

Contents lists available at ScienceDirect

Ecological Indicators



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Original Articles

Ecosystem functioning is linked to microbial evenness and community composition along depth gradient in a semiarid lake

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ARTICLE INFO

Keywords: Water depth Sediment Microbial communities Ecosystem multifunctionality Semiarid region Lake

ABSTRACT

Lake level or water depth are key physical variables known to respond dramatically to climate change, especially in arid regions, and their fluctuations exert substantial influences on lake biodiversity and ecosystem functioning. However, it is unclear how multiple ecosystem functions (i.e., ecosystem multifunctionality, EMF) respond to changes in water depth and how aquatic community attributes, such as species richness, evenness and community compositions, are linked to EMF along water depth gradients. Lake Hulun, a representative of semiaridregion lake in China, has experienced serious lake area shrinkage over the past 20 years. Here, we explored the water-depth patterns of three microbial taxonomic groups of bacteria, archaea and fungi and nine ecosystem functions related to nutrient cycling in Lake Hulun. We further examined the relative importance of different community attributes on EMF variations. We found that the community compositions of bacteria, archaea and fungi showed consistent water-depth decay patterns, and EMF and most individual ecosystem functions involved in C, N, P and S cycling increased with water depth. Further, EMF was predominantly mediated by microbial evenness and community composition, but not species richness, as predicted by the traditional theory of biodiversity-ecosystem functioning relationships. In addition, water depth indirectly affected the relationships between the microbial community and EMF via sediment nutrient contents. These findings indicate that the water depth changes under climate change could substantially alter ecosystem structure and functioning in arid regions. We further emphasize the necessity of including multiple community attributes in biodiversity-EMF relationship research to clarify the biotic and abiotic forces underlying EMF variations.

1. Introduction

Climate change and its impacts on ecosystems have become a major global concern (Parmesan and Yohe, 2003). Owing to climate warming, lake water levels are expected to decrease because of higher lake surface water temperatures and elevated lake evaporation rates (Woolway et al., 2020), especially in arid regions where these effects are exacerbated (Huang et al., 2016). To mitigate the negative impacts of climate change, the consequences of changes in lake level or water depth on lake ecosystems, especially ecosystem structure such as physiochemical conditions and biodiversity, have received increased attention. For instance, declines in water level often result in higher total phosphorus concentrations and higher risks of cyanobacteria blooms (Jeppesen et al., 2015). Lakes with lower water depths are prone to eutrophication, which are more difficult to manage and mitigate than deep water lakes (Zhang et al., 2020). Furthermore, along water depth gradients, biodiversity and community composition usually show clear spatial patterns, such as for benthic microbes in deep water lakes (Zhao et al., 2019). As lake ecosystems are defined not only by their structures but also by the ecosystem functions they perform (Mooney et al., 2004; Rinke et al.,

https://doi.org/10.1016/j.ecolind.2021.108314

Received 13 April 2021; Received in revised form 19 October 2021; Accepted 20 October 2021

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2019), their structure should be complemented by functional metrics to accurately evaluate ecosystem responses to environmental variables such as water depth (Parr et al., 2016). However, the effects of lake water depth changes on ecosystem function remain poorly understood.

Microbial communities, such as bacteria, archaea and fungi, play critical roles in simultaneously maintaining multiple ecosystem functions (that is, ecosystem multifunctionality, EMF) associated with elemental geochemical processes (Miki et al., 2014) and thus driving ecosystem circulation (Bodelier, 2011). In the last two decades, intensive studies mainly in terrestrial ecosystems have shown that the loss of microbial diversity tends to impair EMF and subsequently alter their ability to provide ecosystem services, ultimately threatening human welfare (Cardinale et al., 2012; Garland et al., 2021). For aquatic ecosystems, research on microbial diversity-ecosystem functioning relationships has focused mostly on single functions, such as primary productivity or organic degradation (Ylla et al., 2013; Zimmerman and Cardinale, 2014; Zhang et al., 2017), and has neglected the effects of biodiversity on EMFs. Moreover, the links between environmental stressors and biodiversity may also regulate the relationships between microbial diversity and ecosystem functioning (Bradford et al., 2014; Jing et al., 2015; Bastida et al., 2016). For instance, regional-scale variation in climate can determine, or at least modify, the effects of biodiversity on EMF in a study combining the effects of plant and soil microbial biodiversity on EMF in the Tibetan Plateau (Jing et al., 2015). Given the intensification of climate warming, understanding the effects of water depth variations on the relationship between microbial diversity and EMF is critical for the management of lake ecosystems in the future, especially in arid regions.

Biodiversity involves multiple components, such as species richness, evenness and community composition (Maestre et al., 2012a). Although richness has been widely used in studies of microbial biodiversity-ecosystem functioning relationships (Lefcheck et al., 2015; Schuldt et al., 2018; Hu et al., 2020), evenness (or the complementary term, dominance) and species composition have also been increasingly shown to affect ecosystem functioning (Hillebrand et al., 2008; Wagg et al., 2014; Zheng et al., 2019). Furthermore, the positive effects of species richness on EMF may be largely modulated by other community attributes, such as species evenness and composition (Maestre et al., 2012a). There is thus a need to further disentangle the relative importance of these microbial community attributes in driving ecosystem functioning upon deciphering the water-depth patterns of the microbial community and ecosystem function.

In this study, we explored the water-depth patterns of three community attributes, including the species richness, evenness and community compositions of benthic bacteria, archaea and fungi in Lake Hulun, and the nine ecosystem functions associated with carbon (C), nitrogen (N), phosphorus (P) and sulfur (S) cycling; in addition, we examined the effects of water depth on microbial biodiversity-ecosystem functioning relationships. Lake Hulun is located in the northern semiarid region and plays a crucial role in maintaining the ecological security of northern China (Li et al., 2008). In the last 20 years, however, Lake Hulun experienced the largest lake area shrinkage among the 10 largest lakes in China (Zhang et al., 2013), indicating a substantial decline in its lake water depth. We proposed the following three hypotheses: i) There are predictable water-depth patterns in the community attributes of bacteria, archaea, fungi and EMF; ii) EMF could be affected not only by species richness but also by other primary microbial attributes, such as evenness and community composition; and iii) water depth indirectly impacts EMF via microbial community attributes.

2. Materials and methods

2.1. Study sites

Lake Hulun (48°30′40″–49°20′40″N, 117°00′10″–117°41′40″E), with an area of $\sim 2,030 \text{ km}^2$ in 2017, is the largest lake in northern

China. In June 2020, 19 sampling sites across Lake Hulun were selected along a water depth gradient of 3.8-6.4 m with an average depth of 5.7 m. We did not sample shallow regions with water depths<3 m because of the heavy contents of sand near the lake bank. At each site, water depth and Secci depth (SD) were monitored by a bathymeter and a Secchi disk, respectively. We collected 1 L of overlying water from the upper 50-cm lake surface layer by a 5-L Schindler sampler for chemical analyses. We did not sample the water over the sediment, but only surface water, for environmental measurement largely due to the maximum depth of only 6.4 m, and the well-mixing water conditions of this lake (Li et al., 2021). The upper 5-cm surface sediments were sampled with a box sampler. The surface sediment samples were separated into two subsamples: one was immediately stored at -20 °C for microbial community and enzyme activity analyses, and the other was stored at 4 °C for physiochemical measurements. While fresh sediment is ideal for enzymatic activity assays, freezing is proved a suitable alternative preventing further decomposition and preserving extracellular enzymes of the sediment when logistically necessary (Hewins et al., 2016). The sediment measurements above were conducted within one week after sampling. For each site, the water temperature, dissolved oxygen concentration, pH and conductivity in the water column were monitored in situ using a multiparameter water quality detector (YSI Incorporated, Yellow Springs, USA). The surface sediment pH and conductivity were also measured in situ.

2.2. Physiochemical variables

For surface water, total nitrogen (TN), total phosphorus (TP) and dissolved nitrogen, including nitrate (NO₃⁻), nitrite (NO₂⁻) and ammonium (NH₄⁺), and dissolved phosphorus (PO₄^{3–}), were measured according to standard methods (Huang et al., 2000).

For surface sediments, the samples were freeze-dried for four days to constant weights and ground into fine powder. The samples passed through a 100-mesh sieve were used for elemental analyses. Sediment total carbon (TC) and TN were determined by an elemental analyzer (Flash EA 1112 series, CE instruments, Italy). Sediment TP was first digested by hydrofluoric acid (HF)-perchloric acid (HClO₄) and then determined by molybdenum blue colorimetry (Sparks, 1996). The dissolved inorganic nutrients and dissolved organic carbon (DOC) in sediments were extracted by filtering the aqueous suspension of the freeze-dried surface sediment (1:20 sediment:water ratio, g/ml) using a 0.45- μ m cellulose acetate membrane. The dissolved nitrogen (NO₃⁻, NO₂⁻ and NH₄⁺) and phosphorus (PO₄³⁻) in sediments were measured according to standard methods (Huang et al., 2000), and DOC was measured by a total organic carbon analyzer (ET1020A, USA) using the combustion oxidation method.

We also obtained the annual mean water level data in Lake Hulun during 1991–2015 from the literature (Zhang et al., 2016; Li et al., 2019), and the annual TN and TP in the surface water during 1991–2015 based on Chuai et al (2012) and Liang et al (2016). The lake water level, that is the elevation of the lake water surface, was determined according to the principle of satellite altimetry and calculated by the Basic Radar Altimetry Toolbox software based on the geoid height, which is described in details in previous literature (Li et al., 2019). Due to the synchrony of changes in lake annual mean water depth and annual mean water level, the changes in ecosystem structures and functioning along water depth in spatial are expected to predict those along water level in temporal.

2.3. Bacterial and fungal communities

The DNeasy PowerSoil Kit (QIAGEN, Germany) was used to extract genomic DNA from the sediment samples according to the manufacturer's protocols. The identifications of bacterial and fungal communities were performed according to previously reported descriptions (Yeh et al., 2019). For bacterial and archaeal communities, the V4 hypervariable region of the 16S ribosomal RNA gene was amplified in triplicate via polymerase chain reaction (PCR) using universal primers [515F, 5'-GTGYCAGCMGCCGCGGTAA-3' and 806R, 5'-GGAC-TACNVGGGTWTCTAAT-3'], and the replicates were then mixed. The barcoded PCR products were normalized at equal molality and sequenced with 2×250 bp paired-end on the Illumina HiSeq sequencing platform (Illumina Inc.). The sequences were then processed using the Quantitative Insights into Microbial Ecology (QIIME, v1.9) pipeline (Caporaso et al., 2010b). Briefly, a denoiser algorithm was used to denoise the sequences (Reeder and Knight, 2010), and then the seedbased UCLUST algorithm was used to cluster the sequences into operational taxonomic units (OTUs) at \geq 97% pairwise identity (Edgar, 2010). Singletons, that is OTUs with only one read in the entire dataset were excluded and chimeras were removed by ChimeraSlayer (Haas et al., 2011). Then, the representative sequences from each OTU were aligned to the Greengenes imputed core reference alignment (DeSantis et al., 2006) using PyNAST (Caporaso et al., 2010a). After gaps and hypervariable regions were removed using a Lane mask, the alignments were used to construct an approximately maximum-likelihood phylogenetic tree based on Jukes-Cantor distance using FastTree (Price et al., 2010). Finally, a naive Bayesian model with the RDP classifier was used to determine the taxonomic identity of each representative sequence (Wang et al., 2007).

For fungal communities, the nuclear ribosomal internal transcribed spacer 2 (ITS2) region was amplified using the primers [gITS7F, 5'-GTGARTCATCGARTCTTTG-3' and ITS4R, 5'-TCCTCCGCTTATTGA-TATGC-3']. The purified amplicons were pooled in equimolar amounts and sequenced in the same HiSeq run. The sequences were processed by a published pipeline (Nilsson et al., 2009). Briefly, the chimeric sequences were removed using USEARCH (Edgar, 2010). Similar to bacterial identification, the UCLUST algorithm was used to cluster fungal sequences in the UNITE database (Abarenkov et al., 2010), and the RDP classifier (Wang et al., 2007) was used to further identify the taxonomies against the UNITE database (Abarenkov et al., 2010).

The 16S and ITS rRNA raw reads were deposited into the NCBI Sequence Read Archive database (Accession Number: SRR13611586 to SRR13611623).

2.4. Ecosystem function measurements

To quantify the ecosystem functions, the potential activities of nine extracellular enzymes related to carbon and nutrient (nitrogen, phosphorus and sulfur) cycling were determined using a fluorimetric microplate enzyme assay (Pritsch et al., 2004) following a previous protocol (Liu et al., 2021). Among the nine extracellular enzymes, β -glucosidase (BG), cellobiohydrolase (CBH), xylanase (BX) and β -galactosidase (GAL) are related to carbon cycling. Leucine aminopeptidase (LAP) is related to nitrogen cycling. β -N-acetylglucosaminidase (NAG) and N-acetyl- β -galactosaminidase (SUL) and acid phosphatase (AP) are related to sulfur and phosphorous cycling, respectively. The detailed functions of these nine enzymes are listed in Table S1.

2.5. Microbial community analyses and ecosystem multifunctionality

Before microbial community analyses, we rarefied bacterial, archael and fungal communities at 65813, 444 and 1288 OTUs respectively to avoid variation in abundance or deviation in sampling intensity. We confirmed that the rarefied microbiome data were reliable by testing the effects of sequencing depths on microbial diversity estimates (Fig. S1). We then calculated species richness (the number of observed OTUs), Pielou's evenness (Pielou, 1969) and community compositions of bacteria, archaea and fungi in surface sediments. The community compositions were represented by Bray-Curtis dissimilarity matrices.

Ecosystem multifunctionality (EMF) was quantified with an

averaging approach to indicate the simultaneous performance of multiple functions (Manning et al., 2018). This approach is widely used in the multifunctionality literature (Maestre et al., 2012b; Bradford et al., 2014; Wagg et al., 2014; Lefcheck et al., 2015) and measures EMF by collapsing multiple ecosystem functions into a single metric. Specifically, we calculated Z-scores for all of the ecosystem functions measured and averaged them for each site, and the averaged value indicated EMF (Maestre et al., 2012b).

2.6. Statistical analyses

We fitted ordinary least squares (OLS) regressions for the waterdepth patterns of abiotic environmental factors, the species richness and evenness of the three taxonomic groups, EMF, and the nine single ecosystem functions; we also fitted OLS regressions for the relationships between EMF and richness or evenness. In addition, OLS regression was used to explore the relationships between annual mean water level change and annual mean water TN or TP. The significance of OLS regressions was evaluated by the analysis of variance method with pseudo-F statistics. Then, linear models were used to visualize the relationships of the Bray-Curtis dissimilarity of the three taxonomic groups with water-depth distances or the differences in ecosystem functions. And the significance of the linear models was determined using Mantel test (999 permutations). The Euclidean distance of ecosystem functions were computed between all pairs of sampling sites to represent differences in ecosystem functions. Furthermore, we used the Procrustean matrix superimposition (Jackson, 1995) to assess the degree of association or concordance between microbial community composition and ecosystem function composition using the first two axes of the principal coordinate analysis. We obtained the metric of association $(m^2, ranging from 0 \text{ to } 1)$ indicated by the sum of squared residuals between the scaled and rotated configurations of each ordination solution (Jackson, 1995). The smaller the m^2 metric is, the stronger the concordance between the two data sets (Peres-Neto and Jackson, 2001). The significance was then assessed by permutation tests (999 permutations).

We then performed random forest (RF) analysis to quantify the relative contributions of abiotic factors in explaining the species richness, evenness and PCoA1 of bacterial, archaeal and fungal communities or the nine single ecosystem functions. RF is a machine-learning algorithm that aims to compute the importance of each predictor variable by averaging the decrease in prediction accuracy over 2000 trees in a forest. Such a decrease in each tree for each predictor is determined by calculating the increase in the mean square error between observations and the out-of-bag predictions when the data for that predictor are randomly permuted. We did not apply cross validation for model development or valuation largely because the small sample numbers and no further need of the resulting statistical models for prediction or extrapolation. Because of bagging and random features, RF produces accurate classifications and effective predictions (Breiman, 2001). We also used Pearson correlation analysis to confirm the correlation significance of abiotic factors with species richness, evenness, PCoA1, or the nine single ecosystem functions.

Finally, we used a structural equation model (SEM) (Grace et al., 2012) to further evaluate the relationships among water depth, other abiotic factors, microbial community attributes and EMF. SEM is a modeling framework that aims to develop and evaluate models to quantitatively investigate the underlying causal relationships among variables using statistical principles along with causal assumptions (Grace et al., 2012). In the SEMs, abiotic factors except for water depth were included as a composite variable: that is, the first axis of principal component analysis of these abiotic variables (Envi.PCA1). We combined the three taxonomic groups of bacteria, archaea and fungi into a synthetic community, and then included the species richness, evenness and the first axis of principal coordinate analysis of the synthetic community (PCoA1_{sc}, accounting for 65.1% of the community variation) as microbial community attributes in SEMs. To clarify the role of dominant

environmental factors, we constructed another SEM in which the Envi. PCA1 was replaced by three dominant environmental factors proved to be important for changes in ecosystem functions in the RF analysis above: that is sediment NH₄⁺, sediment TP and sediment DOC. All the initial input variables were Z-score transformed to allow comparisons among the explanatory variables. After modeling, we tested the overall goodness of fit for the SEMs and screened the models with a nonsignificant χ^2 test ($\chi^2 < 2, p > 0.05$), a high comparative fit index (CFI > 0.95) and a nonsignificant root mean square error of approximation (RMSEA < 0.05, p > 0.05) (Grace et al., 2012). The overall significance of the SEMs and standardized effects (standardized path coefficient, β) were assessed by analysis of variance (ANOVA). The model with the lowest Akaike information criterion (AIC) value was selected as the final best model.

The statistical analyses, including the Bray-Curtis dissimilarity matrices of microbial communities (and Procrustean matrix superimposition), PCoA, Pearson correlation analyses, OLS regressions (and PCA), RF and EMF, were performed in R version 3.6.1 using the packages vegan V2.5–6, ape V5.3, Hmisc V4.3–0, Stats V3.6.1, random-ForestSRC V2.9.2 (Breiman, 2001) and lavaan V.0.6–5 (Rosseel, 2012), respectively. Before OLS regressions, the linear model assumptions, including normality, independence, linearity, and homoscedasticity, were tested using the R package gvlma V1.0.0.3 (Peña and Slate, 2006). Before RF analyses, we excluded abiotic environmental variables with strong collinearity based on high variance inflation factors (>10) using the R package usdm V1.1–18 (Naimi et al., 2014). We summarized the overall statistical analyses in the framework in Fig. S2.

3. Results

3.1. Water-depth patterns of physiochemical factors

Across the whole lake, the mean values of TN and TP in the water column were 1.12 and 0.12 mg L⁻¹, respectively (Table S2). Although the water depth range was only 2.6 m, there were large variations in several sediment nutrient variables, such as TC, TN and TP contents, with ranges of $2.3 \sim 56.8$, $0.2 \sim 1.1$, and $0.2 \sim 3.4$ g kg⁻¹, respectively (Table S2). Some physiochemical variables, especially nutrients, showed significant trends along the water-depth gradient, as indicated by OLS

regression analysis (Figs. 1, S3). For instance, in surface water, SD showed a decreasing water-depth pattern (p < 0.05, Fig. 1b), whereas TN and PO₄³⁻ were significantly positively correlated with water depth (p < 0.05, Fig. 1c, d). In surface sediments, the relationships between water depth and abiotic factors, including TC, TN, TP, DOC, NH₄⁺, and PO₄³⁻, were consistently positive (p < 0.001, Figs. 1e–g, S3), indicating that these nutrient levels increased with water depth.

3.2. Water-depth patterns of microbial community attributes and ecosystem function

For microbial community attributes, the community composition, but not species richness and evenness, of the three taxonomic groups showed consistently significant water-depth patterns. Specifically, we only found decreasing patterns of bacterial evenness and fungal richness (p < 0.001, Fig. 2a, Fig. S4c) with water depth. However, the Bray-Curtis similarities of all three taxonomic groups had significant water-depth decay patterns (that is, the increasing Bray-Curtis dissimilarities with greater water depth differences) (p < 0.001, Fig. 2d–f). To better visualize the distribution of the microbial communities along water depth, we ordinated each community using principal coordinate analysis (PCoA) and then the scores on the first and second axis were extracted (that is, PCoA1 and PCoA2) for further analyses. Similarly, the PCoA1 of bacteria, archaea and fungi accounting for 63.6%, 48.3% and 41.8% of community variations, respectively, also clearly varied with water depth (Fig. 2g–i).

For ecosystem functions, EMF (p < 0.001, Fig. 3a) and most of the measured potential enzymatic activities significantly increased with water depth, such as BG, CBH, and BX for C cycling; NAG and NAGA for C and N cycling; and SUL for S cycling (Fig. 3). Potential enzymatic activities showing nonsignificant water-depth patterns were GAL, LAP and AP for C, N and P cycling, respectively (p > 0.05, Fig. 3e, h, i).

3.3. Relationships among microbial community attributes, EMF, and abiotic variables

Only EMF was significantly (p < 0.05) negatively correlated with bacterial evenness, fungal richness and evenness (Figs. 4a, c, S4f). However, EMF differences were significantly positively correlated with



Fig. 1. Sampling map (a) and water-depth patterns of main chemical factors (b-g) in Lake Hulun. In the sampling map, fathom lines are depicted. The lines in (b)-(g) represent the fitted ordinary least squares (OLS) regressions. The *p* and adjusted R^2 values of the OLS regressions are shown. * *p* < 0.05, *** *p* < 0.001.



Fig. 2. Water-depth patterns of microbial community attributes. Microbial community attributes of the bacteria, archaea and fungi in surface sediments include evenness and community compositions. The community compositions include Bray-Curtis dissimilarity matrices and the first and second axes of community principal coordinate analysis (that is, PCoA1 and PCoA2). For the water-depth patterns of evenness, the significant (p < 0.05) regression line were shown with 95% confidence intervals indicated by the shaded areas (a-c). For the Bray-Curtis dissimilarity of community compositions along water-depth distances, the significant regression lines were shown with 95% confidence intervals indicated by the shaded areas (d-f). The F statistic p and adjusted R² values, and the Mantel statistic p and r values are shown. * p < 0.05, ** p < 0.01, *** p < 0.001. Besides, the biplots of PCoA1 and PCoA2 of each taxonomic group was also plotted with the fathom lines added (g-i).

Bray-Curtis dissimilarities for all three taxonomic groups (p < 0.001, Fig. 4d–f). Furthermore, the Procrustean matrix superimposition showed that the community variations of bacteria, archaea and fungi were consistent with the composition variations of ecosystem functions (p < 0.001, Fig. 4g–i).

The analyses of random forest and Pearson correlation showed that the DOC, TP and NH₄⁺ in sediments, in addition to water depth, consistently dominated the variation in the community composition (PCoA1) (p < 0.01, Fig. 5) of the three taxonomic groups. Regarding species richness and evenness, similar results were only observed for fungal richness and bacterial evenness (p < 0.01, Fig. 5). Sediment DOC and water pH were the two abiotic factors that most significantly affected fungal evenness (p < 0.01, Fig. 5). In addition to water depth, DOC, TP and NH₄⁺ in sediments made the greatest contributions (p < 0.05, Fig. 5) to variation in the potential activities of six enzymes (BG, CBH, BX, NAG, NAGA, and SUL). However, GAL activity primarily increased with sediment NO₂⁻ and NH₄⁺ (p < 0.05, Fig. 5), and LAP activity increased with SD (p < 0.05, Fig. 5); AP activity was mainly explained by sediment NO₃⁻, the pH of sediments and water (p < 0.05, Fig. 5).

3.4. Water depth regulated the links between microbial community composition and EMF

The final two SEMs accounted for 84% and 84.6% of the variation in EMF, respectively (Figs. 6 and S4). The first axis of principal component analysis of abiotic environmental variables except for water depth (Envi. PCA1) accounted for 40.05% of the total variations, and the first four loadings of Envi.PCA1 were sediment variables including PO_4^{3-} , TC, TN and TP (Fig. S5). Water depth had significant effects on Envi.PCA1 (p <0.001, $\beta = 0.606$, Fig. 6), and on the sediment NH₄⁺, TP and DOC (p < 10000.001, $\beta = 0.616$, 0.751, 0.735, respectively, Fig. S6). Envi.PCA1 substantially affected microbial evenness (p < 0.001, $\beta = -0.927$, Fig. 6) and community composition PCoA1_{sc} (p < 0.001, $\beta = 1.307$, Fig. 6) but had a nonsignificant effect on species richness (p > 0.05, $\beta = 0.146$, Fig. 6). Specifically, microbial species richness and evenness were mainly affected by sediment NH₄⁺ and TP, respectively (p < 0.001, $\beta =$ 0.943, 0.932, respectively, Fig. S6), while microbial PCoA1sc was simultaneously affected by sediment NH₄⁺ (p < 0.01, $\beta = 0.584$), TP (p $< 0.01, \beta = 0.684$) and DOC ($p < 0.05, \beta = 0.327$, Fig. S6). In the first model, EMF significantly (P < 0.001) decreased with microbial evenness and increased with PCoA1_{sc}, with β values of -0.454 and 0.557,



Fig. 3. Water-depth patterns of ecosystem multifunctionality (EMF, a) and single functions (b-j). Single functions include β -glucosidase (b), cellobiohydrolase (c), xylanase (d), β -galactosidase (e), β -N-acetyl-glucosaminidase (f), N-acetyl- β -galactosaminidase (g), leucine amino peptidase (h), phosphatase (i), aryl sulfatase (j). MUF: 4-methylumbelliferyl. AMC: 7-amino-4-methylcoumarin. The lines represent the fitted ordinary least squares (OLS) regressions, with 95% confidence intervals indicated by the shaded areas. The *p* and adjusted R² values of the OLS regressions are shown. * *p* < 0.05, ** *p* < 0.001, *** *p* < 0.001.

respectively, but was not affected by species richness (p > 0.05, Fig. 6). And the similar results were found in the second model (Fig. S6).

4. Discussion

Although lake water depth responds dramatically to climate change (Woolway et al., 2020), little is known about how changes in water depth influence lake ecosystem function and biodiversity-EMF relationships. Our study addressed these questions by thoroughly analyzing the community attributes of three benthic microbial taxonomic groups and measuring nine microbial ecosystem functions across the water depth gradient in Lake Hulun. Compared with previous studies reporting ecosystem functioning and the ecological consequences of lake water depth changes, our findings are unique in showing that (1) EMF increased with water depth, (2) the evenness and community composition, but not species richness, determined EMF variations, and (3) the causal relationships between the microbial community and EMF were indirectly affected by lake water depth.

In the semiarid Lake Hulun, we unexpectedly found that bacteria, archaea and fungi showed nonsignificant water-depth patterns in species richness or evenness, except for fungal richness and bacterial evenness, but had consistent water-depth decay patterns in community composition. The water-depth decay patterns are consistent with previous studies indicating the generality of spatial distance-decay relationships for both micro- and macroorganisms (Nekola and White, 1999; Hanson et al., 2012). The distance decay of biotic similarity can be attributed to a decrease in environmental similarity with spatial distance (e.g., the water depth gradient in our study) (Nekola and White, 1999). However, our results regarding the increasing water-depth patterns of archaeal richness and bacterial evenness are inconsistent with previous findings in other habitats, such as deep lakes (Zhao et al., 2019) and marine environments (Brown et al., 2009; Kosobokova et al., 2011; Liu et al., 2018). For instance, the species richness of bacteria and chironomids nonlinearly decreases with water depth, and diatom richness shows hump-shaped patterns in deep-water Lake Lugu (Zhao et al., 2019). Bacterial richness in the water column also decreases with depth in marine environments, such as the North Pacific Ocean (Brown et al., 2009) and New Britain Trench (Liu et al., 2018). The richness and evenness of zooplankton show hump-shaped patterns with water depth in the Arctic's central basins (Kosobokova et al., 2011). Generally, the water-depth patterns of aquatic community attributes are frequently observed in marine environments (Smith and Brown, 2002; Brown et al., 2009; Kosobokova et al., 2011; Bryant et al., 2012; Liu et al., 2018) but are less frequently reported in freshwater ecosystems, the latter of which probably stems from the relatively smaller spatial scales and increased levels of disturbance (Gushulak et al., 2017; Zhao et al., 2019). The water-depth biodiversity gradient is not only an understudied biogeographical pattern (Zhao et al., 2019), but it is also an urgent issue regarding the responses of lakes to climate change, especially in arid regions (Huang et al., 2016). Our results highlight that climate change in arid regions with decreasing water levels may increase the driving forces of changes in water depth on aquatic microbial community dynamics, even with a relatively narrow range of water depths, which is further explained in the following discussion.

As we expected, EMF and most individual ecosystem functions were positively correlated with water depth. For instance, the potential enzymatic activities of BG, CBH, BX, NAG, NAGA and SUL increased with water depth. To the best of our knowledge, studies on the waterdepth patterns of single ecosystem functions in sediments are rare; furthermore, this is the first study to reveal the water-depth pattern of EMF. For single ecosystem functions, our increasing water-depth patterns are generally different or even contrary to those of most previous studies, regardless of the differences in ecosystem functional indicators considered (Hunt et al., 2003; Fu et al., 2014; Rober et al., 2014; Martínez et al., 2016), habitat types (Bourgeois et al., 2017) or research scales (Mahmoudi et al., 2020). In previous EMF studies, ecosystem functional indicators such as primary production and nutrient cycling (e. g., enzyme activities, microbial activity and decomposition rate) have received the most attention (Garland et al., 2021). For instance, macrophyte productivity shows a unimodal distribution along a lake water depth gradient with a peak at intermediate depth (Fu et al., 2014). The decomposition rate in streams (Martínez et al., 2016) and the denitrification in bulrush wetlands (Hunt et al., 2003) are negatively correlated with water depth. For oceanic habitats, the benthic remineralization function represented by sediment oxygen demand was also observed to decline with water depth in a Pan-Arctic review (Bourgeois et al., 2017). Along larger depth gradients, for example, from 800 to 2,200 m, the potential enzymatic activities of BG, CBH, BX, NAG and AP decrease in marine environments (Mahmoudi et al., 2020). For EMF, the increasing water-depth pattern further confirms the importance of water depth for microbes in the maintenance of multiple ecosystem functions.

We speculated that the water-depth patterns of microbial communities and ecosystem functioning may be characterized by the uniqueness of lakes in arid regions with intensive human activities. In our analyses of RF and Pearson correlation, most of the ecosystem functions and microbial community attributes were primarily correlated with



Fig. 4. Relationships between microbial community attributes and ecosystem multifunctionality (EMF). Microbial community attributes of the bacteria, archaea and fungi in surface sediments include evenness and community compositions. The community compositions include Bray-Curtis dissimilarity matrices and the first and second axes of community principal coordinate analysis (that is, PCoA1 and PCoA2). For the relationships of the evenness with EMF, the significant (p < 0.05) regression line were shown with 95% confidence intervals indicated by the shaded areas (a-c). For the relationships of community compositions with EMF, the significant regression lines were shown with 95% confidence intervals indicated by the shaded areas (d-f). In addition, the procrustean matrix superimposition was performed to assess the degree of concordance between the spatial patterns in community composition (PCoA1 and PCoA2) of each taxonomic group and ecosystem function composition (g-i). The arrows indicate the rotation directions from rotated configurations (ecosystem functions, solid square) to scaled configurations (bacterial/archaeal/fungal communities, hollow circle). The F statistic *p* and adjusted R² values, the Mantel statistic *p* and r values, and permutation statistic *p* and m² values are shown. * p < 0.05, ^{***} p < 0.01, ^{****} p < 0.001.

sediment nutrient status, including the DOC, TP and NH₄-N in sediments. Interestingly, sediment TN and water depth are also the first two main factors affecting the spatial distribution of diatom assemblages in the surface sediments of Lake Hulun (Wang et al., 2015). Lake Hulun has suffered from eutrophication since the 1980s (Li et al., 2008), and the situation is aggravated because of water level declines under a warming and drying climate (Chuai et al., 2012). Under extreme water level declines, internal nutrient cycling increases, and nutrient retention capacity decreases, with nutrients being available to the biota and thereby enhancing the risk of algal blooms (Jeppesen et al., 2015). The enrichments in internal C, N, and P sources inevitably reshuffle the spatial layouts of microbial communities and ecosystem functions related to nutrient cycling. Therefore, the water-depth patterns of microbial community attributes and ecosystem functioning result from the waterdepth nutrient variation associated with climate warming. This conclusion is supported by the fact that there were no direct effects of water depth on microbial community attributes and EMF when other

environmental and biotic factors were considered in the SEM analysis. Generally, global arid regions are expected to increase in lake area by 11–23% and to warm at twice the rate of humid regions by the end of this century (Huang et al., 2016). Our findings imply that climate change will likely amplify the negative effects of eutrophication or other human stressors on the structure and function of lake ecosystems, especially in arid regions.

Inconsistent with our hypothesis, microbial community attributes, including evenness and community composition but not species richness, dominated EMF variations. The nonsignificant effects of microbial richness on EMF in our study contradicted previous findings in biodiversity-EMF relationship research (Lefcheck et al., 2015; Schuldt et al., 2018; Hu et al., 2020), indicating that the capability of sustaining multiple ecosystem functions is enhanced with increasing species richness because different species often influence different functions (Hector and Bagchi, 2007). Indeed, we revealed the substantial impacts of evenness and community composition on EMF but not those of species



richness. Evenness was negatively correlated with EMF, indicating that greater species dominance is expected to increase EMF because dominance is inversely proportional to evenness. This implies that specific microbial species could simultaneously influence multiple functions, and thus, the increases in their relative abundances would promote EMF compared with other species. Such a phenomenon was also observed in a microcosm experiment exploring the impacts of lichen evenness on EMF related to C, N and P cycling (Maestre et al., 2012a). The strong effects of community composition on EMF might result from the unique assemblage of microbial species (Zhang et al., 2019a; Zhang et al., 2019b). That is, particular assemblages with unique suites of species might be responsible for the great changes in EMF, and communities that are highly dissimilar to these assemblages would exhibit lower EMF. In addition, the relationships between community compositions and EMF may also depend on the sets of ecosystem functions examined. For instance, a study in soil ecosystems showed significant effects of bacterial and archaeal community composition on soil N processes but not on soil C processes (Zheng et al., 2019). These findings shed light on the mechanisms by which microbial communities alter EMF related to aquatic nutrient cycling and emphasize the importance of integrating multiple community attributes to improve predictions of EMF.

We further found that the associations between the microbial community and EMF were indirectly affected by water depth. As speculated in previous discussions, microbial communities mediate their capability to simultaneously alter multiple ecosystem functions through variation in evenness and composition resulting from the dynamics in specific species and particular assemblages with unique functions. The spatial variations in these species or assemblages were likely attributed to alterations in environmental variables, including DOC, TP and NH₄⁺ in sediments, which increased with water depth. Therefore, water depth not only determined the spatial patterns of sediment microbial communities and ecosystem functioning but also indirectly affected microbial community-EMF relationships via nutrient enrichment. The TP concentration in the overlying water of Lake Hulun is close to that of Lake Taihu, which has been highly publicized for its eutrophication in the last decade (Zhang et al., 2021). Lake Hulun has experienced continuous water level declines in 2001-2012 (Fig. S7a), and in extremely arid years such as 2009, the concentrations of TN, TP and dissolved organic matter in Lake Hulun could be 0.5, 6.0 and 8.8 times higher than those in Lake Taihu, respectively (Chen et al., 2012; Chuai

Fig. 5. The environmental factors related to microbial community attributes and single functions. The relative contributions (%) of these factors to each response variable was estimated with Random Forest and represented by the color and size of solid circles. The significance of environmental factors on response variables were confirmed by using Pearson correlation analyses and labeled with asterisks. Red and black asterisk indicate positive and negative correlation, respectively. * p < 0.05, ** p < 0.01, * v < 0.001. Microbial community attributes include species richness, evenness and community compositions (the first axis of the community's principal coordinate analysis, PCoA1) of the bacteria, archaea and fungi in surface sediments. Single functions include β-glucosidase (BG), cellobiohydrolase (CBH), xylanase (BX) and β-galactosidase (GAL) involving in carbon cycling, leucine amino peptidase (LAP) involving in nitrogen cycling, p-Nacetylglucosaminidase (NAG) and N-acetyl-β-galactosaminidase (NAGA) involving in carbon and nitrogen cycling, aryl sulfatase (SUL) involving in sulfur cycling and acid phosphatase (AP) involving in phosphorous cycling. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

et al., 2012). In Lake Hulun, the TN and TP contents in the overlying water increase linearly with declining water level (p < 0.001, Fig. S7b). Thus, the continuous water level decline under climate warming might not only accelerate eutrophication and promote the formation of microbial water-depth patterns but also weaken whole-lake ecosystem functioning because of the dual loss of aquatic microbial biogeochemical cycling functionality in deep water zones and coastal zones driven by the conversion to drvlands. Moreover, biodiversity-EMF links have also recently been proposed to be indirectly controlled by human stressors and climate change (Jing et al., 2015). For example, the positive linear relationship between microbial diversity and EMF is mediated by the contents of heavy metals and phosphorus via changes in sediment microbial communities (Zhang et al., 2021). Regional-scale precipitation and temperature changes can modify the impacts of biodiversity on EMF in alpine grasslands (Jing et al., 2015). Our findings thus provide new evidence for the joint effects of human stressors (i.e., eutrophication) and climate change (i.e., decreased water depth associated with lake shrinkage) on lake microbial diversity-EMF relationships in arid regions.

5. Conclusions

Our study revealed consistent water-depth decay patterns in the community composition of benthic microbes, including bacteria, archaea and fungi, and increasing water-depth patterns of ecosystem multifunctionality and individual ecosystem functions related to C, N, P and S cycling in semiarid Lake Hulun. We further found that microbial community attributes, including evenness and community composition rather than species richness, dominated the variations in ecosystem multifunctionality. Furthermore, these links between the sediment microbial community and ecosystem multifunctionality were indirectly affected by lake water depth via nutrient enrichment and the microbial attributes of evenness and community composition. Our findings in semiarid lakes provide a new perspective on the growing literature in our understanding of the mechanisms underlying the responses of aquatic ecosystems to human stressors and climate change and highlight the urgency of protecting freshwater biodiversity from the joint influence of human activities and climate change to sustain lake ecosystem functioning in arid regions.



 $Df = 2; \chi^2 = 0.397; p = 0.82; CFI = 1; RMSEA = 0; p = 0.828$

Fig. 6. Structural equation models (SEM) of ecosystem multifunctionality (EMF) (a). In the SEMs, we obtained the best-fit model depicting the effects of abiotic and biotic factors on EMF by overall model fit statistics with the lowest Akaike information criterion (AIC) value. Abiotic factors included water depth and a parameter indicating all other physiochemical environmental factors measured in overlying water and surface sediments, which was represented by the first axis of their principal component analysis (Envi.PCA1). Biotic factors included the alpha diversities (richness and evenness) and composition (the first axis of the community's principal coordinate analysis, PCoA1sc) of the synthetic community of the three taxonomic groups including bacteria, archaea and fungi. In the model, the black and grey arrows represent significant (* p <0.05, ** p < 0.01, *** p < 0.001) and nonsignificant (p > 0.05) effects, respectively. The arrow widths and accompanying values denote standardized path coefficients (β). R² indicates the proportion of variance explained for the model variables. The fitting parameters of the model are listed below the model diagram.

CRediT authorship contribution statement

Weizhen Zhang: Investigation, Software, Formal analysis, Visualization, Writing – original draft. Ruirui Chen: Resources, Methodology, Data curation, Writing – review & editing. Fanfan Meng: Software, Formal analysis, Data curation. Haijun Yuan: Validation, Formal analysis. Mengdie Geng: Formal analysis. Longjuan Cheng: Investigation, Validation. Huaqun Yin: Writing – review & editing. Bin Xue: Writing – review & editing. Jianjun Wang: Supervision, Conceptualization, Resources, Writing – review & editing, Data curation, Funding acquisition.

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.

Acknowledgements

We appreciate Jingkui Tao for the help in sample collections. This study was supported by National Key R&D Program of China (2019YFA0607100), National Natural Science Foundation of China (91851117), CAS Key Research Program of Frontier Sciences (QYZDB- SSW-DQC043) and National Natural Science Foundation of China (41977045).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.108314.

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