MICROBIOLOGY OF AQUATIC SYSTEMS

Seasonal Variability of Conditionally Rare Taxa in the Water Column Bacterioplankton Community of Subtropical Reservoirs in China

Pascaline Nyirabuhoro[1,2] · Min Liu[1,2] · Peng Xiao[1] · Lemian Liu[1,3] · Zheng Yu[1] · Lina Wang[1,2] · Jun Yang[1]

Abstract

Conditionally rare bacteria are ubiquitous and perhaps the most diverse of microbial lifeforms, but their temporal dynamics remain largely unknown. High-throughput and deep sequencing of the 16S rRNA gene has allowed us to identify and compare the conditionally rare taxa with other bacterioplankton subcommunities. In this study, we examined the effect of season, water depth, and ecological processes on the fluctuations of bacterial subcommunities (including abundant, conditionally rare, moderate, and rare taxa) from three subtropical reservoirs in China. We discovered that the conditionally rare taxa (CRT) made up 49.7 to 71.8% of the bacterioplankton community richness, and they accounted for 70.6 to 84.4% of the temporal changes in the community composition. Beta-diversity analysis revealed strong seasonal succession patterns among all bacterioplankton subcommunities, suggesting abundant, conditionally rare, moderate, and rare taxa subcommunities have comparable environmental sensitivity. The dominant phyla of CRT were Proteobacteria, Actinobacteria, and Bacteroidetes, whose variations were strongly correlated with environmental variables. Both deterministic and stochastic processes showed strong effect on bacterioplankton community assembly, with deterministic patterns more pronounced for CRT subcommunity. The difference in bacterial community composition was strongly linked with seasonal change rather than water depth. The seasonal patterns of CRT expand our understanding of underlying mechanisms for bacterial community structure and composition. This implies their importance in the function and stability of freshwater ecosystem after environmental disturbance.

Keywords Community ecology · Bacterioplankton · Subtropical reservoir · Conditionally rare species · Rare species

Introduction

Bacterial communities typically contain a high number of rare species, which were difficult to detect by culture-based method until the discovery of molecular methods along with a

✉ Jun Yang
jyang@iue.ac.cn

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

2 University of Chinese Academy of Sciences, Beijing 100049, China

3 Technical Innovation Service Platform for High Value and High Quality Utilization of Marine, Fuzhou University, Fuzhou 350116, China

small portion of abundant taxa, which are easier to identify [1]. They play crucial roles in the biogeochemical cycle of the aquatic ecosystem [2, 3]. Rare taxa sustain a high proportion of microbial richness or diversity at low relative abundance, while abundant taxa consist of a few taxa at high relative abundance [4–6]. The rare microbial community can stay in long-term dormancy due to environmental disturbance; this is likely to increase the rate of immigration or reduce the rate of extinction of some taxa [7, 8]. Thus, rare taxa are potential members of a "seed bank," which can maintain the stability of community due to their high diversities and rapid response to the environmental changes [9]. Many taxa of microorganisms can fluctuate between being abundant and rare [10, 11], and the majority of them are active while others are dormant or inactive [6, 9]. The active members of rare taxa are called conditionally rare taxa (CRT) because they can become abundant across space and time. However, the dynamics and mechanisms of the distribution of these CRT remain largely unknown in freshwater ecosystems [12–15].

Sogin et al. [16] first recognized and described the rare biosphere by using the deep sequencing of microbes from the deep sea. Indeed, the idea that many aquatic microbes would be too rare to find with classical (non-molecular) methods was suggested for lakes decades earlier [17]. Fuhrman [2] further showed that few rare taxa might be found in culture-based cloning library; however, he suggested that high-throughput sequencing (HTS) is a more reliable method for exploring the rare microbial community. High-throughput sequencing has not only narrowed the efforts spent on the culture-based methods, but also improved surveying techniques in freshwater molecular ecological studies [18–20]. These modern approaches that allow the exploration of rare microbial communities are more compelling because they can help to analyze the variability of bacterial community diversity and its dynamic composition on an unprecedented scale [1, 14, 15, 20]. Therefore, it is now possible to trace the link of rare microbial communities associated with the seasonal variation and the potential biogeochemical process using datasets that would have been impossibly large only two decades ago.

To investigate mechanisms regulating the microbial community structure across space and time, two main ecological processes (deterministic and stochastic) were found to be shaping the coexistence of microbial taxa [6, 21]. The concept of deterministic process implies that niche-based processes (e.g., biotic interactions and environmental filtering) generate the patterns of species diversity and composition in community ecology. In contrast, ecological process (including migration, drift, and extinction) producing the community diversity patterns identical to those created by random chance is called stochastic or neutral process [6, 22]. Several studies have revealed the importance of physicochemical forces in shaping microbial community in the aquatic ecosystems [23–25]. According to Galand et al. [26], the assembly of rare and abundant microbial species is largely controlled by both stochastic and deterministic processes. Both environmental filtering and neutral/stochastic processes drive the microbial communities simultaneously, but to different degrees [27, 28]. It has been suggested that sampling intensity should also be taken into consideration when exploring dynamics of microbial taxa. The key reason underlying this argument is that different members of microorganisms do not contribute equally to the community variation due to different assembly processes and mechanisms [23, 26]. Thus, both deterministic and stochastic processes need to be considered when investigating the microbial community assembly.

Although many studies have been conducted to understand rare microorganisms [18, 20, 29], little is known about the importance of the conditionally rare biosphere in ecology [11, 12]. In the subtropical region, most of the studies on freshwater reservoirs focused on the phytoplankton and trophic state [30–32], with less attention to bacterioplankton communities in those ecosystems. In the context of

bacterioplankton in freshwater, the studies were conducted on bacterioplankton community structure and composition [20, 33, 34], but seasonal succession patterns of conditionally rare bacterioplankton in those ecosystems have not yet been addressed [25]. Hence, an important question is whether the abundant taxa and the conditionally rare taxa exhibit similar or different responses to seasonal change along the water depths.

This study concentrates on the seasonal dynamics of conditionally rare taxa in bacterioplankton community of subtropical reservoirs. In this study, we hypothesized that (i) different taxa categories of bacterioplankton in the reservoirs respond distinctively to seasonal changes and that (ii) CRT explain large percentage of dissimilarity in whole bacterioplankton communities of the studied reservoirs. We aimed to answer the following questions: (1) Do bacterioplankton subcommunities exhibit similar or distinctive responses to seasonal variation in subtropical reservoirs? (2) What is the contribution of CRT in bacterial communities' variation within the studied reservoirs? (3) What are the key ecological processes that driving rare and abundant bacterioplankton subcommunity's assembly: deterministic or stochastic processes? To test our hypotheses, we studied three reservoirs over 1-year period (4 seasons) along the water depth gradient (5 layers) with a total of 60 samples and used high-throughput sequencing of the 16S rRNA gene as well as 15 measured environmental variables.

Material and Methods

Study Area and Sampling

This study was conducted in Dongzhen (DZ), Tingxi (TX), and Shidou (SD) reservoirs in Fujian Province, southeastern China (Fig. 1). All these reservoirs are subjected to subtropical humid monsoonal climate and have similar seasonal precipitation patterns [35]. These three deep reservoirs are characterized by warm monomictic waters and exhibit a thermal stratification throughout the year except for a short mixing period in winter. From 2011 to 2014, three subtropical reservoirs were seasonally sampled near the dam at five different depths from surface to bottom waters (Table S1). The sampling was conducted in January for winter, in April/May for spring, in July for summer, and in October for autumn, respectively. In each reservoir, 20 water samples were collected, and a total of 15 physicochemical parameters were determined as described in our previous study [25]. More details on study area, sampling, and physicochemical measurements have been provided in Liu et al. [36], Yang et al. [30], and Yu et al. [25], respectively, and the results are summarized in Table S2.

After the fieldwork, water samples were immediately transported into the laboratory. We filtered the water onto 0.22-μm-pore polycarbonate membrane (47 mm diameter;
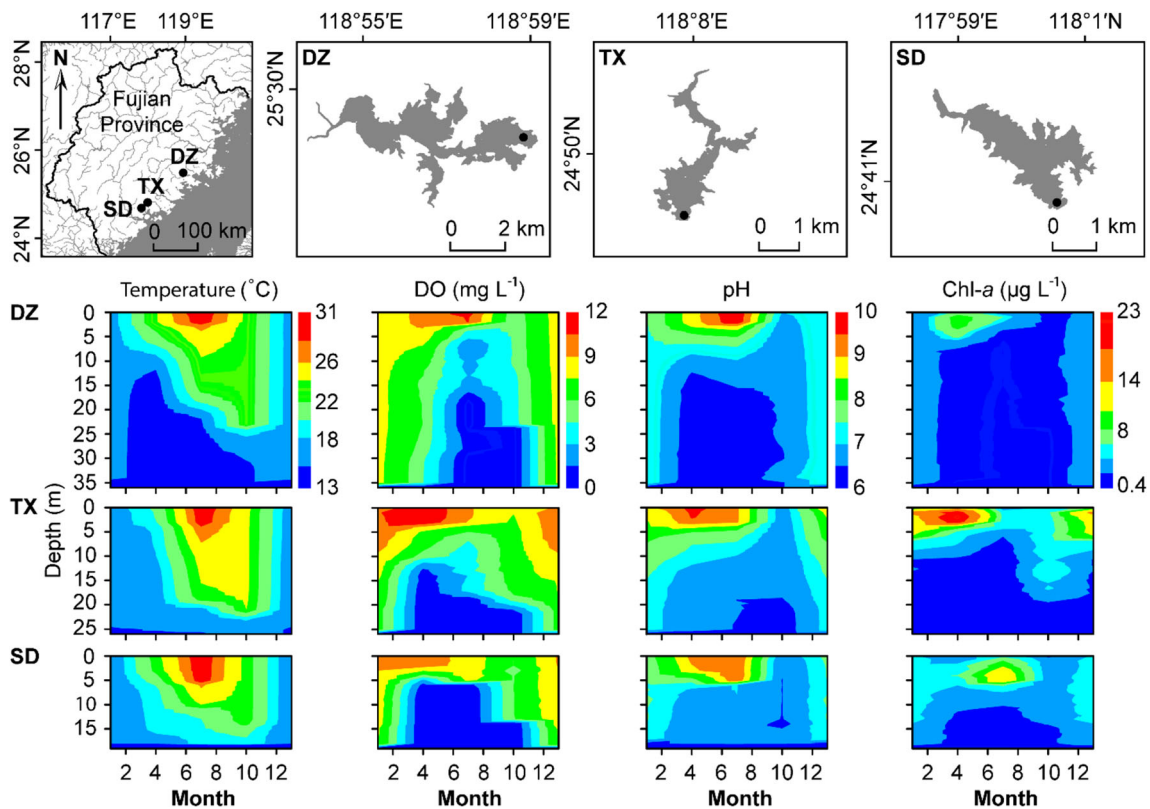
Fig. 1 The geographical location of the sampling sites in Dongzhen (DZ), Tingxi (TX), and Shidou (SD) reservoirs in the southeast China (top maps), and the contour plots showing the variation of temperature, dissolved oxygen (DO), pH, and chlorophyll *a* (Chl-*a*) across the water depth and sampling time

Millipore, Billerica, MA, USA). The filter membranes containing bacterioplankton were stored at − 80 °C until deoxyribonucleic acid (DNA) extraction.

DNA Extraction, PCR Amplification, and Sequencing

Microbial DNA was extracted following Liu et al. [20] and Yu et al. [25]. Total DNA was extracted using a FastDNA spin kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. We summarized the characteristics of these high-throughput sequencing-based community data (Table S3). For Dongzhen samples, we used PCR primers 357F and 926R and targeted the V3–V5 regions of the bacterial 16S rRNA gene. The PCR reaction conditions were 94 °C for 4 min, followed by 25 cycles of 30 s at 94 °C, 50 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. The V4 and V3–V4 of 16S rRNA genes were amplified for bacteria from Tingxi and Shidou reservoirs, respectively, following our previous studies [15, 20]. The bacteria from Dongzhen Reservoir were sequenced with Roche 454 GS FLX+ Instrument according to the manufacturer's protocols (454 Life Sciences, Branford, CT, USA) [25]. The bacteria from Tingxi and Shidou reservoirs were sequenced with an Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA) using a paired-end (2 × 250 bp) approach [20].

Sequence Analysis

Raw sequences obtained from 454 pyrosequencing were processed using QIIME software package [37] to remove barcodes on the sequences and low-quality reads (sequence lengths < 200 or > 1000 bp, quality scores < 25, homopolymer length > 6, and ambiguous base > 1). Chimeric sequences were identified by the Chimera Slayer [38] and removed before downstream analysis. High-quality sequences were clustered into operational taxonomic units (OTUs) using the pick_otus.py script with the UCLUST method (97% sequence similarity) [39]. Taxonomic classification of the reads was performed in the Silva (Silva 123) database and RDP classifier at a bootstrap cutoff of 80% [37].

The Illumina raw paired-end reads from Tingxi and Shidou reservoirs were quality trimmed in QIIME bioinformatics pipeline and assembled by the FLASH software [37]. Assembled sequences underwent quality control in the following way: sequences were eliminated if they contain ambiguous bases, if average quality is < 25, if homopolymer length is > 6, if there was a mismatch in primers, or if sequence length was < 200 bp and get the final sequences for downstream analysis [40]. Further, chimeric sequences were detected using UCHIME [41] and discarded before downstream analysis. The high-quality sequences were classified into multiple

OTUs at 97% similarity level using the UCLUST algorithm. Their taxonomic assignment was performed using Silva 123 database and RDP classifier at a bootstrap cutoff of 80% [37, 42].

For these three datasets, all reads related to Archaea, chloroplasts, eukaryote, and mitochondria were removed before the OTU table was generated. To minimize the possible sequences errors, the singleton OTUs were discarded in the final dataset. To maintain the equal sequences in the samples, all samples were normalized using the "subsample" command in the mothur software (version 1.33.3) [43]. For sequences, quality and OTUs yield after normalization (see Tables S3 and S4, respectively). The rarefaction curve was determined in the R software and sufficient observations have been made in Fig. S1. Diversity indices such as OTUs richness, ACE, Chao 1, Shannon-Wiener, Simpson index, and Pielou's evenness were computed using the vegan package to predict the biodiversity of the samples [44]. The differences among season groups' means were tested using a one-way analysis of variance (Tukey's post hoc test) (Fig. S2).

Accession Numbers

All raw sequences generated in the present study can be accessed through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) Sequence Read Archive (SRA) database under the following BioProject accession numbers: SRP121029: PRJNA415267; SRP124388: PRJNA416267; and SRP121029: PRJNA415267 for DZ, TX, and SD reservoirs, respectively.

Definition of Abundant and Rare Taxa

The cutoff for defining the rare biosphere is artificial so that there is no fixed threshold of relative abundance to differentiate abundant taxa from rare ones [1, 12, 45]. The choice of thresholds could depend on the technology used to find out species or the sake of the study [46]. Some researchers used a cutoff of 0.1% local relative abundance for rare taxa definition [2, 47]; others used 0.01% [20, 26, 48–50]. Furthermore, a threshold of 1% of relative abundance has been widely used in numerous studies to define abundant species [1, 20, 47, 49]. For our study, we classified bacterial community into different categories of taxa by setting a local relative abundance threshold of 1% for abundant taxa and 0.01% for rare taxa. Our community was artificially classified into six categories [14, 15, 21, 51]: (i) always abundant taxa (AAT), OTUs with a relative abundance ≥ 1% in all samples; (ii) conditionally abundant taxa (CAT), OTUs with relative abundance ≥ 1% in some samples and ≥ 0.01% in other samples, but never being rare; (iii) conditionally abundant or rare taxa (CRAT), OTUs with relative abundance from rare < 0.01% to abundant ≥ 1% in samples; (iv) moderate taxa (MT), OTUs with relative abundance < 1% and ≥ 0.01% in samples; (v) conditionally rare taxa (CRT), OTUs with < 0.01% local relative abundance in some samples and ≥ 0.01% in others but never ≥ 1%; (vi) rare taxa (RT), OTUs with relative abundance < 0.01% in all samples (Fig. S3). To simplify the analysis, OTUs with relative abundance > 1% at least once in a sample (i.e., AAT, CAT, and CRAT) were combined together as abundant taxa (AT) in this study [21].

Statistical Analyses

Analyses of Beta Diversity

The difference among group means was tested using a one-way analysis of variance (Tukey's post hoc test). Non-metric multidimensional scaling (NMDS) ordination analysis based on the Bray-Curtis distance was used to investigate seasonal variation of bacterioplankton community. The degree of separation among subcommunities was tested using an analysis of similarity (ANOSIM) ($R = 1$ and $R = 0$ indicate complete separation of communities and homogeneous community, respectively). The percentage contribution of community dissimilarity of the taxa categories was estimated using dissimilarity percentages (SIMPER) in Past 3. All NMDS and ANOSIM were performed using the package "vegan" in R environment (version 3.5.0) [52].

Relationship Between Bacterial Communities and Environmental Variables

To assess the effect of environmental variables on community composition, the profiles of water temperature, dissolved oxygen, pH, and chlorophyll a in each of three reservoirs were computed using OriginPro 2017. Environmental data were log $(x + 1)$ transformed except pH to standardize and improve their normal distributions. Detrended correspondence analysis (DCA) was carried out to determine the main gradients in the microbial assemblages [53]. Because the longest length of the gradient in the data was < 3 standard deviation DCA, redundancy analysis (RDA) was performed to examine the relationships between environmental variables and bacterial communities. The environmental variables with variance inflation factor (VIF) values > 20 were excluded, and forward selection was conducted to select important and significant variables ($P < 0.05$) [54]. All ordination analyses were performed using the package "vegan" in the R environment (version 3.5.0) [52].

Neutral Community Model for Bacterioplankton

We assessed the potential importance of the neutral or stochastic process in shaping bacterial community based on the neutral community model [55]. The model gives an account of patterns of bacterial communities, but in respect of the scale,

they are normally found. An essential supporting skeleton of the model predicts that the bacterial composition is regulated by the species diversity, species abundance, probability of bacterial migration, compositional drift, and extinction [55]. Practically, the proportion of variability R^2 can quantify the fit level of detection frequency to the model. Nm is an estimate of dispersal between communities in which N determines the metacommunity size, while m expresses the rate of the individuals immigrating from the source community into the local community [55]. Neutral community model was calculated using the package "Hmisc," "minpack.lm," and "stats4" in the R environment (version 3.5.0) [52].

C-score Checkerboard

The checkerboard score (C-score) [56] was carried out to test the actual distributions for non-randomness of bacterial OTUs. C-score was selected based on the reason that the matrix is relatively unaffected by the minor changes in the data. The sequence table was converted into a binary matrix of presence (1) and absence (0), and then analyzed for different combinations [56, 57]. The standardized effect sizes for C-score were estimated as the difference between the observed index and the mean of the stimulated index over the standard deviation of the stimulated index [58]. C-score was calculated based on a burn-in of 30,000 simulations and using sequential swap randomization algorithm in the package "EcoSimR" and the R environment (version 3.5.0) [52].

Results

Seasonal Change of Vertical Profiles of the Environmental Variables

The thermal stratification was pronounced in all reservoirs from spring to autumn. In winter, the water column in all reservoirs was well mixed and oxygenated. During the stratification period, the water became anoxic in the bottom layer of the reservoirs. In spring and summer, pH and chlorophyll a were significantly higher in epilimnetic surface than in hypolimnetic bottom waters (Fig. 1).

### Alpha Diversity and Seasonal Succession of Bacterial Communities

Our high-throughput sequencing yielded 415,023, 825,369, and 948,333 quality sequences in DZ, TX, and SD reservoirs, respectively (Table S3); these sequences were subsampled to 16,636, 28,822, and 24,009 sequences per sample, respectively. After normalization, sequences yielded 3429, 8551, and 1353 OTUs at 97% similarity level, respectively (Table S4). The global rarefaction curves showed that 20 samples were

sufficiently close to saturation of bacterioplankton richness, although the rarefaction curves were not saturated for single samples (Fig. S1). The comparison of bacterioplankton alpha diversity among four seasons revealed that the Shannon-Wiener index was significantly different among seasons in TX Reservoir at $P < 0.05$. The high values were estimated during spring ($n = 5$, mean = 5.59 ± 0.03) and then decreased during autumn ($n = 5$, mean = 5.27 ± 0.07). The similar trends were observed in Simpson and Pielou's evenness indices (Fig. S2), which demonstrated a high value in spring and low value during the summer. Our NMDS analysis revealed four distinct bacterial community successions, which corresponded well to four different seasons, especially in DZ and TX reservoirs (Fig. S4). The significant difference among seasons in three reservoirs was detected by ANOSIM test, whereas R values of almost all groups were closer to 1 at $P < 0.05$ in DZ and TX reservoirs (Table S5).

Dynamics of CRT and Its Contribution to Bacterial Community Dissimilarity

The CRT showed a great contribution in species richness, and they accounted for 71.80, 49.71, and 60.83% of OTUs richness in DZ, TX, and SD reservoirs, respectively (Table S4). The proportion of CAT range (0.50–2.44%) in species richness was much smaller than that of CRT range (49.71–71.80%); however, CAT were more abundant in all reservoirs than any other category of bacterioplankton (32.72 to 53.07% of the total sequences) (Fig. 2a, b; Fig. S3). The relative abundance of AT (including AAT, CAT, and CRAT) was much higher in comparison with other categories of taxa ($P < 0.05$) (Fig. 2c). Both AT and MT showed higher occurrence than CRT or RT (Fig. 2d). SIMPER test indicated that CRT contributed 84.38%, 70.57%, and 73.19% of bacterial community dissimilarity in DZ, TX, and SD reservoirs, respectively (Table 1). For the ten most contributed taxa, our results revealed AT as the main contributor to the community variation. For the top 50 and 100 taxa, the CRT were the greater contributors (Table S6).

The taxonomy of bacterial community composition showed that CRT were most diverse in all reservoirs (Figs. S5–S6). For CRT phylum number, we found 21, 30, and 29 taxa in DZ, TX, and SD, respectively. Proteobacteria were the dominant group of CRT specifically with high relative abundance in DZ Reservoir (range 17.6–48.6% in CRT subcommunity). Its dominance was high during autumn and low during summer stratification. CRT Bacteroidetes phylum in SD Reservoir exhibited a variation according to seasonal change; its dominance was clear during spring period and dropped down in autumn. At the class level, both Actinobacteria and Acidimicrobiia exhibited a seasonal variation (Fig. S6). In DZ Reservoir, the relative abundance of the class Actinobacteria varied between the lowest value (54.39%) in spring and the
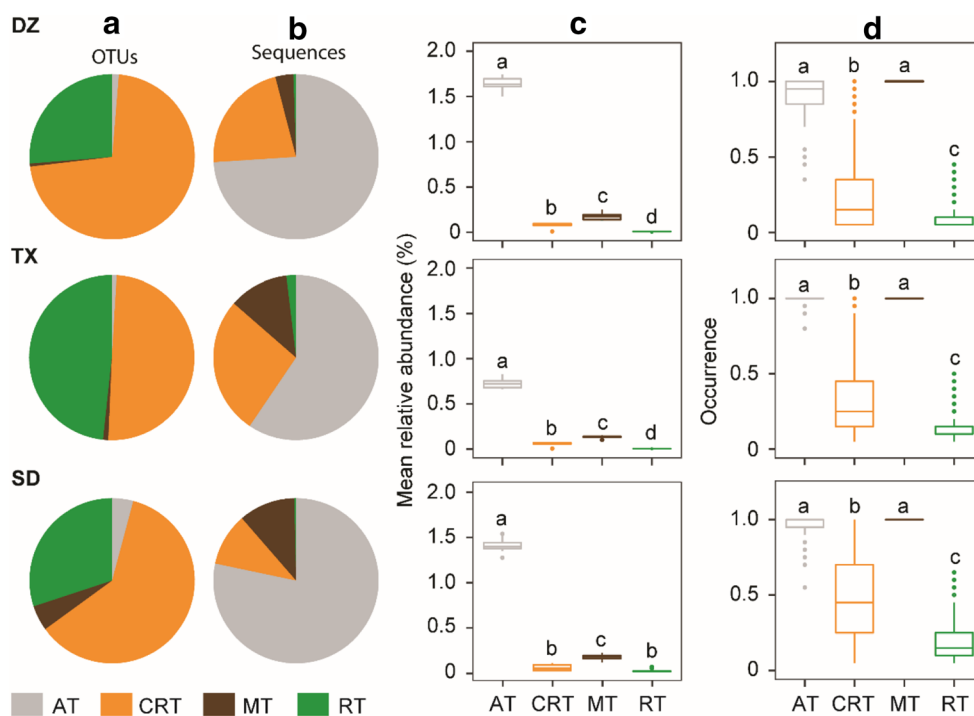
Fig. 2 Contributions of the four categories of taxa to the overall compositional abundances in three subtropical reservoirs. From the left to the right, proportion of OTUs (**a**), sequences associated to those OTUs (**b**), the mean relative abundance (**c**), and occurrence of the OTUs (**d**), belonging to each category of taxa. Significant differences ($P < 0.05$) between categories are indicated by different letters of the alphabet. Statistical analysis is ANOVA with Tukey's post hoc test. Reservoirs: DZ, Dongzhen; TX, Tingxi; SD, Shidou. AT, abundant taxa; CRT, conditionally rare taxa; MT, moderate taxa; RT, rare taxa. In this study, AT is the combination of three categories of abundant taxa including AAT (always abundant taxa), CAT (conditionally abundant taxa), and CRAT (conditionally rare and abundant taxa)

highest value (75.79%) in winter. In SD Reservoir, Acidimicrobiia's relative abundance ranged from 7.79 to 36.64%, and the highest value was observed in summer and the lowest one in winter. At the genus level, the most abundant genera were *Methylocystis* (Proteobacteria) in DZ Reservoir, *Sediminibacterium* and *Terrimonas* (Bacteroidetes) in TX Reservoir, and *Prochlorococcus* (Cyanobacteria) in SD Reservoir. For CRT, *Fluviicola* in DZ Reservoir and *Prochlorococcus* in SD Reservoir revealed high relative abundance during spring. In contrast, an increasing relative abundance of *Terrimonas* was observed from the summer to autumn in TX Reservoir (Fig. S7). Both abundance (sequence

number) and richness (OTU number) of bacterioplankton sub-communities' dynamics across different seasons and across five water layers are shown in Fig. S8.

Relationships Between Bacterial Community and Environmental Factors

Our RDA results revealed the important environmental variables which were closely related with bacterial community across four seasons ($P < 0.05$) (Fig. 3). In DZ Reservoir, the variation in bacterial subcommunities' composition was related to five environmental factors: water temperature, chlorophyll *a*,

Table 1 SIMPER test of four categories of bacterial OTUs in three reservoirs

Taxa	Dongzhen		Tingxi		Shidou	
	Average dissimilarity	Contribution (%)	Average dissimilarity	Contribution (%)	Average dissimilarity	Contribution (%)
AT	3.53	6.09	2.27	4.17	3.26	9.20
CRT	48.89	84.38	38.43	70.57	25.89	73.19
MT	0.71	1.23	1.48	2.72	2.23	6.29
RT	4.81	8.30	12.27	22.54	4.00	11.31

AT, abundant taxa; *CRT*, conditionally rare taxa; *MT*, moderate taxa; *RT*, rare taxa. AT is the combination of three categories of abundant taxa including AAT (always abundant taxa), CAT (conditionally abundant taxa), and CRAT (conditionally rare and abundant taxa)
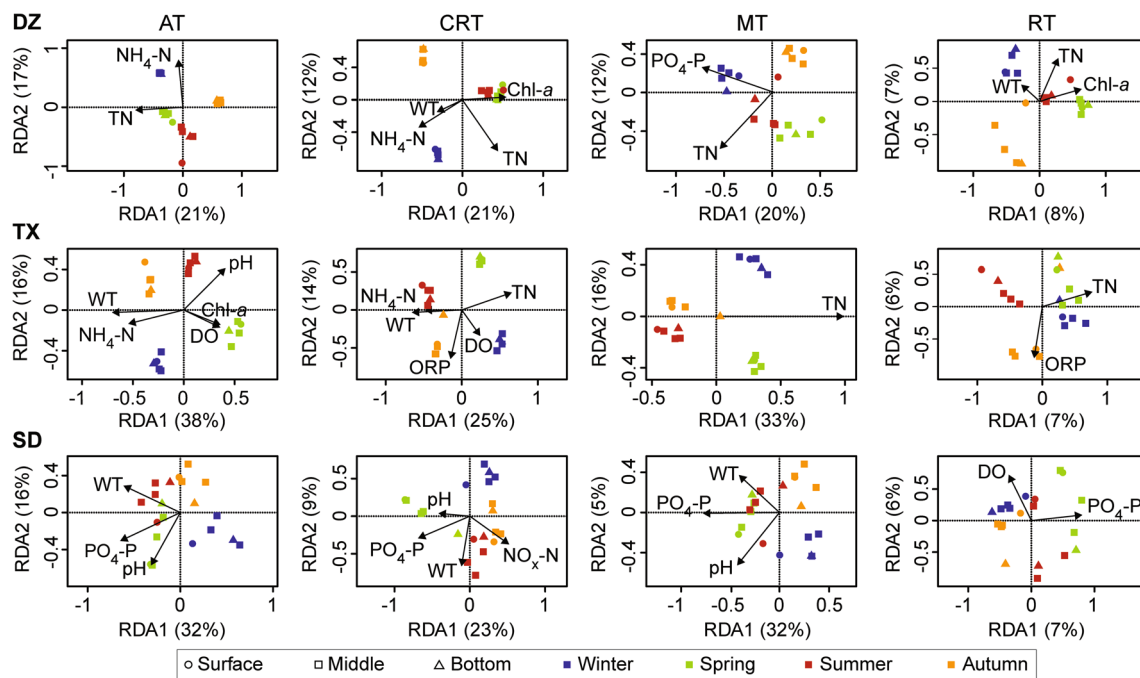
Fig. 3 Redundancy analysis (RDA) indicating seasonal dynamics of bacterioplankton community in relation to significant environmental parameters from three subtropical reservoirs. Only significant environmental factors are plotted ($P < 0.05$). Reservoirs: DZ, Dongzhen; TX, Tingxi; and SD, Shidou. AT, abundant taxa; CRT, conditionally rare taxa; MT, moderate taxa; RT, rare taxa. Environmental parameters: WT, water temperature; Chl-a, chlorophyll a; DO, dissolved oxygen; ORP, oxidation-reduction potential; TN, total nitrogen; NH_4-N, ammonium nitrogen; NO_x-N, nitrite and nitrate nitrogen; PO_4-P, phosphate phosphorus. Taxa categories: AT, abundant taxa; CRT, conditionally rare taxa; MT, moderate taxa; RT, rare taxa. AT is the combination of three categories of abundant taxa including AAT (always abundant taxa), CAT (conditionally abundant taxa), and CRAT (conditionally rare and abundant taxa)

total nitrogen, ammonium nitrogen, and phosphate phosphorus. Total nitrogen was found to be the most important environmental variable. It significantly correlated with the bacterial community composition ($P = 0.002$ for AT, $P = 0.004$ for CRT, $P = 0.006$ for MT, and $P = 0.006$ for RT). In TX Reservoir, seven environmental variables (water temperature, chlorophyll a, pH, total nitrogen, ammonium nitrogen, dissolved oxygen, and oxidation-reduction potential) were strongly correlated with the bacterial subcommunities ($P < 0.05$). Most of subcommunities from SD Reservoir were significantly correlated with phosphate phosphorus, water temperature, and pH. However, dissolved oxygen was also in association with RT ($P = 0.028$), while nitrite and nitrate nitrogen significantly correlated with CRT ($P = 0.002$).

Fit to the Neutral Model of Bacterioplankton

The neutral community model revealed a great proportion of the variation in the frequency of occurrence of entire bacterial communities ($R^2 = 0.69$, 0.61, and 0.76 in DZ, TX, and SD reservoirs, respectively), suggesting an important role for neutral or stochastic process in shaping the bacterial community assembly. The CRT showed the moderate fit to the neutral model whose variances R^2 were 0.63, 0.51, and 0.60 in DZ, TX, and SD reservoirs, respectively. The neutral model

explained a very low fraction of the variation in the frequency of occurrence in AT with an exception in SD Reservoir. RT subcommunities showed no fit to the neutral model due to a negative value of the variation in the frequency of occurrence (Fig. 4).

C-score of Co-occurrence Patterns

C-score analysis indicated species segregation in all CRT and RT subcommunities (standardized effect sizes > 2, $P < 0.001$) (Table 2). In CRT subcommunities of DZ, TX, and SD reservoirs, observed C-score values (7.61, 10.49, and 11.72, respectively) were slightly higher than simulated ones (7.42, 10.34, and 11.41, respectively). Overall, C-scores for CRT were the high values among the four categories of taxa. By contrast, MT subcommunities in all reservoirs and AT from TX Reservoir exhibited species co-occurrence because their C-scores were almost equal to 0. The highest and lowest SES values of CRT subcommunities appeared in SD Reservoir (SES = 16.00) and TX Reservoir (SES = 5.88), respectively, while in DZ Reservoir, the highest SES value (8.16) was found in AT subcommunity. Likewise, C_{var}-score and SES of C_{var}-score showed the similar patterns, suggesting the strength of the deterministic mechanism for structuring bacterioplankton communities in the three reservoirs.
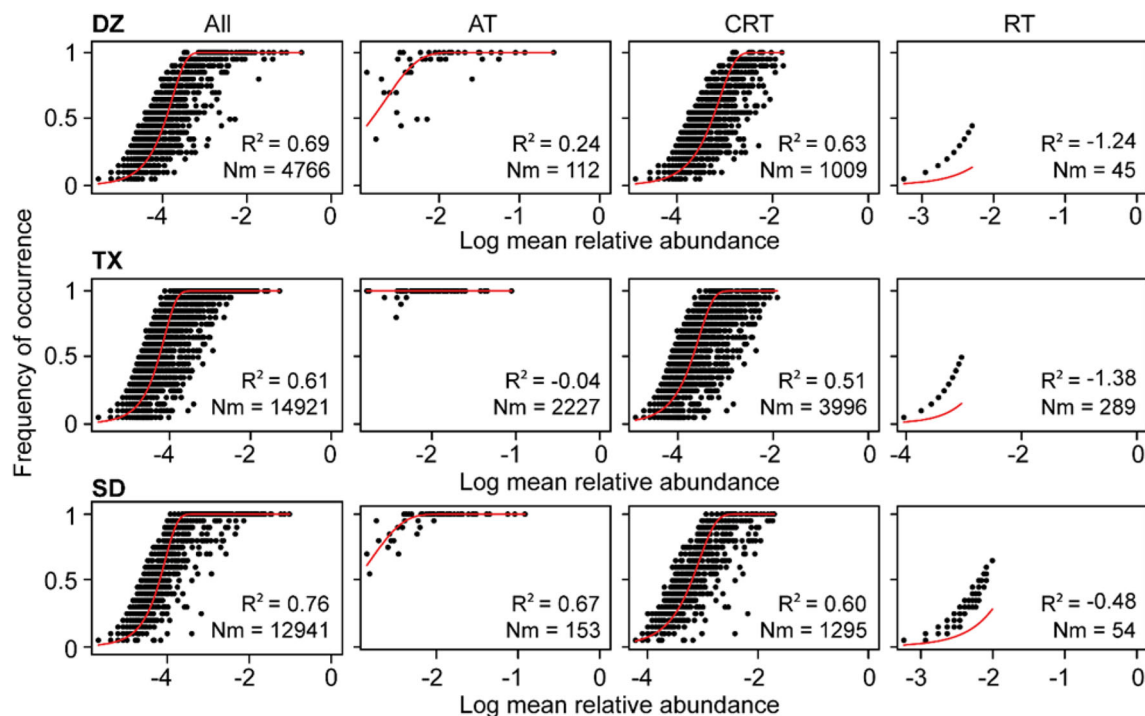
Fig. 4 Frequency of occurrence of bacterial OTUs as a function of mean relative abundance in communities from Dongzhen (DZ), Tingxi (TX), and Shidou (SD) reservoirs. Red lines indicate the best fit to the neutral community model and Nm indicates the metacommunity size (*m*) multiplied with immigration rate (*N*). The positive R^2 values indicate the fit to the neutral model, while the negative R^2 values indicate no fit to the neutral model. The mean relative abundance of OTUs was log-transformed. AT, abundant taxa; CRT, conditionally rare taxa; MT, moderate taxa; RT, rare taxa. AT is the combination of three categories of abundant taxa including AAT (always abundant taxa), CAT (conditionally abundant taxa), and CRAT (conditionally rare and abundant taxa)

Discussion

Rare taxa are important in maintaining ecosystem stability and function after environmental fluctuations due to their capability to awaken from a dormant state to active one [8, 13]. In the present study, we examined bacterioplankton communities in three subtropical drinking water reservoirs to understand their seasonal dynamics with special emphasis on conditionally rare taxa. We also explored the ecological processes driving spatial-temporal dynamics of those bacterial communities.

Responses of Bacterioplankton Subcommunities' Diversity to Seasonal Variation

Normally, seasonal succession patterns of microbial communities in the aquatic ecosystem are driven by numerous environmental variables such as nutrients, water temperature, oxygen concentration, pH, and chlorophyll *a* [58–61]. Based on our results, Shannon-Wiener, Simpson, and Pielou's evenness calculations showed a significant difference among seasons especially during the period of water stratification (Fig. S2), during which pH and water temperature were higher in the surface waters (Fig. 1). Further, the Shannon-Wiener and Simpson indices are reported to be more accurate in the analysis of rare phylotypes [62, 63]. Reasonably, our results were consistent with foregoing findings that highlighted some seasonal variabilities in bacterial community of freshwater lakes [60, 64] and the great importance of thermal stratification in shaping bacterial diversity changes [25, 33]. Therefore, we assumed that seasonal succession could have a large influence on the beta diversity of bacterioplankton community and composition dynamics [65]. Our findings showed seasonal succession patterns of the bacterial community in all reservoirs and categories of taxa (Fig. S4), but the strength was different across reservoirs (Table S5) probably due to unmeasured variables such as land use and water-level fluctuation. The SD was the shallowest reservoir and exhibited weaker seasonal patterns than DZ and TX reservoirs. Our results did not show a big change in bacterial community along the vertical gradient (Figs. S4, S8, and S9).

Dynamics and Contribution of Conditionally Rare Taxa in Bacterial Community Variation

In this study, we found that CRT richness was high in all reservoirs (Fig. S3; Table S4). This result may reflect the fact that about half of the bacterial community circulated between rare and abundant taxa [9, 12]. Moreover, this agreed with studies suggesting that rare taxa, with capacity to become dominant under favorable conditions, are considered as seed

Table 2 Observed C-scores, C_{var}-scores and simulated C-scores under null models and standardized effect sizes for bacterial communities in three subtropical reservoirs

Reservoir	Taxa	C-score			C_{var}-score		
		Obs	Sim	SES	Obs	Sim	SES
Dongzhen	All	6.10	5.96	6.10	61.37	51.74	5.95
	AT	1.85	1.50	8.16	31.29	18.02	6.38
	CRT	7.61	7.42	7.50	86.47	69.60.	7.15
	MT	0.00	0.00	NA	0.00	0.00	NA
	RT	3.13	3.12	5.52	6.77	6.59	4.59
Tingxi	All	7.32	7.28	3.93	57.67	53.62	3.81
	AT	0.01	0.01	-0.53	0.03	0.03	-0.25
	CRT	10.49	10.34	5.88	101.83	88.10	5.70
	MT	0.00	0.00	NA	0.00	0.00	NA
	RT	5.14	5.13	5.22	16.76	16.53	4.80
Shidou	All	8.30	8.10	8.95	99.92	84.35	9.79
	AT	0.37	0.26	10.36	7.32	1.93	13.88
	CRT	11.72	11.41	16.00	134.17	106.55	15.75
	MT	0.00	0.00	NA	0.00	0.00	NA
	RT	6.83	6.74	6.30	37.36	34.14	4.96

C-score significantly larger than expected by chance and high standardized effect size (SES) value (> 2) suggests greater degrees of species segregation. C-scores that are smaller than expected by chance and SES value (< -2) indicate species aggregation distribution. C-score that is equal to 0 determines species always occurring with others

Higher SES value of C_{var}-score indicates greater degrees of species segregation and/or aggregation

Obs, observed index; *Sim*, simulated index; *AT*, abundant taxa; *CRT*, conditionally rare taxa; *MT*, moderate taxa; *RT*, rare taxa. AT is the combination of three categories of abundant taxa including AAT (always abundant taxa), CAT (conditionally abundant taxa), and CRAT (conditionally rare and abundant taxa)

bank [66–68]. It has been suggested that rare taxa could be considered active when they are rare in certain samples and become abundant elsewhere in other samples [69]. The analysis of the mean relative abundance and occurrence contribution within bacterial subcommunities shows that both AT and MT had elevated mean relative abundance and high occurrence (Fig. 2d). These results suggest their wide distribution across the sampling sites compared with rare taxa that were only found in a small number of samples (Fig. 2). Our results were consistent with adaptation theory attributing a greater capacity of adaptation to core species than rare species if they are subjected to the environmental disturbances [70]. Normally, species with a large local population are widely distributed, while rare species are likely to occupy less proportion of sample sites [69, 71]. For example, in TX Reservoir, CRT showed high and low relative abundance during spring and summer, respectively (Fig. S8). In another example illustrated in Fig. S9, the phyla dominating CRT

subcommunities were slightly distinct across water layers. Therefore, the active rare species (e.g., CRT) can be the main cause of changes in microbial community structure and their contribution to community dissimilarity may grow up to 97% [9, 12]. Likewise, CRT contributed great proportions in the overall dissimilarities observed (Table 1). It is plausible that this is because of their capacity of competitiveness, commensalism, predation, and other ecological relationships [72, 73]. This indicates that CRT were potential drivers behind biogeochemical processes in the reservoir ecosystems [13].

In our study, the taxonomy at the phylum level revealed that many phyla exhibited seasonal dynamics in relative abundance rather than sampling depth (Fig. S5). Our results were almost identical with previous studies which showed that Proteobacteria, Actinobacteria, and Bacteroidetes were dominant phyla in the freshwater habitats [3, 74–77]. Seasonal variations in CRT and RT were highlighted with high diversity and phyla appearing in particular seasons. This was clear for Acidobacteria, Fusobacteria, and Planctomycete (Fig. S5). The seasonal trends were stronger at the genus level because some genera were dominant in one season and tended to be rare or absent in others (Fig. S7). Some reasons underlying such rarity might be attributed to nutrient availability and metabolites produced by rare taxa, which are critical in reproduction of abundant taxa [78]. Another reason might be the thresholds of rareness below which dormant taxon could not grow even if it is exposed to favorable conditions [79].

In this study, we used different sequencing techniques with different sequencing depths and target regions of 16S rRNA gene and this may affect our bacterial community results [15, 80]. Consequently, the diversities of bacterioplankton communities in different reservoirs were not compared directly. However, we can use three cases to demonstrate the general patterns and mechanisms in CRT dynamics in subtropical reservoirs across space and time.

Ecological Processes Driving Bacterioplankton Community Assembly

Microbial community ecology is now advancing from the elucidation of patterns in microorganisms' composition in the direction of explaining the mechanisms underlying the community assembly [22]. Bacterial communities inhabiting aquatic ecosystems are affected by nutrients, water temperature, pH, and chlorophyll *a* [25, 58, 61]. Here, we expected to find the same factors driving seasonal change in the bacterial community composition of subtropical reservoirs. We found that all subcommunities were significantly correlated with very similar environmental variables. The observed seasonal succession patterns were controlled by total nitrogen, nitrite and nitrate nitrogen, ammonium nitrogen, phosphate phosphorus, water temperature, chlorophyll *a*, pH, dissolved oxygen, and oxidation-reduction potential (Fig. 3). This shows

that the variability of bacterioplankton communities based on environmental parameters unskewed deterministic ecological process shaping bacterioplankton community in tropical stratified lakes [23]. Even though there was no specific environmental variable underlying dynamics of CRT in the reservoirs, we suspected unmeasured environmental factors, which need further investigation.

The deterministic process is likely to have an effect on temporal changes in microbial community structure; however, recent studies indicated that both environmental filtering and stochastic dynamics can shape bacterial communities simultaneously [55, 81, 82]. In our results, the entire community and CRT subcommunities showed a good fit to the neutral community model (Fig. 4), suggesting a stronger influence of the neutral or stochastic process to the CRT subcommunities. There is an ongoing debate on the impact of stochastic and deterministic processes on microbial community structure [83, 84]. Moreover, Shade and Gilbert [85] suggested that the dynamics of CRT community is influenced by either deterministic or stochastic processes and that deterministic changes should be linked to environmental drivers and microbial species interactions [14].

So far, it has been still a challenge to determine the relative influence of stochastic and deterministic processes on the distribution of microbial communities [86, 87]. Here, we additionally tested the actual distributions for non-randomness of bacterioplankton with checkerboard score [56, 57].

The C-score showed segregated patterns within CRT and RT subcommunities, random patterns of co-occurrence in MT subcommunities, and complex processes (random and non-random) within AT subcommunities (Table 2). Further, C-score results suggested that CRT and RT were strongly mediated by the deterministic process. Although CRT fitted the neutral model, our RDA and C-score results showed strong influence of environmental filtering on bacterial distribution. This shows that the neutral model is unable to directly decide the mechanism shaping microorganism unless it is combined with other models [88]. In partial conclusion, both CRT and RT assemblages in subtropical reservoirs were strongly regulated by deterministic processes. The MT assemblage was greatly controlled by the stochastic process, while the AT were concurrently driven by both stochastic and deterministic processes. Even though both deterministic and stochastic ecological processes have great importance in shaping microbial occurrence, CRT communities that are influenced by deterministic process are possibly more suitable to forecast community change [85].

Conclusions

This study demonstrated the seasonal dynamics of conditionally rare taxa in the bacterioplankton community from the three subtropical reservoirs of China. These data highlight the importance of examining both rare and conditionally rare taxa to provide a more complete view of microbial diversity. Conditionally rare taxa contributed 49.7 to 71.8% of total community membership over 1 year and accounted for 70.6 to 84.4% temporal variability in bacterioplankton community. The major phyla in the three reservoirs were Actinobacteria and Proteobacteria. The abundant, conditionally rare, moderate, and rare taxa subcommunities showed similar seasonal patterns, suggesting that these four subcommunities did not exhibit a large difference in their environmental sensitivity. However, the significant controlling factors were different among the reservoirs. Deterministic and stochastic processes simultaneously affected bacterial community assembly, whereas the deterministic process was more pronounced within CRT and RT subcommunities than the abundant one. Altogether, our results indicate that conditionally rare taxa can be a seed bank of bacterial community in the reservoirs, and they might play a crucial role in maintaining stability of bacterioplankton community in freshwater ecosystems under a changing environment.

Compliance with Ethical Standards

Conflict of Interest   The authors declare that they have no conflicts of interest.

References

1. Pedrós-Alió C (2012) The rare bacterial biosphere. Annu Rev Mar Sci 4:449–466

2. Fuhrman JA (2009) Microbial community structure and its functional implications. Nature 459:193–199

3. Newton RJ, Jones SE, Eiler A, McMahon KD, Bertilsson S (2011) A guide to the natural history of freshwater lake bacteria. Microbiol Mol Biol Rev 75:14–49

4. Huse SM, Welch DM, Morrison HG, Sogin ML (2010) Ironing out the wrinkles in the rare biosphere through improved OTU clustering. Environ Microbiol 12:1889–1898

5. Sjöstedt J, Koch-Schmidt P, Pontarp M, Canbäck B, Tunlid A, Lundberg P, Hagström Å, Riemann L (2012) Recruitment of members from the rare biosphere of marine bacterioplankton communities after an environmental disturbance. Appl Environ Microbiol 78:1361–1369

6. Logares R, Lindström ES, Langenheder S, Logue JB, 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

7. Epstein SS (2009) Microbial awakenings. Nature 457:1083

8. Jones SE, Lennon JT (2010) Dormancy contributes to the maintenance of microbial diversity. Proc Natl Acad Sci U S A 107:5881–5886

9. Lennon JT, Jones SE (2011) Microbial seed banks: the ecological and evolutionary implications of dormancy. Nat Rev Microbiol 9:119–130

10. Campbell BJ, Yu L, Heidelberg JF, Kirchman DL (2011) Activity of abundant and rare bacteria in a coastal ocean. Proc Natl Acad Sci U S A 108:12776–12781

11. Jousset A, Bienhold C, Chatzinotas A, Gallien L, Gobet A, Kurm V, Küsel K, Rillig MC, Rivett DW, Salles JF, Van Der Heijden MGA, Youssef NH, Zhang XW, Wei Z, Hol WHG (2017) Where less may be more: how the rare biosphere pulls ecosystems strings. ISME J 11:853–862

12. Shade A, Jones SE, Caporaso JG, Handelsman J, Knight R, Fierer N, Gilbert JA (2014) Conditionally rare taxa disproportionately contribute to temporal changes in microbial diversity. mBio 5:e01371–e01314

13. Lynch MD, Neufeld JD (2015) Ecology and exploration of the rare biosphere. Nat Rev Microbiol 13:217

14. Xue Y, Chen H, Yang JR, Liu M, Huang B, Yang J (2018) Distinct patterns and processes of abundant and rare eukaryotic plankton communities following a reservoir cyanobacterial bloom. ISME J 12:2263–2277

15. Liu M, Liu L, Chen H, Yu Z, Yang JR, Xue Y, Huang B, Yang J (2019) Community dynamics of free-living and particle-attached bacteria following a reservoir *Microcystis* bloom. Sci Total Environ 660:501–511

16. Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". Proc Natl Acad Sci U S A 103:12115–12120

17. Hutchinson GE (1964) The lacustrine microcosm reconsidered. Am Sci 52:334–334

18. Huse SM, Dethlefsen L, Huber JA, Welch DM, Relman DA, Sogin ML (2008) Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing. PLoS Genet 4:e1000255

19. Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, Mills DA, Caporaso JG (2013) Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat Methods 10:57–59

20. Liu L, Yang J, Yu Z, Wilkinson DM (2015) The biogeography of abundant and rare bacterioplankton in the lakes and reservoirs of China. ISME J 9:2068–2077

21. Chen W, Pan Y, Yu L, Yang J, Zhang W (2017) Patterns and processes in marine microeukaryotic community biogeography from Xiamen coastal waters and intertidal sediments, Southeast China. Front Microbiol 8:1912

22. Stegen JC, Lin X, Konopka AE, Fredrickson JK (2012) Stochastic and deterministic assembly processes in subsurface microbial communities. ISME J 6:1653–1664

23. Barberan A, Casamayor EO (2011) Euxinic freshwater hypolimnia promote bacterial endemicity in continental areas. Microb Ecol 61:465–472

24. Gonzalez A, King A, Robeson II MS, Song S, Shade A, Metcalf JL, Knight R (2012) Characterizing microbial communities through space and time. Curr Opin Biotechnol 23:431–436

25. Yu Z, Yang J, Amalfitano S, Yu X, Liu L (2014) Effects of water stratification and mixing on microbial community structure in a subtropical deep reservoir. Sci Rep 4:5821

26. Galand PE, Casamayor EO, Kirchman DL, Lovejoy C (2009) Ecology of the rare microbial biosphere of the Arctic Ocean. Proc Natl Acad Sci U S A 106:22427–22432

27. Glöckner FO, Zaichikov E, Belkova N, Denissova L, Pernthaler J, Pernthaler A, Amann R (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of Actinobacteria. Appl Environ Microbiol 66:5053–5065

28. Logue JB, Langenheder S, Andersson AF, Bertilsson S, Drakare S, Lanzén A, Lindström ES (2012) Freshwater bacterioplankton richness in oligotrophic lakes depends on nutrient availability rather than on species-area relationships. ISME J 6:1127–1136

29. Low-Décarie E, Kolber M, Homme P, Lofano A, Dumbrell A, Gonzalez A, Bell G (2015) Community rescue in experimental metacommunities. Proc Natl Acad Sci U S A 112:14307–14312

30. Yang J, Yu X, Liu L, Zhang W, Guo P (2012) Algae community and trophic state of subtropical reservoirs in southeast Fujian, China. Environ Sci Pollut Res 19:1432–1442

31. Cunha DG, do Carmo Calijuri M, Lamparelli MC (2013) A trophic state index for tropical/subtropical reservoirs (TSItsr). Ecol Eng 60:126–134

32. Wojciechowski J, Heino J, Bini LM, Padial AA (2017) Temporal variation in phytoplankton beta diversity patterns and metacommunity structures across subtropical reservoirs. Freshw Biol 62:751–766

33. Zwart G, Crump BC, Kamst-van Agterveld MP, Hagen F, Han SK (2002) Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquat Microb Ecol 28:141–155

34. Humbert JF, Dorigo U, Cecchi P, Le Berre B, Debroas D, Bouvy M (2009) Comparison of the structure and composition of bacterial communities from temperate and tropical freshwater ecosystems. Environ Microbiol 11:2339–2350

35. Ndayishimiye JC, Ju L, Li H, Yang X, Liu Z, Yang J (2019) Temperature transfer functions based on freshwater testate amoebae from China. Eur J Protistol 69:152–164

36. Liu L, Yang J, Lv H, Yu Z (2014) Synchronous dynamics and correlations between bacteria and phytoplankton in a subtropical drinking water reservoir. FEMS Microbiol Ecol 90:126–138

37. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336

38. Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, Giannoukos G, Ciulla D, Tabbaa D, Highlander SK, Sodergren E, Methé B (2011) Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Res 21:494–504

39. Fortunato CS, Herfort L, Zuber P, Baptista AM, Crump BC (2012) Spatial variability overwhelms seasonal patterns in bacterioplankton communities across a river to ocean gradient. ISME J 6:554–563

40. Magoč T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963

41. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200

42. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006) Greengenes, a

chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72:5069–5072

43. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541

44. Magurran AE (1988) Ecological diversity and its measurement. Princeton University Press, Princeton, USA

45. Youssef NH, Couger M, Elshahed MS (2010) Fine-scale bacterial beta diversity within a complex ecosystem (Zodletone Spring, OK, USA): the role of the rare biosphere. PLoS One 5:e12414

46. Reid A, Buckley M (2011) The rare biosphere. American Academy of Microbiology, Washington, pp 1–32

47. Vergin KL, Done B, Carlson CA, Giovannoni SJ (2013) Distributions of rare bacterioplankton populations indicate adaptive strategies in the oligotrophic ocean. Aquat Microb Ecol 71:1–13

48. Mangot JF, Domaizon I, Taib N, Marouni N, Duffaud E, Bronner G, Debroas D (2013) Short-term dynamics of diversity patterns: evidence of continual reassembly within lacustrine small eukaryotes. Environ Microbiol 15:1745–1758

49. Logares R, Audic S, Bass D, Bittner L, Boutte C, Christen R, Claverie JM, Decelle J, Dolan JR, Dunthorn M, Edvardsen B (2014) Patterns of rare and abundant marine microbial eukaryotes. Curr Biol 24:813–821

50. Logares R, Mangot JF, Massana R (2015) Rarity in aquatic microbes: placing protists on the map. Res Microbiol 166:831–841

51. Dai T, Zhang Y, Tang Y, Bai Y, Tao Y, Huang B, Wen D (2016) Identifying the key taxonomic categories that characterize microbial community diversity using full-scale classification: a case study of microbial communities in the sediments of Hangzhou Bay. FEMS Microbiol Ecol 92:fiw150

52. R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

53. Lepš J, Šmilauer P (2003) Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge, UK

54. Blanchet FG, Legendre P, Borcard D (2008) Forward selection of explanatory variables. Ecology 89:2623–2632

55. Sloan WT, Lunn M, Woodcock S, Head IM, Nee S, Curtis TP (2006) Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environ Microbiol 8:732–740

56. Stone L, Roberts A (1990) The checkerboard score and species distributions. Oecologia 85:74–79

57. Gotelli NJ, McCabe DJ (2002) Species co-occurrence: a meta-analysis of J.M. Diamond's assembly rules model. Ecology 83:2091–2096

58. Crump BC, Peterson BJ, Raymond PA, Amon RM, Rinehart A, McClelland JW, Holmes RM (2009) Circumpolar synchrony in big river bacterioplankton. Proc Natl Acad Sci U S A 106:21208–21212

59. Rösel S, Allgaier M, Grossart HP (2012) Long-term characterization of free-living and particle-associated bacterial communities in Lake Tiefwaren reveals distinct seasonal patterns. Microb Ecol 64:571–583

60. Ávila MP, Staehr PA, Barbosa FA, Chartone-Souza E, Nascimento A (2016) Seasonality of freshwater bacterioplankton diversity in two tropical shallow lakes from the Brazilian Atlantic Forest. FEMS Microbiol Ecol 93:fiw218

61. Bunse C, Pinhassi J (2017) Marine bacterioplankton seasonal succession dynamics. Trends Microbiol 25:494–505

62. Bent SJ, Forney LJ (2008) The tragedy of the uncommon: understanding limitations in the analysis of microbial diversity. ISME J 2:689–695

63. Haegeman B, Hamelin J, Moriarty J, Neal P, Dushoff J, Weitz JS (2013) Robust estimation of microbial diversity in theory and in practice. ISME J 7:1092–1101

64. Diao M, Sinnige R, Kalbitz K, Huisman J, Muyzer G (2017) Succession of bacterial communities in a seasonally stratified lake with an anoxic and sulfidic hypolimnion. Front Microbiol 8:2511

65. Shade A, Kent AD, Jones SE, Newton RJ, Triplett EW, McMahon KD (2007) Interannual dynamics and phenology of bacterial communities in a eutrophic lake. Limnol Oceanogr 52:487–494

66. Kunin V, Engelbrektson A, Ochman H, Hugenholtz P (2010) Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. Environ Microbiol 12:118–123

67. Aanderud ZT, Vert JC, Lennon JT, Magnusson TW, Breakwell DP, Harker AR (2016) Bacterial dormancy is more prevalent in freshwater than hypersaline lakes. Front Microbiol 7:853

68. Newton RJ, Shade A (2016) Lifestyles of rarity: understanding heterotrophic strategies to inform the ecology of the microbial rare biosphere. Aquat Microb Ecol 78:51–63

69. Shade A, Hendelsman J (2012) Beyond the Venn diagram: the hunt for a core microbiome. Environ Microbiol 14:4–12

70. Hanski I (1982) Dynamics of regional distribution: the core and satellite species hypothesis. Oikos 1:210–221

71. Bock CE, Ricklefs RE (1983) Range size and local abundance of some North American songbirds: a positive correlation. Am Nat 122:295–299

72. Magurran AE, Henderson PA (2003) Explaining the excess of rare species in natural species abundance distributions. Nature 422:714–716

73. Jiao S, Luo Y, Lu M, Xiao X, Lin Y, Chen W, Wei G (2017) Distinct succession patterns of abundant and rare bacteria in temporal microcosms with pollutants. Environ Pollut 225:497–505

74. Warnecke F, Amann R, Pernthaler J (2004) Actinobacterial 16S rRNA genes from freshwater habitats cluster in four distinct lineages. Environ Microbiol 6:242–253

75. Šimek K, Horňák K, Jezbera J, Mašín M, Nedoma J, Gasol JM, Schauer M (2005) Influence of top-down and bottom-up manipulations on the R-BT065 subcluster of β-Proteobacteria, an abundant group in bacterioplankton of a freshwater reservoir. Appl Environ Microbiol 71:2381–2390

76. Allgaier M, Grossart HP (2006) Diversity and seasonal dynamics of Actinobacteria populations in four lakes in northeastern Germany. Appl Environ Microbiol 72:3489–3497

77. Zhang H, Feng J, Chen S, Zhao Z, Li B, Wang Y, Jia J, Li S, Wang Y, Yan M, Lu K (2019) Geographical patterns of nirS gene abundance and nirS-type denitrifying bacterial community associated with activated sludge from different wastewater treatment plants. Microb Ecol 77:304–316

78. Morris JJ, Lenski RE, Zinser ER (2012) The Black Queen Hypothesis: evolution of dependencies through adaptive gene loss. mBio 3:e00036–e00012

79. Ruiz-González C, Niño-García JP, del Giorgio PA (2015) Terrestrial origin of bacterial communities in complex boreal freshwater networks. Ecol Lett 18:1198–1206

80. Engelbrektson A, Kunin V, Wrighton KC, Zvenigorodsky N, Chen F, Ochman H, Hugenholtz P (2010) Experimental factors affecting PCR-based estimates of microbial species richness and evenness. ISME J 4:642–647

81. Fernández A, Huang S, Seston S, Xing J, Hickey R, Criddle C, Tiedje J (1999) How stable is stable? Function versus community composition. Appl Environ Microbiol 65:3697–3704

82. Hubbell SP, Borda-de-Água L (2004) The unified neutral theory of biodiversity and biogeography: reply. Ecology 85:3175–3178

83. Ulrich W, Ollik M (2004) Frequent and occasional species and the shape of relative-abundance distributions. Divers Distrib 10:263–269

84. Isabwe A, Yang JR, Wang Y, Liu L, Chen H, Yang J (2018) Community assembly processes underlying phytoplankton and bacterioplankton across a hydrologic change in a human-impacted river. Sci Total Environ 630:658–667

85. Shade A, Gilbert JA (2015) Temporal patterns of rarity provide a more complete view of microbial diversity. Trends Microbiol 23: 335–340

86. Jia X, Dini-Andreote F, Salles JF (2018) Community assembly processes of the microbial rare biosphere. Trends Microbiol 26: 738–747

87. Mo Y, Zhang W, Yang J, Lin Y, Yu Z, Lin S (2018) Biogeographic patterns of abundant and rare bacterioplankton in three subtropical bays resulting from selective and neutral processes. ISME J 12: 2198–2210

88. Gotelli NJ, McGill BJ (2006) Null versus neutral models: what's the difference? Ecography 29:793–800