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Eutrophication causes microbial community homogenization via modulating generalist species

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ABSTRACT

Eutrophication substantially influences the community structure of aquatic organisms and has become a major threat to biodiversity. However, whether eutrophication is linked to homogenization of microbial communities and the possible underlying mechanisms are poorly understood. Here, we studied bacterial and fungal communities from water and sediments of 40 shallow lakes in the Yangtze-Huaihe River basin, a representative area characterized by intensifying eutrophication in China, and further examined the beta diversity patterns and underlying mechanisms under eutrophication conditions. Our results indicate that eutrophication generally caused biotic homogenization of bacterial and fungal communities in both habitats showing decreased community variations for the sites with a higher trophic state index (TSI). In the two habitats, community dissimilarities were positively correlated with TSI changes for both taxonomic groups, while the local contribution to beta diversity (LCBD) remarkably declined with increasing TSI for the fungal community. These phenomena were consistent with the pivotal importance of the TSI in statistically accounting for beta diversity of bacterial and fungal communities in both habitats. In addition, we found that physicochemical factors such as water temperature and pH were also important for bacterial and fungal communities in water, while heavy metal elements were important for the communities in sediments. Interestingly, generalist species, rather than specialist species, were revealed to more dominantly affect the variations in beta diversity along the trophic gradient, which were quantified by Bray-Curtis dissimilarity and LCBD. Collectively, our findings reveal the importance of generalist species in contributing to the change of beta diversity of microbial communities along trophic gradients, which have profound implications for a comprehensive understanding of the effects of eutrophication on microbial community.

1. Introduction

Eutrophication has become a global aquatic environmental issue, often resulting in the swamping of lakes, deterioration of water quality, loss of diversity and changes of ecosystem structure and function (Alexander et al., 2017; Ansari et al., 2010). One of the main consequences caused by eutrophication is biotic homogenization (McKinney and Lockwood, 1999), a complex process in which the compositional similarity between communities increases (Otto et al., 2020), as has previously been observed in macroorganisms such as fish (Menezes et al., 2015), macrophytes (Salgado et al., 2018), macroinvertebrates (Donohue et al., 2009) and zooplankton (Liu et al., 2020b). Theoretically, the occurrence of biotic homogenization could be simply summarized as the following three aspects: (1) extinction of native species, (2) invasion of widespread nonnative species, and (3) range expansion of native generalists (McKinney and Lockwood, 2001; Olden et al.,

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2004). For example, in the case of fish, it has been reported that worldwide freshwater fish homogenization is strongly driven by the species translocated within a realm and by only a few nonnative generalists whereas most nonnative species contribute to the differentiation effect (Toussaint et al., 2016). Similarly, the increased community similarity of aquatic macrophytes may be caused by invasive species via their own presence in multiple sites and influence on the composition of native species (Muthukrishnan et al., 2020). However, current understandings of homogenization of aquatic biological communities, particularly microbes, remain elusive.

Biotic homogenization can be quantified by beta diversity, which can be calculated using the dissimilarity indices such as Bray-Curtis and Jaccard dissimilarity (Bray and Curtis, 1957; Jaccard, 1900) and the ecological uniqueness of local site (Legendre and De Caceres, 2013). The selection of indicators is important for quantifying biotic homogenization because they can capture different aspects of information about the community (Petsch, 2016). The most straightforward and commonly used beta-diversity indicator for quantifying biotic homogenization is the dissimilarity index, among which the Bray-Curtis and Jaccard dissimilarity are used most frequently in homogenization studies (Baeten et al., 2012). Jaccard's dissimilarity index is used most frequently for occurrence data, while the Bray–Curtis dissimilarity index is appropriate for the data with abundance information (Olden and Rooney, 2006). Biotic homogenization can be measured by comparing the dissimilarity among biological communities at two distinct times or the average pairwise dissimilarity between sites in different environmental states, and the degree of homogenization can be evaluated by comparing the change rate of the dissimilarity index with temporal or spatial distance (Petsch, 2016). In addition, as another beta diversity that is based on each local site, local contributions to beta diversity (LCBD) are proposed to quantify the community uniqueness of each site; a lower LCBD value indicates a site with a smaller difference in species composition (Legendre and De Caceres, 2013). The LCBD can explain the site from a biological conservation perspective, and large LCBD values represent the sites of high conservation values (Legendre, 2014). Thus, although rarely used to quantify biotic homogenization in literature, we proposed that the LCBD could be a complementary metric to pairwise similarity to help understand biotic homogenization and provide a theoretical reference for ecological conservation.

Here, we studied bacterial and fungal communities from water and sediments of 40 shallow lakes in the Yangtze-Huaihe River basin, China, and investigated the patterns of beta diversity quantified by the pairwise similarity and LCBD along the trophic gradient. This basin has the most abundant water sources in China, with the highest density of lakes and number of freshwater lakes. It is an area with a relatively high level of urbanization and agricultural development (Li et al., 2019). Due to extensive human activities and accelerated urbanization, it has become a representative area characterized by increasing eutrophication (Liu et al., 2020a). Our main aims were to determine the following questions: (1) Are there general patterns of beta diversity along the trophic gradient for bacteria and fungi in water and sediments? If so, do these patterns prove the existence of biological homogenization? (2) What are the main environmental drivers of microbial community compositions considering eutrophication, heavy metal pollution or other physicochemical factors? (3) What kind of species, that is, specialists or generalists, contribute more to beta diversity and explain the observed biological homogenization?

2. Materials and methods

2.1. Sampling

From August to September 2019, we collected paired samples from water and surface sediments at 98 sites in 40 lakes in the Yangtze-Huaihe River basin, China (28.55-33.29°N, 113.00-119.80°E; Fig. 1A). We selected 1-4 sampling sites according to the area of each lake and avoided the sites with obvious human disturbance such as dredging, hydrological engineering and cage aquaculture. At each site, the water from the upper 50-cm lake surface layer was collected with the Schindler sampler and then the surface sediment (0-1 cm) was collected with a 6-cm diameter gravity core. It should be noted that surface sediments could not be retrieved with our sampling cores in several sites, and thus we finally obtained 98 and 80 samples for water and sediment habitats, respectively. In situ, latitude and longitude were recorded using a GPS device. We measured environmental variables using a portable multiparameter water quality analyzer (ProPlus, YSI, USA), including water depth (WD), water conductivity (SPC), pH, transparency (SD), turbidity (TUB), dissolved oxygen (DO) and water temperature (WT). After thorough mixing, all samples were placed in sealed containers and stored at -20°C before further analysis including DNA extraction and chemical analyses.

For the water sample analysis, we used the unfiltered water to analyze the total nitrogen (TN) and total phosphorus (TP) and the filtered water with a 0.7 μm GFF membrane to measure the dissolved total nitrogen (DTN), ammonium nitrogen (NH_4^+), nitrate nitrogen (NO_3^-), nitrite nitrogen (NO_2^-), dissolved total phosphorus (DTP) and dissolved phosphate ion (PO_4^{3-}). We measured main ions such as K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^- and SO_4^{2-} using ion chromatography (Dionex DX-600, USA). In addition, chlorophyll a (Chl-a) was extracted by acetone solution and quantified by spectrophotometry (Steinman et al., 2017).

For surface sediments, 0.7 g freeze-dried sediment was mixed with 30 mL of deionized water, followed by ultrasonic treatment for 2 h and centrifugation at 4,000 rpm for 40 min. The centrifuged supernatant was filtered through a 0.45 μm membrane to obtain the sediment extract. The ammonium nitrogen (NH_4^+), nitrate nitrogen (NO_3^-), nitrite nitrogen (NO_2^-), total phosphorus (TP), dissolved phosphate ion (PO_4^{3-}) and dissolved total organic carbon (DOC) of the sediment extract were measured. The conductivity (SPC) and pH were obtained by measuring the liquid obtained by mixing 0.3 g freeze-dried sample with 6 ml of deionized water. In addition, we used ICP-AES to measure metal elements, including Al, Ba, Be, K, Mg, Mn, Na, Ca, Fe, Sr, Ti, V, Zn, Cr, Co, Ni, Cu, As, Mo, Cd, Sb, Tl and Pb.

To evaluate the trophic status of each site, we calculated a trophic state index (TSI) based on the concentrations of TN, TP, Chl-a and SD in the water environment (Carlson, 1977; Zhang et al., 2018b). Specifically, the calculation of the TSI was as follows:

$$\begin{aligned}\mathrm{TSI} =& 0.326 * \mathrm{TSI(Chl-a)} + 0.219 * \mathrm{TSI(TN)} + 0.230 * \mathrm{TSI(TP)} \\ &+ 0.225 * \mathrm{TSI(SD)}\end{aligned} \quad (1)$$

$$\mathrm{TSI(Chl-a)} = 10 * [2.5 + 1.086 * \ln(\mathrm{Chl-a})] \quad (2)$$

$$\mathrm{TSI(TP)} = 10 * [9.436 + 1.624 * \ln(\mathrm{TP})] \quad (3)$$

$$\mathrm{TSI(TN)} = 10 * [5.453 + 1.694 * \ln(\mathrm{TN})] \quad (4)$$

$$\mathrm{TSI(SD)} = 10 * [5.118 - 1.94 * \ln(\mathrm{SD})] \quad (5)$$

where TSI(Chl-a), TSI(TN), TSI(TP), and TSI(SD) are the trophic state index in relation to Chl-a (mg/L), TN (mg/L), TP (mg/L), and SD (m), respectively. Five trophic levels were defined: oligotrophic (TSI < 30), mesotrophic (30 ≤ TSI ≤50), slightly eutrophic (50 < TSI ≤ 60), moderately eutrophic (60 < TSI ≤70) and highly eutrophic (TSI >70) (Huo et al., 2013).

For detailed information on the environmental variables of the four trophic groups, see Supporting Information Tables S1 and S2 for water and sediments, respectively.

2.2. Sequence analyses

We extracted the bacterial and fungal DNA from the filtered water and sediment samples using a FastDNA spin kit for soil (MoBio

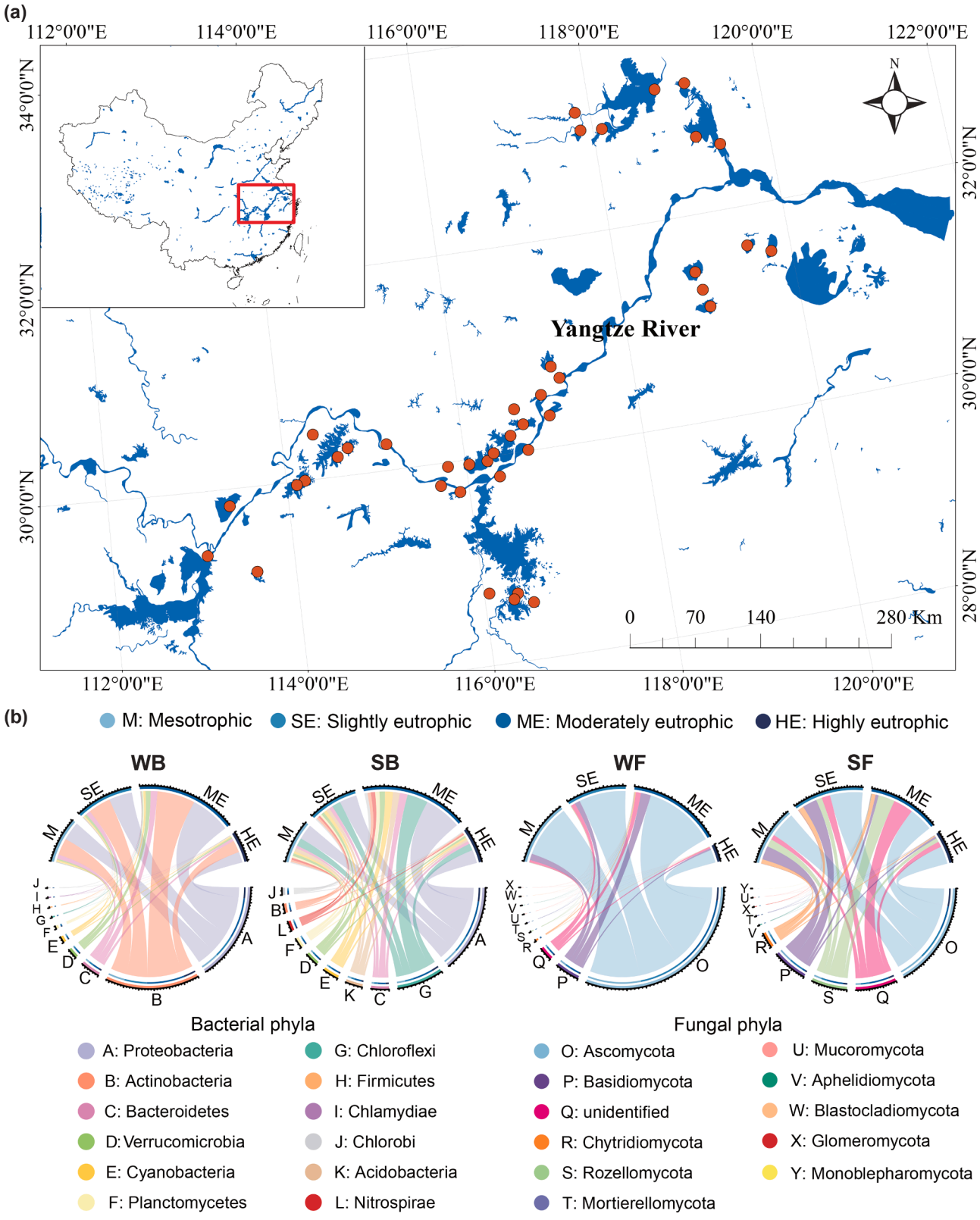

Fig. 1. Sample information. (a) Distribution of the 40 shallow lakes in the middle and lower reaches of the Yangtze and Huaihe Rivers, China. A total of 98 water samples and 80 sediment samples were collected. (b) Circular visualization of the dominant bacterial and fungal phyla in four trophic types for water and sediment samples. The inner circular diagram shows the relative abundance of different bacterial and fungal phyla in four sample types. Only the dominant phylum with the top 10 most abundant phyla is shown. The width of different colored ribbons represents relative abundance of the corresponding phyla in each sample type and the width is directly proportional to their relative abundance. WB: bacteria in water samples; SB: bacteria in sediment samples; WF: fungi in water samples; SF: fungi in sediment samples.

Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. For bacteria, the V4 region of 16S rRNA genes was amplified in triplicate by PCR with primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). For fungi, the ITS2 region was amplified using primers gITS7F (5′- GTGARTCATCGARTCTTTG-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′). The PCR products of triplicate reactions were combined and quantified with PicoGreen (Eugene, OR, USA) and pooled in equal molar amounts to maximize the even-sequencing efforts for all samples. The pooled mixture was purified with a QIAquick Gel Extraction Kit (QIAGEN Science, Germantown, MD, USA) and requantified with PicoGreen. Sample libraries for sequencing were prepared according to the MiSeq Reagent Kit Preparation Guide (Illumina, San Diego, CA, USA) and sequenced on the Illumina MiSeq platform.

For bacteria, the sequences were processed using the script 'pick_open_reference_otus.py' in QIIME v1.9.1 (Caporaso et al., 2010b). Sequences longer than 450 bp were denoised with the Denoiser algorithm (Reeder and Knight, 2010) and clustered into operational taxonomic units (OTUs) at a 97% similarity level with the seed-based UCLUST algorithm (Edgar, 2010). Representative sequences were extracted from each OTU, and a sequence alignment was performed with PyNAST (Caporaso et al., 2010a), and a taxonomy assignment was performed using the Greengenes database (DeSantis et al., 2006). For fungi, the clustering of OTUs was the same as for bacteria whereas taxonomic identification of each OTU was determined using the UNITE database (Nilsson et al., 2019). Finally, the bacterial and fungal sequences were both rarefied at the minimum sequence abundance to ensure that the biodiversity was not influenced by variation in abundance or sampling intensity.

2.3. Statistical analyses

First, we performed Kruskal–Wallis and Wilcoxon tests to examine the significant differences in each environmental variable for water or sediment samples across different trophic groups. In addition, the linear and quadratic models were used to explore the relationships between the TSI and nutrient-related environmental variables in water (TN, DTN, NH_4^+, NO_3^-, NO_2^-, TP, DTP, PO_4^{3-}, Chl-a and SD) and sediment (NH_4^+, NO_3^-, NO_2^-, TP and PO_4^{3-}) environments, and the better models were selected based on lower values of the Akaike information criterion (AIC) (Yamaoka et al., 1978). To analyze the bacterial and fungal community composition, we visualized the relative abundances of the top 10 bacterial and fungal phyla in four sample types and explored the relationships between the TSI and relative abundance of these phyla with linear or quadratic models based on the lower value of AIC (Yamaoka et al., 1978).

Second, we explored the differences between different trophic groups of bacterial and fungal community structures in water and sediment samples by nonmetric multidimensional scaling (NMDS) based on the Bray-Curtis similarity. We tested for homogeneity of dispersion using the permutational analysis of multivariate dispersions (PERMDISP) (Anderson et al., 2006), which assessed the within-group differences using the average value of the individual observation distances to the centroid of the own group. In addition, we calculated the mean values of beta diversity for each trophic group based on the Bray-Curtis similarity (Anderson et al., 2006) and compared them by Kruskal–Wallis and Wilcoxon tests. We also used Kruskal–Wallis and Wilcoxon tests to determine the variation in alpha diversity across different trophic groups, namely, the species richness, Pielou's evenness (Pielou, 1966), Shannon (Shannon, 1948) and Chao1 (Chao, 1984) indices.

Third, to explore the patterns of beta diversity, we calculated the Bray-Curtis dissimilarity among different sites and LCBD for each site (Legendre and De Caceres, 2013). Mantel tests were used to determine the relationships between the TSI change and Bray-Curtis dissimilarity matrices with Pearson's correlation and 999 permutations. The turnover rate of communities was calculated as the slope of the ordinary least-squares regression line fitted to the relationship between the TSI distance and community dissimilarity. Additionally, we explored the relationships between the TSI distance and Bray-Curtis dissimilarity for each trophic group using Mantel tests and compared the turnover rate of communities for each trophic group. Linear or quadratic models were applied to analyze the relationships between LCBD and TSI and the most appropriate models were selected based on AIC (Yamaoka et al., 1978).

Fourth, to analyze the important drivers of beta diversity, we used distance-based redundancy analysis (db-RDA) (Legendre and Anderson, 1999), multiple regression on distance matrices (MRM) (Legendre et al., 1994) and multiple linear regression (MLR). Before these analyses, we excluded the variables with Pearson's correlation coefficients larger than 0.7 (Leathwick et al., 2006). RDA was used to select the important drivers for community composition with Hellinger-transformation (Legendre and Gallagher, 2001). MRM analyses were conducted to quantify the relative importance of the environmental variables on the Bray-Curtis distance for bacteria and fungi. All environmental variables were z-score standardized (i.e., mean = 0, SD = 1) before the MRM analyses, as these standardizations can make the importance of all environmental variables comparable. We chose the final models using forward selection of explanatory variables based on AIC that best accounted for variation in beta diversity and used the partial regression coefficients of these variables as the measure of the relative importance of variables on response variables. Furthermore, MLR was employed to examine environmental drivers of LCBD. For the MLR analyses, the selection method of the final models was similar to MRM, and the partial regression coefficients of each variable were used to compare the effect of each variable on the LCBD.

Finally, to test to what degree the homogenization is affected by generalist and specialist species, we estimated each species' niche breadth along the TSI gradients (that is, TSI range size) by the absolute difference between the maximum and minimum of species TSI values across all samples and then gradually removed the species with large or small niche breadths from the observed communities (i.e., TSI range size), respectively (Wang et al., 2020). For each taxonomic group in both water and sediments, we classified the species into 20 range-size categories according to their TSI range size. For example, for generalist species, we removed the species starting from the category of the largest TSI range size for the observed community and then recalculated the Bray-Curtis dissimilarity and LCBD, respectively. We used the Bray-Curtis dissimilarity as the response variable for all subcommunities and assessed the effects of TSI on beta diversity in each scenario by linear models and MRM analyses. The slopes in the linear model and partial regression coefficients of TSI in the MRM model were used to quantify the effects of removal of generalist species on the degrees of biotic homogenization. Additionally, we selected the LCBD as response variables, and linear models and MLR analyses were used to determine the influences of the TSI on beta diversity for all sub-communities. The effects of generalist species removal on the degrees of biotic homogenization can be compared by the slopes in the linear model and partial regression coefficients of TSI in the MLR model. We used the novel approach to explore the relative importance of generalists and specialists on biotic homogenization, which has no specific bias in thresholds in relative abundance and/or species occurrence for selecting or grouping species. To confirm the reliability of our results, we also classified the species into 20 categories based on Levins' niche breadth (*B*) (Levins, 1968), a traditional niche breadth index, and then performed the same analyses. Furthermore, we divided all species into generalist, neutral taxa and specialist groups according to neutral model (Sloan et al., 2006) and explored the relationships for each group between the TSI distance and Bray-Curtis dissimilarity and between LCBD and TSI using Mantel tests and linear models, respectively. More detailed information about traditional statistical analyses based on Levins' niche breadth (Supplementary Materials and Methods) and the corresponding results (Figs. S1–S4) are provided in supplementary materials. Moreover, we

calculated the species contribution of beta diversity (SCBD) to verify the importance of generalist and specialist species to biotic homogenization (Legendre and De Caceres, 2013). We quantified the relative contributions of deterministic processes and stochastic processes by using null model analysis based on the Raup-Crick metric of beta diversity (β_{RC}) (Chase et al., 2011) for all sub-communities. We were unable to distinguish detailed ecological processes combining both phylogenetic beta diversity and β_{RC} (Ning et al., 2020; Stegen et al., 2015) because the ITS-based fungal phylogenetic information is not reliable (Schoch et al., 2012).

All of the above statistical analyses were conducted with stats V3.6.0, base V3.6.0, MASS V7.3-54 (Ripley et al., 2013), circlize V0.4.13 (Gu et al., 2014), vegan V2.5-7 (Oksanen et al., 2013) and adespatial 0.3-14 () in R 3.6.0.

3. Results

The 98 sampling sites had a mean TSI of 60.13, ranging from 38.62 to 76.89, and most sites were eutrophic according to a TSI threshold larger than 50 (Zhang et al., 2018b). We categorized the sites into four trophic groups based on the calculated TSI: mesotrophic ($30 \leq TSI \leq 50$, 19 sites), slightly eutrophic ($50 < TSI \leq 60$, 24 sites), moderately eutrophic ($60 < TSI \leq 70$, 42 sites), and highly eutrophic ($TSI > 70$, 13 sites). We found that the environmental variables related to eutrophication or nutrient levels generally showed significant relationships with TSI in both water and sediment habitats (Fig. S5; $P < 0.05$). For instance, chlorophyll a, total nitrogen and dissolved total nitrogen in water tended to increase with increasing TSI, while the transparency decreased. The phosphorus content in water (total phosphorus, dissolved total phosphorus, and dissolved PO_4^{3-}) and sediments (total phosphorus, and

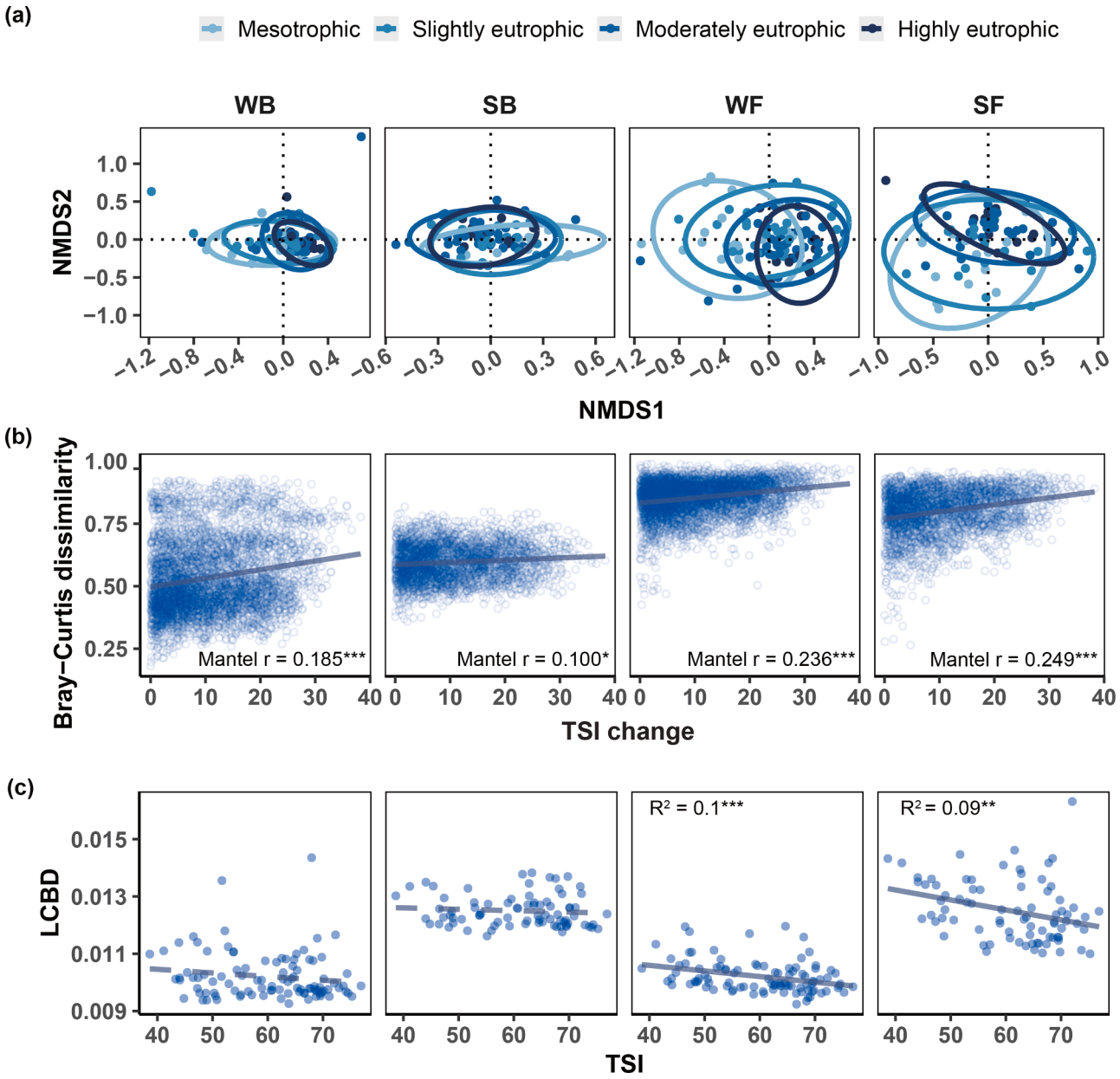

Fig. 2. Beta diversity patterns along the trophic gradient. (a) Nonmetric multidimensional scaling (NMDS) based on the Bray-Curtis dissimilarity matrices, showing the differences in community composition among the four lake trophic groups. (b) Pairwise relationships between the Bray-Curtis dissimilarity and TSI distance based on the Euclidean distance for observed communities. Statistically significant relationships ($P \leq 0.05$) indicated by Mantel tests are illustrated by solid lines, while dashed lines indicate non-significant relationships ($P > 0.05$). (c) Linear regression relationships between the LCBD and TSI for observed communities. Statistically significant relationships are shown with solid lines ($P \leq 0.05$), while dashed lines show non-significant relationships ($P > 0.05$).

dissolved PO_4^{3-}) exhibited consistent U-shaped patterns along the TSI gradient, reaching a minimum at TSI values between 45 and 60. However, the dissolved NH_4^+, NO_3^-, and NO_2^- showed non-significant patterns for both habitats (Fig. S5; $P > 0.05$). Furthermore, the above variables of both habitats generally supported the classification of trophic groups by showing significant differences among the four groups (Tables S1 and S2; $P < 0.05$).

The main phyla of the two taxonomic groups also showed predictable patterns along the TSI gradient. For bacteria, the most abundant phyla in water were *Proteobacteria* and *Actinobacteria*, followed by *Bacteroidetes*, while *Proteobacteria* was most abundant in sediments, followed by *Chloroflexi* (Fig. 1B). In water, the relative abundances of *Chlamydiae* and *Planctomycetes* increased with TSI (Fig. S6; $P < 0.05$); in sediments, the relative abundance of *Acidobacteria* and *Actinobacteria* in sediments decreased with increasing TSI, while that of *Bacteroidetes* increased toward high TSI (Fig. S6; $P < 0.05$). For fungi, the most abundant phylum

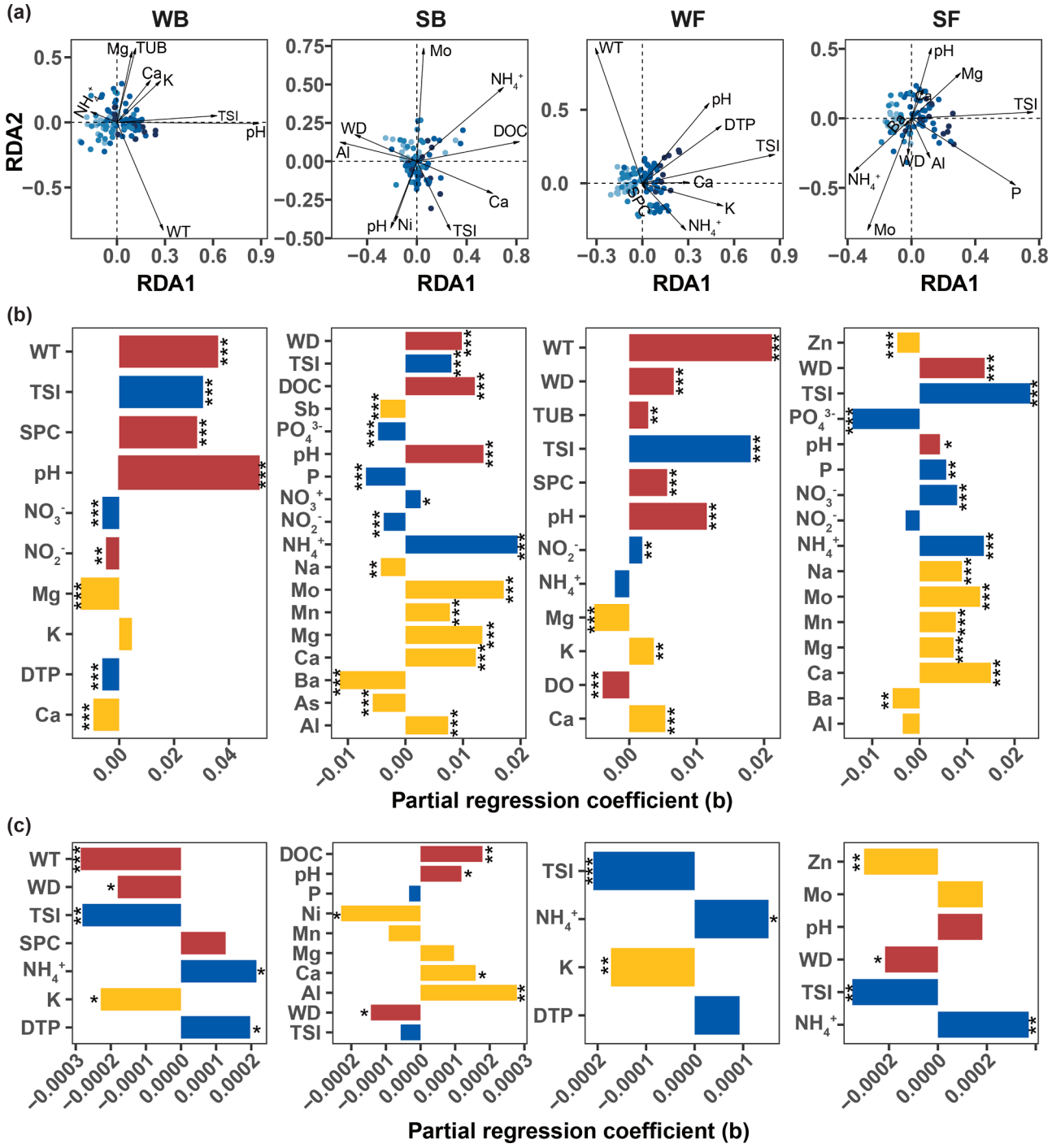

Fig. 3. Environmental factors affecting the beta diversity, identified with distance-based redundancy analysis (a), multiple regression on distance matrices (b) and multiple linear regression (c). (a) Arrows show the significant environmental variables after forward selection. The point colors represent different trophic groups. (b) The Bray-Curtis dissimilarity was calculated as a response variable and forward selection was used to determine the final model. The partial regression coefficients show the relative importance of each variable for the Bray-Curtis dissimilarity. (c) We calculated the LCBD as a response variable and then used forward selection to determine the final model. The partial regression coefficients show the relative importance of each variable for LCBD. Significance levels: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

in both habitats was *Ascomycota*, followed by *Basidiomycota* in water and by *Rozellomycota* and *Basidiomycota* in sediments (Fig. 1B). The relative abundances of *Ascomycota, Basidiomycota* and *Chytridiomycota* in sediments and *Ascomycota* in water decreased with increasing TSI (Fig. S6; $P < 0.05$).

Along the trophic gradient, we observed taxonomic homogenization for bacteria and fungi. Bacterial and fungal communities in water and sediments generally became more clustered in highly eutrophic lakes, measured by the distance to group centroid (Fig. 2A; Table S3). Such a phenomenon can be supported by the average Bray-Curtis dissimilarity for each trophic group, which showed lower values in highly eutrophic lakes than in lakes with lower trophic status (Table S4). Moreover, the average richness increased toward higher trophic groups for bacteria and fungi in water but not in sediments (Table S5).

The taxonomic homogenizations were also confirmed by quantifying the changes in beta diversity indicated by the LCBD and Bray-Curtis dissimilarity along TSI. For bacteria or fungi in both habitats, there were consistent environmental distance-decay relationships along TSI changes (Fig. 2B; $P < 0.05$), and the communities in mesotrophic lakes showed the fastest turnover rates with the highest slopes (Fig. S7). In both habitats, LCBD showed significantly negative correlations with the TSI for fungi (Fig. 2C, $P < 0.01$) but non-significant correlations for bacteria (Fig. 2C, $P > 0.05$).

The TSI was further revealed to be a primary driver of bacterial and fungal community compositions in water or sediments. We used RDA to select environmental variables affecting bacterial and fungal community compositions and MRM analyses and multiple linear regressions for LCBD to investigate the drivers of beta diversity (pairwise dissimilarity and LCBD). In these three analyses, TSI was generally selected as a significant predictor for both taxonomic groups in water and sediments (Fig. 3), although the first and second axes of the RDA (WB: 10.8% and 4.8%; SB: 6.7% and 4.5%; WF: 4.2% and 3.0%; SF: 5.6% and 4.5%) only explained a small amount of the variation observed, and R^2 values of the final models of the MRM (WB: 0.211; SB: 0.393; WF: 0.180; SF: 0.190) and multiple regression (WB: 0.295; SB: 0.395; WF: 0.167; SF: 0.319) were also low (Fig. 3). Additionally, RDA analyses showed that pH and water temperature were the main drivers for both bacterial and fungal communities in water, while water depth, pH and NH_4^+ and metal elements such as Mo were the main drivers for sediment communities (Fig. 3A). Notably, the community composition in water was significantly impacted by the turbidity for bacteria and the dissolved total phosphorus for fungi, while the community composition in sediments was significantly impacted by the dissolved organic carbon for bacteria and the total phosphorus for fungi (Fig. 3A). In line with the RDA results, MRM analyses revealed that community dissimilarity was largely driven by water temperature and pH for bacteria and fungi in water and by metal elements, water depth, pH and NH_4^+ for sediments. In the multiple linear regression for LCBD, the most important drivers were water temperature and TSI for bacteria in water, followed by the potassium and electrical conductivity, while TSI was a driver for fungi in water, followed by potassium (Fig. 3C). In sediments, the most important driver was aluminum for the bacterial LCBD, followed by nickel, while the NH_4^+ and TSI were the most important drivers for fungi.

Generalist species, quantified by large TSI range sizes, generally showed stronger environmental affinity along the trophic gradient and contributed more to biotic taxonomic homogenizations. For instance, the slopes of the relationships between the community dissimilarity and TSI changes decreased when generalist species were gradually removed for bacteria and fungi in both habitats, except for bacteria in sediments (Figs. 4A and S8A). In the MRM analyses, the removal of generalist species resulted in lower absolute values of the partial regression coefficients of TSI with the exception of bacteria in sediments (Fig. 4B). The above importance of generalist species to the beta diversity was further supported by the sequential removal of specialist species, which showed that the slopes of the relationships between the community dissimilarity and TSI changes or partial regression coefficients of TSI in MRM analyses increased, albeit very slightly except for bacteria in sediments (Fig. S9A and B). Therefore, biotic homogenization with increasing TSI is largely driven by generalist species rather than specialist species.

Notably, when we used the LCBD to quantify beta diversity, the results were highly consistent with the above results using pairwise dissimilarity. For instance, the removal of generalist species led to decreased slopes of the relationships between LCBD and TSI and lower absolute values of the partial regression of TSI in multiple regression analyses except for bacteria in sediments (Fig. 4C and D), while the removal of specialist species resulted in the increase of above two parameters for both taxonomic groups in water, but not in sediments (Fig. S9C and D). These results indicate that generalist species contribute more to the decreases in ecological uniqueness along the trophic gradient, and LCBD is a valuable indicator to reveal biological homogenization at regional scales. Additionally, deterministic processes generally decreased when generalist species were removed but increased when specialist species were removed, albeit very slightly (Fig. S10).

4. Discussion

Rapid eutrophication of natural freshwater environments has become a major global concern threatening water environmental quality and biodiversity (Ansari et al., 2010; Kiersztyn et al., 2019). Understanding the effects of eutrophication on microbes is crucial for aquatic ecosystems (Falkowski et al., 2008) due to their key roles in biogeochemical cycling and ecosystem functioning. In this study, we used pairwise dissimilarity and LCBD to explore the beta diversity patterns for bacterial and fungal communities in lake water and sediments along the trophic gradient and investigated the drivers underlying the observed beta diversity. We conclude three main findings: (1) Eutrophication causes the alteration of physicochemical factors and leads to bacterial and fungal community homogenization. (2) Although the TSI plays pivotal roles in bacterial and fungal community variation, other physicochemical variables and heavy metal elements have nonnegligible influences. (3) Generalist species generally explain more variations in beta diversity along the TSI gradient and contribute more strongly to biotic homogenization.

We generally observed biotic homogenization toward high trophic levels for bacterial and fungal communities in both water and sediments, which is supported by several lines of evidence. First, there were reduced similarities among bacterial and fungal communities with higher trophic status. This is indicated by a more concentrated cluster in highly eutrophic lakes than in other trophic statuses in ordination plots (Fig. 2A), which is the predominant approach to quantify biotic homogenization with the dispersion metric calculated by the distance to group centroid in PERMDISP analysis (Hawkins et al., 2015; Holman et al., 2021; Huber et al., 2020). Second, there were generally lower mean dissimilarities for bacteria or fungi in both water and sediments in the highly eutrophic lake group, suggesting that the overall trend of community composition became similar along with the intensification of eutrophication. Finally, there were significant declines in LCBD with increasing TSI for fungi. Such patterns have also been documented for macroorganisms such as the benthic macroinvertebrates in 41 lakes of the middle and lower reaches of the Yangtze and Huaihe Rivers (Zhang et al., 2018a) and fish communities in 53 Danish lakes (Menezes et al., 2015).

For macroorganisms, the mechanisms of biotic homogenization are generally explored from three aspects: (1) extinction of native species, (2) invasion of widespread nonnative species, and (3) range expansion of native generalists (Holmes and Webster, 2010; Muthukrishnan et al., 2020; Villeger et al., 2011). However, it is challenging to apply these three mechanisms for microbes largely due to the lack of feasibility in a clear definition of native and nonnative species. We thus developed an approach by gradual removal of generalists from observed communities,

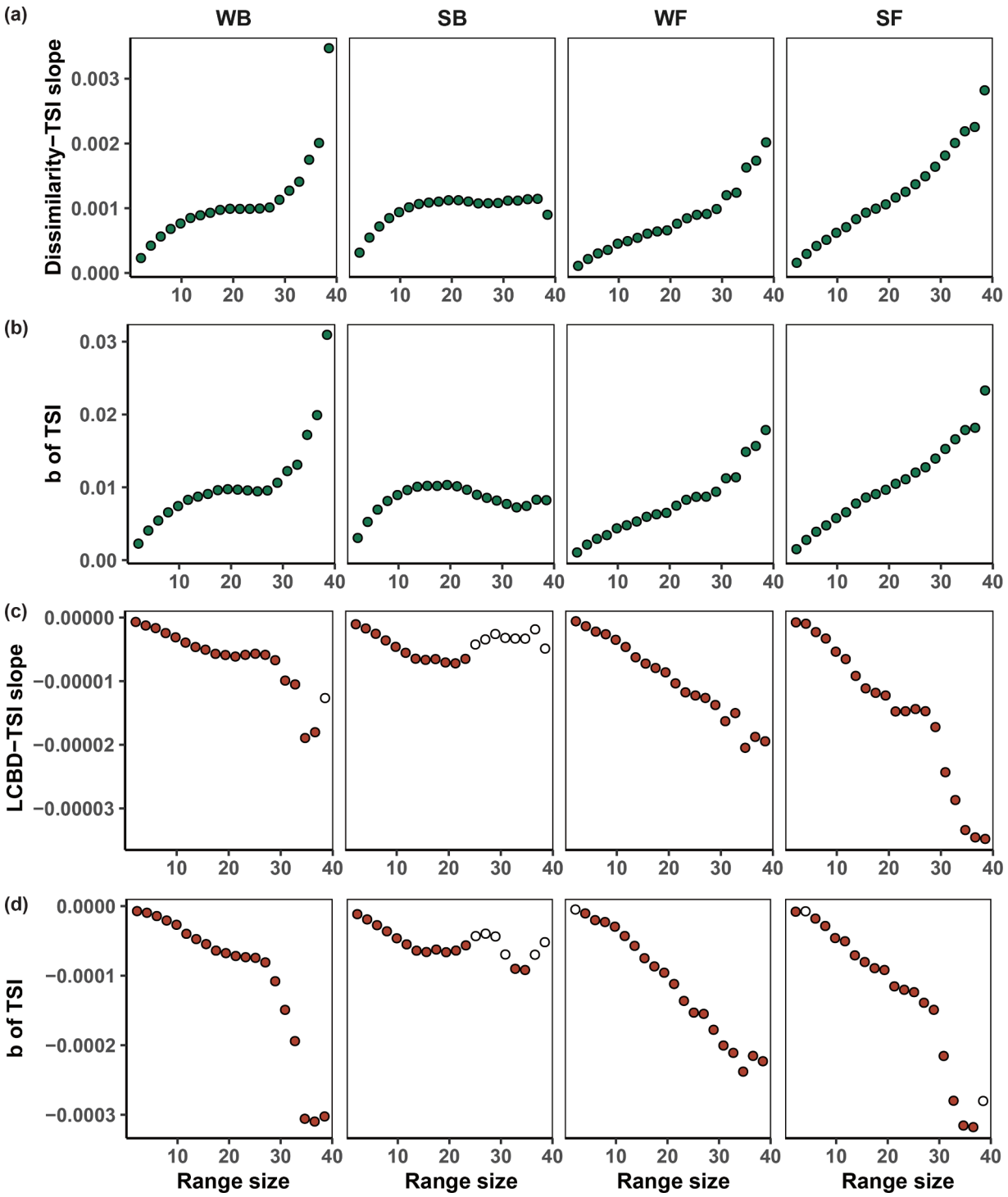

Fig. 4. Effects of the species TSI range size on the degree of variation in beta diversity due to TSI changes. The TSI range size of each species was calculated by the difference between the maximum and minimum of TSI values in which the species occurred across all samples. For each habitat and taxonomic group, we classified the species into 20 range-size categories according to their TSI range size. For generalist species, we removed the species starting from the category of the largest TSI range size for the observed community, and then recalculated LCBD or Bray-Curtis dissimilarity. That is, all species with a TSI range size larger than specific cut-offs (x-axes) were removed from the observed community matrix. (a) The Bray-Curtis dissimilarity was recalculated for all sub-communities and the relationships between the Bray-Curtis dissimilarity and TSI change were quantified with linear models. The slope indicates the degree of variation in beta diversity along the trophic gradient. (b) The Bray-Curtis dissimilarity was recalculated for all sub-communities and the relative contribution of the TSI to the beta diversity was quantified with MRM. The partial regression coefficients of TSI indicate the degree of variation in beta diversity along the trophic gradient. (c) The LCBD was recalculated for all sub-communities and the relationships of LCBD and TSI were quantified with linear models. The slope indicates the degree of variation in beta diversity along the trophic gradient. (d) The LCBD was recalculated for all sub-communities and the relative contribution of TSI to the LCBD was quantified with MLR. The partial regression coefficients of TSI indicated the degree of variation in beta diversity along the trophic gradient. The significantly ($P \leq 0.05$) negative and positive relationships are shown by filled red and blue circles, respectively, while open circles show non-significant ($P>0.05$) relationships.

which could provide a scenario to mimic the gradual extinction of generalists to some extent and examine the roles of generalists in biotic homogenization. We proposed that the observed biotic homogenization caused by eutrophication may primarily result from the following three reasons. First, habitat heterogeneity, referring to the spatial and temporal variations in environmental variables (Shade et al., 2008), could be the main cause of biotic homogenization. This is because the habitat heterogeneity is a major determinant of beta diversity (Astorga et al., 2014), and its reduction suggests fewer niches available in an ecosystem (Shade et al., 2008), increased competition among species for limited resources and possible extinction of competitively inferior native species. Macrophytes can provide habitats and food for other organisms and have vital roles in structuring communities in aquatic environments (Declerck et al., 2005). In highly eutrophic systems, the loss of macrophytes and subsequent dominance of phytoplankton tend to decrease the overall habitat heterogeneity.

Second, environmental filtering has a high probability of being a powerful mechanism for biotic homogenization because harsh environments such as eutrophication could decrease the importance of stochastic processes in structuring assemblages, leading to biotic homogenization (McGoff et al., 2013; Zhang et al., 2018a). Environmental filtering tends to exclude more sensitive species and favor more adapted, pollution-tolerant species, ultimately leading to biotic homogenization via the range expansion of pollution-tolerant species and the decline or extinction of pollution-intolerant species.

Third, eutrophication can result in homogenization by regulating interspecies interactions (Langenheder and Jurgens, 2001). For instance, interactions between phytoplankton and bacteria have been shown to influence bacterioplankton community composition (Currie, 1990; Paver et al., 2013; Su et al., 2017). Phytoplankton can play a vital role in shaping the bacterial community by providing a source of organic matter, as dissolved organic carbon produced by different species of phytoplankton leads to selection for different bacterial communities that have different utilization capacities for organic matter (Li et al., 2017; Sarmento and Gasol, 2012). Additionally, phytoplankton can also negatively affect the bacterial community through nutrient competition (Rivkin and Anderson, 1997).

Our results reveal that eutrophication plays a vital role in microbial community composition and beta diversity in water and sediments, which may be driven by multiple environmental variables. The microbial community composition is influenced by environmental variables related to nutrient enrichment, such as organic carbon, pH and heavy metals. Dissolved organic carbon produced by the photosynthesis of phytoplankton is an important carbon source for planktonic bacteria (Cole et al., 1982). Eutrophication causes the production of large amounts of algae-derived organic matter, leading to changes in organic mass sources and influencing bacterial community structure (Han et al., 2020). Additionally, pH is a major environmental driver of the microbial community by regulating the relative importance and interplay between niche-related and neutral processes (Ren et al., 2015). Nutrient enrichment tends to accompany heavy metal pollution in many human-impacted aquatic ecosystems (Jaiswal and Pandey, 2019). Heavy metals often exhibit negative impacts on microbial community such as the declines in microbial diversity and enzyme activities (Hoostal et al., 2008). Moreover, the responses of microbial community structure and function to heavy metals may be stronger than those to nutrient enrichment (Zhang et al., 2021). On the other hand, microbial community composition can be synergistically driven by eutrophication and other environmental variables such as temperature. It has been reported that the effect of temperature on species richness is greatest at extreme nutrient levels whereas the effects of nutrients on species richness are strongest at intermediate temperatures (Wang et al., 2016). Moreover, slight warming may not significantly change the bacterial community composition in the mesocosms by itself but the bacterial community composition shifts when warming acts in concert with nutrient enrichment (Ren et al., 2017). It should be noted that nutrients and temperature may influence lake microbial communities largely independently (Schulhof et al., 2020). Collectively, the microbial community structure is the combined effect of multiple environmental variables, but knowledge on the interaction mechanisms of these factors remains poor.

Most importantly, we observed that generalist species with larger TSI range size may contribute more to beta diversity than specialists and play a key role in biotic homogenization along trophic gradients. The importance of generalists is also supported by the metric SCBD, which was higher than that of specialists (Fig. S11). This importance may be explained by the following reasons. First, our collected samples covered a relatively large trophic gradient, along which nutrient enrichment conditions could result in a decline in the competitive advantage of specialists, leading to the dominance of generalists in community assembly (Cook et al., 2018). Generalist species can tolerate a wide environmental range such that they have a decreased probability of local extinction in the whole study area (Szekely and Langenheder, 2014), and the variation in beta diversity may be mainly due to the change in their own relative abundance (Fig. S12). Second, specialist species usually occur in specific habitats and are low in abundance (Lindh et al., 2016). They are more sensitive to environmental changes than generalists, and very slight environmental changes may cause the loss of species (Monard et al., 2016). In addition, we found that there was a lower proportion of deterministic processes in community assembly for specialists (Fig. S10), which indicates that species gain and loss are likely to result from stochastic processes such as random birth and death events (Jiao et al., 2020). Such frequent species gain and loss is likely responsible for the low contribution of specialists to variations in beta diversity, leading to the relatively stable uniqueness of specialist community compositions at all sites, with consistently high dissimilarity between any two sites. Collectively, most of the variations in beta diversity are attributed to the response of generalists to eutrophication, and thus generalists could be better indicator species of lake trophic status than specialists.

5. Conclusion

To our knowledge, our findings for the first time revealed the occurrence of biotic homogenization of microbes in water and sediments along trophic gradients. This suggests that biotic impoverishment might become more severe in the coming years if eutrophication is not effectively improved. Therefore, we believe that further observation of the microbial community under eutrophication is necessary. The mechanism of biotic homogenization caused by eutrophication should be identified before biotic impoverishment worsens. Moreover, our study reveals that generalist species contribute more to the variation in beta diversity than specialist species. To obtain a comprehensive understanding of the effects of eutrophication on the microbial community, we suggest that generalists and specialists should be distinguished to explore the mechanism of variation in beta diversity in future studies.

Data availability

The 16S rRNA sequences were deposited in the NODE project under accession number OEP002777 (https://www.biosino.org/node/project/detail/OEP002777).

CRediT authorship contribution statement

Mengdie Geng: Resources, Data curation, Formal analysis, Writing – review & editing. **Weizhen Zhang:** Writing – original draft, Writing – review & editing. **Ting Hu:** Writing – original draft, Resources, Data curation, Writing – review & editing. **Rong Wang:** Writing – review & editing. **Xiaoying Cheng:** Writing – review & editing. **Jianjun Wang:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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