

Characterization Free-living (FL) and Particle-attached (PA) bacterial communities of a canyon river reservoir on Yungui Plateau, China

Yang Yang (✉ gznuyy@126.com)

Guizhou Normal University School of Life Sciences <https://orcid.org/0000-0002-9881-3952>

Chen Chen

Guizhou Normal University School of Life Sciences

Xu Tao

Guizhou Normal University

Research Article

Keywords: River reservoir, bacterioplankton, community composition, bacterial diversity, gene functions, environmental drivers

Posted Date: May 2nd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1535823/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Bacteria play an important role in nutrient recycling and energy conversion in any aquatic ecosystem. Revealing the composition of free-living (FL) and particle-attached (PA) bacterial communities provide insights into their roles for biogeochemical processes and algal bloom dynamics. This study revealed the structure of FL and PA bacterial communities in a canyon reservoir in the Yungui-Plateau region, southern China. Firstly, we compared the difference using 3.0 μm and 5.0 μm filter for PA and FL bacterial communities (defined as PA3, FL3 and PA5, FL5), in order to choose the better sampling protocol. Secondly, we identified the differences between FL and PA in respect of their composition, diversity as well as function. Results showed that (1) dominant taxa did not change between FL3 and FL5, PA3 and PA5, but the relative abundance varied. Community composition and predicted gene functions showed significant differences between PA3 and PA5, while insignificant difference was noticed between FL3 and FL5; (2) FL was dominated by *Actinobacteria* and *Proteobacteria*, while PA was dominated by *Planctomycetes* and *Proteobacteria*; (3) there was insignificant difference on α -diversity between PA3 and PA5, but PA5 community exhibited greater dissimilarity (β -diversity) than PA3; (4) differences were detected between FL and PA community, with PA community exhibiting greater diversity and metabolic versatility than FL; (5) nitrogen metabolism related genes were abundant, implying an important role of both FL and PA in aquatic nitrogen cycling and hence in the microbial loop in this reservoir. This study provided evidence for using 5.0 μm filter to separate PA bacterial community, and also presented fundamental information on FL and PA bacterioplankton in Wujiangdu reservoir.

1. Introduction

Bacteria, are a major component of the microbial loop, and play an important role in biogeochemical processes. They mineralize dissolved organic matter, such as algal exudate and transfer carbon and energy to higher trophic levels, and play an essential role in the microbial food web (Azam et al., 1983). Furthermore, bacterioplankton can play different roles in the initiation, development and termination of a broad range of algal blooms (Paver et al., 2013; Buchan et al., 2014). Thereby, the bacterioplankton community can, to some extent, indicate trophic status and certain organic or heavy metal pollution, mirroring the state of water quality (Pinto et al., 2021). Therefore, it was proposed that bacteria act as sentinels of environmental change due to their sensitivity to changing environmental conditions including trophic status and anthropogenic pollution (Harnisz, 2013; Savio et al., 2015). Based on their life style, there are two bacterial fractions, i.e. free-living (FL), with a size range between 0.22 to 3.0 μm or 5 μm (Crespo et al., 2013; Grossart, 2010), and particle-attached (PA), which can be separated from FL by filtration on 3.0 μm / 5 μm filters. FL refers to bacteria floating freely in the water column, while the PA fraction comprises bacteria attached to organic particles and living organisms. Several studies revealed that there are significant phylogenetic differences between FL and PA fractions, whereby some bacteria can rapidly exchange between the size fractions (Tang et al., 2015; Bizic-Ionescu et al., 2014). The first step to study FL and PA assemblages is to collect the most representative PA samples. Generally, the prevalent method is filtration and centrifugation, earlier study has proposed that centrifugation was

proper in eutrophic and turbulent lakes, but it could enrich some taxa disproportionately due to various sinking velocity (Xie et al., 2020). Filtration with 3.0 μm (ImLiu et al., 2019; Wang et al., 2020) or 5.0 μm (Allgaier and Grossart, 2006; Parveen et al., 2011; Zhao et al., 2017; Hu et al., 2020) pore size filter is the most frequently used. Different pore sizes may affect the structure of community. Moreover, the methods applied for collection may be trophic-dependent because nutrients are associated with the composition and size of particles in the water column (Tang et al., 2017).

Research about bacterioplankton in river reservoirs on Yungui-Plateau are scarce. Zhang et al. (2020) investigated the relationship between phytoplankton and bacterioplankton in Dianchi Lake. Wu et al. (2019) revealed the vertical bacterial profile in Lake Lugu. Both studies were conducted in Yunnan Province. Wu River is the largest southern tributary of Yangtze River and also the largest river in Guizhou Province. Wujiangdu reservoir was the first major dam established in the middle and upper reaches of the Wu River. There are many streams supplying water to the impoundment, such as Xifeng River and Pianyan River. Due to the karstification characteristics in this region, Wu River water is abundant with carbonated weathering (Han and Liu, 2004). Yue et al. (2021) investigated the vertical distribution of bacterial community in Wujiangdu reservoir and emphasized the role of stratification on the vertical pattern of bacterial community structure. Cascade dams along Wu River could affect overall microbial community via altering hydrological features. However, their community structure and function, classified as FL and PA fraction, in such plateau reservoirs are still poorly understood. Information about the bacterial distribution, assembly, diversity and functioning in this reservoir could help us understand the mechanisms underlying microbial food web and phytoplankton succession in such plateau freshwater ecosystems. Therefore, it is necessary to unveil the bacterial community structure in reservoirs of Yungui Plateau.

We collect samples from 14 sites along Wujiangdu reservoir, a typical canyon river reservoir. Based on 16S rRNA marker gene, we firstly compared the results of PA bacterioplankton by using 3.0 μm and 5.0 μm filter, and simultaneously investigate both FL and PA bacterial community structure, diversity and gene function. We hypothesized that (1) different pore size filters would result in different PA bacterioplankton community; (2) FL and PA bacterioplankton differ in composition, diversity and functions in Wujiangdu reservoir.

2. Materials And Methods

2.1 Site description and sampling

Wujiangdu Reaservoir, built in 1979, is located at the lower basin of Wu River, and at the conjunction of Xifeng Town and Zunyi city, Guizhou Province. It has a watershed of 27790 km^2 , a mean depth of 154 m, and volume of $23 \times 10^8 \text{ m}^3$. The average water residence time is 53 days. This reservoir has a history of cage aquaculture activities since 1999.

Water samples were collected from 14 locations (HS, YL, YLr, KC, JK, JKr, DTD, TL, TLR, XT, XF, XFr, PY, and PYr) in Wujiangdu Reservoir on Aug 19th, 2021 (Fig. 1). These locations were chosen due to their position at the confluence of small rivers and the main river. Water Temperature, Dissolved Oxygen (DO), pH and Conductivity (Cond) were measured on site via a portable YSI probe (HANNA HI98194), Secchi depth (SD) was measured by a Secchi disk at each station. Based on the temperature profile, there was weak thermal stratification. Thus, we regard that the surface samples could represent the epilimnion. For chemical analyses, 1L of surface water (0.5 m) was taken using a water sampler. And for microbial analyses, an additional 2 liter was filled in a sterile PE bottle which was kept at in site temperature for transport to the lab.

2.2 Water chemistry analysis

Total phosphorus (TP), phosphate (PO_4^{3-}), total nitrogen (TN), nitrate (NO_3^-), nitrite (NO_2^-), silicate (SiO_2), total suspended solids (TTS), and Chlorophyll a (Chl a) were measured according to standard methods (A.P.H.A., 2012). Based on these measurements, trophic status index (TSI) was calculated according to the equations below (Carlson, 1991; Matthews et al., 2002; An & Park, 2003):

$$\text{TSI}_{\text{SD}} = 60 - 14.42 \ln(\text{SD, m})$$

$$\text{TSI}_{\text{TN}} = 54.45 + 14.43 \ln(\text{TN, mg/L})$$

$$\text{TSI}_{\text{TP}} = 14.42 \ln(\text{TP, } \mu\text{g/L}) + 4.15$$

$$\text{TSI}_{\text{CHL}} = 9.81 \ln(\text{CHL, } \mu\text{g/L}) + 30.6$$

2.3 Bacterial community analysis

For bacterial DNA, two types of polycarbonate filter (3.0 μm and 5.0 μm) were applied to collect PA bacteria (PA3 and PA5). 400 mL water were filtrated on a 47 mm diameter filter firstly, and then filtered through a 0.22 μm filter to collect FL fraction (FL3 and FL 5). All filters were stored at -80 °C for further analyses. The ALFA-seq Advanced water Kit was used for DNA extraction according to the manufacturer's instruction. DNA concentration and purity were measured using the NanoDrop One. V4-V5 region of 16S rRNA gene were amplified used the primer 806R/515F. Based on NEBNext Ultra™ DNA library Prep kit for Illumina standard protocol, a library was established before sequencing on an Illumina Hiseq2500 platform and 250bp paired-end reads were generated. Quality filtering on the paired-end raw reads were performed according to the Trimmomatic (V0.33) quality-controlled process. Paired-end clean reads were merged using FLASH (V1.2.11). Then 16S rRNA gene sequences were analyzed by usearch software (V8.0.1517). sequences with $\geq 97\%$ similarity were assigned to the same OTU. For each representative sequence, the GreenGene database was used based on RDP classifier algorithm and the assign_taxonomy.py script in Qiime to annotate taxonomic information. Rarefaction curve and rank abundance curve were seen in Fig. S1.

Diversity index was calculated in R, including OTU reads number, Chao1 and Abundance-based coverage estimator (ACE), Simpson index, Shannon index, and Evenness. Chao1 and ACE are used to estimate richness. Shannon and Simpson index provide more inference about the community composition than simple species richness or evenness. Analysis of variance (ANOVA) tests were used to test for significant differences in diversity between FL and PA fractions. Community dissimilarity was calculated using Bray-Curtis dissimilarity. Non-parametric multidimensional scaling (NMDS) was performed to display the distance of bacterial communities. Permutational multivariate analysis of variance (PerMANOVA) was used to test whether there was significant difference on community composition between groups (Anderson, 2008). PERMDISP, a common test completed in conjunction with PerMANOVA, a multivariate analog of Levene's test for homogeneity of multivariate variances, was performed to test the null hypothesis of 'no difference in dispersion between groups' (Anderson and Walsh, 2013). Distance decay pattern was tested using Mantel tests, which calculates geographic distance based on sampling coordinates, and Euclidean distance using environmental parameters. Redundancy analysis (RDA) was used to summarize the relationship between bacterial community and measured environmental factors. PerMANOVA, PERMDISP, NMDS and RDA were performed using the 'vegan' package in R (Oksanen et al., 2020).

OTU data was used for Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSts2) and for gene function prediction based on clusters of orthologous groups (COG) database and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Douglas et al, 2020).

3. Results

3.1 Water physio-chemical parameters

Water transparency, indicated by SD, ranged from 2.6 m to 5.3 m. Nutrient concentrations, including total nitrogen (TN), total phosphorus (TP), phosphate (PO_4^{3-}), nitrate (NO_3^-) and total suspended solid (TSS) and Chlorophyll a (Chl a), exhibited spatial heterogeneity in this reservoir with various degrees. TN ranged from 2.31 mg/L to 3.25 mg/L, and TP from 0.01 mg/L to 0.02 mg/L. Nitrate (NO_3^-) was the major component of dissolved nitrogen, ranging from 1.72 mg/L to 2.55 mg/L, followed by NO_2^- (0.008 mg/L-0.035 mg/L) and NH_4^+ (0.008 mg/L-0.110 mg/L) (nitrogen and phosphorus concentration seen in Fig. S2). According to Trophic Status Index (TSI), Wujiangdu reservoir was mesotrophic to eutrophic (Table 1).

Table 1
Summary of Trophic Status Index
(TSI) in Wujiagndu Reservoir

TSI	Mean	Min	Max
TSI _{SD}	46.99	40.37	51.52
TSI _{TN}	68.68	66.55	71.47
TSI _{TP}	45.05	43.31	47.19
TSI _{CHL}	50.54	43.09	56.62

3.3 FL and PA bacterioplankton community in surface water

In the surface waters of Wujiangdu reservoir, the detected bacterial OTUs belonged to 37 phyla and 73 class. Within FL or PA, the dominant taxa were the same, but with varying relative abundance. Dominant phyla in FL include *Actinobacteria* and *Proteobacteria*, whereas dominant phyla in PA were *Planctomycetes* and *Proteobacteria*. On class level, *Actinobacteria* and *Acidimicrobia* were dominant taxa in FL. *Planctomycetes* predominant in PA, followed by *α-Proteobacteria*. On order level, *Frankiales* and *Microtrichales* (*Actinobacteria*) were dominant in FL, whereas *Pirellulales* (*Planctomycetes*) predominated in PA. On family level, the dominant group in FL fraction were *Sporichthyaceae* and *Illumatobacteraceae* (*Actinobacteria*) and *Burkholderiaceae* (*Proteobacteria*). Whereas *Pirellulaceae* (*Planctomycetes*) dominated the PA bacterial communities (Fig. 2). ANOVA tests showed that there were insignificant differences on the relative abundance of dominant taxa between FL3 and FL5 fraction at phylum, class, order and family level ($p > 0.05$). While significant differences were noticed between PA3 and PA5 fraction on relative abundance ($p < 0.05$). Beside, significant different was detected between PA 3 and PA5 ($p < 0.05$), and in-significant difference was noticed between FL 3 and FL5 by PerMANOVA test ($p > 0.05$).

Community structure differed between FL and PA, with taxa showing various relative abundance. For community structure, PerMANOVA suggested that there was significant difference between FL and PA bacterial community (both 3.0 and 5.0) ($p = 0.001$). SIMPER analysis showed that significant different phyla between FL and PA were *Actinobacteria* and *Planctomycetes*.

3.3 Bacterial community diversity

Reads number, Chao1, ACE, Shannon index and Simpson index, and Evenness index were calculated. It showed that all richness (reads number, Chao1 and ACE) and diversity indexes (Simpson index, Shannon index and evenness) were greater in PA5 than PA3. But no such pattern was observed for FL3 and FL5 (Fig. 3). ANOVA tests suggested that there was insignificant difference on diversity indexes between FL3 and FL5, whereas significant difference was detected on Shannon index between PA3 and PA5 ($p = 0.04$). Besides, richness showed higher values in PA than in FL. Whereas diversity indexes exhibited various

patterns in PA and FL, with FL (both FL3 and FL5) greater than PA3, but similar with PA5. Significant differences on diversity indices were detected between FL3 and PA3 diversity, and FL5 and PA5.

NMDS was performed to identify the β -diversity of both FL and PA bacterioplankton community using Bray-Curtis dissimilarity index. FL5 had greater dissimilarity than FL3, and PA5 was more dissimilar than PA3 (Fig. 4a). PERMDISP test suggested that there was significant difference on β -diversity between PA3 and PA5 ($p = 0.02$) (Fig. 4b).

3.4 Functional analysis of bacterial communities

Dominant gene functions clustered on metabolism based on KEGG pathways for both FL and PA bacterial community, primarily including carbohydrate, amino acid, cofactor and vitamins metabolism. An overview on the relevant diseases is given in Fig. 5a and b. In addition, sub-dominant functions were associated with genetic information processing, such as replication and repair, folding, sorting and degradation, translation, transcription, transport and catabolism. Other less-dominant categories of KEGG pathways were related to cellular processes and environmental information processes. It was worthy noticing that several OTUs associated with human diseases were detected in this study, such as neurodegenerative disease, parasitic and bacterial infectious diseases, immune systems and cancer. Significant differences were noticed on gene function between PA3 and PA5 ($p < 0.01$).

Wujiangdu reservoir exhibited a relatively high level of nitrogen, as indicated by TN, nitrate, nitrite and ammonium concentration. Genes associated with nitrogen metabolism, clustered as KO, were observed based on KEGG pathway analysis. There were 47 nitrogen-relevant KO noticed in this reservoir. Among them, the predominant gene is ko01915 (glnA, GLUL), coding for glutamine synthetase (GS).

3.5 Bacterioplankton community with environmental factors

Mantel tests suggested that environmental matrix had a strong relationship with the FL bacterial communities ($p < 0.01$), while weak relationship with PA bacterial communities ($p = 0.02$). in other words, bacterial communities became more dissimilar along with the environmental conditions. Moreover, both FL and PA bacterioplankton communities exhibited significant relationship with geographic separation ($p = 0.001$) (Fig. 6). Therefore, distance decay pattern occurs in this reservoir for FL and PA bacteria (Table 2).

Table 2
 Results of Mantel tests (Env: environmental factors; Geo: Geographical factor, R: correlation coefficient; p: probability value)

	FL3	FL5	PA3	PA5
Env-R	0.65	0.45	0.44	0.24
Env-p	0.001	0.001	0.001	0.02
Geo-R	0.61	0.56	0.61	0.60
Geo-p	0.001	0.002	0.001	0.001

RDA showed that FL and PA bacterioplankton, dominated by varying relative abundance of different phyla, were affected by different environmental factors (Fig. 7a). FL fraction was present in the right panel on the biplot, along the NH_4^+ , Chl a, TP, TN and NO_3^- gradients, whereas PA fraction, distributed on the left side, was associated with pH, TSS, and SD. Correlation analysis results suggested that dominant phyla (top 10) exhibiting different correlation relationships with environmental factors (Fig. 7b and c). In addition, *Cyanobacteria* showed positively correlation with *Planctomycetes* in FL, whereas negatively correlation in PA. *Proteobacteria* negatively correlated with *Cyanobacteria* in both FL and PA.

4. Discussions

4.1 Comparison of PA3 and PA5 bacterioplankton composition, diversity and gene functions

Actinobacteria, small and slow-growing (Allgaier et al., 2007), are ubiquitous in lakes. they are ultramicrobacteria, able to decompose high molecular weight compounds, such as cellulose, lignin, are generally free-living, open-water defense specialists (Newton et al., 2011; Koblizek, 2015). This group represents a unique pelagic freshwater lineage, and they are uniform in the metabolism and ecological requirements.

Proteobacteria appeared to be comprised more from PA (27.15%) than from FL (23.4%) bacterioplankton, with *alpha-Proteobacteria* and *gamma-Proteobacteria* as the most dominant subphylum of *Proteobacteria* in both FL and PA. *Proteobacteria* with these two patterns implying their adaptation strategy to the environment (Dang and Lovell, 2016). *Alpha-Proteobacteria* are phototrophic bacteria with preference for low-nutrient conditions and resistant to predation (Imhoff, 2006, Newton et al., 2011). Whereas *gamma-Proteobacteria* prefer nutrient-rich environment, with the capacity to rapidly exploit the extra nutrient available.

Planctomycetes, predominant in PA community, normally present in low abundance in lakes and can be abundant in nutrient-enriched waters (Liu et al., 2014; Newton et al., 2011). They usually have large cell

sizes due to their cellular structures and budding cell division. This group prefer to be attached than free-living (Allgaier and Grossart, 2006). Earlier studies suggested observed its high abundance associated with diatom (Morris et al., 2006) and cyanobacterial blooms (Woodhouse et al., 2016; Guedes et al., 2018). Ruber et al. (2017) reported high *Planctomycetes* abundance in a lake dominated by *Synechococcus*, and Salmaso et al. (2017) observed that *Planctomyces* was the most abundant genus within *Planctomycetes* in a bacterioplankton with *Synechococcus* accounted for 30% of *Cyanobacteria*. Consistent with other studies, we observed that *Synechococcus* was relatively high in this reservoir. This observation might imply the co-occurrence pattern of *Plantomycetes* and *Synechococcus* in this reservoir. But their functional roles in association with phytoplankton, particularly cyanobacterial bloom remain unclear and need to be further researched. One possible mechanism could be their degradation capacity of sulfated polysaccharides produced by cyanobacteria (Cai et al. 2013).

Richness and diversity were greater in PA5 than PA3. Besides, greater dispersion of PA5 was observed than PA3, which can be interpreted as higher dissimilarity of PA5 community structure. For comparison between FL and PA, the richness was higher in PA than FL, but diversity was lower in PA3 than FL, and PA5 was similar with FL. This indicated that more abundance but less diverse bacteria were collected on 3.0 μm filter. The lower diversity of PA3 suggested that filtration through 5.0 μm filter was better than 3.0 μm filter for collecting PA bacteria in respect of diversity. Therefore, filtration using 5.0 μm was better at separating PA bacterioplankton in this reservoir when diversity was the priority for research.

Significant differences were also observed for predicted gene functions between PA3 and PA5. In this reservoir, the observed abundant metabolism function suggested that bacterioplankton, both FL and PA, involved in the decomposition and recycling of nutrients and other compounds. Furthermore, the predominant nitrogen-relevant gene was coding for glutamine synthetase. This enzyme catalyzes the ATP-dependent conversion of glutamate and ammonia to glutamine. It uses the bioavailable nitrogen in the form of ammonium into cellular metabolism. The abundant nitrogen metabolism gene, as well as relatively high level of nitrogen observed in Wujiangdu reservoir implies the productivity of bacterioplankton in the recycling of nitrogen.

4.2 Comparison of filter with different pore size for PA collection

Different porosity may be one of the reasons for low diversity in PA samples on 3.0 μm filters than 5.0 μm filters in Wujiangdu reservoir. This might be associated with the filter clogging and cell retention. During filtration process, clogging could lead to a progressive increase in the size range and diversity of the retained particles (Padilla et al., 2015). FL bacteria and bacteria attached to smaller particles could be detained on filters due to clogging as the increase of filtration time. Therefore, when the filtration time using 3.0 μm filters increases, smaller cells less than 3.0 μm and potentially free-living cells could also be retained. This explains that the relative abundances of some taxa were greater in PA3 than PA5. Besides, it would accumulate more dominant taxa, even though rare species could be collected using 3.0 μm . Thus, the diversity of PA3 was lower than PA5.

Meanwhile, PA bacteria could also enter into FL due to detachment from particles (Padilla et al., 2015). Clogging and detachment would increase the similarity between FL and PA fraction. Additionally, the lifestyle of FL and PA was not absolutely defined, many taxa possess an alternative lifestyle (Grossart, 2010). In our results, the higher diversity in PA5 than PA3 suggested that using 5.0 μm filter was better choice for PA separation.

Particles in deep lakes are generally rich in organic detrital matter which is densely colonized by bacteria, while particles in turbulent estuaries tend to be smaller with significant content of inorganic matter (Simon et al., 2002; Zhang et al., 2016). Wujiangdu reservoir is a typical riverine reservoir, with the water residence time about 0.14 year (Wang et al., 2019), and the particulate organic matter was mainly from phytoplankton-derived particulate organic carbon (Shi et al., 2018). The choice of pore size to separate PA from FL fractions is dependent on the size of the particles present in the aquatic ecosystem. Therefore, heterotrophic bacteria were mainly colonized on large organic particles in Wujiangdu reservoir. Our results suggested that using 5.0 μm filters is the better choice to separate PA bacteria. (1) There are significant differences between PA3 and PA5 on community composition; (2) diversity of PA5 is greater than PA3; (3) bacterial community of PA5 was significantly different from FL community, indicating a distinct discrimination of FL and PA.

4.3 The relationship between environmental factors and bacterioplankton in Wujiangdu reservoir

Distance decay pattern refers that communities become increasingly different as the distance increases (Nekola and White, 1999). The underlying mechanism for this phenomenon is that environmental conditions are more similar with shorter distance. Therefore, species adapted to such habitats would also appear and colonize in similar habitats. Another reason is dispersal limitation, meaning that individuals tend to arrive at nearby sites. For microbes, however, it is generally regarded as no dispersal limitation, but the environment selects (Becking, 1934). In this reservoir, distance decay pattern was observed for both FL and PA, implying that both spatial and environmental factors affect the bacterioplankton community. For PA5 community, the influence of environment was relatively weak, indicating other environmental factors might play more important role. For PA bacterioplankton, the particle was crucial for determining the specific species attached. Thus, the composition of PA is dependent on the composition of particle in this reservoir.

5. Conclusions

This study proposed that 5.0 μm pore size filter was better to collect PA bacterioplankton because it could retain more diverse PA community. Moreover, it investigated both FL and PA bacterioplankton community structure in this typical Plateau reservoir in Yungui Plateau. FL and PA bacterioplankton exhibited different composition, diversity, and gene functions. The mechanisms underlying the community assemble and the observed human disease relevant gene need to be further researched in the next step.

Declarations

Funding

This study was funded by National Scientific Funding of China (No. 32060270), the Science and Technology Foundation of Guizhou Province (No. Qian ke he ji chu [2020]1Y072), and the Doctor Scientific Research Foundation of Guizhou Normal University for 2017 (No. 11904/0519087).

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Chen Chen and Xu Tao. The first draft of the manuscript was written by Yang Yang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical approval - Not applicable

Consent to participate – Not applicable

Consent to publish – Not applicable

Availability of data and materials

Readers interested in this study may contact the corresponding author for dataset access.

Acknowledgements

We thank Professor Hans-Peter Grossart from Leibniz Institute for Freshwater Ecology and Inland Fisheries for commenting on the manuscript.

This study was funded by National Scientific Funding of China (No. 32060270), the Science and Technology Foundation of Guizhou Province (No. Qian ke he ji chu [2020]1Y072), and the Doctor Scientific Research Foundation of Guizhou Normal University for 2017 (No. 11904/0519087).

References

1. A.P.H.A. Standard Methods for the examination of water and wastewater, 22nd. American Water Works Association and Water Pollution Control Federation, Washington, DC
2. Allgaier M, Grossart HP (2006) Seasonal dynamics and phylogenetic diversity of free-living and particle-associated bacterial communities in four lakes in northeastern Germany. *Aquat Microb Ecol* 45:115–128

3. Allgaier M, Bruckner S, Jaspers E, Grossart HP (2007) Intra- and inter-lake variability of free-living and particle-associated Actinobacteria communities. *Environ Microbiol* 9:2728–2741
4. An K, Park SS (2003) Influence of seasonal monsoon on the trophic state deviation in an Asian reservoir. *Water Air Soil Pollution* 145:267–287
5. Anderson MJ (2008) A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 26:32–46
6. Anderson MJ, Walsh DCI (2013) PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecol Monogr* 83:557–574
7. Azam F, Fenchel T, Field JG, Gray JS, Meyerreil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. *Mar Ecol Prog Ser* 10:257–263
8. Bass Becking LGM (1934) Geobiologie of inleiding tot de milieukunde. W.P. Van Stockum & Zoon. The Hague, the Netherlands (in Dutch)
9. Bizic-Ionescu M, Zedae M, Ionescu D, Orlic S, Fuchs BM, Grossart HP, Amann R (2015) Comparison of bacterial communities on limnic versus coastal marine particles reveals profound differences in colonization. *Environ Microbiol* 17:3500–3514
10. Buchan A, LeCleir GR, Gulvik CA, Gonzalez JM (2014) Master recyclers: features and functions of bacteria associated with phytoplankton blooms. *Nat Rev Microbiol* 12:686–698
11. Cai H, Yan Z, Wang A (2013) Analysis of the attached microbial community on mucilaginous cyanobacterial aggregates in the eutrophic lake Taihu reveals the importance of Planctomycetes 66: 73–83
12. Carlson RE (1991) Expanding the trophic state concept to identify non-nutrient limited lakes and reservoirs. Proceeding of a National Conference on Enhancing the States' Lake Management Programs. Monitoring and Lake Impact Assessment, Chicago, pp, 59–71
13. Crespo BG, Pommier T, Fernandez-Gomez B, Pedros-Alio C (2013) Taxonomic composition of the particle-attached and free-living bacterial assemblages in the Northwest Mediterranean Sea analyzed by pyrosequencing of the 16S rRNA. *Microbiology*, 2: 541–552
14. Dang H, Lovell CR (2016) Microbial surface colonization and biofilm development in marine environments. *Microbiologu and Molecular Biology Review* 80:91–138
15. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Tayloe CM, Huttenhower C, Langille MG (2020) PICRUSt2 for prediction of metagenome functions. *Nat Biotechnol* 38:685–588
16. Grossart HP (2010) Ecological consequences of bacterioplankton lifestyles: changes in concepts are needed. *Environ Microbiol Rep* 2:706–714
17. Guedes IA, Rachid CTCC, Rangel LM, Silva LH, Bisch PM, Azevedo SMFO, Pacheco ABF (2018) Close link between harmful cyanobacterial dominance and associated bacterioplankton in a tropical eutrophic reservoir. *Front Microbiol* 9:424
18. Han GL, Liu CQ (2004) Water geochemistry controlled by carbonate dissolution: A study of the river waters draining karst-dominated terrain, Guizhou Province, China. *Chem Geol* 204:1–21

19. Harnisz M (2013) Total resistance of native bacteria as an indicator of changes in the water environment. *Environ Pollut* 174:85–92
20. Hu Y, Xie G, Jiang X, Shao K, Tang X, Gao G (2020) The relationship between the free-living and particle-attached bacterial communities in response to elevated eutrophication. *Front Microbiol* 11:423
21. Imhoff JF (2006) Chapter 3.1.1. The phototrophic Alpha-Proteobacteria. In: Dworkin M, Flakow S, Rosenberg B, Schleifer KH, Stackebrandt E, editors. *The prokaryotes*. Springer; New York: pp. 41–64. 2006
22. Koblizek M (2015) Ecology of aerobic anoxygenic phototrophs in aquatic environments. *FEMS Microbiol Rev* 39:854–870
23. Liu L, Yang J, Wilkinson DM (2015) The biogeography of abundant and rare bacterioplankton in the lakes and reservoirs of China. *ISME J* 9:2068–2077
24. Liu M, Liu LM, Chen HH, Yu Z, Yang JR, Xue YY, Huang BQ, Yang J (2019) Community dynamics of free-living and particle-attached bacteria following a reservoir *Microcystis* bloom. *Sci Total Environ* 660:501–511
25. Matthews R, Hilles M, Pelletier G (2002) Determining trophic state in Lake Whatcom, Washington (USA), a soft water lake exhibiting seasonal nitrogen limitation. *Hydrobiologia* 468:107–121
26. Morris RM, Longnecker K, Giovannoni SJ (2006) *Pirellula* and OM43 are among the dominant lineages identified in an Oregon coast diatom bloom. *Environ Microbiol* 8:1361–1370
27. Nekola JC, White PS (2004) The distance decay of similarity in biogeography and ecology. *J Biogeogr* 26:867–878
28. Newton RJ, Jones SE, Elier A, McMahon KD, Bertilsson S (2011) A guide to the natural history of freshwater lake bacteria. *Microbiol Mol Biology Rev* 75:14–49
29. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MH, Szoecs E, Wagner H 2020. *vegan: Community Ecology Package*. R package version 2.5-6
30. Padilla CC, Ganesh S, Gantt S, Huhman A, Parris DJ, Sarode N, Stewart FJ (2015) Standard filtration practices may significantly distort planktonic microbial diversity estimates. *Front Microbiol* 6:547
31. Parveen B, Reveilliez JP, Mary I, Ravet V, Bronner G, Mangot JF, Domaizon I, Debroas D (2011) Diversity and dynamics of free-living and particle-attached Betaproteobacteria and Actinobacteria in relation to phytoplankton and zooplankton communities. *FEMS Microbiol Ecol* 77:461–476
32. Paver SF, Hayek KR, Gano KA, Fagen JR, Brown CT, Davis-Richardson AG, Crabb DB, Rosario-Passapera R, Giongo A, Triplett EW, Kent AD (2013) Interactions between specific phytoplankton and bacteria affect lake bacterial community succession. *Environ Microbiol* 15:2489–2504
33. Pinto I, Calisto R, Serra CR, Lage OM, Antunes SC (2021) Bacterioplankton community as a biological element for reservoirs water quality assessment. *Water* 13:2836

34. Ruber J, Bauer FR, Millard AD, Raeder U, Geist J, Zwirglmaier K (2017) Synechococcus diversity along a trophic gradient in the Osterseen Lake District, Bavaria. *Microbiology* 162:2053–2063
35. Salmaso N, Albanese D, Capelli C, Boscaini A, Pindo M, Donati C (2017) Diversity and cyclical seasonal transitions in the bacterial community in a large and deep perialpine lake. *Microbiol Aquat Syst* 76:125–143
36. Savio D, Sinclair L, Ijaz UZ, Parajka J, Reischer GH, Stadler P, Blaschke AP, Blöschl G, Mach RL, Kirschner AKT, Farnleitner AH, Eiler A (2015) Bacterial diversity along a 2600 km river continuum. *Environ Microbiol* 17:4994–5007
37. Shi J, Wang BL, Wang FS, Peng X (2018) Sources and fluxes of particulate organic carbon in the Wujiang cascade reservoirs, southwest China. *Inland Waters* 8:141–147
38. Simon M, Grossart HP, Schweitzer B, Ploug H (2002) Microbial ecology of organic aggregates in aquatic ecosystems. *Aquat Microb Ecol* 28:175–211
39. Tan gXM, Li LL, Shao KQ, Wang BW, Cai XL, Zhang L, Chao JY, Gao G (2015) Pyrosequencing analysis of free-living and attached bacterial communities in Meiliang Bay, Lake Taihu, a large eutrophic shallow lake in China. *Can J Microbiol* 61:22–31
40. Tang XM, Chao JY, Gong Y, Wang YP, Wilhelm SW, Gao G (2017) Spatiotemporal dynamics of bacterial community composition in large shallow eutrophic Lake Taihu: high overlap between free-living and particle-attached assemblages. *Limnol Oceanogr* 62:1366–1382
41. Wang BL, Zhang HT, Liang X, Li X, Wang FS (2019) Cumulative effects of cascade dams on river water cycle: Evidence from hydrogen and oxygen isotopes. *J Hydrol* 568:604–610
42. Wang Y, Pan J, Yang J, Zhou Z, Pan Y, Li M (2020) Patterns and processes of free-living and particle-associated bacterioplankton and archaea-plankton communities in a subtropical river-bay system in South China. *Limnol Oceanogr* 65:S161–S179
43. Woodhouse JN, Kinsela AS, Collins RN, Bowling LC, Honeyman GL, Holliday JK, Neilan BA (2016) Microbial communities reflect temporal changes in cyanobacterial composition in a shallow ephemeral freshwater lake. *ISME* 10:1337–1351
44. Wu K, Zhao W, Wang Q, Yang X, Zhu L, Shen J, Cheng X, Wang J (2019) The relative abundance of benthic bacterial phyla along a water-depth gradient in a Plateau Lake: physical, chemical, and biotic drivers. *Front Microbiol* 10:1521
45. Xie GJ, Tang XM, Gong Y, Shao KQ, Gao G (2020) How do planktonic particle collection methods affect bacterial diversity estimates and community composition in oligo-, meso- and eutrophic lakes? *Front Microbiol* 11:593589
46. Yue YH, Cai L, Tang Y, Zhang YY, Yang M, Wang FS (2021) Vertical distribution of bacterial community in water columns of reservoirs with different trophic conditions during thermal stratification. *Front Environ Sci* 9:632089
47. Zhang Y, Xiao W, Jiao NZ (2016) Linking biochemical properties of particle-attached and free-living bacterial community structure along the particle density gradient from freshwater to open ocean. *J Geophys Res Biogeoscience* 121:2261–2274

48. Zhang Y, Zuo J, Salimova A, Li AJ, Li L, Li D (2020) Phytoplankton distribution characteristics and its relationship with bacterioplankton in Dianchi Lake. Environ Sci Pollution Res 27:40592–40603
49. Zhao DY, Xu HM, Zeng J, Cao XY, Huang R, Shen F, Yu ZB (2017) Community composition and assembly processes of the free-living and particle-attached bacteria in Taihu Lake. FEMS Microbiol Ecol 93:fix062

Figures

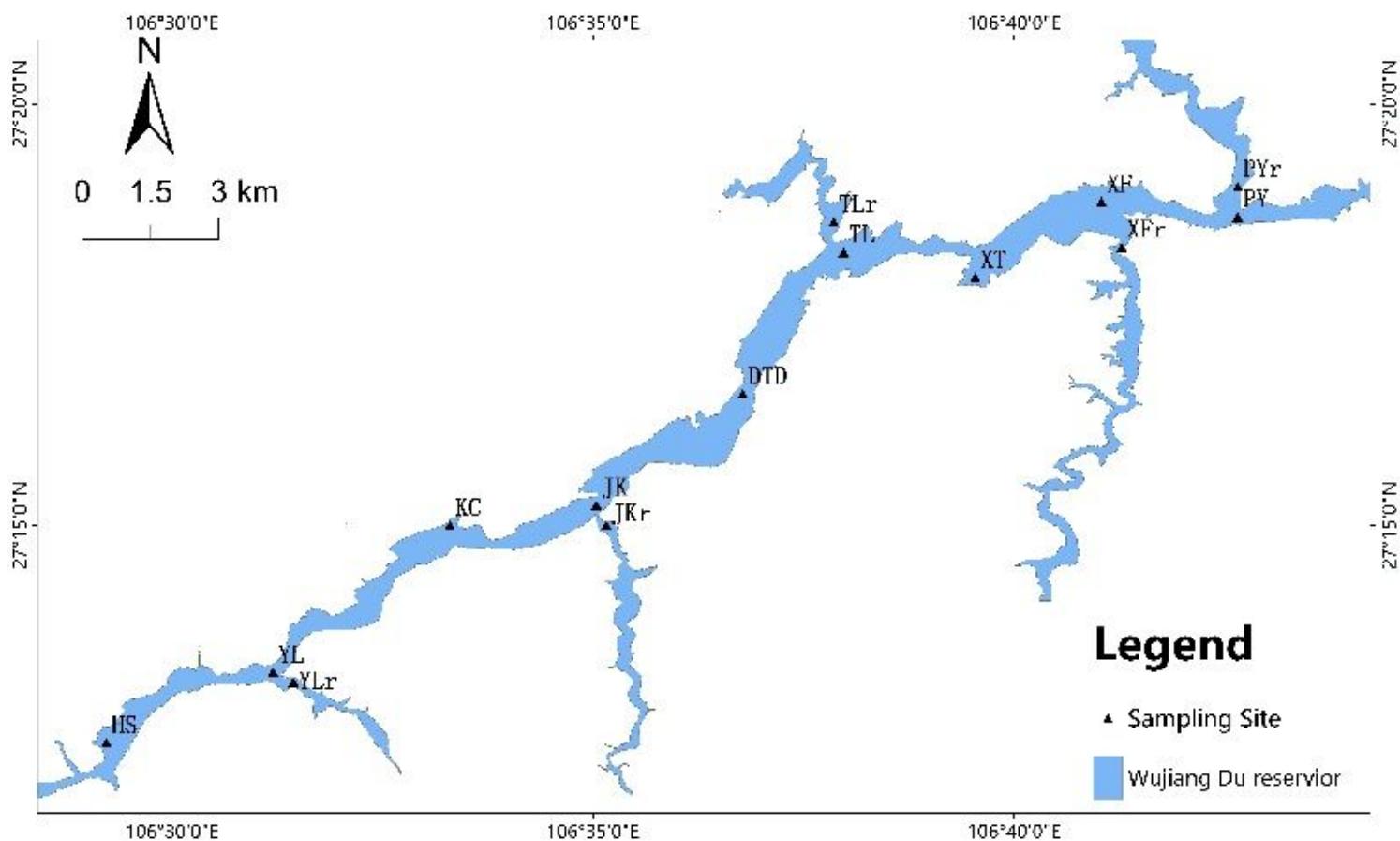


Figure 1

Sampling locations in Wujiangdu Reservoir.

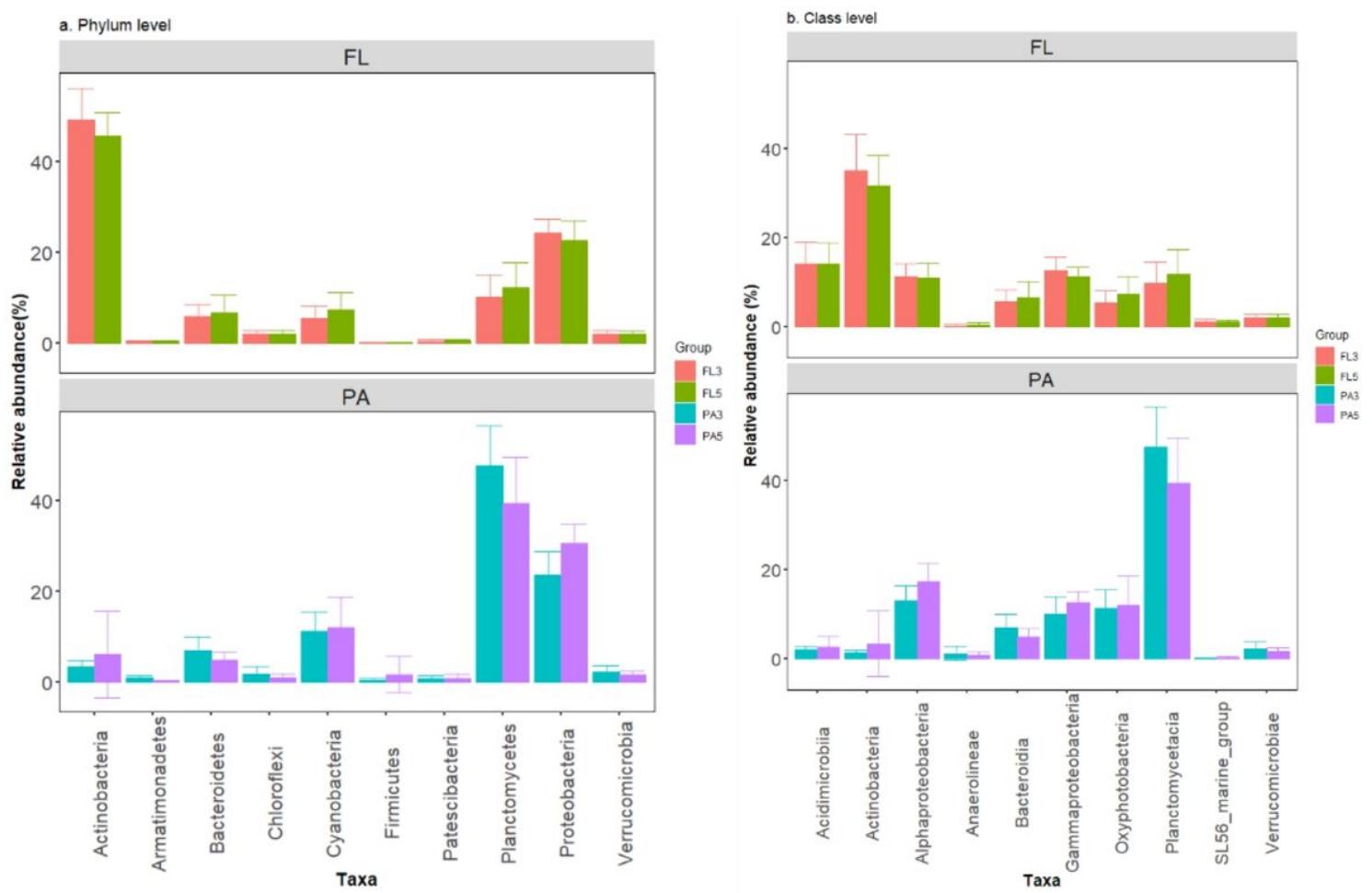


Figure 2

Relative abundance of top 10 taxa in FL and PA at phylum (a) and class level (b) in Wujiangdu Reservoir.

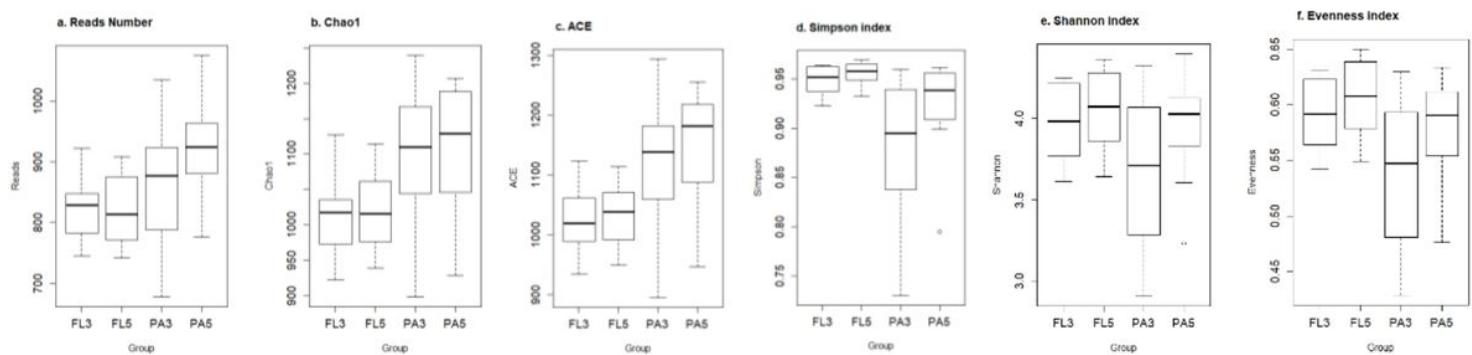


Figure 3

Diversity indexes (a. Reads; b. Chao1; c. ACE; d. Simpson index; e. Shannon index; and f. evenness index) of Free-living (FL3 and FL5) and Particle-attached (PA3 and PA5) bacterial community.

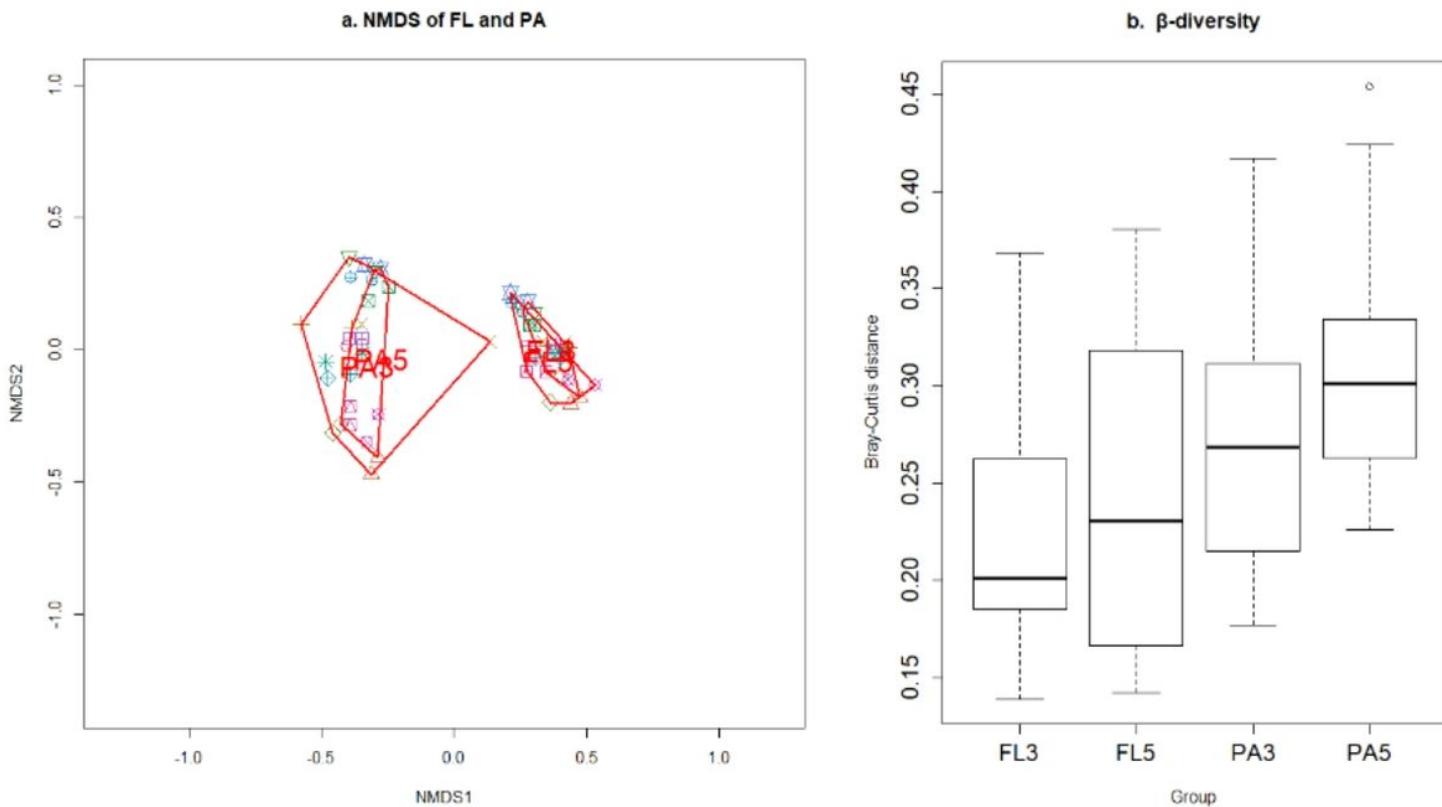


Figure 4

NMDS plot (a) and β -diversity (b) of FL and PA bacterioplankton community in Wujiangdu Reservoir.

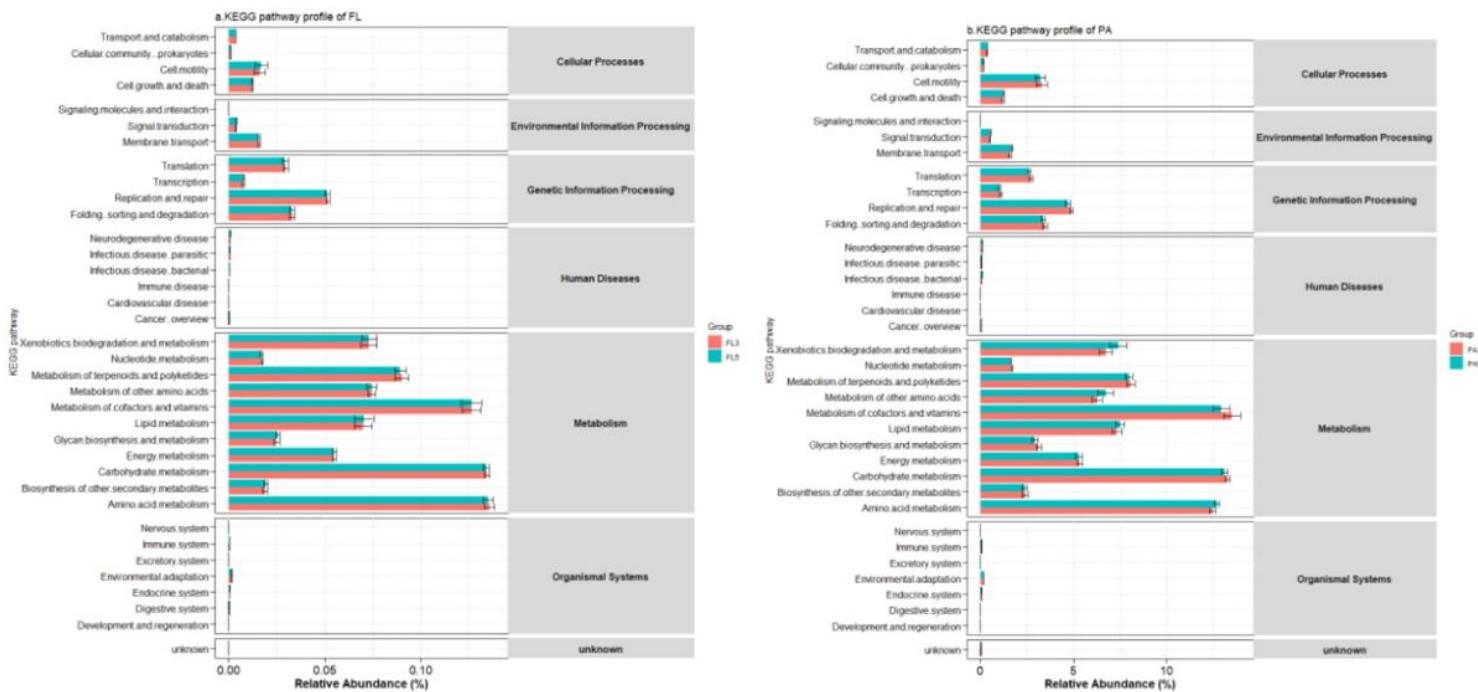


Figure 5

Functional profiles of FL (a) and PA (b) bacterioplankton communities in Wujiangdu Reservoir.

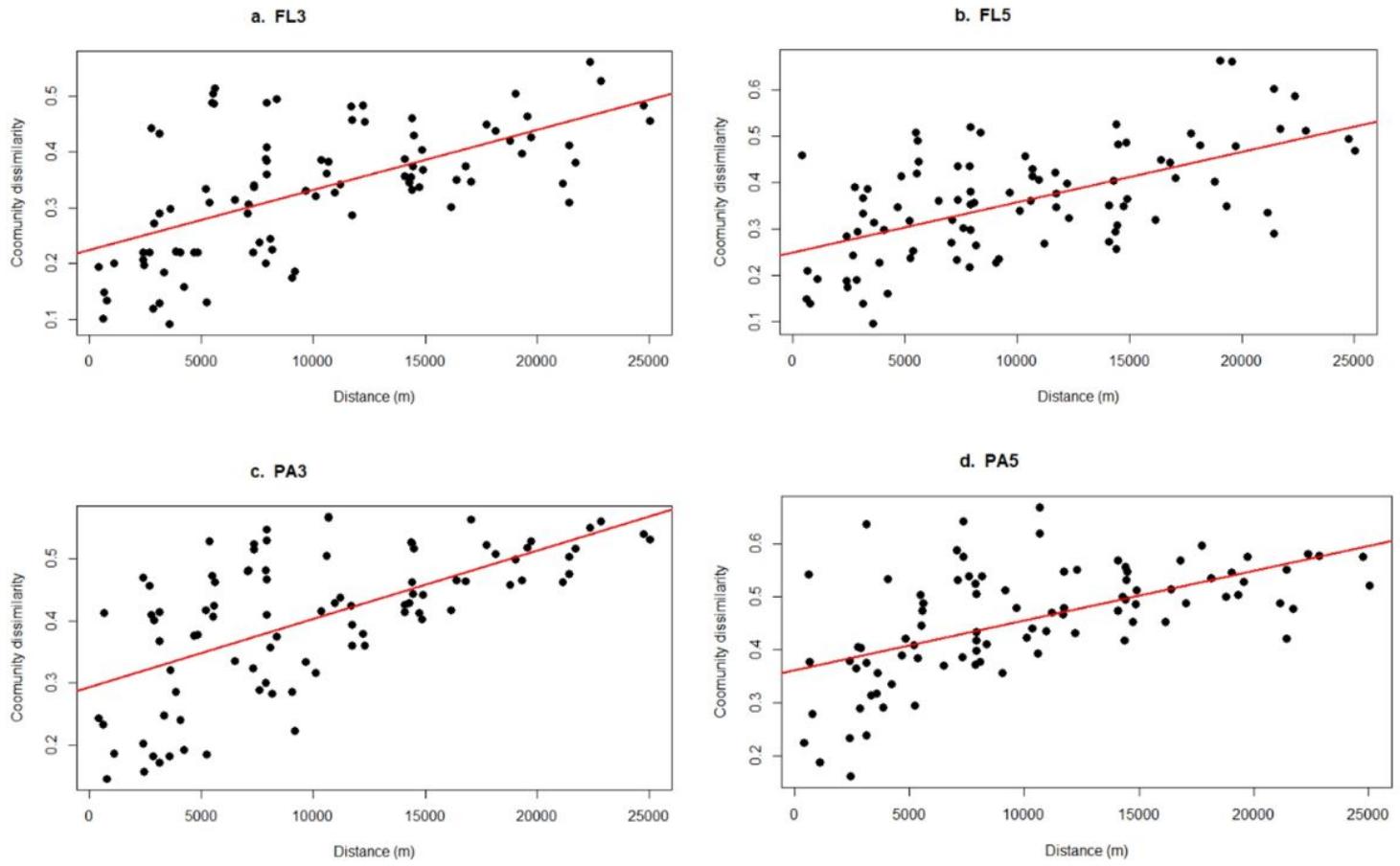


Figure 6

Relationship between geographic distance and Bray-Curtis Dissimilarity of FL3 (a), FL5 (b), PA3 (c) and PA5 (d) bacterial communities in Wujiangdu Reservoir.

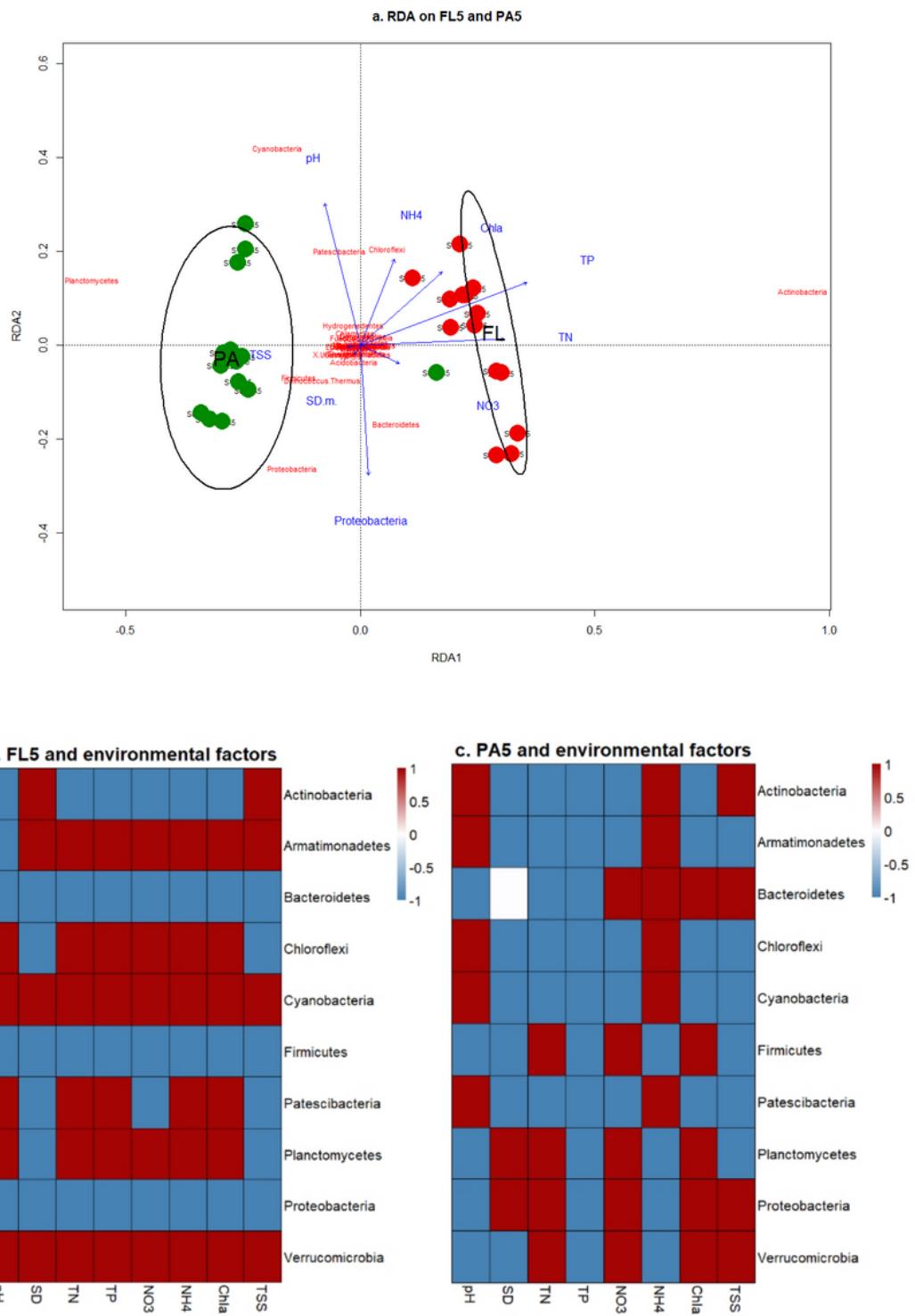


Figure 7

RDA on FL5 and PA5 bacterial community (a), and correlations between environmental factors and dominant phyla of FL5 (b) and PA5 (c).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterials.docx](#)