13
γ -proteobacteria	Copiotroph	2.64
Family		
Bacillaceae (Firmicutes)	Copiotroph	10.51
Longimicrobiaceae	Oligotroph	1.84
lamiaceae	Oligotroph	1
Alcaligenaceae	Copiotroph	2.99
Flavobacteriaceae(Bacteroidota)	Copiotroph	3.81
Genera		
Longimicrobium	Oligotroph	1.84
Achromobacter(gamma_pro)	Copiotroph	3.49
Alkalihalobacillus	Copiotroph	8.75
Egibacter	Oligotroph	1
Actinomarinicola(Actinobacteriota)	Oligotroph	1

106 **Table S4.** The PERMANOVA significance test, for environmental factor parameters and the grouping of different samples, is based
 107 on the Bray-Curtis distance matrix of community function and bacterial composition.
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PERMANOVA						
Environment Factor	FAPROTAX Funcation			Bacterial Community		
	R ²	p.value	Sig	R ²	p.value	Sig
DOC	0.01452409	0.301	n.s	0.02474795	0.083	n.s
TA	0.01422211	0.307	n.s	0.04941776	0.002	**
DO	0.05581084	0.015	*	0.03573343	0.02	*
Salinity	0.02476948	0.119	n.s	0.03746952	0.017	*
Temperature	0.38796891	0.001	***	0.05467499	0.001	***
pH	0.01086555	0.423	n.s	0.03456495	0.031	*
Windspeed	0.09684211	0.001	***	0.03688769	0.022	*
Rainfall	0.11406667	0.002	**	0.04372162	0.007	*
Time	0.57522595	0.001	***	0.13909388	0.001	***
Typhoon/Non- Typhoon	0.22212107	0.001	***	0.05221431	0.005	**
Region	0.05762233	0.056	n.s	0.03986367	0.112	n.s
Time and Region	0.76414918	0.001	***	0.31910269	0.001	***

Table S5. The empirical network and random network parameters of the bacterial community using the MENA pipeline.

Typhoon periods		BT	BT	3DPT	3DPT	24DPT	24DPT	60DPT	60DPT
Sample Area		QZB	JJR	QZB	JJR	QZB	JJR	QZB	JJR
Total nodes		685	180	914	347	753	109	805	136
Total edges		3464	1019	10559	593	5569	247	3867	437
Empirical networks	Ave_degree	10.114	11.322	23.105	3.418	14.792	4.532	9.607	6.426
	Average path distance (GD)	5.885	3.188	6.175	5.623	3.4	3.7	3.537	NA
	Density	0.015	0.063	0.025	0.01	0.02	0.042	0.012	0.048
	Natural Connectivity	26.274	26.091	83.159	5.076	35.802	4.506	16.18	8..539
	AUC (random remove)	10.426	11.563	42.126	2.271	15.071	1.908	7.990	3.017
	AUC (targeted remove)	2.138	2.097	6.717	0.269	13.877	1.220	8.169	2.379
	Modularity	0.82	0.308	0.5	0.685	0.694	0.605	0.874	0.523
	Clustering_Coefficient	0.398	0.469	0.442	0.157	0.584	0.362	0.368	0.473
	Pos/Neg_Rate	0.523	7.352	0.497	1.864	1.201	9.292	0.428	7.404
	K/r_Rate	1	1.232	1.339	1.464	1.954	2.217	1.890	1.327
	Activated Rare Taxa (CRT)	291	111	354	294	323	64	277	62
Random networks	Average clustering coefficient (avgCC $\pm$ SD)	0.040 $\pm$ 0.003	0.279 $\pm$ 0.015	0.994 $\pm$ 0.0004	0.026 $\pm$ 0.006	0.083 $\pm$ 0.004	0.075 $\pm$ 0.013	0.108 $\pm$ 0.014	0.423 $\pm$ 0.004
	Modularity(M $\pm$ SD)	0.262 $\pm$ 0.003	0.182 $\pm$ 0.005	0.865 $\pm$ 0.0001	0.551 $\pm$ 0.006	0.198 $\pm$ 0.003	0.403 $\pm$ 0.011	0.317 $\pm$ 0.009	0.542 $\pm$ 0.004
	Average path distance (GD $\pm$ SD)	3.137 $\pm$ 0.016	2.606 $\pm$ 0.032	2.581 $\pm$ 0.021	4.115 $\pm$ 0.062	2.717 $\pm$ 0.010	3.135 $\pm$ 0.060	2.856 $\pm$ 0.046	1.655 $\pm$ 0.001

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Table S6. Variables related to PLS-PM and latent variables, and the loadings of the variables

Variables	Block	Weight	Loading	Communality	Redundancy
DOC	Nutrient_input	-0.308179	-0.7021346	0.492993	0
Phosphate	Nutrient_input	0.2597057	0.8738404	0.763597	0
Silicate	Nutrient_input	0.2346489	0.8504217	0.7232171	0
Nitrite	Nutrient_input	0.3812427	0.9367392	0.8774802	0
Salinity	Hydrological_stability	0.341116	0.9701972	0.9412827	0
TA	Hydrological_stability	0.3633753	0.9550964	0.9122091	0
pH	Hydrological_stability	0.3412928	0.9434471	0.8900925	0
Shannon	Bacterial diversity	0.5144315	0.9800427	0.9604837	0.2700472
Pielou	Bacterial diversity	0.5062708	0.9793873	0.9591995	0.2696862
r_Strategists	r_Strategists	0.3676691	0.8635992	0.7458036	0.6567853
CRT	r_Strategists	0.707208	0.9650362	0.9312949	0.8201366
K_Strategists	K_Strategists	0.5457261	0.8159184	0.6657228	0.4548907
AAT	K_Strategists	0.6381857	0.8692329	0.7555658	0.5162807
Community_avd	Bacterial AVD	0.6243549	0.9143822	0.8360947	0.6022667
Function_avd	Bacterial AVD	0.4980172	0.861619	0.7423873	0.5347661
Carbon_function	CNS_cycle	-0.13917	-0.5669149	0.3213925	0.2187021
Nitrogen_function	CNS_cycle	0.4432868	0.8583985	0.736848	0.5014124
Sulfur_function	CNS_cycle	0.5849407	0.9241717	0.8540934	0.5811959

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Supplementary Figure:

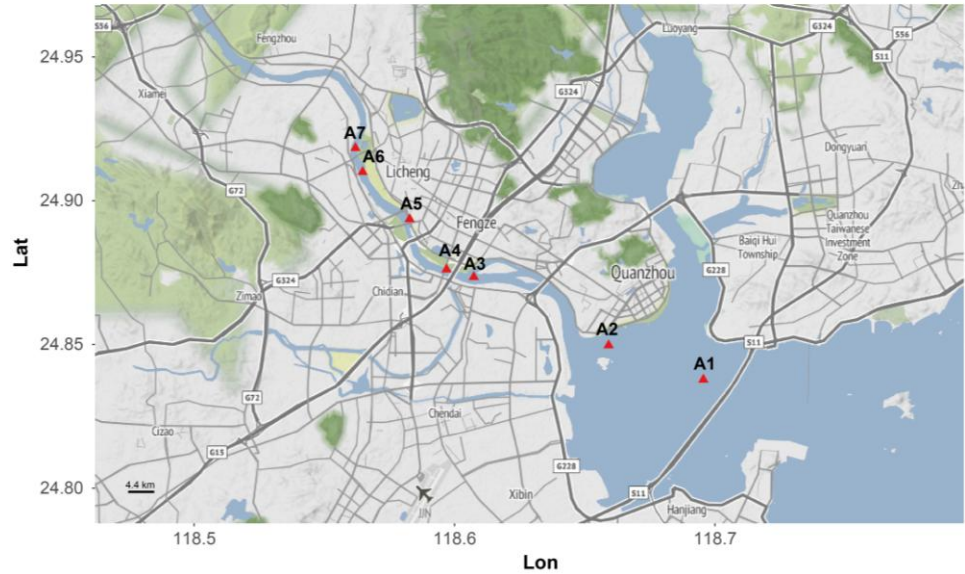


Figure S1. Map of the longitude and latitude coordinates of the sample collection points. A1-A2 are areas with higher salinity (QZB, >20ppt), while A3-A7 are areas with lower salinity (JJR, <10ppt).

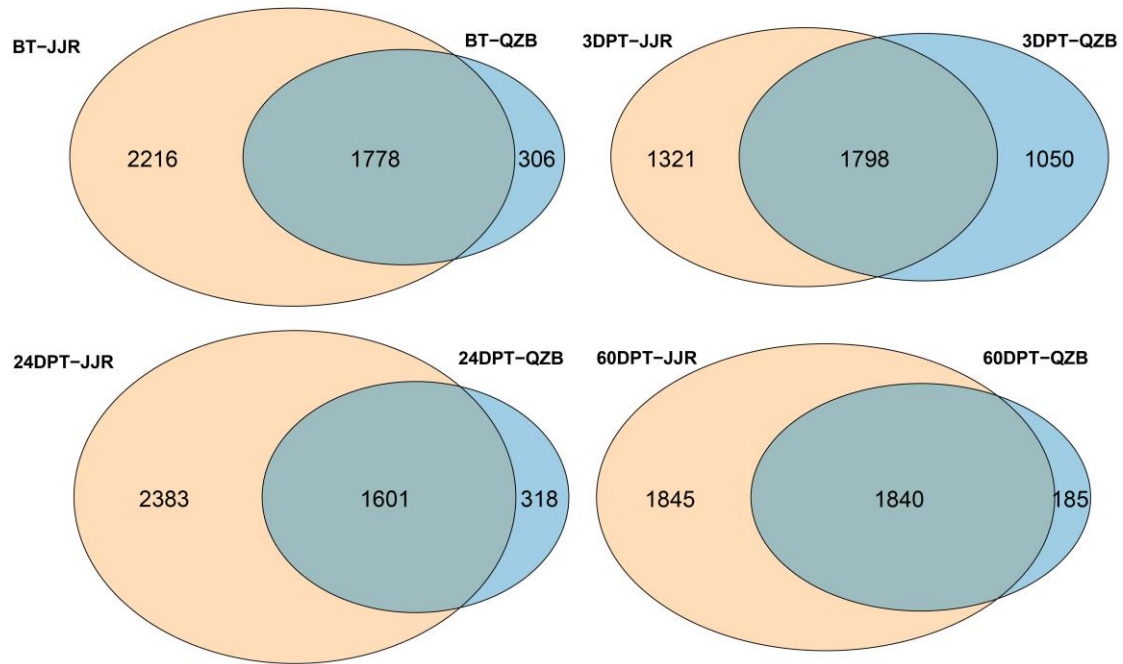


Figure S2. Venn analysis of the bacterial communities of QZB and JJR.

During the 3DPT period, the number of unique bacteria of JJR (A3-A7) decreased significantly, while the number of unique bacteria of QZB (A1-A2) increased significantly. After 3DPT, the bacterial population gradually recovered.

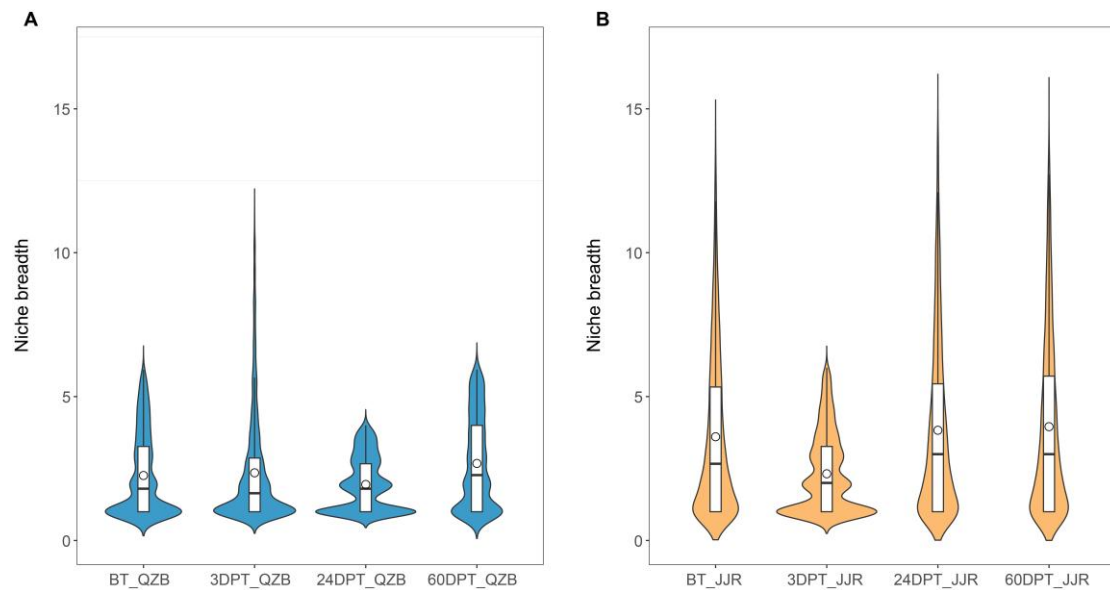


Figure S3. Analysis of the ecological niche width of bacterial communities in the QZB and JJR regions. During the 3DPT period, the ecological niche width of some bacteria in QZB (A1-A2) increased, while the ecological niche width of JJR (A3-A7) bacteria generally decreased. After 3DPT, the bacterial ecological niches gradually recovered.

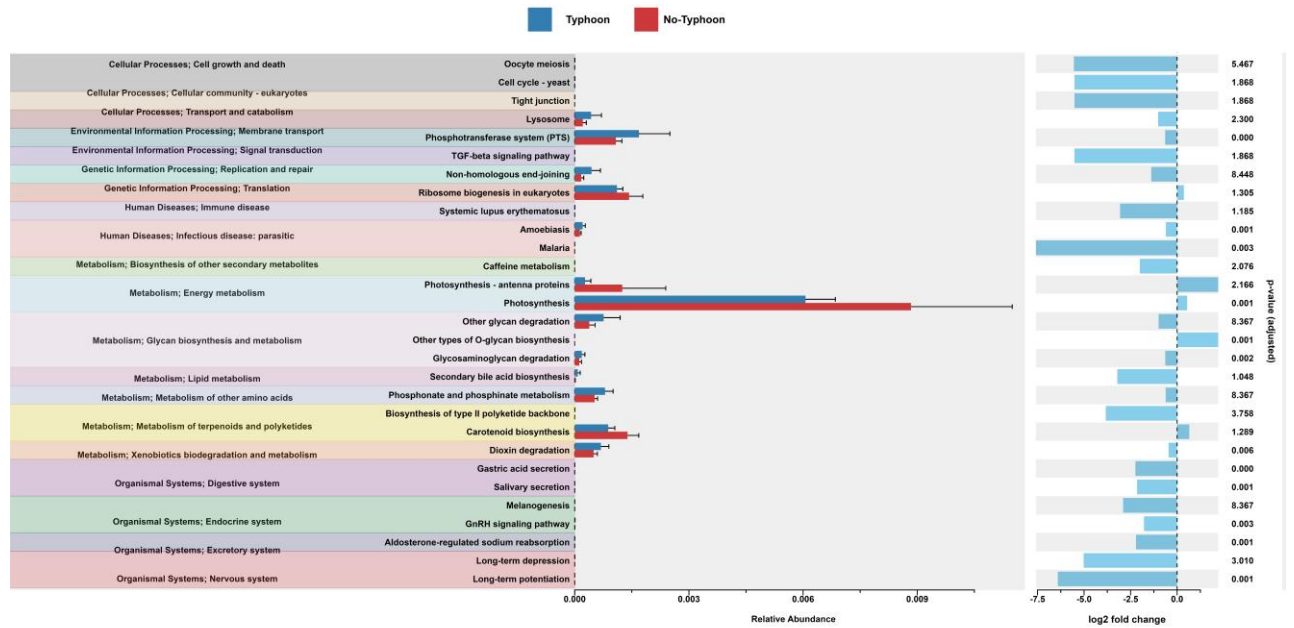


Figure S4. Community function analysis (PICRUST2). The leftmost part of the figure was the annotation of community functions, the abundance bar chart of functions in the middle, and the abundance ratio of functions during non-typhoon periods and typhoon periods (after log2 transformation) on the right (A).

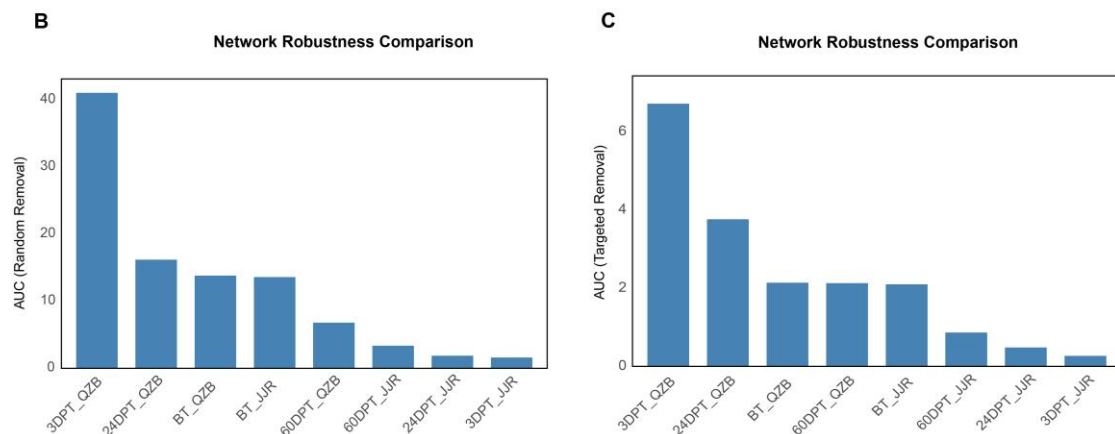
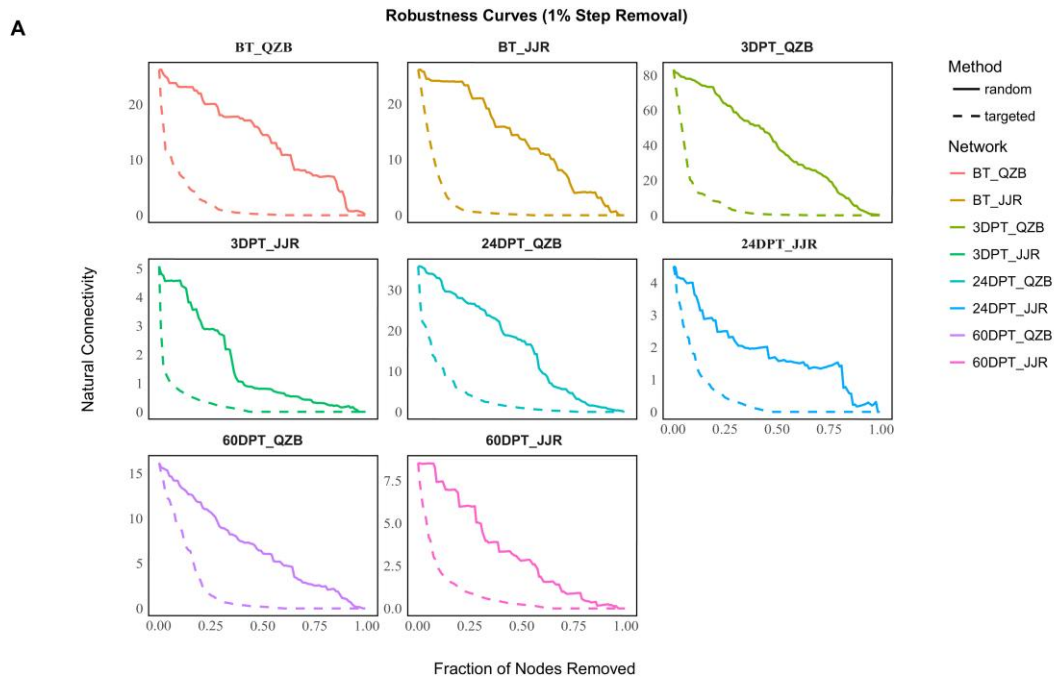


Figure S5. Network Robustness Test of QZB (A1-A2) and JJR (A3-A7).

The robustness of the network is evaluated based on two methods: random node removal and the removal of nodes with higher degrees in priority (A). Subsequently, the area under curve (AUC) values for both random removal (B) and non-random removal (C) were calculated. Regardless of the evaluation method used, QZB demonstrated relatively excellent robustness characteristics.

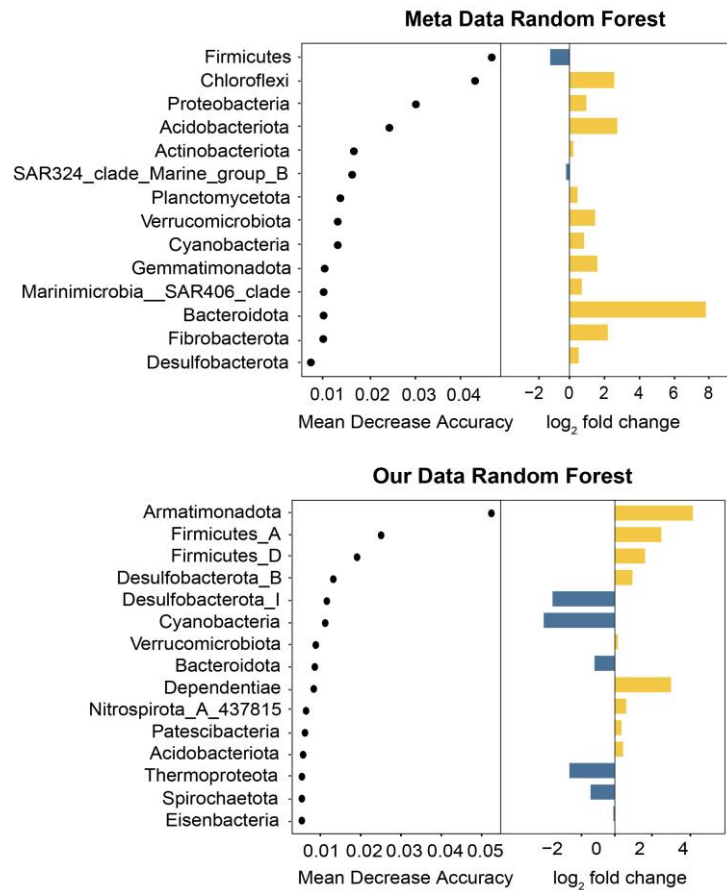


Figure S6. Random forest model analysis for identifying biomarkers with log₂ relative abundance ratio by Typhoon and Non-Typhoon.

154 **References:**

- 155 1. Xue Y, Chen H, Yang JR, Liu M, Huang B, and Yang J. Distinct patterns and processes of abundant
156 and rare eukaryotic plankton communities following a reservoir cyanobacterial bloom. *The*
157 *ISME Journal*, 2018; 12(9): 2263-2277.
- 158 2. Wang Y, Qu M, Wang J, Zhu W, and Li J. Recovery of bacterial network complexity and stability
159 after simulated extreme rainfall is mediated by K-/r-strategy dominance. *Appl. Soil Ecol.*, 2024;
160 203: 105657.
- 161