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# Eutrophication in subtropical lakes reinforces the dominance of balanced-variation component in temporal bacterioplankton community heterogeneity by lessening stochastic processes

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**One sentence summary:** This study first elucidates the shifts in the relative contributions of balanced-variation and abundance-gradient components to total temporal  $\beta$ -diversity of bacterioplankton communities along the trophic state gradient, which are linked to the changes in the balance between deterministic and stochastic processes.

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## Abstract

Unveiling the rules of bacterioplankton community assembly in anthropogenically disturbed lakes is a crucial issue in aquatic ecology. However, it is unclear how the ecological processes underlying the seasonally driven bacterioplankton community structure respond to varying degrees of lake eutrophication. We, therefore, collected water samples from three subtropical freshwater lakes with various trophic states (i.e. oligo-mesotrophic, mesotrophic, and eutrophic states) on a quarterly basis between 2017 and 2018. To innovatively increase our understanding of bacterioplankton community assembly along the trophic state gradient, the total bacterioplankton community dissimilarity was subdivided into balanced variation in abundances and abundance gradients. The results indicated that balanced-variation component rather than abundance-gradient component dominated the total temporal  $\beta$ -diversity of bacterioplankton communities across all trophic categories. Ecological stochasticity contributed more to the overall bacterioplankton community assembly in the oligo-mesotrophic and mesotrophic lakes than in the eutrophic lake. The reduced bacterioplankton network complexity at the eutrophic level was closely associated with the enhancement of environmental filtering, showing that bacterioplankton communities in eutrophic lakes are likely to be less stable and more vulnerable to water quality degradation. Together, this study offers essential clues for biodiversity conservation in subtropical lakes under future intensified eutrophication.

**Keywords:** abundance-gradient component, bacterioplankton community, balanced-variation component, environmental filtering, lake eutrophication, network complexity

## Introduction

Bacterioplankton in freshwater lakes are critical players underpinning ecological functions, such as their involvements in nutrient cycling and organic matter transformation (Wang et al. 2020), but they are of high sensitivity to multiple environmental disturbances (Philippot et al. 2021). Increasing eutrophication triggered by intensive anthropogenic activities associated with excessive nutrients (Le Moal et al. 2019) has profound effects on water quality and ecological communities in lake ecosystems (Qin et al. 2013). It has been well-demonstrated that eutrophication as an extrinsic disturbance strongly alters the abundance, diversity, composition, metabolism, and taxon interactions of bacterioplankton communities in freshwater lakes (Chróst and Siuda 2006, Kiersztyn et al. 2019, Zhou et al. 2021), thereby leading to the changes in ecological functions. However, less is known about how the ecological processes underlying the observed structure

of bacterioplankton communities respond to the varying degrees of lake eutrophication.

A comprehensive understanding of how the assembly mechanisms shape and maintain the diversity and composition of microbial communities at different spatial and temporal scales is a central concern in microbial ecology (Xu et al. 2020). A widely prevailing view is that stochastic (neutral) and deterministic (niche-based) processes collectively affect the assembly of microbial communities (Ning et al. 2019, Jiao et al. 2021). Stochastic processes are dependent on the assumption that all taxa are ecologically equivalent (Hubbell 2001), emphasizing the importance of colonization and extinction dynamics, ecological drift (i.e. random changes in the relative abundance of species due to stochastic birth, death, and reproduction events), and dispersal limitation (Chave 2004). Deterministic processes are any ecological processes that involve nonrandom, niche-based mechanisms, such

as environmental filtering (i.e. selection of taxa by the prevailing abiotic environmental conditions) and different biotic interactions (e.g. predation, mutualism, parasitism, and competition; Zhou and Ning 2017). Previous experimental studies have revealed that the relative importance of stochastic assembly processes increased under nutrient-enriched conditions in freshwater ecosystems (Chase 2010, Ren et al. 2017). Conversely, opposite results were observed in other field studies by indicating that deterministic processes played a stronger role in the assembly of bacterioplankton communities in enriched nutrient environments (Langenheder et al. 2012, Liu et al. 2021). These divergent findings further suggest that a clear elucidation of the relative importance of stochastic vs. deterministic processes is essential to evaluate the impact of eutrophication on bacterial community assembly. Here, we are expected that the relative importance of stochastic processes governing the spatiotemporal assembly patterns of bacterioplankton communities is likely to show a unimodal trend along the eutrophication gradient. This is because nutrient inputs could dampen deterministic assembly processes by reducing species competition for resources and strengthen drift by promoting the growth of rare microbial taxa (Zhou et al. 2014), but anthropogenic enrichment of ecosystems with nutrients would increase the harshness of environmental conditions, thus enhancing environmental filtering (Donohue et al. 2009, Zeng et al. 2019).

Spatial and temporal variations in community composition provide significant insights into community assembly mechanisms and biodiversity maintenance (Mori et al. 2018, López-Delgado et al. 2020). Total biotic dissimilarity between two or more assemblages (i.e. total  $\beta$ -diversity) in terms of variation in taxon abundances can be partitioned into two components: dissimilarity due to balanced variation in abundances (i.e. taxon abundances change from site to site with different signs for different taxa and change balance each other) and dissimilarity due to abundance gradients (i.e. all taxa that change their abundances from one site to the other make it with the same sign; Baselga 2013). Partitioning these two components of abundance-based dissimilarity provides novel perspectives for exploring the causality of the processes underlying biodiversity (Baselga 2017); however, it is much less considered in microbiology relative to animals and plants. Seasonal shifts in microbial assemblage composition (known as temporal  $\beta$ -diversity, Magurran et al. 2019) have been widely observed in freshwater lakes with various trophic states (Jiao et al. 2021) due to the seasonal fluctuations in abiotic conditions. Furthermore, it has been documented that temporal  $\beta$ -diversity of different aquatic communities can be strongly influenced by nutrient enrichment (Langenheder et al. 2012, Steiner 2014, Cook et al. 2018). Multiple macroecological studies demonstrated that eutrophication in freshwater ecosystems homogenizes the biotic assemblages over space and time, which is characterized as reductions in spatiotemporal  $\beta$ -diversity (Cook et al. 2018, Salgado et al. 2018, Zhang et al. 2019); however, there is no consensus on the response of  $\beta$ -diversity of bacterial communities to lake eutrophication (Jiao et al. 2021). As well, our understanding of the contributions of balanced-variation and abundance-gradient components to the overall temporal  $\beta$ -diversity of bacterioplankton assemblages along the eutrophication gradient in freshwater lakes remains largely unknown. Nevertheless, it is expected that when bacterioplankton community assemblages are subject to increasing environmental filtering under eutrophic conditions (Zhang et al. 2021), the total temporal  $\beta$ -diversity of bacterioplankton communities may be driven largely by balanced variation in abundances, as bacterial taxa are

categorized into copiotrophic and oligotrophic groups (Fierer et al. 2007) that respond differently to increased nutrient enrichment.

In this study, we aimed to elucidate (i) how the balance between stochastic and deterministic processes varied with lake trophic states; and (ii) whether and how lake eutrophication altered the contributions of balanced-variation and abundance-gradient components to total temporal  $\beta$ -diversity of bacterioplankton communities. We, therefore, tested two hypotheses that (i) the relative contributions of stochastic processes to the assembly of total bacterioplankton communities initially increase and then decrease along the trophic state gradient; and (ii) a weakening of ecological stochasticity at the eutrophic level (or in other words, the enhanced environmental filtering) leads to the greater balanced-variation component of total temporal  $\beta$ -diversity. To test our hypotheses, the field sampling was executed by selecting three subtropical lakes with various trophic states (i.e. oligo-mesotrophic, mesotrophic, and eutrophic states) in eastern China to obtain water samples. To ensure that we could observe seasonally driven variation in community composition, we started water sampling in April 2017 and revisited the sampled lakes at several-month intervals until January 2018. Bacterioplankton community composition along the trophic state gradient was determined using the 16S rRNA gene amplicon sequencing. Temporal  $\beta$ -diversity of bacterioplankton communities was quantified by using the Baselga partitioning statistical framework (Baselga 2013). To tease apart the relative role of stochastic vs. deterministic mechanisms underlying community assembly along the trophic state gradient, a null model analysis was conducted based on the community-level data. Moreover, microbial co-occurrence network analysis was executed in this study to provide further insight into bacterioplankton assembly patterns (Jiao et al. 2020) by investigating the variation in neutrally and non-neutrally distributed taxa along the eutrophication gradient.

## Materials and methods

### Study lakes and field sampling

To verify our hypotheses within the same regional species pool and consider the accessibility of samples, three freshwater lakes (i.e. Lake Meihua, Lake Pipa, and Lake Qianhu) with diverse trophic states (Table S1, Supporting Information) in the subtropical monsoon climate zone of eastern China were selected as our study lakes (Figure S1, Supporting Information). Lake Meihua (area, 0.04 km<sup>2</sup>; maximum depth, 2.5 m) is a macrophyte-dominated lake. Lake Pipa (area, 0.055 km<sup>2</sup>; maximum depth, 1.9 m) is a mesotrophic lake with the majority of macrophytes in the littoral zone. Compared with Lake Meihua and Lake Pipa, Lake Qianhu (area, 0.067 km<sup>2</sup>; maximum depth, 2.2 m) is free of macrophytes and highly subject to anthropogenic activities (e.g. sewage discharge and tourism disturbances; Jiao et al. 2018). These lakes are not distant from each other (maximum distance between lakes less than 1.5 km), and they are not connected to the river. We conducted four sampling campaigns during spring, summer, autumn, and winter between 2017 and 2018 (Table S1, Supporting Information). According to the survey specification for Chinese lake eutrophication proposed by Jin and Tu (1990), where sampling sites should be good representativeness of the abiotic and biotic parameters of the whole study lake under the premise of meeting the requirement of statistical number of samples, we thus determined six sampling sites distributed randomly in each lake (Figure S1, Supporting Information). Water samples were collected in

triplicate from a depth of 0.5 m at each site using a plexiglass water sampler. Triplicate samples were then mixed together to make a composite sample for each site and stored in 1.5 l sterile plastic bottles. The collected water samples were temporarily stored at  $\sim 4^{\circ}\text{C}$  in a portable icebox and then immediately transported to the laboratory. Finally, we obtained a total of 72 water samples from our four field sampling sessions. Each water sample was further divided into two subsamples: one for bacterioplankton analysis and the other for water physicochemical measurements. In the laboratory, we filtered 200 ml of water per sample through a 0.22- $\mu\text{m}$  pore size polycarbonate membranes with a 47-mm diameter (Millipore) to obtain bacterioplankton samples. The filter membranes were stored at  $-80^{\circ}\text{C}$  in an ultralow temperature refrigerator until further DNA extraction.

### Physicochemical measurements of water samples and calculation of lake trophic level index

Conductivity (Cond), dissolved oxygen (DO), water temperature (WT), and pH were measured at a depth of 0.5 m underwater in the field by a multisensor sonde (YSI 6600, Yellow Springs Instruments). Unfiltered water samples were used for the measurements of total phosphorus (TP), total nitrogen (TN), and water turbidity. Water turbidity was determined using a portable turbidimeter (WZB-170, Shanghai INESA Scientific Instrument Co., Ltd.). Filtered water samples (through a 0.22- $\mu\text{m}$  pore size polycarbonate membranes with a 47-mm diameter) were employed to determine the concentrations of ammonia nitrogen ( $\text{NH}_3\text{-N}$ ), nitrate nitrogen ( $\text{NO}_3\text{-N}$ ), and nitrite nitrogen ( $\text{NO}_2\text{-N}$ ). TP, TN,  $\text{NH}_3\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and  $\text{NO}_2\text{-N}$  were measured using continuous flow analysis (Skalar San++ system, Skalar Analytical BV). Moreover, filtered water samples (through a 0.45- $\mu\text{m}$  pore size polycarbonate membranes with a 47-mm diameter) were used for measuring the concentrations of dissolved organic carbon (DOC) and the filter membranes were used for the extraction of chlorophyll *a* (Chl*a*). Chl*a* was measured spectrophotometrically with ethanol as the extraction solvent (Jespersen and Christoffersen 1987). DOC was measured with a TOC analyzer (Multi N/C 2100, Analytic Jena). To estimate the seasonal variation in water environmental attributes within lakes, we calculated the coefficients of variation (CVs) of all measured environmental parameters based on the means of each season of each lake, i.e. determined the extent to which the means measured in individual seasons deviated from the overall mean for the given lake. To compare the differences in environmental variables across seasons within each lake, one-way analysis of variance (ANOVA) with Turkey HSD *post hoc* test was performed in SPSS (V20.0; IBM Corp). Principal component analysis (PCA) was further applied to visualize the variation in physicochemical properties across seasons in different lakes in R (v3.6.1; R Core Team 2020). Prior to PCA, environmental data were standardized using the 'scale' argument in the 'rda' function of the R package 'vegan' (v2.5–6).

The trophic state assessment of three subtropical lakes was implemented based on the comprehensive trophic level index (TLIc), which has been widely used to assess the degree of eutrophication of Chinese lakes (including macrophyte-dominated and phytoplankton-dominated lakes; Hu et al. 2014, Lin et al. 2021). In this study, three water environmental parameters (i.e. Chl*a* ( $\mu\text{g l}^{-1}$ ), TN ( $\text{mg l}^{-1}$ ), and TP ( $\text{mg l}^{-1}$ )) were applied to calculate TLIc (Lin et al. 2021). The formula for the calculation of TLIc was detailed in the Supporting Information.

### Bacterioplankton DNA extraction, PCR amplification, and amplicon sequencing

Total bacterioplankton DNA were extracted from filter membranes using E.Z.N.A.<sup>®</sup> Water DNA Kit (Omega Biotek, Doraville, USA) following the manufacturer's instructions. The V4–V5 variable regions of the bacterioplankton 16S rRNA gene were PCR-amplified using universal primer sets 515F and 907R (Caporaso et al. 2012). The PCR reaction system and the thermal cycling conditions for PCR amplification were identical to our previous publication (Jiao et al. 2018). We performed high-throughput amplicon sequencing using  $2 \times 250$  bp paired end sequencing on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) at the Shanghai BIOZERON Biotechnology Co., Ltd. (Shanghai, China). Moreover, raw sequencing data have been uploaded to the Sequence Read Archive (SRA) database under the NCBI BioProject number of PRJNA780039.

### Bioinformatics of 16S rRNA gene sequencing data

As described previously (Jiao et al. 2020), raw sequencing data downloaded from MiSeq platform were processed to obtain high-quality sequences. Briefly, the paired-end sequences were first merged by using FLASH (v1.2.11; Magoč and Salzberg 2011). Low-quality sequences (i.e. those with an average quality score  $< 25$ , a homopolymer  $> 6$  bp or ambiguous bases, or a sequence length  $< 200$  bp) and chimeric sequences were discarded. The generated 3126 102 high-quality sequences were subsequently clustered into operational taxonomic units (OTUs) at 97% similarity level using the UCLUST algorithm (Edgar 2010). The taxonomic classification information of representative sequences from each OTU was obtained using the RDP classifier with a bootstrap cutoff of 80% (Wang et al. 2007). In addition, archaea, mitochondria, chloroplast, unassigned, unknown, and singleton (i.e. sequence appearing only once over all samples) sequences were removed. After that, a total of 2904 691 sequences and 5651 OTUs were obtained from 72 DNA samples. An approximately maximum-likelihood phylogenetic tree was constructed using FastTree (v2.1.10; Price et al. 2009). To address variation in sampling depth (ranging from 23 370 to 55 595 sequences) and avoid potential biases resulting from rarefaction (Willis 2019), the OTU matrix was normalized using metagenomeSeq's cumulative-sum scaling (CSS) method (Paulson et al. 2013). The CSS-normalized OTU table was then used for the bacterioplankton  $\alpha$ - and  $\beta$ -diversity analyses in R.

### Analyses of bacterioplankton community composition and diversity

To unravel the seasonal variation in bacterioplankton community composition at the phylum level in different lakes, a stacked bar graph was plotted using GraphPad Prism (v8.0.2). Bacterioplankton taxonomic  $\alpha$ -diversity characterized by taxon richness (i.e. the number of observed OTUs with a threshold of 97% similarity), Shannon index, Simpson index, and Pielou's evenness were calculated with R package 'vegan'. One-way ANOVA with Turkey HSD test ( $n = 24$  denoting the number of samples for this statistical test, the same below) was used to compare the differences in bacterioplankton  $\alpha$ -diversity indices between lakes. A nonmetric multidimensional scaling (NMDS) analysis of bacterioplankton communities was conducted based on Bray–Curtis pairwise dissimilarity using the function 'metaMDS' in the R package 'vegan' to visualize the community dissimilarity across samples ( $n = 72$ ). To investigate the environmental drivers of bacterioplankton community structure, we applied the function 'envfit' to map all the measured environmental variables as vectors onto the NMDS or-

dinations. We evaluated the significance of these environmental variables with 999 random permutations, and kept the significant ones for plotting. In addition, to investigate whether the variation in bacterioplankton communities was spatially or temporally dominated for each lake category (i.e. lakes with diverse trophic states), we used a two-way permutational multivariate analysis of variance (PERMANOVA,  $n = 24$ ) with the function 'adonis' in the R package 'vegan' (Anderson 2017) to reveal the effects of season and spatial sampling site on bacterioplankton community dissimilarity. A one-way PERMANOVA ( $n = 12$ ) was further applied to assess the differences in bacterioplankton communities between lakes and between seasons within lakes.

### Partitioning abundance-based $\beta$ -diversity of bacterioplankton communities into balanced-variation and abundance-gradient components

To evaluate the contributions of balanced-variation and abundance-gradient components to the seasonal variation in bacterioplankton community composition, the Baselga partitioning framework (Baselga 2013) was used to partition temporal  $\beta$ -diversity of bacterioplankton communities based on Bray–Curtis pairwise dissimilarity. Total Bray–Curtis dissimilarity ( $\beta_{bc}$ ) was separated into balanced-variation component ( $\beta_{bc,bal}$ ) and abundance-gradient component ( $\beta_{bc,gra}$ ) using the function 'beta.pair.abund' in the R package 'betapart' (v1.5.4). Here, a total of three pairwise dissimilarity matrices were generated:  $\beta_{bc}$ ,  $\beta_{bc,bal}$ , and  $\beta_{bc,gra}$ .

Two-way PERMANOVA (permutations = 999; R package 'vegan') was used to examine the effects of lake category, season and their interaction on total Bray–Curtis dissimilarity and its two components of bacterioplankton communities ( $n = 72$ ). One-way ANOVA with Turkey HSD test ( $n = 24$ ) was used to test for the significant differences in Bray–Curtis pairwise dissimilarity and its two components between lakes. To identify the relative impact of environmental variables on the seasonal variation in bacterioplankton communities within each lake, we conducted an analysis of multiple regression on distance matrices (MRM,  $n = 24$ ) based on  $\beta_{bc}$ ,  $\beta_{bc,bal}$ , and  $\beta_{bc,gra}$  using the function 'MRM' in the R package 'ecodist' (v2.0.7), which offers significant advantages over traditional partial Mantel analysis to explore linear, nonlinear, or nonparametric relationships between a response distance matrix and any number of explanatory distance matrices (Lichstein 2007, Wang et al. 2017). Given the possible multicollinearity among the explanatory variables, prior to MRM analysis, variable clustering was employed to evaluate the collinearity of the environmental factors with the function 'varclus' in the R package 'Hmisc' (v4.5–0). The variables with high correlations (Spearman's  $\rho^2 > 0.7$ ) were removed from the MRM analysis, but all other variables were retained in the model. We ran the MRM analysis twice to reduce the effect of spurious relationships between variables. The first run was to remove insignificant variables and then the MRM analysis was rerun. We reported the analysis results of the second run.

### Null model analysis

To estimate the relative importance of stochastic and deterministic processes on bacterioplankton community assembly, taxonomic normalized stochasticity ratio (NST), a metric proposed by Ning et al. (2019), was calculated using the function 'tNST' (dist.method = 'bray'; 1000 randomizations) in the R package 'NST' (v2.0.4). We used the null model algorithm of proportional

taxa occurrence frequency and fixed taxa richness, which has been more often used in previous publications (Chase et al. 2011, Stegen et al. 2013, 2015) and is also the default algorithm in the NST calculation. The NST index estimates the average ecological stochasticity within a set of samples ( $n \geq 6$ ), which was developed with 50% as the cutoff point between more deterministic ( $< 50\%$ ) and more stochastic ( $> 50\%$ ) community assembly (Ning et al. 2019). NST is a valuable metric for quantitatively assessing ecological stochasticity based on the whole community (Ning et al. 2019), but it fails to directly reflect the relative contributions of various ecological processes to the bacterioplankton community assemblages. The various ecological processes that regulate the assembly of bacterial communities include homogeneous selection (i.e. selection under homogeneous abiotic and biotic conditions leads to low compositional turnover), heterogeneous selection (i.e. selection under heterogeneous abiotic and biotic conditions results in high compositional turnover), homogenizing dispersal (i.e. high dispersal rates can homogenize community composition and hence lead to low compositional turnover), dispersal limitation (i.e. low dispersal rates can cause high compositional turnover), and drift (i.e. stochastic changes in taxon relative abundances within a community over time due to random birth and death events that occur with respect to taxon identity; Stegen et al. 2013).

To assess which ecological processes dominantly governed bacterioplankton community assembly, we applied a statistical framework developed by Ning et al. (2020) from a previous framework (Stegen et al. 2013) to quantitatively infer the relative importance of different ecological assembly processes through a phylogenetic-bin-based null model analysis (iCAMP). In the iCAMP framework, the observed bacterial taxa were first divided into different groups (i.e. bins) based on their phylogenetic relationships. Then, the ecological processes governing each bin were quantified based on an abundance-based null model analysis of the phylogenetic  $\beta$ -diversity metric using beta net relatedness index ( $\beta$ NRI) and taxonomic  $\beta$ -diversity metric using modified Raup–Crick metric based on Bray–Curtis dissimilarity ( $RC_{Bray}$ ). Subsequently, the proportions of individual processes across all bins were weighted by the relative abundance of each bin and summarized to calculate the relative importance of individual processes at the entire community level (Ning et al. 2020). We performed statistical analyses described above in the R package 'iCAMP' (v1.3.4). The R code for iCAMP used to quantify various ecological processes was detailed in Ning et al. (2020).

Based on the iCAMP framework, the fraction of pairwise comparisons with  $\beta$ NRI  $> +1.96$  or  $< -1.96$  was considered as the percentages of heterogeneous selection or homogeneous selection on the assembly of bacterioplankton communities, respectively.  $RC_{Bray}$  was then used to separate the pairwise comparisons that were not part of selection (i.e.  $|\beta$ NRI  $\leq 1.96$ ). The fraction of pairwise comparisons with  $|\beta$ NRI  $\leq 1.96$  and  $RC_{Bray} > +0.95$  or  $< -0.95$  indicated that compositional turnover was governed by dispersal limitation or homogenizing dispersal, respectively. The fraction of pairwise comparisons with  $|\beta$ NRI  $\leq 1.96$  and  $|RC_{Bray}| \leq 0.95$  was quantified as the effect of drift (Ning et al. 2020). In this study, we calculated the relative importance of selection (i.e. homogeneous selection and heterogeneous selection), dispersal (i.e. homogenizing dispersal and dispersal limitation), and drift for each pair of samples and obtained an average of percentage of ecological processes for each lake category. Moreover, ecological stochasticity of bacterioplankton community assembly can be also reflected by the sum of the relative importance of dispersal and drift (Ning et al. 2020).

## Neutral community model

For bacterioplankton communities, the relationship between occurrence frequency of bacterioplankton OTUs and their average relative abundance in the metacommunity (i.e. the sum of all samples for each trophic category) was predicted by the neutral community model (NCM) proposed by Sloan et al. (2006) to validate the potential influence of neutral processes on the assembly of bacterioplankton communities. In the NCM, the parameters  $R^2$  and  $Nm$  values denote the overall fit to the NCM and the metacommunity size times immigration, respectively, with  $N$  representing the metacommunity size and  $m$  denoting the immigration rate.  $R^2 > 0$  indicates that bacterioplankton communities conform to the NCM (subject to neutral processes), and greater  $R^2$  values suggest that stochastic dispersal and ecological drift contribute more to the community assembly. The  $Nm$  value is an estimate of dispersal between communities (Sloan et al. 2006). The calculation of 95% confidence intervals around all fitting statistics was performed by bootstrapping with 1000 bootstrap replicates (Burns et al. 2016). The R code used to fit the NCM and calculate the goodness-of-fit statistics was detailed in Burns et al. (2016). In addition, bacterioplankton OTUs were partitioned into three different partitions depending on whether they occurred more frequently than (above prediction), less frequently than (below prediction), or within (neutral distribution) the 95% confidence interval of the NCM predictions. OTUs assigned to the above and below partitions were non-neutrally distributed.

## Co-occurrence network analysis of bacterioplankton communities

To compare the co-occurrence patterns of bacterioplankton OTUs, networks of three subtropical lakes were generated using Sparse Correlations for Compositional data (SparCC), a robust network analysis method, i.e. especially suited for compositionally diverse microbial data (Friedman and Alm 2012). Bacterioplankton OTUs were selected by frequency of occurrence (> 50%) to simplify our data sets and address the bias caused by too many matching zeros across samples (Faust 2021). Only robust (SparCC  $|r| > 0.80$ ) and statistically significant ( $P < .01$ ) correlations were included in the network analysis (Jiao et al. 2020). The constructed networks were visualized using Gephi (v0.9.2). Network nodes represent neutrally or non-neutrally distributed OTUs, and network links connecting two nodes signify significant correlations between OTUs. Network topological parameters (i.e. average degree, graph density, network diameter, modularity, average path length, and average clustering coefficient) were described collectively in our previous studies (Huang et al. 2020, Jiao et al. 2020) and calculated using the R package 'igraph' (v1.2.4.1). Furthermore, 1000 random Erdős–Rényi networks (Erdős and Rényi 1960) sharing the same number of nodes and links as empirical networks were constructed using the R 'igraph' package and their topological parameters were calculated as described above. The differences in topological parameters between random and empirical networks was compared by Z-test in R (Zhao et al. 2016).

## Results

### Physicochemical properties of three subtropical lakes with diverse trophic states

The TLIc index, which represents the degree of eutrophication, revealed that Lake Qianhu characterized as a eutrophic state was more eutrophic than Lake Meihua and Lake Pipa, which were in oligo-mesotrophic and mesotrophic states, respectively (Table S1,

Supporting Information). PCA based on water environmental variables showed that the water samples were clearly differentiated by both lake category and season (Figure S2, Supporting Information), but season exerted a greater effect on the variation in water environment attributes than lake category (Table 1). We, thus observed significant seasonal variations in the measured environmental variables in the oligo-mesotrophic lake (Figure S3, Supporting Information), mesotrophic lake (Figure S4, Supporting Information), and eutrophic lake (Figure S5, Supporting Information). Of all the measured water physicochemical parameters, Chla,  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and turbidity concentrations were higher but their CVs were relatively lower in the eutrophic lake compared to the oligo-mesotrophic and mesotrophic lakes (Table S2, Supporting Information), indicating that seasonal fluctuations in these environmental variables were lower in the eutrophic lake than in the other two lakes.

### Seasonally driven variation in bacterioplankton community structure along the trophic state gradient

The overall bacterioplankton communities in the eutrophic lake showed lower Shannon index, Simpson index, and Pielou's evenness than in the oligo-mesotrophic and mesotrophic lakes (Figure S6, Supporting Information). However, no difference in taxon richness was found among the three lake categories (Figure S6, Supporting Information). Actinobacteria (average relative abundance, 29.36%), Gammaproteobacteria (26.59%), and Cyanobacteria (37.20%) were the most abundant bacterial phyla/classes based on all seasonal datasets in the oligo-mesotrophic, mesotrophic, and eutrophic lakes, respectively (Figure S7, Supporting Information). For each lake category, we observed that seasonality explained much greater amount of the variability in bacterioplankton communities than spatial sampling site (Table S3, Supporting Information), indicating that overall bacterioplankton community dissimilarity within lakes was dominantly driven by seasonality. In addition, lake category and season and their interactions contributed to significant composition variation in total bacterioplankton communities (Table 1). Total bacterioplankton community structure for all lake categories displayed a remarkable seasonal variation in community composition (Fig. 1A), which was also strongly validated by the PERMANOVA results that bacterioplankton community structure differed significantly between seasons within each lake ( $P < .05$  in all cases; Table S4, Supporting Information).

Balanced-variation and abundance-gradient components jointly contributed to the observed seasonally driven variation in bacterioplankton community structure for each lake; however, we found that balanced-variation component contributed more to total temporal  $\beta$ -diversity than abundance-gradient component (Fig. 1B). In comparison with oligo-mesotrophic and mesotrophic lakes, bacterioplankton communities in the eutrophic lake exhibited significantly higher overall  $\beta$ -diversity and balanced-variation component but lower abundance-gradient component ( $P < 0.05$ , one-way ANOVA with Turkey HSD test; Fig. 1B).

### Environmental drivers of bacterioplankton community structure of three subtropical lakes

Among bacterioplankton assemblages from all the lake categories, the NMDS ordinations of overall  $\beta$ -diversity partitioned into balanced-variation and abundance-gradient components revealed the important environmental drivers of spatiotemporal

**Table 1.** Permutational multivariate analysis of variance (PERMANOVA) showing the effects of lake category, season and their interaction on environmental variables (Euclidean distance), and bacterioplankton community structure (Bray–Curtis dissimilarity and its two components).

Source of variation	Df	SS	MS	F. Model	R <sup>2</sup>	P-value
<i>Environmental variables</i>						
Lake category	2	252.03	126.02	83.65	<b>0.296</b>	<b>.001</b>
Season	3	349.89	116.63	77.42	<b>0.411</b>	<b>.001</b>
Interaction	6	159.68	26.61	17.67	<b>0.187</b>	<b>.001</b>
Residuals	60	90.39	1.51		0.106	
Total	71	852.00			1	
<i>Bacterioplankton communities (<math>\beta_{bc}</math>)</i>						
Lake category	2	4.44	2.22	19.92	<b>0.225</b>	<b>.001</b>
Season	3	4.74	1.58	14.16	<b>0.240</b>	<b>.001</b>
Interaction	6	3.90	0.65	5.84	<b>0.197</b>	<b>.001</b>
Residuals	60	6.69	0.11		0.338	
Total	71	19.77			1	
<i>Bacterioplankton communities (<math>\beta_{bc, bal}</math>)</i>						
Lake category	2	4.50	2.25	27.50	<b>0.264</b>	<b>.001</b>
Season	3	4.45	1.48	18.12	<b>0.261</b>	<b>.001</b>
Interaction	6	3.18	0.53	6.48	<b>0.187</b>	<b>.001</b>
Residuals	60	4.91	0.08		0.288	
Total	71	17.04			1	
<i>Bacterioplankton communities (<math>\beta_{bc, gra}</math>)</i>						
Lake category	2	-0.17	-0.087	-10.62	-0.562	1
Season	3	-0.03	-0.009	-1.08	-0.086	.954
Interaction	6	0.02	0.003	0.38	0.061	.744
Residuals	60	0.49	0.008		1.587	
Total	71	0.31			1	

Note: Df, degrees of freedom; SS, sum of squares; MS, mean squares; F. Model, F statistic. Lake category includes oligo-mesotrophic, mesotrophic, and eutrophic lakes. Sampling season includes spring, summer, autumn, and winter.  $\beta_{bc}$ , total Bray–Curtis pairwise dissimilarity;  $\beta_{bc, bal}$ , balanced-variation component of Bray–Curtis pairwise dissimilarity; and  $\beta_{bc, gra}$ , abundance-gradient component of Bray–Curtis pairwise dissimilarity. Statistical significance was calculated based on 999 permutations. Bold values indicate statistical significance at the  $P < .05$  level.

variation in bacterioplankton community structure (Fig. 1A). We found that the spatiotemporal variation in overall bacterioplankton communities was most closely linked to DO ( $r^2 = 0.70$ ) and WT ( $r^2 = 0.64$ ), whereas community dissimilarity due to balanced-variation and abundance-gradient components was most strongly explained by NO<sub>2</sub>-N ( $r^2 = 0.76$ ) and DOC ( $r^2 = 0.23$ ), respectively. WT, DO, and pH were all significantly correlated with three pairwise dissimilarity matrices (i.e.  $\beta_{bc}$ ,  $\beta_{bc, bal}$ , and  $\beta_{bc, gra}$ ). Moreover, differences among bacterioplankton assemblages were also significantly related to TN, TP, NO<sub>3</sub>-N, Chl<sub>a</sub>, Cond, and turbidity for  $\beta_{bc}$  and  $\beta_{bc, bal}$  [see Table S5 (Supporting Information) for details].

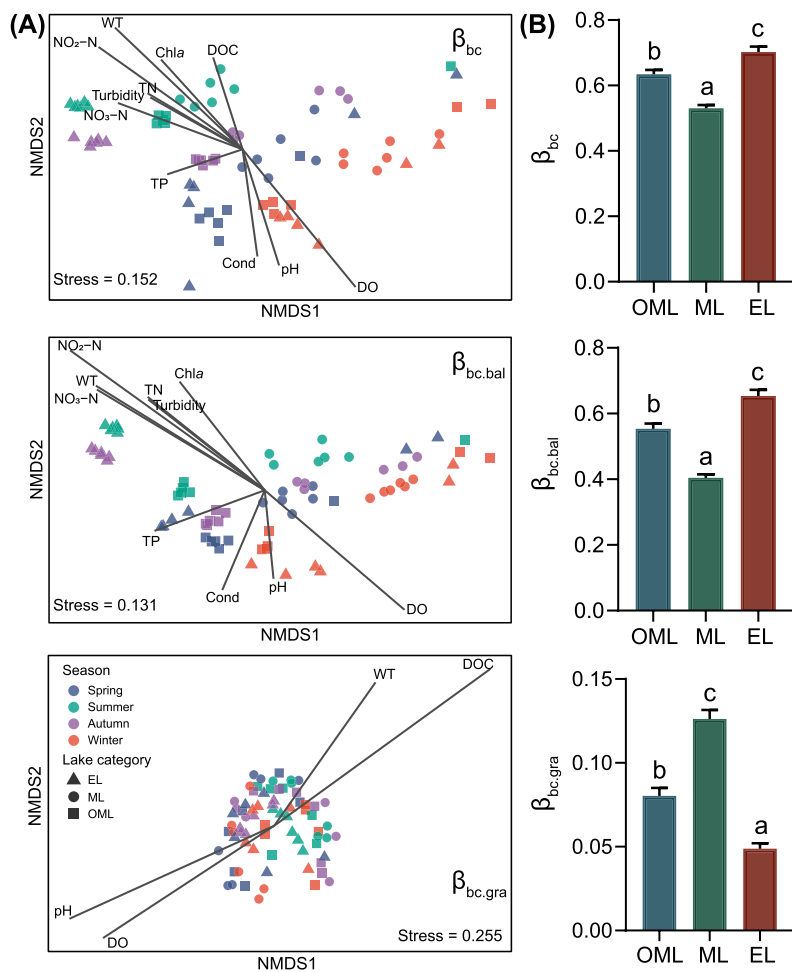
Within each lake category, MRM model was performed to further examine the relative contributions of different environmental variables to temporal  $\beta$ -diversity of bacterioplankton communities. MRM model showed that WT was the most influential factor driving the seasonal variation in overall  $\beta$ -diversity across all lake categories (partial regression coefficient  $b = 0.088$  for OML,  $b = 0.062$  for ML, and  $b = 0.122$  for EL;  $P < .001$ ; Table S6, Supporting Information). Community dissimilarity due to balanced-variation component ( $\beta_{bc, bal}$ ) was most explained by WT in the oligo-mesotrophic ( $b = 0.117$ ,  $P < .001$ ) and eutrophic ( $b = 0.135$ ,  $P < .001$ ) lakes. In the mesotrophic lake, pH contributed the greater partial regression coefficient ( $b = 0.067$ ,  $P < .001$ ) to the variability in  $\beta_{bc, bal}$  than other variables such as WT ( $b = 0.056$ ,  $P < .01$ ), TP ( $b = 0.043$ ,  $P < .01$ ), and DOC ( $|b| = 0.032$ ,  $P < .05$ ). Community dissimilarity due to abundance-gradient component ( $\beta_{bc, gra}$ ) was significantly explained by DOC ( $b = 0.028$ ,  $P < .01$ ), turbidity ( $b = 0.024$ ,  $P < .05$ ), and pH ( $|b| = 0.023$ ,  $P < .05$ ) in the mesotrophic lake, while only NO<sub>3</sub>-N ( $|b| = 0.018$ ,  $P < .001$ ) made a significant contribution to  $\beta_{bc, gra}$  in the eutrophic lake. However, no variable showed

a significant impact on  $\beta_{bc, gra}$  in the oligo-mesotrophic lake (Table S6, Supporting Information).

### The balance between deterministic and stochastic assembly processes along the trophic state gradient

Our NST results showed that seasonal change in the relative importance of ecological stochasticity was closely associated with lake trophic states (Fig. 2A). For example, the influence of ecological stochasticity on the assembly of bacterioplankton communities in the oligo-mesotrophic, mesotrophic, and eutrophic lakes was greatest in autumn (61.3%), summer (76.6%), and winter (68.0%), respectively. Compared to those in the oligo-mesotrophic lake, stronger ecological stochasticity (71.9%) regulated the total temporal variation in bacterioplankton communities in the mesotrophic lake; however, the relative contribution of ecological stochasticity to the total community assemblages decreased at the eutrophic level (45.0%; Fig. 2A).

To further disentangle the variation in the relative importance of different ecological processes along the trophic state gradient in subtropical freshwater lakes, iCAMP analysis was performed. The iCAMP results indicated that the relative role of drift on the assembly of the overall bacterioplankton communities increased first and then decreased along the trophic state gradient (Fig. 2B). The importance of ecological stochasticity (i.e. dispersal and drift) was lower in the eutrophic lake (77.1% on average) than in the oligo-mesotrophic (87.4%) and mesotrophic (87.7%) lakes. In turn, selection played a greater role in shaping seasonally driven bacterioplankton community assemblages in the eutrophic lake



**Figure 1.** (A) NMDS plots of bacterioplankton communities based on total Bray–Curtis dissimilarity and its two components (i.e. balanced variation in abundances and abundance gradients). The function envfit maps environmental variables onto the NMDS ordination space as vectors. Only environmental vectors with a significance of  $P < .05$  (999 permutations) are displayed. The environmental variables with a longer line segment are more strongly correlated with the ordination than those with a shorter line segment. Chla, chlorophyll a; Cond, conductivity; DO, dissolved oxygen; DOC, dissolved organic carbon; NO<sub>2</sub>-N, nitrite nitrogen; NO<sub>3</sub>-N, nitrate nitrogen; TN, total nitrogen; TP, total phosphorus; and WT, water temperature.  $\beta_{bc}$ , total Bray–Curtis dissimilarity;  $\beta_{bc.bal}$ , balanced-variation component; and  $\beta_{bc.gra}$ , abundance-gradient component. (B) Comparisons of total temporal  $\beta$ -diversity and its two components across lake categories. Total temporal  $\beta$ -diversity and its two components within lakes are calculated from four seasonal data sets. Data are presented as mean  $\pm$  standard error of mean (SEM). Different lowercase letters over the bars denote statistically significant differences ( $P < .05$ , one-way ANOVA with Turkey HSD test). OML, oligo-mesotrophic lake; ML, mesotrophic lake; and EL, eutrophic lake.  $n$  represents the number of samples.

(22.9% on average) compared to oligo-mesotrophic (12.6%) and mesotrophic (12.3%) lakes (Fig. 2B).

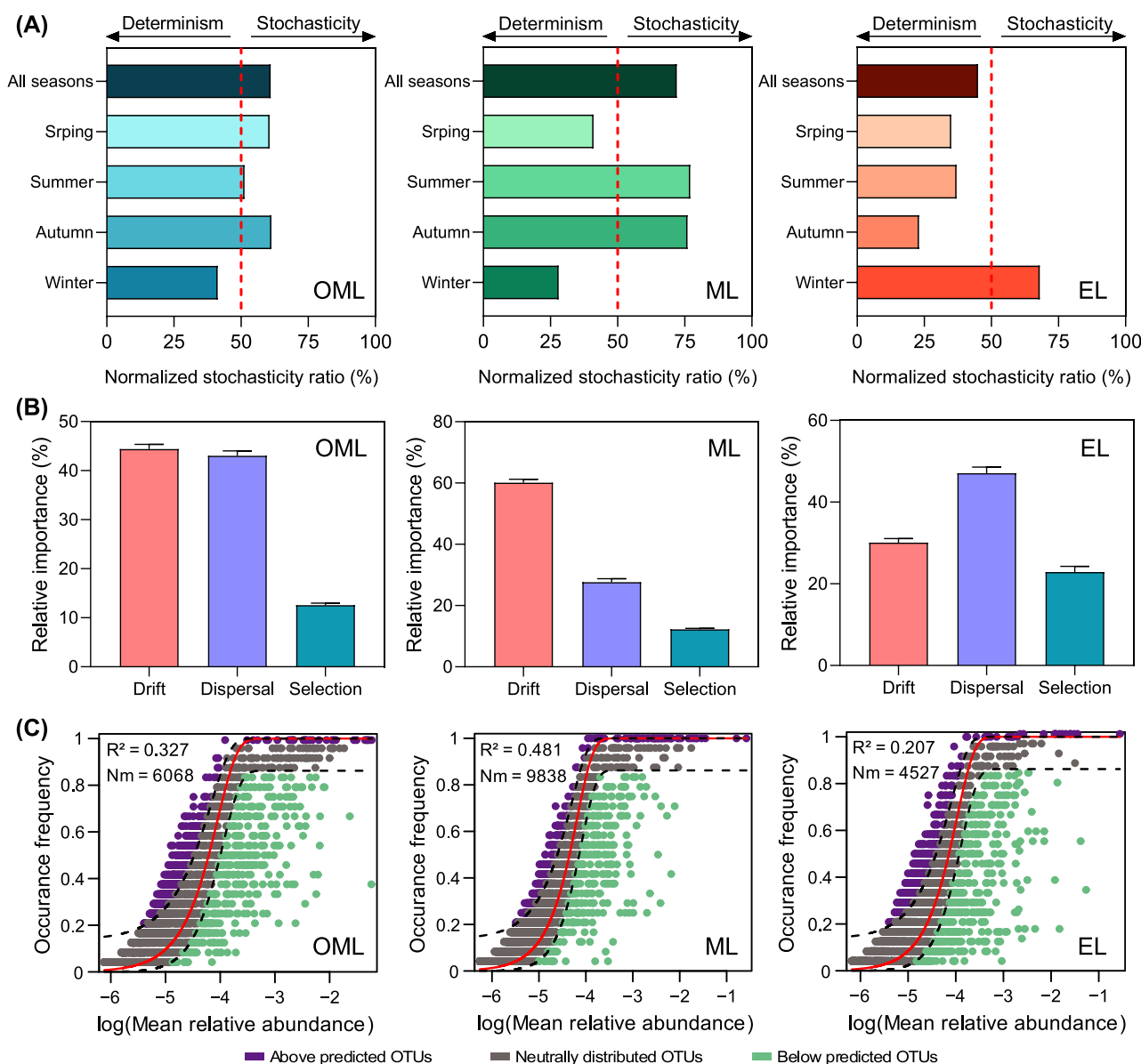
The relationship between the occurrence frequency of total bacterioplankton OTUs and their relative abundance was well-described by Sloan's NCM (Fig. 2C). The relative contributions of neutral processes first increased and then decreased with increasing eutrophication, explaining 32.7%, 48.1%, and 20.7% of the variation in bacterioplankton metacommunity structure for the oligo-mesotrophic, mesotrophic, and eutrophic levels, respectively.

### Co-occurrence networks of bacterioplankton communities along the trophic state gradient

Three metacommunity co-occurrence networks along the lake trophic gradient (i.e. oligo-mesotrophic, mesotrophic, and eutrophic states) were constructed based on all datasets from four seasons (Fig. 3), and four subnetworks for each lake during four seasons were analyzed (Table S7, Supporting Information). The observed network parameters (i.e. network diameter, modularity, average clustering coefficient, and average path length) were sig-

nificantly greater than those of the corresponding random networks (Z-test,  $P < .001$ , Table 2; Table S7, Supporting Information), suggesting that the co-occurrence networks were nonrandom, and they exhibited modular structures and small-world properties (i.e. high interconnectivity and efficiency). In addition, the average degree, average clustering coefficient, and modularity of bacterial communities in the mesotrophic lake were greater than those in the oligo-mesotrophic and eutrophic lakes (Table 2), indicating that the complexity of co-occurrence networks increased first and then decreased along the trophic state gradient. In comparison with the oligo-mesotrophic level, the co-occurrence networks at the higher trophic levels had a shorter average path length, which indicates an increased sensitivity of bacterioplankton networks to eutrophication. The metacommunity networks from three lakes primarily consisted of non-neutrally distributed nodes (Fig. 3A), suggesting that the metacommunity networks were strongly influenced by non-neutral processes. In the three lake trophic categories, we further observed that the proportion of non-neutrally distributed nodes increased as eutrophication





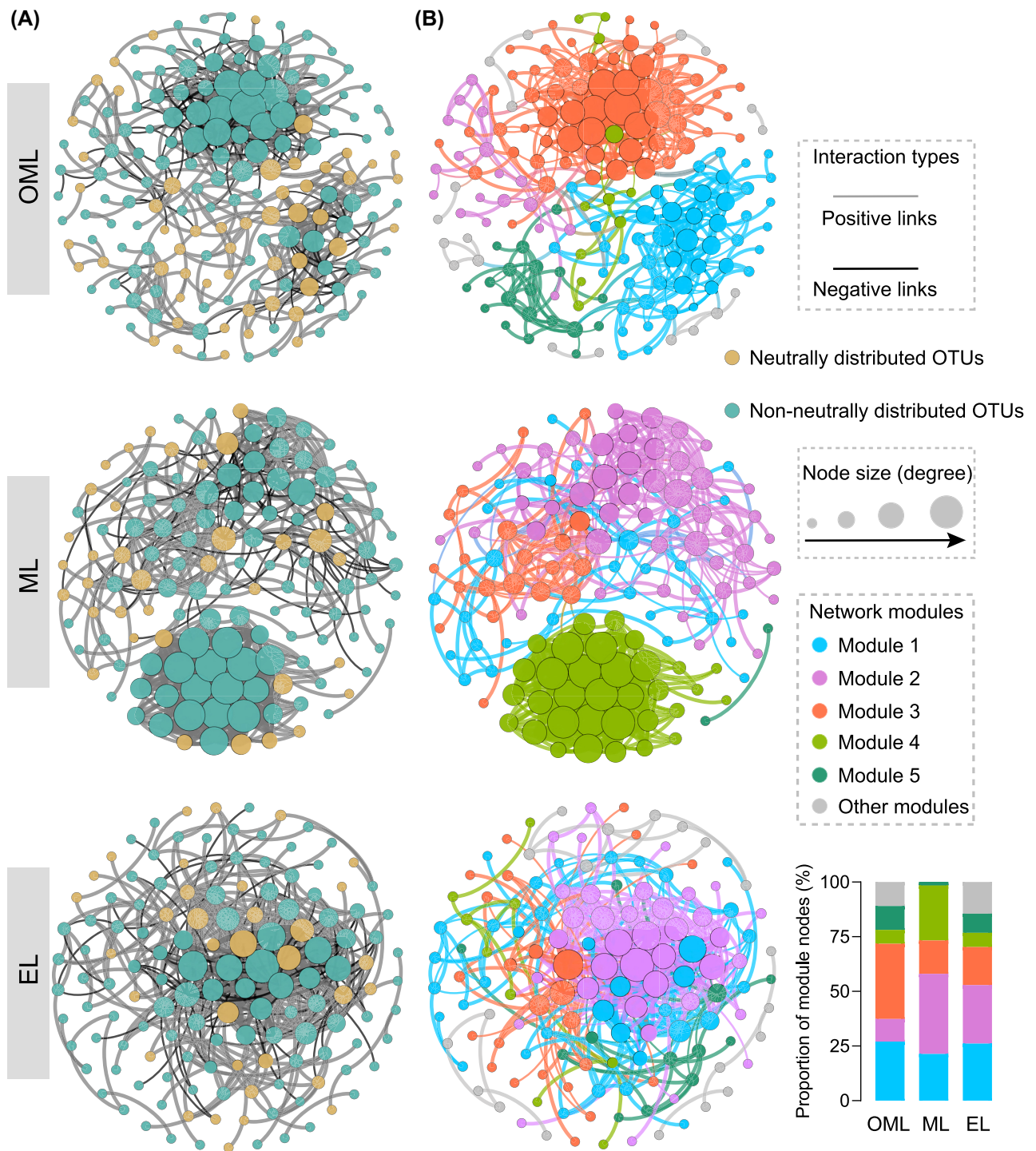
**Figure 2.** Ecological processes governing the assembly of bacterioplankton communities in subtropical lakes assessed by the normalized stochasticity ratio (NST), phylogenetic-bin-based null model analysis (iCAMP), and Sloan's NCM. **(A)** The NST index (ranging from 0 to 100%) of bacterioplankton communities was developed based on Bray–Curtis dissimilarity metric with 50% as the boundary point between more deterministic (< 50%) and more stochastic (> 50%) community assembly. **(B)** Relative importance (%) of different ecological processes (i.e. drift, dispersal, and selection) in the seasonal assembly of bacterioplankton communities. Data are presented as mean values  $\pm$  standard error of mean (SEM). Error bars represent SEM. Note the different scales of the y-axis among panels. **(C)** Fit of the NCM for total bacterioplankton communities in three lakes. In this model, OTUs were separated into three categories (above predicted OTUs, neutrally distributed OTUs, and below predicted OTUs). OTUs that occur more or less frequently than predicted by the NCM are shown in purple and green, respectively. OTUs that occur within prediction are shown in gray. Dashed black lines represent 95% confidence intervals around the model prediction (solid red line).  $R^2$  indicates the fit to the NCM; Nm indicates the metacommunity size times immigration. OML, oligo-mesotrophic lake; ML, mesotrophic lake; and EL, eutrophic lake.

increased (69.5% for the oligo-mesotrophic lake, 72.5% for the mesotrophic lake, and 75.4% for the eutrophic lake). Bacterioplankton co-occurrence networks in three lakes were characterized by modular structures, and they were all clearly divided into five major modules (Fig. 3B). These modules were specific to each of four seasons (Figure S8, Supporting Information). For instance, module IV in the oligo-mesotrophic and eutrophic lakes as well as modules I and V in the mesotrophic lake were specific to spring. Module II in the oligo-mesotrophic and mesotrophic lakes and module III in the eutrophic lake were specific to summer. Modules I, II, and V in the eutrophic lake as well as module IV in the mesotrophic lake were specific to winter.

## Discussion

### The contribution of ecological stochasticity to the temporal assembly of bacterioplankton communities first increased and then decreased along the trophic state gradient

Bacterioplankton community assemblages were jointly influenced by niche-based (deterministic) and neutral (stochastic) processes; however, our NST results based on null model analysis exhibited that the relative influence of ecological stochasticity on governing total bacterioplankton community assembly showed a first upward and then downward trend with increasing eutrophication. This finding supported our first hypothesis. The



**Figure 3.** Ecological networks of total bacterioplankton communities in three different subtropical lakes. **(A)** Co-occurrence patterns of bacterioplankton communities. Each node represents a bacterioplankton OTU; each link connecting two nodes represents a positive (gray line) or negative (black line) correlation. The nodes are colored according to their distributed types resulting from the NCM. **(B)** Network analysis showing the modular associations between bacterioplankton OTUs. The nodes are colored by their affiliated modules. A stacked bar graph exhibits the proportion of module nodes in different bacterioplankton networks. Node size is proportional to the node degree (i.e. the number of connections). A connection between two nodes indicates a robust (SparCC  $|r| > 0.80$ ) and statistically significant ( $P < .01$ ) correlation; the thickness of each connection is proportional to the absolute magnitude of the SparCC correlation coefficients. OML, oligo-mesotrophic lake; ML, mesotrophic lake; and EL, eutrophic lake.

**Table 2.** Topological features of the empirical networks of bacterioplankton communities in different trophic lakes and their associated random networks.

Network topological parameters		OML	ML	EL
Empirical network	Nodes	174	131	138
	Neutrally distributed nodes	53 (30.5%)	36 (27.5%)	34 (24.6%)
	Non-neutrally distributed nodes	121 (69.5%)	95 (72.5%)	104 (75.4%)
	Links	546	584	480
	Positive links	370 (67.8%)	496 (84.9%)	350 (72.9%)
	Negative links	176 (32.2%)	88 (15.1%)	130 (27.1%)
	Average degree	6.276	8.916	6.957
	Graph density	0.036	0.069	0.051
	Network diameter	12***	11***	11***
	Modularity	0.556***	0.579***	0.434***
	Average path length	4.853***	4.443***	3.713***
	Average clustering coefficient	0.545***	0.645***	0.571***
	Random network	Network diameter (SD)	5.668 (0.508)	4.132 (0.339)
Modularity (SD)		0.360 (0.009)	0.280 (0.008)	0.330 (0.009)
Average path length (SD)		2.998 (0.007)	2.457 (0.007)	2.737 (0.012)
Average clustering coefficient (SD)		0.036 (0.007)	0.069 (0.007)	0.051 (0.008)

Erdős-Rényi random networks (repeating 1000 times) are generated by rewiring all of the links with the same number of nodes and links as the corresponding empirical networks. The values highlighted with asterisks indicate significant differences between empirical and random networks (Z-test,  $P < .001$ ). SD, standard deviation. OML, oligo-mesotrophic lake; ML, mesotrophic lake; and EL, eutrophic lake.

possible interpretations for the upward trend could be that lake eutrophication increases the relative importance of ecological stochasticity by (i) promoting priority effects by enhancing the growth of earlier random colonizers (Fukami 2015), (ii) strengthening ecological drift by increasing stochastic variation in colonization or extinction across localities (i.e. sampling sites; Chase 2010), and (iii) weakening niche selection by providing greater resource availability (Zhou et al. 2014). The potential interpretations of the latter two points are confirmed by our iCAMP results. As for the downward trend, it is presumably that increased eutrophication could serve as an environmental filter (Zeng et al. 2019) that regulates bacterioplankton communities by reinforcing deterministic processes as the degradation of the aquatic environment leads to variations in abiotic environmental conditions and biotic interactions (Zhou et al. 2021), with a reduction in the relative importance of stochasticity (Cao et al. 2021, Zhang et al. 2021). This suggestion is further supported by our NCM analysis in which the relative contributions of neutral processes (e.g. stochastic dispersal and ecological drift) to the community assembly decreased in the eutrophic category.

In addition, we also found that the overall bacterioplankton assemblages in the eutrophic lake exhibited significantly greater variation in community dissimilarity (i.e.  $\beta$ -diversity) compared to those in the oligo-mesotrophic and mesotrophic categories, which agrees with a recent finding on freshwater reservoirs (Zhang et al. 2021). A stochasticity-dominated view suggests in aquatic ecosystems that higher  $\beta$ -diversity at higher nutrient levels is attributed to the greater role for stochastic relative to deterministic processes as eutrophication increases (Chase 2010), while another argument holds that increased eutrophication enhances the deterministic selection processes, resulting in greater variation in bacterial community composition (Langenheder et al. 2012). It is generally considered that the spatiotemporal  $\beta$ -diversity is explained by community assembly mechanisms involving (i) purely deterministic assembly processes, (ii) purely stochastic assembly processes, and (iii) the interaction between deterministic and stochastic assembly processes (Chase 2010, Zhang et al. 2021). In this study, bacterioplankton community assembly was less stochastic in the eutrophic lake than in the oligo-mesotrophic and mesotrophic lakes. Accordingly, higher overall temporal  $\beta$ -

diversity in the eutrophic category may be the result of the increased importance of deterministic processes. However, we should not ignore the contributions of neutral stochastic processes to total temporal  $\beta$ -diversity, as stochastic processes made more contributions to the community assembly in the oligo-mesotrophic and mesotrophic lakes.

### Balanced-variation component rather than abundance-gradient component dominated the temporal $\beta$ -diversity of bacterioplankton communities

Although both balanced-variation and abundance-gradient components contributed to total temporal  $\beta$ -diversity, we observed that balanced-variation component overwhelmed abundance-gradient component in dominating the total temporal  $\beta$ -diversity of bacterioplankton communities across all trophic categories. This finding was in agreement with the results for stream algae and macrophyte in freshwater ecosystems (Fernández-Aláez et al. 2020, Cook et al. 2022), suggesting that the dominance of the balanced-variation component in overall  $\beta$ -diversity is potentially prevalent in both microbial and macrobial taxa. Moreover, greater balanced-variation component was found in the eutrophic lake compared to oligo-mesotrophic and mesotrophic lakes, an indication of the importance of environmental filtering (López-Delgado et al. 2020). This finding supported our second hypothesis. However, opposite to our finding, a recent study on lotic algal assemblages has shown that increased nutrient enrichment suppressed balanced-variation component of total temporal  $\beta$ -diversity by reducing the importance of seasonal fluctuations in aquatic environmental conditions (Cook et al. 2022). The contradictory results yielded between the two studies may be linked to three factors: first, the geographical scale investigated (lake- vs. watershed-scale) and aquatic ecosystems (lentic vs. lotic ecosystems) of the two studies are greatly different; second, two biological groups (bacterioplankton vs. benthic algae) respond differently to eutrophication. For example, Cook et al. (2022) reported that taxon richness of benthic algal assemblages increased positively with nutrient enrichment, whereas no significant difference in taxon richness of bacterioplankton assemblages was observed along the

trophic state gradient; third, there may be dissimilar community assembly mechanisms between algae and bacteria due to their differences in body size. Compared to algal assemblages, bacterial assemblages that are characterized by higher dispersal rates and faster population growth rates due to a smaller body size, respond more rapidly to environmental fluctuations and tend to be governed by relatively stronger deterministic assembly processes (Aslani et al. 2022).

Among all lake categories, our results indicated that the spatiotemporal variability in overall  $\beta$ -diversity of bacterioplankton communities was most importantly explained by DO and WT that are highly associated with seasonal variability, while balanced-variation and abundance-gradient components were most largely correlated with  $\text{NO}_2\text{-N}$  and DOC, respectively. This implies that fluctuations in nutrient loading may contribute to changes in the relative roles of balanced-variation and abundance-gradient components on the structure of bacterioplankton communities. Consistent with our results, a field study on macrophyte community assemblages has also reported that balanced-variation and abundance-gradient components were shaped by dissimilar locally measured environmental drivers (Fernández-Aláez et al. 2020). These findings collectively suggest that environmental filtering exerts an indispensable role in the variation of overall  $\beta$ -diversity and its two components in aquatic environments (Fernández-Aláez et al. 2020, López-Delgado et al. 2020).

At the level of individual lake categories, we found a significant correlation between WT and the total temporal  $\beta$ -diversity and balanced-variation component in all lake categories; however, no significant correlation between WT and abundance-gradient component was found within lake categories, probably due to the small contribution of abundance-gradient component to the observed seasonally driven variation in bacterioplankton communities (Fernández-Aláez et al. 2020). It is also possible that the abundance-gradient component of total temporal  $\beta$ -diversity has greater seasonal stability. Hence, the overall bacterioplankton communities in response to thermal fluctuations depended mainly on the balanced-variation component in this study. In addition, a laboratory study has suggested that elevated nutrient concentrations reduced the stabilizing effects of biodiversity in aquatic microcosms of algae exposed to thermal fluctuations (Zhang and Zhang 2006). Thus, we would suggest that the overall bacterioplankton communities in the eutrophic lake characterized by lower community diversity (e.g. Shannon and Simpson indices) and greater balanced-variation component compared to the other two lakes may be at higher risk of community destabilization. This suggestion can be further supported by our co-occurrence network analysis we discussed below.

### Shifts in the balance between stochastic and deterministic processes induced changes in the complexity of bacterioplankton co-occurrence networks

Bacteria do not usually live in isolation but interact with a myriad of other bacteria to form complex ecological networks (Faust and Raes 2012). Based on microbial network construction analysis, our results indicated that network complexity (reflected by average degree, average clustering coefficient, and modularity) initially increased and then decreased along the trophic state gradient. This pattern could be explained by the shifts in the balance between stochastic and deterministic processes. It has been reported that the bacterial network complexity was closely associated with the relative importance of stochastic vs. deterministic assembly pro-

cesses (Wang et al. 2020, Cao et al. 2021). It is typically that non-random co-occurrence patterns of bacterial communities are of environmental preferences (Comte et al. 2016, Zeng et al. 2019), indicating that environmental filtering is an important determinant of taxa coexistence. It has been indicated that nutrient enrichment could enhance the environmental filtering to weaken the ecological interactions among bacteria taxa, thereby reducing network complexity (Wang et al. 2020). In this study, the proportion of non-neutrally distributed OTUs in our networks increased as eutrophication increased, suggesting the greater role of non-neutral processes on bacterioplankton network structure along the eutrophication gradient. Some evidence from freshwater ecosystems show that environmental stress filters the most sensitive taxa (Mo et al. 2021) and favors the occupation of more generalist ones (Zorzal-Almeida et al. 2021), leading to lower  $\alpha$ -diversity. In the present study, the greater environmental filtering at the eutrophic level compared to other two trophic levels potentially contributed to a reduction in taxon diversity, which may in turn result in a decrease in bacterioplankton network complexity.

Recent study has been empirically demonstrated that network stability was strongly and positively correlated with network complexity in microbial ecology (Yuan et al. 2021), which also supported the classical ecological belief that increasing complexity begets increased stability (MacArthur 1955). We are, thus expected that the stability of bacterioplankton co-occurrence networks would be reduced under eutrophic conditions. In agreement with our expectation, a related study has also suggested that aquatic eutrophication tends to destabilize bacterial co-occurrence networks (Zhou et al. 2021). Moreover, it has been documented that small-world network properties were more robust to environmental changes (Comte et al. 2016). Our constructed co-occurrence networks had small-world properties across all trophic categories, but the eutrophic network had a shorter average path length than networks in other two trophic categories. This indicates that bacterioplankton communities could be more susceptible to variations in environmental conditions in the eutrophic context and, thus weaker to resist environmental disturbances. Moreover, given that networked communities are strongly linked to ecosystem function (Wang et al. 2020, Yuan et al. 2021), preserving the structure of bacterioplankton networks is critical for future aquatic ecosystem conservation, especially in eutrophic lakes.

### Conclusions

Our study revealed that the relative role of stochastic processes on total bacterioplankton community assembly elevated first and then declined along the trophic state gradient in subtropical freshwater lakes. Moreover, balanced variation in abundances made a larger contribution than abundance gradients to total temporal  $\beta$ -diversity of bacterioplankton communities across all trophic categories. Lake eutrophication did not lead to an increase in abundance-gradient component of bacterioplankton communities; conversely, greater balanced-variation component was observed in the eutrophic category. Within each lake category, unlike total temporal  $\beta$ -diversity and balanced-variation component, there was no significant relationship between WT and abundance-gradient component, an indication of a possible greater seasonal stability of abundance-gradient component. The network complexity of bacterioplankton communities along the eutrophication gradient was associated with the shifts in the balance between deterministic and stochastic processes. Freshwater eutrophication tended to reduce the complexity and, thus the stability of the bacterioplankton community network. Our study

provides novel insights into the ecological impacts of freshwater eutrophication on bacterioplankton community assemblages in subtropical lake ecosystems. This research further suggests that the conservation of microbial diversity and network structure is of great significance for subtropical lakes with different trophic states.

## Supplementary data

Supplementary data are available at [FEMSEC](https://femsec.org) online.

## Data availability

The raw sequencing data have been submitted to the NCBI Sequence Read Archive (SRA) database under the BioProject number PRJNA780039.

## Authors' contributions

D.Z., J.Z., and Q.L.W. designed the experiment. C.J. and D.Z. conducted the experiment. C.J., D.Z., and J.Z. analyzed the data and visualized the figures. C.J. and D.Z. wrote the manuscript. C.J., D.Z., and J.Z. contributed to the discussion of the manuscript. D.Z., J.Z., and Q.L.W. revised the manuscript. D.Z., J.Z., and Q.L.W. contributed reagents and materials. All authors approved the final submitted manuscript.

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**Conflicts of interest statement.** None declared.

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