



Community stability of free-living and particle-attached bacteria in a subtropical reservoir with salinity fluctuations over 3 years

Xue Yan^{a,b}, Shuzhen Li^{a,*}, Mamun Abdullah Al^a, Yuanyuan Mo^{a,c,d}, Jun Zuo^{a,1},
Hans-Peter Grossart^{e,f}, Hongteng Zhang^a, Yigang Yang^a, Erik Jeppesen^{g,h,i,j,k}, Jun Yang^{a,d,*}

^a Aquatic EcoHealth Group, Fujian Key Laboratory of Watershed Ecology, Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

^b University of Chinese Academy of Sciences, Beijing 100049, China

^c Key Laboratory of Urban Environment and Health, Ningbo Urban Environment Observation and Research Station, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

^d Zhejiang Key Laboratory of Urban Environmental Processes and Pollution Control, CAS Haixi Industrial Technology Innovation Center in Beilun, Ningbo 315830, China

^e Department of Plankton and Microbial Ecology, Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Stechlin 16775, Germany

^f Institute of Biochemistry and Biology, Potsdam University, Potsdam 14469, Germany

^g Department of Ecoscience, Aarhus University, Aarhus 8000, Denmark

^h Sino-Danish Centre for Education and Research, Beijing 100049, China

ⁱ Limnology Laboratory, Department of Biological Sciences and Centre for Ecosystem Research and Implementation, Middle East Technical University, Ankara 06800, Turkey

^j Institute for Ecological Research and Pollution Control of Plateau Lakes, School of Ecology and Environmental Science, Yunnan University, Kunming 650091, China

^k Institute of Marine Sciences, Middle East Technical University, Erdemli, Mersin 33731, Turkey

¹ National and Local Joint Engineering Research Center of Ecological Treatment Technology for Urban Water Pollution, Zhejiang Provincial Key Lab for Water Environment and Marine Biological Resources Protection, Institute for Eco-Environmental Research of Sanyang Wetland, Wenzhou University, Wenzhou 325035, China

ARTICLE INFO

Keywords:

Plankton
Bacterial community
Bacterial lifestyle
Rare taxa
Species interaction
Precipitation

ABSTRACT

Changes in salinity have a profound influence on ecological services and functions of inland freshwater ecosystems, as well as on the shaping of microbial communities. Bacterioplankton, generally classified into free-living (FL) and particle-attached (PA) forms, are main components of freshwater ecosystems and play key functional roles for biogeochemical cycling and ecological stability. However, there is limited knowledge about the responses of community stability of both FL and PA bacteria to salinity fluctuations. Here, we systematically explored changes in community stability of both forms of bacteria based on high-frequency sampling in a shallow urban reservoir (Xinglinwan Reservoir) in subtropical China for 3 years. Our results indicated that (1) salinity was the strongest environmental factor determining FL and PA bacterial community compositions – rising salinity increased the compositional stability of both bacterial communities but decreased their α -diversity. (2) The community stability of PA bacteria was significantly higher than that of FL at high salinity level with low salinity variance scenarios, while the opposite was found for FL bacteria, i.e., their stability was higher than PA bacteria at low salinity level with high variance scenarios. (3) Both bacterial traits (e.g., bacterial genome size and interaction strength of rare taxa) and precipitation-induced factors (e.g., changes in salinity and particle) likely contributed collectively to differences in community stability of FL and PA bacteria under different salinity scenarios. Our study provides additional scientific basis for ecological management, protection and restoration of urban reservoirs under changing climatic and environmental conditions.

1. Introduction

Global warming triggers global sea level rise with potential

profound, yet partly unknown negative impacts on coastal cities, e.g., saltwater intrusion (Ward et al., 2020), with implications for the supply of clean drinking water for residents and the quality of water for

* Corresponding authors at: Aquatic EcoHealth Group, Fujian Key Laboratory of Watershed Ecology, Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China.

E-mail addresses: szli@iue.ac.cn (S. Li), jyang@iue.ac.cn (J. Yang).

<https://doi.org/10.1016/j.watres.2024.121344>

Received 29 October 2023; Received in revised form 22 January 2024; Accepted 19 February 2024

Available online 20 February 2024

0043-1354/© 2024 Elsevier Ltd. All rights reserved.

agricultural and industrial demands (Ma et al., 2022). Similarly, microbial communities, which play pivotal roles in freshwater ecosystems, are also seriously threatened by saltwater intrusion in the form of biodiversity loss (Mo et al., 2021). Numerous studies have shown that the diversity, complexity and stability of microbial networks decrease with increasing salinity (Corsi et al., 2010; Mo et al., 2021), and salinity has long been recognised as a major factor controlling community composition of freshwater microbes (Cañedo-Argüelles et al., 2019), either directly or indirectly by salinity-induced changes in the trophic structure (Jeppesen et al., 2020; Cunillera-Montcusi et al., 2022). In particular, salinity fluctuations in inland waters are predicted to increase with a higher frequency and intensity of weather extremes such as heavy precipitation events (Gulev et al., 2023), which may have marked implications for microbial community composition and related ecosystem functions (Giling et al., 2017; Stockwell et al., 2020; Talbot et al., 2018).

Bacterioplankton has the potential to rapidly reproduce and respond to environmental fluctuations, making them suitable models for capturing small changes in environmental fluctuations (Allison and Martiny, 2008; Cram et al., 2015; Logares et al., 2013). Therefore, many ecologists have started to use bacterioplankton as the appropriate organisms to investigate the resistance and resilience of microbial communities to disturbances in freshwater ecosystems (Jones et al., 2008; Li et al., 2012; Shade et al., 2011; Zhang et al., 2024). Particulate matter in water bodies appears in a continuum of sizes and can generally be divided into organic and inorganic particulate matter (Mestre et al., 2017). Particulate organic matter (POM) is mainly composed of living plankton and the remains of dead organisms, serving as “hotspots” for microbial decomposition (Farnelid et al., 2019; Simon et al., 2014; Zoccarato and Grossart, 2019). Consequently, bacterioplankton can be divided into two lifestyles depending on their relationship with the particulate matter: free-living (FL) and particle-attached (PA) bacteria (Grossart, 2010). PA bacteria normally colonize the surfaces of particulate matter, resulting in biofilm formation, and play an important role in the degradation and remineralisation of POM (Grossart, 2010; Landa et al., 2016). In contrast, FL bacteria are freely moving and mainly rely on dissolved organic matter (DOC) (Mestre et al., 2017). Given the profound effect of POM in aquatic biogeochemical cycling, a large number of studies have focused on exploring the roles of bacterial lifestyles (Tang et al., 2010; Villalba et al., 2022). Our previous studies revealed that co-occurrence between different lifestyles of bacterioplankton and microeukaryotes is closely linked to changes in ecological processes and functions (Xue et al., 2022). Generally, bacterioplankton is vital for the matter cycling and energy flow in freshwater ecosystems (Chow et al., 2014; Shade et al., 2012; Villalba et al., 2022). Therefore, it is of major importance to know how bacterial communities respond to salinity fluctuations in inland waters. In particular, knowledge about the responses of bacterial community stability to salinity is crucial for predicting the future bacterial community dynamics and functionality in a changing environment and for improving management, protection and restoration of coastal urban ecosystems (Cañedo-Argüelles et al., 2019). To date, however, research on the community stability of FL and PA bacteria under salinity fluctuations is lacking.

Free-living (FL) and particle-attached (PA) bacteria show significant differences in their diversity and community composition in inland waters (Shen et al., 2022; Zoccarato and Grossart, 2019). Recently, GeoChip 5.0 analysis revealed that the richness and abundance of functional genes of PA bacteria, especially those related to nutrient cycling and stress response, were much higher than those of FL bacteria (Liu et al., 2020). Furthermore, genomic analysis results have revealed that FL and PA bacteria have different metabolic trade-off strategies (Polz and Cordero, 2016), indicating differences in the adaptation of the two groups to the same environmental fluctuations. Along this line, our previous studies showed that abundant PA bacterial subcommunities have a higher variation between cyanobacterial bloom and non-bloom

periods than abundant FL bacterial subcommunities (Liu et al., 2019). This indicates that PA bacteria are more responsive to changes in cyanobacterial bloom-induced environmental changes. So far, however, no study has evaluated the differences in community stability between FL and PA bacteria over time under environmental fluctuations (e.g., salinity) and their underlying mechanisms.

Our previous work showed that the environmental heterogeneity in the Xinglinwan Reservoir was low, presumably due to its small area and shallow water depth. Salinity had strong effects on microeukaryotic plankton communities, highlighting that even small salinity increases are sufficient to cause dramatic changes in microeukaryotic plankton communities (Mo et al., 2021; Yang et al., 2022). Here, we selected one sampling site to further investigate the community stability of FL and PA bacteria under salinity fluctuations using 16S rRNA gene sequencing and multivariate statistical analyses based on high-frequency sampling in the Xinglinwan Reservoir for 3 years. We aimed to answer the following questions: (1) How does salinity affect the community stability of FL and PA bacteria? (2) Do the response processes or mechanisms between FL and PA bacterial community stabilities differ with salinity changes? We proposed two hypotheses: (1) The community stability of both FL and PA bacteria would increase with increasing salinity levels but decrease with increasing salinity variance. (2) Bacterial traits and environmental factors (e.g., precipitation) would both lead to different responses between FL and PA bacterial community stability under different salinity scenarios.

2. Materials and methods

2.1. Study station and sampling

Surface water samples (0.5 m) were collected at station G (24° 36' N, 118° 04' E) in the Xinglinwan Reservoir, Xiamen City, Fujian Province, subtropical China (Fig. 1a). Xinglinwan Reservoir is a shallow and eutrophic urban reservoir with low water transparency (trophic state index = 80.0 ± 4.1 , transparency = 40.0 ± 11.1 cm) (Luo et al., 2024; Yang et al., 2022). A dam separates Xinglinwan Reservoir from the sea, and saltwater intrusions in the bottom water layer are stronger in winter than in summer due to the substantial decrease of incoming freshwater (Mo et al., 2021).

Water samples were collected twice a week from 1st December 2017 to 27th November 2020, giving a 3-year high-resolution time-series dataset. Water samples were divided into two sub-samples and immediately brought to the laboratory for further processing. Detailed information of sample collection and environmental variables measurements is shown in the Supplementary methods.

2.2. Bioinformatics

DNA extraction, PCR, sequencing and quality control processes are described in the Supplementary methods. High-quality sequences were assigned to zero-radius operational taxonomic units (zOTUs) using the unioise3 algorithm in USEARCH v11 (Edgar, 2010, 2016). Unique sequences were compared against the SILVA v138 database (Quast et al., 2013) using the SINTAX algorithm to obtain annotation information. Then, we normalised the zOTU table with a minimum sequence number of 69,735, and a total of 25,259 zOTUs were acquired. Finally, FL and PA bacteria were selected from the total table for subsequent analysis, including 22,263 zOTUs of FL and 20,984 zOTUs of PA bacteria, respectively.

2.3. Definition of different salinity scenarios

To facilitate the comparison of differences in community stability of FL and PA bacteria under different salinity scenarios, we divided our samplings into six periods (Table 1) depending on the cross-point between the mean and coefficient of variation (CV) of salinity (Fig. 1b).

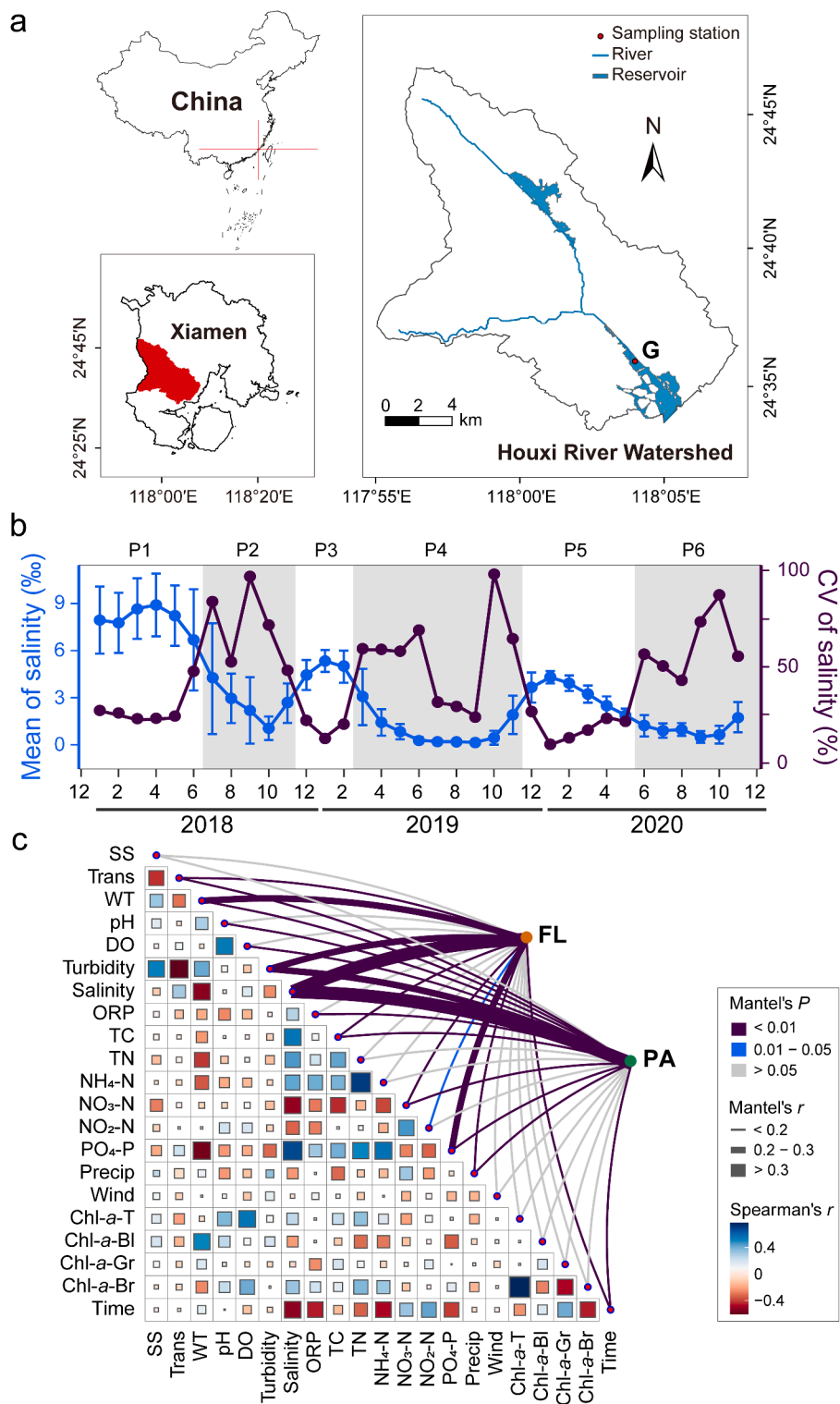


Fig. 1. Sampling station and environmental drivers of free-living (FL) and particle-attached (PA) bacterial community composition. (a) Map of the sampling station in Xinglinwan Reservoir, Xiamen, Southeast China. The water samples were taken from station G twice a week. (b) Trend of the mean and coefficient of variation (CV) of salinity in every two adjacent months from December 2017 to November 2020. For example, the values for February 2018 indicate the mean and CV of salinity for January and February 2018. Error bar represents the standard deviation. (c) Pairwise comparisons between environmental factors are shown in lower figure to the left, with a colour gradient representing Spearman's correlation coefficients. Partial Mantel test was performed for FL or PA bacterial community composition and each environmental factor, respectively. Line width indicates the partial Mantel's *r* statistic for corresponding correlations, with the larger values indicating stronger correlations. Line colour indicates significance based on 999 permutation tests. Grey shadows in (b) represent periods 2, 4 and 6, respectively. FL, free-living bacteria; PA, particle-attached bacteria. SS, suspended solids; Trans, transparency; WT, water temperature; DO, dissolved oxygen; ORP, oxidation–reduction potential; TC, total carbon; TN, total nitrogen; NH₄-N, ammonium nitrogen; NO₃-N, nitrate nitrogen; NO₂-N, nitrite nitrogen; PO₄-P, phosphate phosphorus; Precip, precipitation, 7-day accumulated precipitation before the sampling day; Wind, daily average wind speed; Chl-a-T, total algae chlorophyll-a; Chl-a-BI, cyanobacterial chlorophyll-a; Chl-a-Gr, Chlorophyta chlorophyll-a; Chl-a-Br, Bacillariophyta/Dinophyta chlorophyll-a; Time, sampling span.

Table 1
Salinity properties of Xinglinwan Reservoir across six periods.

Period	Time	Salinity (%)	CV of salinity (%)	Precipitation (mm)
Period1 (P1)	Dec, 2017 – Jun, 2018	8.0 ± 2.6	28.0 ± 9.2	17.3 ± 33.6
Period2 (P2)	Jul, 2018 – Nov, 2018	2.4 ± 1.6	70.5 ± 18.4	21.3 ± 42.3
Period3 (P3)	Dec, 2018 – Feb, 2019	5.1 ± 1.0	18.4 ± 3.8	5.7 ± 9.1
Period4 (P4)	Mar, 2019 – Nov, 2019	0.8 ± 1.0	54.7 ± 22.1	22.6 ± 29.7
Period5 (P5)	Dec, 2019 – May 2020	3.2 ± 1.2	18.3 ± 6.0	13.5 ± 15.3
Period6 (P6)	Jun, 2020 – Nov, 2020	1.1 ± 0.8	60.9 ± 15.1	9.1 ± 12.3

To facilitate the comparison of the community stability of free-living and particle-attached bacteria over time, we divided the study time into six periods according to the mean and CV of the salinity: period 1 (M1–M7), period 2 (M8–M12), period 3 (M13–M15), period 4 (M16–M24), period 5 (M25–M30), period 6 (M31–M36). M, month. CV, coefficient of variation. Precipitation, 7-day accumulated precipitation before the sampling day. Values indicate mean ± standard deviation.

The salinity of 0–2 ‰, 2–4 ‰, > 4 ‰ periods were termed as “low salinity”, “medium salinity”, “high salinity”, respectively, and periods with a CV of salinity more than 50 % were termed as “high variance”, otherwise they were termed as “low variance”. Finally, we obtained the following six periods: P1 (Month (M) 1–7, high salinity & low variance period), P2 (M8–12, medium salinity & high variance period), P3 (M13–15, high salinity & low variance period), P4 (M16–24, low salinity & high variance period), P5 (M25–30, medium salinity & low variance period) and P6 (M31–36, low salinity & high variance period), respectively.

2.4. Statistical analyses

All statistical analyses were performed in R software (v4.1.2) (R Core Team, 2023). Partial Mantel test was applied to reveal the contribution of each of the environmental variables to community composition variation via the “linkET” package (Huang, 2021). To further investigate the relationship between FL and PA bacterial community stability and salinity, we used a generalized linear regression model via the glm function. Besides, we assessed the differences between groups by Wilcoxon rank sum test. All *P* values were adjusted by the Bonferroni method. Visualisation was performed using the packages “ggplot2”, “ggpubr”, “UpSetR” and “patchwork” (Gehlenborg, 2019; Kassambara, 2023; Pedersen, 2022; Wickham et al., 2016). Detailed information of statistical analyses, definition of abundant and rare taxa, network construction, estimation of genome size in bacterial communities and random forest is given in the Supplementary methods.

2.5. Community stability analysis

We calculated multiple quantitative indices of community stability, including community composition stability, node contribution, robustness and interaction strength. We compared the community stability of FL and PA bacteria based on two datasets, including original resampled zOTUs and retained zOTUs after network construction.

Community compositional stability. We used the original resampled zOTUs tables to calculate the community compositional stability of FL and PA bacteria over time (Yuan et al., 2021; Zelikova et al., 2014). Higher values indicate stronger stability of community composition. Moreover, to visually observed trends in FL and PA bacterial community compositional stability, we used a generalized additive model for fitting using the gam function in the “mgcv” package (Auladell et al., 2022). We calculated community compositional stability of FL and PA bacteria

from every two adjacent months.

Node contribution. Node contribution evaluates the relative contribution of each node in the network to the global efficiency of the network (Deng et al., 2012). In ecological networks, it can reflect the spreading rate of species in the network to ecological events or disturbances (Yuan et al., 2021). The equation is as follows:

$$\text{Node contribution} = \frac{1}{d(i, j)}$$

where $d(i, j)$ is the number of edges on the shortest path between node i and node j in the network. The larger the value is, the greater the node's contribution to the overall network.

Robustness. Robustness in a network is defined as the proportion of nodes remaining in the network after random or targeted removal of nodes (Montesinos-Navarro et al., 2017). Larger values normally indicate a more robust network. In order to show the impact of rare and abundant taxa on network robustness, we gradually removed rare or abundant nodes, respectively. To compare the differences in the robustness of rare taxa in FL and PA bacterial communities during different periods, we calculated the robustness of the network with random removal of 50 % of all rare nodes.

Interaction strength. To evaluate the relative importance of one taxon to the maintenance of community composition, we calculated the interaction strength. The interaction strength of one taxon in a network is defined as the average of the association strength of all nodes of the taxon. The equation is as follows:

$$\text{Interaction strength} = \frac{\sum_{i=1}^n [(r_{ipos}) + \text{abs}(r_{ineg})]}{n}$$

where n is the total number of nodes of one taxon in the network; i is the i th node of one taxon, r_{ipos} and r_{ineg} are, respectively, all positive and all negative associations that associate with node i in the network. Larger values normally represent a greater contribution of the taxon to community structure maintenance. We calculated the interaction strength of rare taxa in each network to compare the relative importance of rare taxa to community composition maintenance.

2.6. PLS path modelling

We used the “plsmpm” package in R to construct partial least squares path modelling (PLS-PM) to investigate direct and indirect effects of environmental drivers on the community stability of FL and PA bacteria under different salinity scenarios, respectively. Since the community stability of PA bacteria was significantly higher than that of FL bacteria (i.e., P1, P2, P3, P5 in Fig. 3a) at medium/high salinity levels, for simplicity, we combined the samples of these periods in a “high salinity” group, and periods 4 and 6 were defined as “low salinity” groups. The environmental drivers were categorised into six block variables: precipitation (Precipitation), physical and chemical factors (Physicochemical), nutrients, salinity, algal properties (Chl-*a*) and particulate matter (Particle). In our initial model, precipitation was expressed as 7 days' accumulated precipitation before the sampling day; physical and chemical factors included water temperature, pH, dissolved oxygen and oxidation–reduction potential; nutrients included TC, TN, TP, TOC, NH₄-N and PO₄-P; algal properties included Chl-*a*-Bl (cyanobacteria), Chl-*a*-Gr (chlorophyta) and Chl-*a*-Br (bacillariophyta/dinophyta); salinity included salinity and electrical conductivity; particulate matter included suspended solids, turbidity and transparency (transparency was inverse-transformed). We removed all variables with loadings < 0.7 to ensure model reliability. Finally, model performance was evaluated using the value of goodness of fit (GoF) (Gao et al., 2021).

3. Results

3.1. Environmental variable dynamics and their relationships with bacterial communities

Changes in the environmental variables over three years are given in Fig. S1, major and dramatic changes appearing in both precipitation and salinity. Precipitation significantly ($P < 0.01$) impacted the aquatic environment, including salinity, turbidity and water transparency (Fig. S2). Partial Mantel test showed that salinity was the most important factor shaping the variations in both FL and PA bacterial community compositions (Fig. 1c). Concurrently, random forest analyses further supported that salinity contributed the most to the community composition of FL and PA bacteria, accounting for the maximum mean squared error (Fig. S3). Community dissimilarity exhibited a stronger correlation with salinity ($R = 0.386$, $P < 0.001$ for FL bacteria; $R = 0.383$, $P < 0.001$ for PA bacteria; Fig. S4a–b) than with temperature ($R = 0.316$, $P < 0.001$ for FL bacteria; $R = 0.245$, $P < 0.001$ for PA bacteria; Fig. S4c–d) and time ($R = 0.279$, $P < 0.001$ for FL bacteria; $R = 0.274$, $P < 0.001$ for PA bacteria; Fig. S4e–f).

3.2. Community stability of FL and PA bacteria under salinity fluctuations

The variation in community stability of FL and PA bacteria showed an obvious pattern, being strongly correlated with salinity level and salinity variance, respectively (Fig. 2). Additionally, a significant and negative correlation between salinity level and variance was observed ($R = -0.490$, $P < 0.001$; Fig. 2b). Notably, the FL bacterial community exhibited a strong correlation with salinity level ($R = 0.566$, $P < 0.001$

for FL bacteria; $R = 0.141$, $P = 0.393$ for PA bacteria; Fig. 2c), while the PA bacterial community was more sensitive to salinity variance than FL bacteria ($R = -0.387$, $P < 0.01$ for FL bacteria; $R = -0.447$, $P < 0.001$ for PA bacteria; Fig. 2d). Furthermore, we found different stability patterns of FL and PA bacterial communities under different salinity scenarios. At high salinity level with low salinity variance scenarios, the stability of the PA bacterial community was significantly higher than that of the FL bacteria (e.g., 0.216 ± 0.007 for FL bacteria, 0.230 ± 0.008 for PA bacteria in P1; Fig. 3a), whereas it was lower at the low salinity level with high salinity variance scenarios (e.g., 0.233 ± 0.007 for FL bacteria, 0.219 ± 0.007 for PA bacteria in P4; Fig. 3a). Interestingly, the interaction strength of rare taxa in the FL bacterial communities was significantly higher than in the PA bacterial communities at high salinity level with low salinity variance scenarios (e.g., 0.239 ± 0.003 for FL bacteria, 0.216 ± 0.003 for PA bacteria in P1; Fig. 3b), whereas it was lower at low salinity level with high salinity variance scenarios (e.g., 0.244 ± 0.008 for FL bacteria, 0.250 ± 0.007 for PA bacteria in P4; Fig. 3b). Furthermore, the genome size of FL bacteria was significantly higher than for PA bacteria at family level in both salinity scenarios (e.g., 4.24 Mbp for FL bacteria, 4.20 Mbp for PA bacteria during high salinity period; 4.28 Mbp for FL bacteria, 4.25 Mbp for PA bacteria during low salinity period; Table S1–2).

3.3. α -diversity of FL and PA bacterial communities under salinity fluctuations

The α -diversity indices (richness, Chao1, phylogenetic diversity) of both FL and PA bacterial communities decreased with increasing salinity, whereas they increased with salinity variance (Fig. S5). Both Shannon-Wiener and Pielou's evenness showed a significant correlation

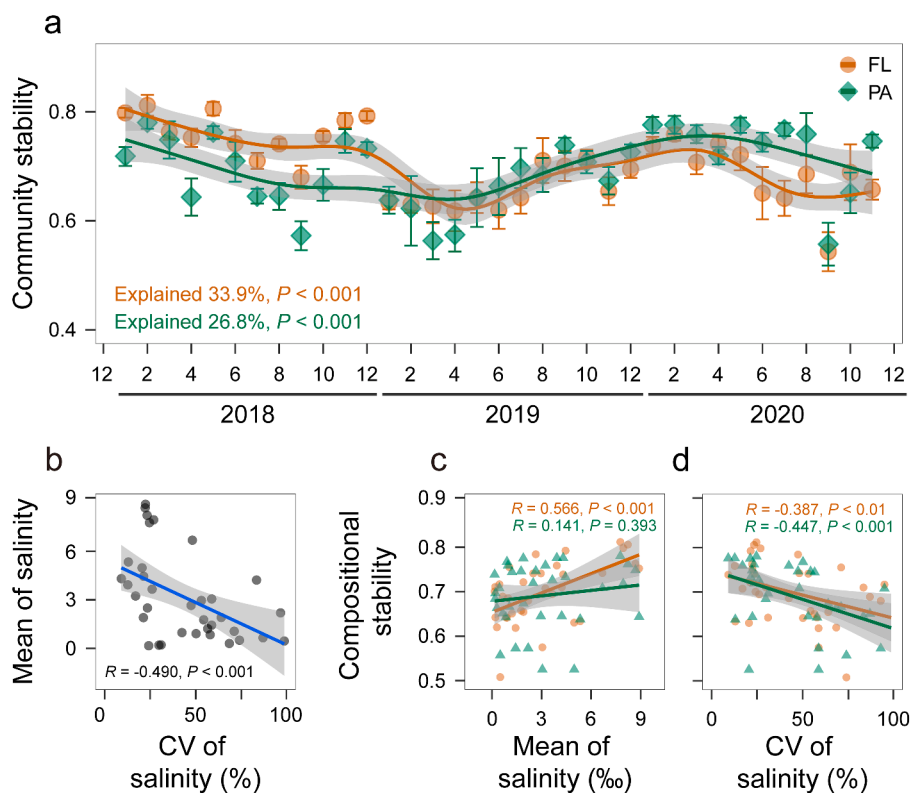


Fig. 2. Dynamics of the community stability of free-living (FL) and particle-attached (PA) bacterial communities under salinity fluctuation. (a) Generalized additive model fits of the compositional stability of FL (orange) and PA (green) bacterial communities over time during two adjacent months from December 2017 to November 2020, respectively. For example, the values for February 2018 indicate the comparison of FL or PA bacterial community composition between January and February 2018. (b) Relationship between the mean and CV of salinity. (c) Relationship between mean salinity and the compositional stability of FL or PA bacterial community, respectively. (d) Relationship between CV of salinity and the compositional stability of FL or PA bacterial community, respectively. Note that the results of (b), (c) and (d) are based on generalized linear regression model. Grey shadows indicate the 95% confidence interval.

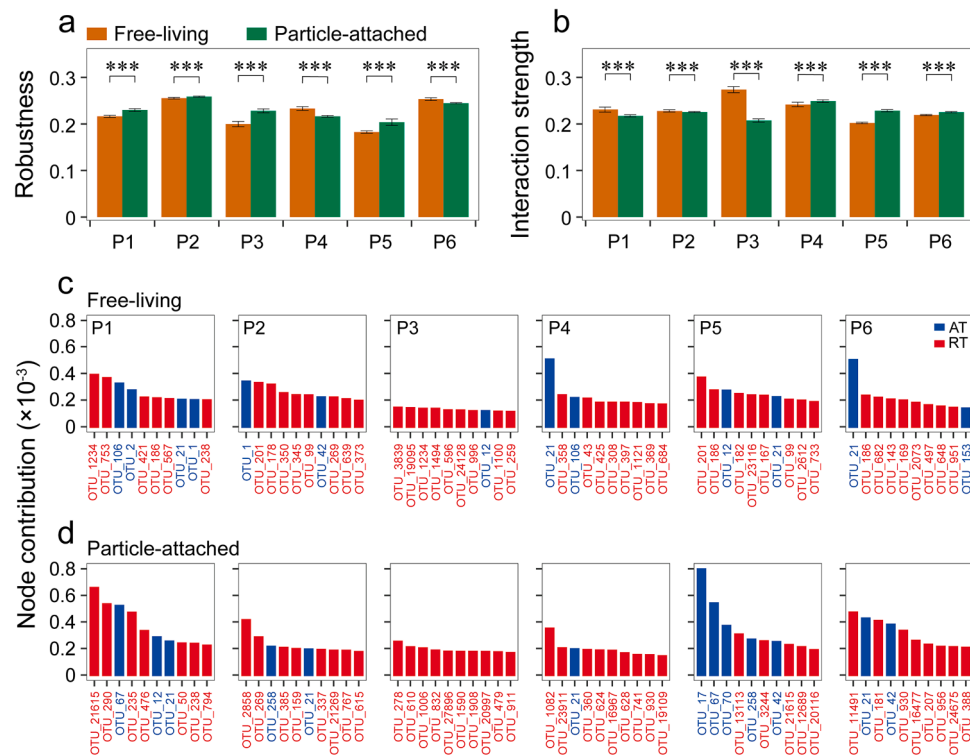


Fig. 3. Differences in the community stability of free-living (FL) and particle-attached (PA) bacteria and the importance of rare taxa for community stability under different salinity scenarios. (a) Robustness is measured as the proportion of nodes remaining in the network after removal of nodes accounting for 50 % of all taxa in each sub-network. (b) Interaction strength including only rare nodes of each sub-network of FL and PA bacterial communities, respectively. Error bar represents the standard error (** $P < 0.001$, Wilcoxon rank sum test). (c) and (d) Column charts showing the top 10 taxa in each sub-network of FL and PA bacterial communities contributing to the global efficiency of the network, respectively. AT, abundant taxa; RT, rare taxa. P1, period 1; P2, period 2; P3, period 3; P4, period 4; P5, period 5; P6, period 6. For details of the six periods see [Table 1](#).

with salinity for PA bacteria ($R = -0.648$, $P < 0.001$; Fig. S5c; $R = -0.500$, $P < 0.01$; Fig. S5d). For FL bacteria, in contrast, no significant relationship was observed between salinity and Shannon-Wiener or Pielou's evenness ($R = -0.200$, $P = 0.249$; Fig. S5c; $R = -0.032$, $P = 0.932$; Fig. S5d), implying that taxa with low abundance had different effects on FL and the PA bacterial communities.

3.4. Importance of rare taxa for community stability of FL and PA bacteria

Both FL and PA bacterial communities were composed of a few abundant taxa and many more rare taxa (Table S3). Rare taxa were the main contributors to community diversity (Fig. S6a–c) and were more sensitive to environmental fluctuations than the abundant taxa (Figs. S6d and S7, Table S4–5). Moreover, FL and PA bacterial networks in the six defined periods revealed that rare taxa were important in maintaining bacterial community stability (Table S6). For instance, about 80 % of the top-10 nodes contributing to the global efficiency of the network were rare (Fig. 3c–d), and 335 of the 554 keystone taxa were identified as rare (i.e., conditionally rare taxa, Table S7). Clearly, the node (zOTU) proportion changed dramatically when rare nodes were removed from the networks, whereas it changed only negligibly when abundant nodes were removed (Fig. S8).

3.5. Linkage between FL and PA bacterial community stability and environmental variables

The partial least squares path modelling revealed that precipitation, physical and chemical condition, salinity, nutrient, Chl-*a* and particulate matter exerted a direct or indirect effect on the community stability of FL and PA bacteria (Fig. 4). At high salinity level with low salinity

variance scenarios, salinity was the most important driver and had a significant direct effect on FL and PA bacterial community stability. Further, the effect of salinity on the stability of FL bacterial community (correlation coefficient = 0.306, Figs. 4a and S9a) was greater than for PA bacteria (correlation coefficient = 0.148, Figs. 4c and S9c). At low salinity level with high salinity variance scenarios, for FL bacteria, precipitation exerted an indirect and significant effect on stability by affecting mainly the particulate matter concentrations in the water body (correlation coefficient = -0.189, Figs. 4b and S9b). For PA bacteria, precipitation had a significant indirect effect on community stability through multiple paths. First, precipitation had a significant positive effect on PA bacterial community stability via changing the physical and chemical conditions in the water body (correlation coefficient = 0.495, Figs. 4d and S9d). Second, a precipitation-driven change in physical and chemical conditions altered nutrient and particulate matter in the water, directly influencing PA bacterial community stability.

4. Discussion

4.1. Increasing salinity promotes community stability but reduces FL and PA bacterial α -diversity

The responses of bacterial communities to environmental changes have a great impact on the entire aquatic ecosystem as they are key players in matter cycling (Chow et al., 2014; Guidi et al., 2016; Shade et al., 2012). We acquired a 3-year high frequency dataset in a subtropical urban reservoir to investigate the community stability of FL and PA bacteria under different salinity scenarios. Our results indicated that the community stability of the FL bacteria increased significantly with increasing salinity, and a similar but non-significant trend was observed for the PA bacteria (Fig. 2). This suggests that increasing salinity

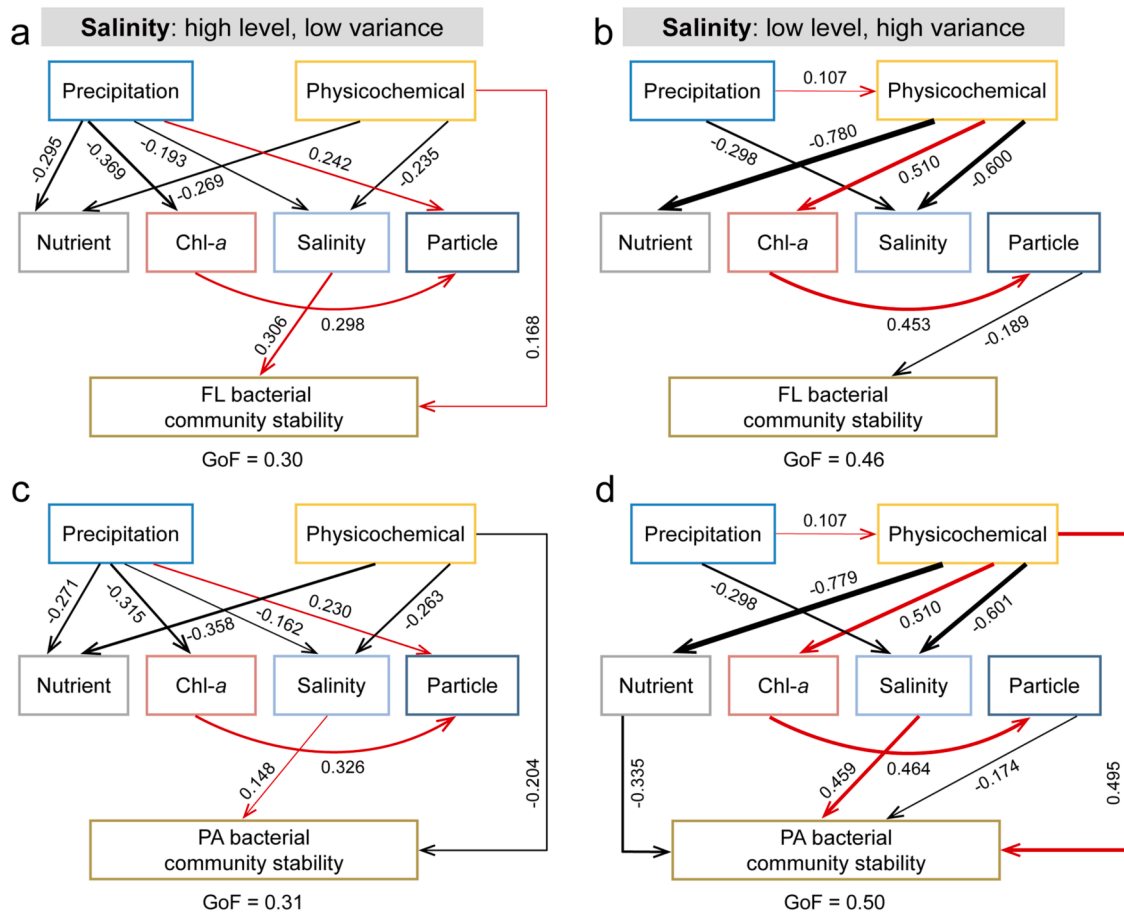


Fig. 4. Partial least squares path models (PLS-PM) showing the relationship between environmental drivers and the community stability of free-living (FL) and particle-attached (PA) bacteria under different salinity scenarios. Red and black lines represent positive and negative effects, respectively. Only significant paths ($P < 0.1$) are shown for the sake of simplicity. The thickness of path lines indicates absolute coefficient values. All data are log-transformed, except pH and stability. GOF means goodness-of-fit; Physicochemical represents physical and chemical factors; Precipitation represents 7-day accumulated precipitation before the sampling day.

generally promotes the community stability of bacteria. However, the richness of the entire bacterial community decreased significantly with increasing salinity (Fig. S5a–b), several explanations are possible to explain this result. First, low salinity periods mainly occur in the wet season or rainy periods (Table 1). Precipitation events increase the input of freshwater and particulate matter, accompanied by a decrease in salinity and often a major change in particulate matter density and composition, which is consistent with our findings (Fig. S2). Meanwhile, bacteria from the surrounding environments (i.e., soil, sediment and watershed) might be washed into reservoir waters due to runoff (Shabarova et al., 2021; Stockwell et al., 2020), which likely results in a faster turnover of the microbial community, leading to decreased compositional stability at low salinity level with high variance scenarios. Second, the responses of bacterioplankton to salinity variances are mainly related to changes in extracellular osmolarity (Mo et al., 2021). For example, bacterioplankton intolerance of high osmotic stress will be filtered out, resulting in low richness at high salinity level. Recently, Li et al. (2023) reported similar results along a gradient of freshwater-to-seawater ecosystems, whereby the majority of retained species in the community being those with strong salt tolerance at increasing salinity. This may reflect a higher resistance to increasing salinity with presumably higher compositional stability. Moreover, we found that the phylogenetic diversity of the FL and PA bacterial communities decreased substantially with increasing salinity (Fig. S5e and j). A recent study on dryland plants highlighted that communities with higher phylogenetic diversity were more likely to provide higher multifunctionality (Le Bagousse-Pinguet et al., 2019). Hence, our results

indicate that increasing salinity might be detrimental to the multifunctionality of freshwater ecosystems. In summary, our results demonstrate that FL and PA bacterial communities maintain higher stability in community composition with enhanced salinity as species with low salt tolerance are increasingly eliminated, which, in turn, may greatly reduce the multifunctionality of freshwater ecosystems.

4.2. Difference in community stability between FL and PA bacteria under different salinity scenarios

Our study indicated that different community stability changes of FL and PA bacteria exist under different salinity scenarios (Fig. 3a). To discuss this further, we focus on internal and external mechanisms, respectively.

4.2.1. Internal mechanisms

Genomic traits, such as genome size, are related to microbial adaptation strategies in nutrient-changing environments (Ngugi et al., 2023; Shenhav and David, 2020). Previous studies have shown that bacteria with larger genomes can better adapt to more variable environments and exhibit high resistance (Scheuerl et al., 2020; Sriswasdi et al., 2017). In this study, we found that genome size was significantly higher for the FL bacteria than for the PA bacteria at the low salinity level with high salinity variance scenarios (Table S3). Therefore, FL bacterial communities show greater stability under higher salinity variance scenarios (e.g., P4 and P6, Fig. 3a). In addition, at the high salinity level with low salinity variance scenarios, we also found a higher genome size of FL

than of PA bacteria (Table S3). A possible reason for this is that salinity fluctuated less during these periods; thus, community stability might not only depend on the ability to resist specific salinity changes but also on other factors, e.g., nutrient availability. Specifically, due to a lower intensity and frequency of precipitation events, the composition and dynamics of particulate matter in water bodies under this scenario might be relatively stable. Moreover, our previous study has shown that relatively warm winters in subtropical regions promote the growth and dominance of phytoplankton in the Xinglinwan Reservoir (Luo et al., 2022), potentially providing more micro-niches for PA bacteria (Roth Rosenberg et al., 2021), and thus making it easier for PA bacteria to colonize on particulate matter surfaces and obtain more nutrients than FL bacteria. Unfortunately, the comparison of FL and PA genome sizes in this study contradicts the results of previous studies, for instance, Ngugi et al. (2023) reported larger genome size of PA than FL bacteria. There might be two reasons for this discrepancy. First, the results of Ngugi et al. were based on a dataset of an estimation of 364 marine metagenomes, while ours were derived from species alignment with more than 200,000 high quality prokaryotic genomes from freshwater and/or brackish water. Second, extracellular or “relic” DNA or dormant individuals in the environment might be considered as FL bacteria, resulting in a larger estimated genome size of FL bacteria (Jones and Lennon, 2010; Lennon et al., 2018). Therefore, comprehensive and standard reference databases of prokaryotic genomes as well as unbiased collection approaches (more rigorous and precise experimental operations) for FL and PA bacteria are of particular importance for genome evaluation of FL and PA bacteria in the future.

In addition to genomic traits, we also focused on the effects of rare taxa on bacterial community stability. Previous studies have suggested that the rare microbial biosphere, which is regarded as a part of the microbial “seed bank” (Lynch and Neufeld, 2015), may drive the bacterial responses to environmental changes, subsequently affecting the stability of microbial communities (Xue et al., 2018). In this study, we not only highlighted the importance of rare taxa to community diversity (Fig. S6, Table S4–5), but also unraveled that rare taxa play a decisive role for community stability of both FL and PA bacteria. Specifically, the higher the community stability is, the weaker is the interaction strength of rare taxa in the community, e.g., the stability was higher while the interaction strength of rare taxa was lower in the PA bacterial community than in the FL bacterial community in period 1 (Fig. 3a and b). Supporting this finding, Hu et al. (2022) observed that increasing interactions between bacteria species correlated with a reduced compositional stability of the community in mesocosm experiments. Furthermore, our results concur with those of Ratzke et al. (2020), who demonstrated that weak microbial interactions increase the stability of total biomass and species composition in a model simulation study. A reasonable explanation is that species with weak interactions have a higher degree of niche differentiation, allowing them to survive when faced with environmental fluctuations, and this may contribute to maintain the stability in bacterial community composition. In conclusion, our results suggest that rare taxa play an irreplaceable role in maintaining community stability of both FL and PA bacteria under salinity fluctuations. Moreover, differences in the community stability of FL and PA bacteria under different salinity scenarios might strongly depend on the interaction strengths of rare taxa.

4.2.2. External mechanisms

At present, an increasing number of studies have focused on the impact of global climate change on ecosystems and their functions (Stockwell et al., 2020; Talbot et al., 2018). Many studies have demonstrated that heavy precipitation can affect the hydrodynamics and particulate matter dynamics of aquatic environments and thus has a large impact on microbial community composition and function (Kasprzak et al., 2017; Shabarova et al., 2021; Xin et al., 2023; Zhang et al., 2024). Our results showed different effects of precipitation on FL and PA bacterial communities, which may be one of the reasons why the

community stability was higher for FL bacteria than for PA bacteria at low salinity with high salinity variance scenarios. This scenario mainly occurs in wet years or wet seasons, i.e., precipitation with strong intensity, high frequency and long duration leads to declining salinity with profound changes in hydrodynamics and particulate matter in water bodies (Talbot et al., 2018). Such changes have a stronger effect on PA bacteria colonising particulate matter than on FL bacteria (Shen et al., 2022; Zoccarato and Grossart, 2019). In addition, Polz and Cordero (2016) argued that microorganisms adapted to high nutrient concentrations (e.g., PA bacteria) typically produce more ribosomal proteins to maximise their growth rate. However, microorganisms in low nutrient environments (e.g., FL bacteria) tend to produce more metabolic enzymes, which enables them to extract more energy from limited resources. Therefore, we speculate that FL bacteria can better obtain energy from more adverse environments than PA bacteria to meet their basic cell metabolism, while most of the PA bacteria that cannot withstand the numerous environmental fluctuations will be eliminated, resulting in greater variation in community composition and thus lower community stability. Furthermore, studies on the functional potential of FL and PA bacteria indicate that PA bacteria occupy a more important position in the biogeochemical cycles than FL bacteria (D’Ambrosio et al., 2014; Grossart, 2010; Liu et al., 2020). Hence, our results suggest that increasing frequency and intensity of heavy precipitation might be detrimental to ecosystem matter cycling and energy flow by control of particles and PA bacteria in surface waters.

At high salinity level with low salinity variance scenarios, we identified salinity as an important driver for the community stability of FL and PA bacteria, while salinity had a greater effect on FL than PA bacteria (Fig. 4a and c). This specific scenario mostly occurred in dry years or dry seasons. Clearly, a low frequency, weak intensity precipitation is the main feature of this scenario, resulting in relatively stable particulate matter concentrations in the water with strong selection due to the high salinity being a major stressor. Previous studies have shown that PA bacteria, in contrast to FL bacteria, usually have larger sizes and are more metabolically active, and they can therefore degrade high-molecular-weight organic compounds to provide energy for physiological cell activities (D’Ambrosio et al., 2014; Li et al., 2021). The relatively stable particulate matter conditions and favourable microenvironment may provide a steady stream of nutrients for PA bacteria (Farnelid et al., 2019; Simon et al., 2014). The obviously higher nutrient availability of PA bacteria augmented the interactions facilitating exchange of energy and information and may render PA bacteria more resistant to enhanced salinity stress than FL bacteria. In contrast, living conditions for FL bacteria appear to be much less favourable than for PA bacteria at high salinity stress. Accordingly, under these conditions, the community stability of PA bacteria was significantly higher than that of FL bacteria. In summary, our results provide direct evidence of the different effects of precipitation on the community stability of FL and PA bacteria by regulating salinity and runoff. Precipitation has a greater negative effect on the community stability of PA bacteria than of FL bacteria in inland surface waters.

5. Conclusion

We first explored the community stability of FL and PA bacteria under salinity fluctuations based on a 3-year high-frequency time series dataset from a subtropical shallow urban reservoir. Our two proposed hypotheses were verified. First, the community stability of both FL and PA bacteria increased with increasing salinity, but decreased with increasing salinity variance, presumably by eliminating salt-sensitive species. Second, the differences of response processes and underlying mechanisms between FL and PA bacterial community stabilities were unraveled. PA bacterial communities were more stable than FL bacterial communities at high salinity level with low salinity variance scenarios, but they were less stable at low salinity level with high salinity variance scenarios, implying that FL bacteria are more susceptible to the absolute

salinity level, whereas PA bacteria are more related to the increased salinity variance during rainy periods. Importantly, these differences in community stability of FL and PA bacteria under different salinity scenarios can be well explained by bacterial genome size, the interaction strength of rare taxa and precipitation events. Given that precipitation-induced salinity changes have a stronger impact on PA than FL bacterial community stability in surface waters, the predicted increase in frequency and intensity of future precipitation events may lead to high risks to aquatic ecosystem functions and services. In summary, our study reveals a strong relationship among precipitation, salinity and community stability of FL and PA bacteria, and provides important insights into the mechanisms underlying the differences in community stability between FL and PA bacteria in a changing climate.

Data availability

Data will be made available from the corresponding author (Jun Yang) on request. The raw sequence data of the 16S rRNA gene in this study have been stored in the NCBI sequence read archive (SRA) database under BioProject number PRJNA1015425 for FL bacteria and BioProject number PRJNA1015423 for PA bacteria, respectively. The raw sequences were also deposited in the National Omics Data Encyclopedia database under the Project ID: OEP004629 for FL bacteria and OEP004628 for PA bacteria, respectively.

CRediT authorship contribution statement

Xue Yan: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Shuzhen Li:** Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Mamun Abdullah Al:** Formal analysis, Writing – review & editing. **Yuanyuan Mo:** Investigation, Writing – original draft. **Jun Zuo:** Formal analysis, Writing – review & editing. **Hans-Peter Grossart:** Writing – review & editing. **Hongteng Zhang:** Investigation, Writing – original draft. **Yigang Yang:** Data curation. **Erik Jeppesen:** Writing – review & editing. **Jun Yang:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Supervision, Visualization, Writing – original draft, Writing – review & editing, Project administration, Resources.

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

Data will be made available on request.

Acknowledgments

We thank the AEHG members for field sampling and experiment assistance in Xiamen Reservoir Time-series (XRT) Project. We also thank Peng Xiao, Kai Feng, Fengjiao Tan and Zijie Xu for statistical support and Anne Mette Poulsen for English editing. This work was supported by the National Natural Science Foundation of China (92251306, 32361133557 and 92047204), the “Fujian STS” Program of the Chinese Academy of Sciences (2021T3015 and 2022T3015). HPG was funded by German Science Foundation projects Zooflux (GR 1540/29-1) and Pycnocline (GR1540/37-1). EJ was supported by the TÜBITAK program BİDEB2232 (Project 118C250).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.watres.2024.121344](https://doi.org/10.1016/j.watres.2024.121344).

References

- Allison, S.D., Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci. U. S. A.* 105, 11512–11519. <https://doi.org/10.1073/pnas.0801925105>.
- Auladell, A., Barberán, A., Logares, R., Garcés, E., Gasol, J.M., Ferrera, I., 2022. Seasonal niche differentiation among closely related marine bacteria. *ISME J.* 16, 178–189. <https://doi.org/10.1038/s41396-021-01053-2>.
- Cañedo-Argüelles, M., Kefford, B., Schäfer, R., 2019. Salt in freshwaters: causes, effects and prospects - introduction to the theme issue. *Phil. Trans. R. Soc. B* 374, 20180002. <https://doi.org/10.1098/rstb.2018.0002>.
- Chow, C-E.T., Kim, D.Y., Sachdeva, R., Caron, D.A., Fuhrman, J.A., 2014. Top-down controls on bacterial community structure: microbial network analysis of bacteria, T4-like viruses and protists. *ISME J.* 8, 816–829. <https://doi.org/10.1038/ismej.2013.199>.
- Corsi, S.R., Graczyk, D.J., Geis, S.W., Booth, N.L., Richards, K.D., 2010. A fresh look at road salt: aquatic toxicity and water-quality impacts on local, regional, and national scales. *Environ. Sci. Technol.* 44, 7376–7382. <https://doi.org/10.1021/es101333u>.
- Cram, J.A., Xia, L.C., Needham, D.M., Sachdeva, R., Sun, F.Z., Fuhrman, J.A., 2015. Cross-depth analysis of marine bacterial networks suggests downward propagation of temporal changes. *ISME J.* 9, 2573–2586. <https://doi.org/10.1038/ismej.2015.76>.
- Cunillera-Montcusí, D., Beklioglu, M., Cañedo-Argüelles, M., Jeppesen, E., Ptacnik, R., Amorim, C.A., Arnott, S.E., Berger, S.A., Brucet, S., Dugan, H.A., Gerhard, M., Horváth, Z., Langenheder, S., Nejtgaard, J.C., Reinikainen, M., Striebel, M., Urrutia-Cordero, P., Vad, C.F., Zadereev, E., Matias, M., 2022. Freshwater salinisation: a research agenda for a saltier world. *Trends Ecol. Evol.* 37, 440–453. <https://doi.org/10.1016/j.tree.2021.12.005>.
- D'Ambrosio, L., Ziervogel, K., MacGregor, B., Teske, A., Arnosti, C., 2014. Composition and enzymatic function of particle-associated and free-living bacteria: a coastal/offshore comparison. *ISME J.* 8, 2167–2179. <https://doi.org/10.1038/ismej.2014.67>.
- Deng, Y., Jiang, Y.H., Yang, Y., He, Z., Luo, F., Zhou, J., 2012. Molecular ecological network analyses. *BMC Bioinform.* 13, 113. <https://doi.org/10.1186/1471-2105-13-113>.
- Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2460–2461. <https://doi.org/10.1093/bioinformatics/btq461>.
- Edgar, R.C., 2016. NOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *BioRxiv* 081257. [10.1101/081257](https://doi.org/10.1101/081257).
- Farnelid, H., Turk-Kubo, K., Ploug, H., Ossolinski, J.E., Collins, J.R., Van Mooy, B.A.S., Zehr, J.P., 2019. Diverse diazotrophs are present on sinking particles in the North Pacific subtropical gyre. *ISME J.* 13, 170–182. <https://doi.org/10.1038/s41396-018-0259-x>.
- Gao, X.F., Chen, H.H., Gu, B.H., Jeppesen, E., Xue, Y.Y., Yang, J., 2021. Particulate organic matter as causative factor to eutrophication of subtropical deep freshwater: role of typhoon (tropical cyclone) in the nutrient cycling. *Water Res.* 188, 116470. <https://doi.org/10.1016/j.watres.2020.116470>.
- Gehlenborg, N., 2019. UpSetR: a more scalable alternative to Venn and Euler diagrams for visualizing intersecting sets. R package version 1.4.0. <https://CRAN.R-project.org/package=UpSetR>.
- Gilling, D.P., Nejtgaard, J.C., Berger, S.A., Grossart, H.P., Kirillin, G., Penske, A., Lentz, M., Casper, P., Sareyka, J., Gessner, M.O., 2017. Thermocline deepening boosts ecosystem metabolism: evidence from a large-scale lake enclosure experiment simulating a summer storm. *Glob. Chang. Biol.* 23, 1448–1462. <https://doi.org/10.1111/gcb.13512>.
- Grossart, H.P., 2010. Ecological consequences of bacterioplankton lifestyles: changes in concepts are needed. *Environ. Microbiol. Rep.* 2, 706–714. <https://doi.org/10.1111/j.1758-2229.2010.00179.x>.
- Guidi, L., Chaffron, S., Bittner, L., Eveillard, D., Larhlimi, A., Roux, S., Darzi, Y., Audic, S., Berline, L., Brum, J.R., Coelho, L.P., Espinoza, J.C.I., Malviya, S., Sunagawa, S., Dimier, C., Kandels-Lewis, S., Picheral, M., Poulain, J., Searson, S., Coordinators, Tara Oceans Consortium, Stemmann, L., Not, F., Hingamp, P., Speich, S., Follows, M., Karp-Boss, L., Boss, E., Ogata, H., Pesant, S., Weissenbach, J., Wincker, P., Acinas, S.G., Bork, P., De Vargas, C., Iudicone, D., Sullivan, M.B., Raes, J., Karsenti, E., Bowler, C., Gorsky, G., 2016. Plankton networks driving carbon export in the oligotrophic ocean. *Nature* 532, 465–470. <https://doi.org/10.1038/nature16942>.
- von Gulev, S.K., Thorne, P.W., Ahn, J., Dentener, F.J., Domingues, C.M., Gerland, S., Gong, D.Y., Kaufman, D.S., Nnamchi, H.C., Quaas, J., Rivera, J.A., Sathyendranath, S., Smith, S.L., Trewin, B., Schuckmann, K., Vose, R.S., 2023. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 287–422. <https://doi.org/10.1017/9781009157896.004>.
- Hu, J.L., Amor, D.R., Barbier, M., Bunin, G., Gore, J., 2022. Emergent phases of ecological diversity and dynamics mapped in microcosms. *Science* 378, 85–89. <https://doi.org/10.1126/science.abm7841>.
- Huang, H.Y., 2021. linkET: everything is linkable. R package version 0.0.7.1. <https://rdrr.io/github/Hy4m/linkET/>.

- Jeppesen, E., Beklioglu, M., Özkan, K., Akyürek, Z., 2020. Salinization increase due to climate change will have substantial negative effects on inland waters: a call for multifaceted research at the local and global scale. *Innovation* 1, 100030. [10.1016/j.xinn.2020.100030](https://doi.org/10.1016/j.xinn.2020.100030).
- Jones, S.E., Chiu, C.Y., Kratz, T.K., Wu, J.T., Shade, A., McMahon, K.D., 2008. Typhoons initiate predictable change in aquatic bacterial communities. *Limnol. Oceanogr.* 53, 1319–1326. <https://doi.org/10.4319/lo.2008.53.4.1319>.
- Jones, S.E., Lennon, J.T., 2010. Dormancy contributes to the maintenance of microbial diversity. *Proc. Natl. Acad. Sci. U. S. A.* 107, 5881–5886. <https://doi.org/10.1073/pnas.0912765107>.
- Kasprzak, P., Shatwell, T., Gessner, M.O., Gonsiorczyk, T., Kirillin, G., Selmezy, G., Padišák, J., Engelhardt, C., 2017. Extreme weather event triggers cascade towards extreme turbidity in a clear-water lake. *Ecosystems* 20, 1407–1420. <https://doi.org/10.1007/s10021-017-0121-4>.
- Kassambara, A., 2023. ggpubr: “ggplot2” based publication ready plots. R package version 0.6.0. <https://CRAN.R-project.org/package=ggpubr>.
- Landa, M., Blain, S., Christaki, U., Monchy, S., Obernosterer, I., 2016. Shifts in bacterial community composition associated with increased carbon cycling in a mosaic of phytoplankton blooms. *ISME J.* 10, 39–50. <https://doi.org/10.1038/ismej.2015.105>.
- Le Bagousse-Pinguet, Y., Soliveres, S., Gross, N., Torices, R., Berdugo, M., Maestre, F.T., 2019. Phylogenetic, functional, and taxonomic richness have both positive and negative effects on ecosystem multifunctionality. *Proc. Natl. Acad. Sci. U. S. A.* 116, 8419–8424. <https://doi.org/10.1073/pnas.1815727116>.
- Lennon, J.T., Muscarella, M.E., Placella, S.A., Lehmkuhl, B.K., 2018. How, when, and where relic DNA affects microbial diversity. *mBio* 9, e00637–18. <https://doi.org/10.1128/mBio.00637-18>.
- Li, C.C., Jin, L., Zhang, C., Li, S.Z., Zhou, T., Hua, Z.Y., Wang, L.F., Ji, S.P., Wang, Y.F., Gan, Y.D., Liu, J., 2023. Destabilized microbial networks with distinct performances of abundant and rare biospheres in maintaining networks under increasing salinity stress. *iMeta* 2, e79. [10.1002/imt2.79](https://doi.org/10.1002/imt2.79).
- Li, H., Xing, P., Wu, Q.L., 2012. The high resilience of the bacterioplankton community in the face of a catastrophic disturbance by a heavy *Microcystis* bloom. *FEMS Microbiol. Ecol.* 82, 192–201. <https://doi.org/10.1111/j.1574-6941.2012.01417.x>.
- Li, J., Gu, L., Bai, S., Wang, J., Su, L., Wei, B., Zhang, L., Fang, J., 2021. Characterization of particle-associated and free-living bacterial and archaeal communities along the water columns of the South China Sea. *Biogeosciences* 18, 113–133. <https://doi.org/10.5194/bg-18-113-2021>.
- Liu, M., Liu, L.M., Chen, H.H., Yu, Z., Yang, R.J., Xue, Y.Y., Huang, B.Q., Yang, J., 2019. Community dynamics of free-living and particle-attached bacteria following a reservoir *Microcystis* bloom. *Sci. Total Environ.* 660, 501–511. <https://doi.org/10.1016/j.scitotenv.2018.12.414>.
- Liu, Y.Y., Lin, Q., Peng, J.R., Yang, F.M., Du, H., Hu, Z., Wang, H., 2020. Differences in metabolic potential between particle-associated and free-living bacteria along pearl river estuary. *Sci. Total Environ.* 728, 138856. <https://doi.org/10.1016/j.scitotenv.2020.138856>.
- Logares, R., Lindström, E.S., Langenheder, S., Logue, J.B., Paterson, H., Laybourn-Parry, J., Rengefors, K., Tranvik, L., Bertilsson, S., 2013. Biogeography of bacterial communities exposed to progressive long-term environmental change. *ISME J.* 7, 937–948. <https://doi.org/10.1038/ismej.2012.168>.
- Luo, A.Q., Chen, H.H., Gao, X.F., Carvalho, L., Xue, Y.Y., Jin, L., Yang, J., 2022. Short-term rainfall limits cyanobacterial bloom formation in a shallow eutrophic subtropical urban reservoir in warm season. *Sci. Total Environ.* 827, 154172. <https://doi.org/10.1016/j.scitotenv.2022.154172>.
- Luo, A.Q., Chen, H.H., Gao, X.F., Carvalho, L., Zhang, H.T., Yang, J., 2024. The impact of rainfall events on dissolved oxygen concentrations in a subtropical urban reservoir. *Environ. Res.* 244, 117856. <https://doi.org/10.1016/j.envres.2023.117856>.
- Lynch, M.D.J., Neufeld, J.D., 2015. Ecology and exploration of the rare biosphere. *Nat. Rev. Microbiol.* 13, 217–229. <https://doi.org/10.1038/nrmicro3400>.
- Ma, R.Q., Yan, M.Q., Han, P., Wang, T., Li, B., Zhou, S.G., Zheng, T., Hu, Y.D., Borwick, A.G.L., Zheng, C.M., Ni, J.R., 2022. Deficiency and excess of groundwater iodine and their health associations. *Nat. Commun.* 13, 7354. <https://doi.org/10.1038/s41467-022-35042-6>.
- Mestre, M., Borrell, E., Sala, M.M., Gasol, J.M., 2017. Patterns of bacterial diversity in the marine planktonic particulate matter continuum. *ISME J.* 11, 999–1010. <https://doi.org/10.1038/ismej.2016.166>.
- Mo, Y.Y., Peng, F., Gao, X.F., Xiao, P., Logares, R., Jeppesen, E., Ren, K.X., Xue, Y.Y., Yang, J., 2021. Low shifts in salinity determined assembly processes and network stability of microeukaryotic plankton communities in a subtropical urban reservoir. *Microbiome* 9, 128. <https://doi.org/10.1186/s40168-021-01079-w>.
- Montesinos-Navarro, A., Hiraldo, F., Tella, J.L., Blanco, G., 2017. Network structure embracing mutualism-antagonism continuums increases community robustness. *Nat. Ecol. Evol.* 1, 1661–1669. <https://doi.org/10.1038/s41559-017-0320-6>.
- Ngugi, D.K., Acinas, S.G., Sánchez, P., Gasol, J.M., Agustí, S., Karl, D.M., Duarte, C.M., 2023. Abiotic selection of microbial genome size in the global ocean. *Nat. Commun.* 14, 1384. <https://doi.org/10.1038/s41467-023-36988-x>.
- Pedersen, T.L., 2022. patchwork: the composer of plots. R package version 1.1.2. <https://CRAN.R-project.org/package=patchwork>.
- Polz, M.F., Cordero, O.X., 2016. Bacterial evolution: genomics of metabolic trade-offs. *Nat. Microbiol.* 1, 16181. <https://doi.org/10.1038/nmicrobiol.2016.181>.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596. <https://doi.org/10.1093/nar/gks1219>.
- R. Core Team, 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.r-project.org>.
- Ratzke, C., Barrere, J., Gore, J., 2020. Strength of species interactions determines biodiversity and stability in microbial communities. *Nat. Ecol. Evol.* 4, 376–383. <https://doi.org/10.1038/s41559-020-1099-4>.
- Roth Rosenberg, D., Haber, M., Goldford, J., Lalzar, M., Aharonovich, D., Al-Ashhab, A., Lehahn, Y., Segre, D., Steindler, L., Sher, D., 2021. Particle-associated and free-living bacterial communities in an oligotrophic sea are affected by different environmental factors. *Environ. Microbiol.* 23, 4295–4308. <https://doi.org/10.1111/1462-2920.15611>.
- Scheuerl, T., Hopkins, M., Nowell, R.W., Rivett, D.W., Barraclough, T.G., Bell, T., 2020. Bacterial adaptation is constrained in complex communities. *Nat. Commun.* 11, 754. <https://doi.org/10.1038/s41467-020-14570-z>.
- Shabarova, T., Salcher, M.M., Porcal, P., Znachor, P., Nedoma, J., Grossart, H.P., Seda, J., Hejzlar, J., Šimek, K., 2021. Recovery of freshwater microbial communities after extreme rain events is mediated by cyclic succession. *Nat. Microbiol.* 6, 479–488. <https://doi.org/10.1038/s41564-020-00852-1>.
- Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Bürgmann, H., Huber, D.H., Langenheder, S., Lennon, J.T., Martiny, J.B.H., Matulich, K.L., Schmidt, T.M., Handelsman, J., 2012. Fundamentals of microbial community resistance and resilience. *Front. Microbiol.* 3, 417. <https://doi.org/10.3389/fmicb.2012.00417>.
- Shade, A., Read, J.S., Welkie, D.G., Kratz, T.K., Wu, C.H., McMahon, K.D., 2011. Resistance, resilience and recovery: aquatic bacterial dynamics after water column disturbance. *Environ. Microbiol.* 13, 2752–2767. <https://doi.org/10.1111/j.1462-2920.2011.02546.x>.
- Shen, Z., Xie, G.J., Zhang, Y.Q., Yu, B.B., Shao, K.Q., Gao, G., Tang, X.M., 2022. Similar assembly mechanisms but distinct co-occurrence patterns of free-living vs. particle-attached bacterial communities across different habitats and seasons in shallow, eutrophic Lake Taihu. *Environ. Pollut.* 314, 120305. <https://doi.org/10.1016/j.envpol.2022.120305>.
- Shenhav, L., David, Z., 2020. Resource conservation manifests in the genetic code. *Science* 370, 683–687. <https://doi.org/10.1126/science.aaz9642>.
- Simon, H.M., Smith, M.W., Herfort, L., 2014. Metagenomic insights into particles and their associated microbiota in a coastal margin ecosystem. *Front. Microbiol.* 5, 466. <https://doi.org/10.3389/fmicb.2014.00466>.
- Sriswasdi, S., Yang, C., Iwasaki, W., 2017. Generalist species drive microbial dispersion and evolution. *Nat. Commun.* 8, 1162. <https://doi.org/10.1038/s41467-017-01265-1>.
- Stockwell, J.D., Doubek, J.P., Adrian, R., Anneville, O., Carey, C.C., Carvalho, L., De Senepont, D.M., Dur, G., Frassl, M.A., Grossart, H.P., Ibelings, B.W., Lajeunesse, M.J., Lewandowska, A.M., Llamas, M.E., Matsuzaki, S.S., Nodine, E.R., Nöges, P., Patil, V.P., Pomati, F., Rinke, K., Rudstam, L.G., Rusak, J.A., Salmazo, N., Seltmann, C.T., Straile, D., Thackeray, S.J., Thiery, W., Urrutia-Cordero, P., Venail, P., Verburg, P., Woolway, R.I., Zohary, T., Andersen, M.R., Bhattacharya, R., Hejzlar, J., Janatian, N., Kpodonu, A.T.N.K., Williamson, T.J., Wilson, H.L., 2020. Storm impacts on phytoplankton community dynamics in lakes. *Glob. Chang. Biol.* 26, 2756–2784. <https://doi.org/10.1111/gcb.15033>.
- Talbot, C.J., Bennett, E.M., Cassell, K., Hanes, D.M., Minor, E.C., Paerl, H., Raymond, P. A., Vargas, R., Vidon, P.G., Wollheim, W., Xenopoulos, M.A., 2018. The impact of flooding on aquatic ecosystem services. *Biogeochemistry* 141, 439–461. <https://doi.org/10.1007/s10533-018-0449-7>.
- Tang, X.M., Gao, G., Chao, J.Y., Wang, X.D., Zhu, G.W., Qin, B.Q., 2010. Dynamics of organic-aggregate-associated bacterial communities and related environmental factors in Lake Taihu, a large eutrophic shallow lake in China. *Limnol. Oceanogr.* 55, 469–480. <https://doi.org/10.4319/lo.2010.55.2.0469>.
- Villalba, L.A., Karnatak, R., Grossart, H.P., Wollrab, S., 2022. Flexible habitat choice of pelagic bacteria increases system stability and energy flow through the microbial loop. *Limnol. Oceanogr.* 67, 1402–1415. <https://doi.org/10.1002/lno.12091>.
- Ward, N.D., Megonigal, J.P., Bond-Lamberty, B., Bailey, V.L., Butman, D., Canuel, E.A., Diefenderfer, H., Ganju, N.K., Goñi, M.A., Graham, E.B., Hopkins, C.S., Khangaonkar, T., Langley, J.A., McDowell, N.G., Myers-Pigg, A.N., Neumann, R.B., Osburn, C.L., Price, R.M., Rowland, J., Sengupta, A., Simard, M., Thornton, P.E., Tzortziou, M., Vargas, R., Weisenborn, P.B., Windham-Myers, L., 2020. Representing the function and sensitivity of coastal interfaces in Earth system models. *Nat. Commun.* 11, 2458. <https://doi.org/10.1038/s41467-020-16236-2>.
- Wickham, H., Chang, W., Henry, L., Pedersen, T.L., Takahashi, K., Wilke, C., Woo, K., Yutani, H., Dunnington, D., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York. <https://CRAN.R-project.org/package=ggplot2>.
- Xin, Y., Zhang, J.Y., Lu, T.D., Wei, Y.S., Shen, P.H., 2023. Response of prokaryotic, eukaryotic and algal communities to heavy rainfall in a reservoir supplied with reclaimed water. *J. Environ. Manag.* 334, 117394. <https://doi.org/10.1016/j.jenvman.2023.117394>.
- Xue, Y.Y., Chen, H.H., Yang, R.J., Liu, M., Huang, B.Q., Yang, J., 2018. Distinct patterns and processes of abundant and rare eukaryotic plankton communities following a reservoir cyanobacterial bloom. *ISME J.* 12, 2263–2277. <https://doi.org/10.1038/s41396-018-0159-0>.
- Xue, Y.Y., Liu, M., Chen, H.H., Jeppesen, E., Zhang, H.T., Ren, K.X., Yang, J., 2022. Microbial hierarchical correlations and their contributions to carbon-nitrogen cycling following a reservoir cyanobacterial bloom. *Ecol. Indic.* 143, 109401. <https://doi.org/10.1016/j.ecolind.2022.109401>.
- Yang, Y.G., Chen, H.H., Abdullah Al, M., Ndayishimiye, J.C., Yang, R.J., Isabwe, A., Luo, A.Q., Yang, J., 2022. Urbanization reduces resource use efficiency of phytoplankton community by altering the environment and decreasing biodiversity. *J. Environ. Sci.* 112, 140–151. <https://doi.org/10.1016/j.jes.2021.05.001>.
- Yuan, M.M., Guo, X., Wu, L.W., Zhang, Y., Xiao, N.J., Ning, D.L., Shi, Z., Zhou, X.S., Wu, L.Y., Yang, Y.F., Tiedje, J.M., Zhou, J.Z., 2021. Climate warming enhances microbial network complexity and stability. *Nat. Clim. Chang.* 11, 343–348. <https://doi.org/10.1038/s41558-021-00989-9>.

- Zelikova, T.J., Blumenthal, D.M., Williams, D.G., Souza, L., LeCain, D.R., Morgan, J., Pendall, E., 2014. Long-term exposure to elevated CO₂ enhances plant community stability by suppressing dominant plant species in a mixed-grass prairie. *Proc. Natl. Acad. Sci. U. S. A.* 111, 15456–15461. <https://doi.org/10.1073/pnas.1414659111>.
- Zhang, H.T., Chen, H.H., Grossart, H.P., Jin, L., Yan, X., Gao, X.F., Zhang, H.H., Xue, Y.Y., Yang, J., 2024. Persistent response of the bottom free-living bacteria to typhoon events in a subtropical reservoir. *Sci. Total Environ.* 908, 168069 <https://doi.org/10.1016/j.scitotenv.2023.168069>.
- Zoccarato, L., Grossart, H-P., 2019. In: Hurst, C. (Ed.). In: *The Structure and Function of Aquatic Microbial Communities*. *Advances in Environmental Microbiology*, 7. Springer, Cham, pp. 13–52. [10.1007/978-3-030-16775-2_2](https://doi.org/10.1007/978-3-030-16775-2_2).