Effects of wind-wave disturbance and nutrient addition on aquatic bacterial diversity, community composition, and co-occurrence patterns: A mesocosm study

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ABSTRACT

Wind-wave disturbance and nutrient input are the major environmental factors that shape bacterial diversity and community composition in lakes. However, knowledge of bacteria as bio-indicators' synergistic response to disturbance patterns and nutrient addition is still poor. To address this knowledge gap, we conducted a mesocosm experiment over 21 days, and collected 90 water samples from tanks under six different patterns of disturbance and nutrient addition; we explored the bacterial communities using high-throughput 16S rRNA gene sequencing. We found significantly increased bacterial diversity in continuously disturbed conditions and in intermittently disturbed conditions, and significantly decreased bacterial diversity under the synergistic effect of disturbances and nutrients. Bacterial community composition (BCC) under conditions of disturbance and nutrient addition exhibited different variations. However, there were no significant differences in BCC between continuously and intermittently disturbed mesocosms. Co-occurrence networks analysis showed that the proportion of positive correlations among edges in nutrient addition conditions were significantly higher than that in disturbed condition, indicating that nutrient input may increase the proportion of niche overlap, and enhance positive interactions among bacteria. Meanwhile, the co-occurrence patterns between algal and bacterial communities suggested shared environmental preferences or potential interactions among these two groups. Our study provides a new insight into the restoration of shallow eutrophic lake ecosystems. Moreover, the different distribution patterns of bacterial community act as ecological indicators of response to disturbances and nutrient input.

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1. Introduction

In lake ecosystems, the accelerating eutrophication induced by climate change and intensive human activities is an increasingly serious problem (Vonlanthen et al., 2012; Zhou et al., 2021), with profound effects on matter recycling and biochemical processes (Davidson et al., 2015; Zhou et al., 2018a). For example, eutrophication facilitates the proliferation of phytoplankton and induces algal blooms, as well as significantly influencing the physicochemical characteristics (such as nitrogen and phosphorus, chlorophyll-a, and dissolved organic matter) of both lake water and sediment (Zhou et al., 2018b; Yang et al., 2021). In shallow lakes, the effects of eutrophication may be compounded, because their sediments are easily suspended due to wind-wave disturbance, releasing nutrients into the water column, further increasing the nutrient level (Huang et al., 2016). In brief, lakes have been increasingly threatened by wind-wave disturbance and eutrophication, and these phenomena have attracted many attentions (Søndergaard et al., 1992; Zhu et al., 2005; Newton and McMahon, 2011; Cai et al., 2016; Chen et al., 2020; Tang et al., 2021).

As an essential ingredient of lake ecosystems, bacteria play a major role in nutrient recycling, energy flow, primary production, and organic matter mineralization (Zhou et al., 2018a; Zhou et al., 2021). Meanwhile, bacteria can provide quantifiable, meaningful and sensitive indicators of ecological change (Paerl et al., 2003). In large shallow lakes,
variations in the physicochemical characteristics of the water caused by wind-wave alters both bacterial activity and nutrient flow (Tang et al., 2009), affecting the decomposition rates of organic matter (Zhou et al., 2017). Therefore, revealing the responses of bacterial to wind-wave could provide novel insights into the mechanisms of disturbance. Furthermore, bacterial community composition (BCC) is also significantly shaped by nutrient composition (Chi et al., 2021). Sudden changes in nutrients accelerate shifts in the diversity, composition, function and co-occurrence pattern of bacterial communities (Newton and McMahon, 2011; Chi et al., 2021). Hence, it is important to have a deeper understanding of how BCC responds to disturbances and nutrient addition in order to better protect and manage lake ecosystems.

Bacterial communities in large shallow lakes are often affected by both wind-wave disturbance and nutrient input (Cai et al., 2016). In the past two decades, many studies have been conducted on BCC in lake ecosystems under diverse disturbance patterns and nutrient levels (Anderson et al., 2002; Newton and McMahon, 2011; Shao et al., 2013; Zhou et al., 2017; Cao et al., 2021). For instance, using 16S rRNA gene sequence combined with microcosm nutrient amendment experiments, Newton and McMahon (2011) reported that bacteria (e.g., Betaproteobacteria class and Bacteroidetes phylum) were favoured following nutrient addition, but bacterial taxa was not universally favoured by increased nutrient input. Zhou et al. (2017) studied bacterial diversity in Lake Taihu with a field simulation experiment, and found that bacterial diversity was not significantly different among low, medium and high nutrient levels, but bacterial community structure exhibited temporal differences in among treatments of the most abundant taxa. However, these studies considered only a single factor (disturbance or nutrient levels). We are not aware of any study that has investigated and revealed how BCC responds to both disturbance and nutrient input.

Furthermore, complex interactions, (such as competition, parasitism, and mutualism), among bacteria in lake ecosystems (Liu et al., 2019a; Liu et al., 2019b), may be indispensable in maintaining bacterial diversity, BCC, and ecosystem functioning (Ramanan et al., 2016; Ratzke et al., 2020). Technical approaches to studying species community structures include not only the routine analysis of sequencing data, such as diversity (e.g., α-diversity), BCC (e.g., analysis of similarity) and bacterial functional composition (e.g., PICRUSt, Tax4Fun, or FAPROTAX), but also addressing the direct and indirect interactions (i.e., co-occurrence patterns) for their communities (Luo et al., 2019). In recent years, co-occurrence networks have revealed the interactions between different species in various ecosystems (Duran et al., 2015; Luo et al., 2019; Zhou et al., 2021). Unfortunately, little is known about the interactions of bacterial communities subjected to simultaneous wind-wave disturbance and nutrient input.

To reveal the synergistic effects of wind-wave disturbances and nutrient addition on bacterial diversity, community composition, and co-occurrence patterns, we used mesocosms to investigate the bacterial and algal communities in shallow, eutrophic, Lake Taihu. We hypothesized the following: (1) bacterial diversity would increase under wind-wave disturbances (continuously disturbed, or intermittently disturbed), but decrease under significant nutrient addition; (2) BCC would exhibit different temporal variations under wind-wave disturbances and nutrient addition; and (3) co-occurrence patterns of bacterial and algal communities, as well as the interaction between them, will differ under wind-wave disturbances and nutrient addition.

2. Materials and methods

2.1. Experiment setup

The mesocosm experiment was conducted on flat ground at Jiangnan University, in Wuxi City in eastern China (Jiangsu Province) from 7 to 31 May 2017. We collected the lake water, containing Microcystis, from the natural freshwater Meiliang Bay in Lake Taihu. This lake, (30°56’-31°33’N, 119°53’-120°36’E), is shallow and eutrophic, and strongly influenced by wind-wave disturbance and nutrient input (Huang et al., 2016; Su et al., 2017).

Water was distributed to a total of eighteen 100 L cylindrical, sterilized water washed high-density polyethylene tanks (d = 50 cm, h = 60 cm), to a depth of 50 cm. We then determined total nitrogen (TN = 1.96 mg/L) and total phosphorus (TP = 0.11 mg/L) in the water, and allowed the tanks to stand undisturbed for three days before the experiment began. Half of the tanks did not have nutrients added, whereas nitrogen and phosphorus were added to the other nine tanks to simulate the hyper-eutrophication of Lake Taihu during cyano-bacterial bloom or ephemeral hypoxia events, so that TN and TP in the water reached 10 and 0.5 mg/L, respectively, with addition of NaNO₃ and K₂HPO₄•3H₂O, (Yang et al. 2020).

Our experiment comprised six treatment groups, with each treatment performed in triplicate (hence 6 × 3 = 18 tanks). Treatments, summarized in Table 1, were: (A) undisturbed with no nutrient addition (control); (B) undisturbed, with nutrient addition; (C) continuously disturbed, no nutrient addition; (D) continuously disturbed, with nutrient addition; (E) intermittently disturbed, no nutrient addition; and (F), intermittently disturbed, with nutrient addition. Disturbance was created by using a wave-making pump (60 W, 2 Hz/Jehao, China). In continuous disturbance treatments (C, D), the disturbance was maintained throughout the experiment. In intermittent disturbance treatments (E, F), the disturbance was applied for 24 h on days 1, 5, 9, 13, 17, and 21 (Yang et al., 2020). Detailed information about the experiment site and simulation system is presented in our previous study (Yang et al. 2020).

2.2. Sample collection and analyses

Intensity of illumination (× 10³ Lux; in 25 cm depth), water temperature (WT), and pH were recorded in each tank, each day, at 9:00 a.m. Seven other physicochemical parameters were recorded less frequently, on days 0, 1, 5, 9, 13, 17, and 21. For these parameters, on measurement day, 1.5 L of water was collected from each tank at 9:00 a.m. By reference to standard methods (Jin and Tu, 1990), we determined: suspended solids (SS), ammonia nitrogen (NH₄-N), orthophosphate (PO₄³⁻-P), chlorophyll-α (Chl-α), nitrogen nitrate (NO₃-N), total dissolved phosphorus (DTP), and total soluble nitrogen (DTN). Phytoplankton samples were fixed with Lugol’s iodine solution (1% final concentration), settled for 48 h, and then counted using a microscope magnification of 200–400 (Jin and Tu, 1990; Yang et al., 2020).

To prepare for sequencing of bacteria, a total of 90 water samples (5 times × 6 treatments × 3 replicates) were collected from the 18 tanks on days 0, 1, 5, 13 and 21. Each sample was filtered through a 0.2 μm pore-size polycarbonate filter (Isopore™, Millipore), and then immediately preserved at -80°C before DNA extraction.

Table 1

| Treatment | Disturbance | Nutrients |
|-----------|-------------|-----------|
| A | Undisturbed | No nutrient addition |
| B | Undisturbed | Nutrient addition |
| C | Continuous | No nutrient addition |
| D | Continuous | Nutrient addition |
| E | Intermittent | No nutrient addition |
| F | Intermittent | Nutrient addition |

Note: A, undisturbed, no nutrient addition; B, undisturbed, nutrient addition; C, continuously disturbed, no nutrient addition; D, continuously disturbed, nutrient addition; E, intermittently disturbed, no nutrient addition; F, intermittently disturbed, nutrient addition.
2.3. DNA extraction, 16S rRNA sequencing, and sequence analysis

The total bacterial DNA for 16S rRNA sequencing was extracted from the filtered water samples using the FastDNA® Spin Kit for Soil (MP Biomedicals, USA), in accordance with the manufacturer’s protocols. The V3–V4 regions of the bacterial 16S rRNA genes from each extracted DNA sample were amplified with the primer sets 338F/806R (Fadrosh et al., 2014). All PCR reactions were carried out in triplicate for each sample using the following program: predenaturing at 98 °C for 3 min; 27 cycles of 98 °C for 45 s, 55 °C for 45 s, and 72 °C for 45 s, and a final elongation at 72 °C for 7 min. Amplicons were extracted and purified using an AsyPrep DNA Gel Extraction Kit (Axxygen Biosciences, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300 bp) on an Illumina MiSeq PE300 platform (Illumina, USA), following the standard protocols by Magi Gene-Biotech Co. Ltd. (Guangzhou, China).

All raw sequences were processed using QIIME v1.9.0 (Caporaso et al., 2010), according to the criteria of Zhang et al. (2021). Chimeras were further identified and discarded using VSEARCH by the de novo method (Rognes et al., 2016). Then, sequences were clustered to generate operational taxonomic units (OTUs) via UPARSE v7.1 with a 97% similarity threshold (Edgar, 2013). To minimize random sequencing error, low abundance OTUs (< 10 reads) were filtered out from subsequent analysis. Finally, representative OTUs were identified by RDP classifier using the SILVA v132 database (Quast et al., 2012; Zhang et al., 2021).

2.4. Co-occurrence networks analysis

To better understand the interaction of bacterial communities, species co-occurrence patterns were constructed using network theory (Gotelli and McCabe, 2002; Zhou and Ning, 2017). Spearman’s correlation coefficients were implemented using the package “psych” in R, with a significant (FDR-adjusted p-value < 0.01) and strong coefficient (|r| ≥ 0.8) was considered in those dominant OTUs (Barberán et al., 2012). Topological properties of the co-occurrence networks and 100 random networks were also examined using the package “igraph” in R (Ju et al., 2016). Nodes with a high value of degrees and a low value of betweenness centrality were determined as keystone bacteria (Berry and Widder, 2014; Zhang et al., 2020). The co-occurrence networks were revealed by the interactive platform Gephi (v0.9.2).

2.5. Statistical analysis

Significant differences in physicochemical parameters among all six treatments were examined by Kruskal-Wallis rank sum tests using R v3.6.3 and the RStudio v1.1.463 interface. Other statistical analyses were implemented using R, unless otherwise specified. Data visualization in this study was carried out by using the packages “ggplot2” and “pheatmap” in R.

To normalize the sequencing depth for each sample, a subset of 9,921 reads were randomly selected, according to those samples with the minimum quantity of sequences. To examine statistical differences in bacterial α-diversity for all treatment groups, we calculated Chao1 and PD whole tree (hereafter PD) for each sample using the “vegan” package in R. To evaluate β-diversity (variation of community structures) among different treatments and time, we performed non-metric multi-dimensional scaling (NMDS) by using the Bray–Curtis distance in R. Analysis of similarity (ANOSIM) was performed to test the compositional differences for each pairwise samples. Taxonomic differences were identified by significantly different biomarkers in different treatment groups using linear discriminant analysis (LDA) effect size (LEfSe) (Segata et al., 2011). To calculate the Spearman’s correlation between BCC and algal community composition (ACC), the Mantel permutation tests were executed using the “vegan” package in R with 9999 permutations (Tang et al., 2021).

2.6. Data availability

All raw sequencing reads in this study have been deposited in the Genome Sequence Archive (GSA) database (http://gsa.big.ac.cn) under accession number CRA005183.

3. Results

3.1. Physicochemical characterization

Physicochemical parameters varied between the six treatments, and for eight of the ten parameters, the difference was significant (Fig. 1 and Table S1); (the two exceptions were WT and intensity of illumination). The pH, and concentrations of Chl-a, DTN, DTP, NO3-N, PO43-, NH4-N, and SS, were significantly different between Nno-add (no nutrient addition treatments; A, C, E) and Nadd (nutrient addition treatments; B, D, F) (all P < 0.05); pH was lower in nutrient addition treatments, and the other five parameters were higher in nutrient addition treatments (Table S1).

Among the three treatments without nutrient addition (Nno-add), there were significant differences in two parameters: the SS in treatment C was significantly higher than in treatment A (P < 0.05), and intensity of illumination in treatment A was significantly higher than in C and E (P < 0.05; Table S1). Among the three treatments with nutrient addition (Nadd), there were significant differences in four parameters: pH in treatment D was significantly higher than those in treatment B and F (P < 0.05); and DTN, DTP and intensity of illumination in treatments B and F were significantly higher than in treatment D (P < 0.05; Table S1).

Over time, DTN decreased gradually in all treatments, and DTN levels in Nno-add were significantly lower than those in Nadd (P < 0.001; Fig. 1). In contrast, the DTP decreased rapidly within 5 days in all treatments; DTP levels in Nno-add were significantly lower than Nadd (P < 0.001; Fig. 1). Chl-a increased slowly in Nno-add, and treatments C and E were larger than treatment A (Fig. 1).

3.2. Bacterial α-diversity and β-diversity

Overall, we generated 2,808,856 high-quality sequences. Among the 90 samples, a total of 3,455 OTUs was obtained, with an average sequencing depth of 31,210. The average PD was 43, and average Chao1 index was 792. The rarefaction curves of richness approached an asymptote after 21,150 sequences (Fig. S1), indicating sufficient sequencing depth.

Bacterial α-diversity in all treatments is presented in Fig. 2. In Nno-add (A, C, and E), Chao1 in treatment A (mean = 712) was significantly lower than those in treatments C and E (mean = 963 and 968, respectively; P < 0.05), and PD in treatments C and E (mean = 50 in both treatments) was significantly higher than in treatment A (mean = 40; P < 0.01). However, Chao1 and PD were not significantly different in treatments C and E. In Nadd (B, D, and F), Chao1 and PD in treatment D (mean = 870 and 47, respectively) were significantly higher than treatment B (mean = 642 and 38; P < 0.01 and 0.05, respectively). Chao1 and PD were not significantly different in treatments A and B. Moreover, Chao1 and PD in Nno-add were significantly higher than that in Nadd.

To evaluate changes in β-diversity between treatments over time, we calculated NMDS (Fig. 3 (bacteria) and Fig. 52 (algae)). The results showed that bacterial community compositions (BCCs) differed significantly in different treatments (r = 0.22, P = 0.001) and time (r = 0.41, P = 0.001). The ANOSIM revealed that the BCCs between Nno-add and Nadd were significantly different (P = 0.001) (Table 2). Within Nno-add, interestingly, BCCs were not significantly different between treatments C and E (Table 2). Within Nadd, there were significant differences in BCCs between each paired treatment (P < 0.05) (Table 2). In addition, the NMDS plot (Fig. S2) revealed that algal community composition (ACC) differed significantly in different treatments (r = 0.06, P = 0.01).
and with time ($r = 0.52$, $P = 0.001$).

3.3. Bacterial taxonomy and community structure

The representative OTU sequences were identified and grouped under 39 phyla. The relative abundances of the first six phyla among the six treatments were exhibited by filtering higher than 1% frequency in at least one sample (Fig. 4). In $N_{\text{no-add}}$ treatments (ACE), the most common bacterial phyla were Proteobacteria (average 31.1%), Cyanobacteria (18.6%), Actinobacteria (17.3%), Bacteroidetes (10.7%), Verrucomicrobia (9.9%), and Planctomycetes (7.4%). In $N_{\text{add}}$ treatments (BDF) the most dominant bacterial phylum was also Proteobacteria (43.9%), then followed by Bacteroidetes (17.0%), Cyanobacteria (13.0%), Planctomycetes (8.5%), Actinobacteria (6.9%), and Verrucomicrobia (5.2%).

In $N_{\text{no-add}}$, on day 5, the dominant phyla were Cyanobacteria and Actinobacteria, which accounted for 30.0% (treatments C) and 30.1% (treatments E) of the total sequences respectively. On day 13, these two phyla continued to be dominant, accounting for 25.6% and 36.7% of the total sequences respectively. In contrast, in $N_{\text{add}}$ on day 5, Proteobacteria was the most abundant phylum, accounting for 79.0% (treatment B), 66.2% (treatment D) and 59.4% (treatment F) of the total sequences, respectively. In contrast, in $N_{\text{add}}$ on day 13, Bacteroidetes was the most dominant phylum accounting for 22.0% (treatment B), 26.1% (treatment D) and 64.0% (treatment F) of the total sequences, respectively.
At the genus level, the BCCs exhibited different and variable patterns over the 21 days of the experiment (Fig. S3). Within N_{no-add} and N_{add}, the taxonomic composition of different treatments varied widely, whereas the BCCs in treatments C and E were similar to each other. The most dominant genera in treatments A–F were Porphyrobacter, Flavobacterium, Prochlorococcus and the hgcI clade.

By carrying out LEfSe using default parameters, we identified 41 bacteria taxa with significant differences between treatments A–F (Fig. 5), and which could therefore be considered as biomarkers. Generally, the mean proportions of Firmicutes and Gammaproteobacteria were significantly higher in treatments F and B than in the other four treatments respectively. Acidimicrobia, Corynebacteriales, Cytophagaceae and some unassigned bacteria were significantly different in treatment C. In treatment E, the genera Ferruginibacter and hgcI clade were the main biomarkers. In addition, the Rhodopirellula of Planctomycetes was enriched in treatment D.

### 3.4. Relationship between BCC and ACC

The NMDS showed that variation in community composition between N_{no-add} and N_{add} was similar in bacteria (Fig. 3) and algae (Fig. S2). In N_{no-add} treatments (A, C, E), the Mantel test revealed that the BCC was significantly positively related to the ACC ($r = 0.26$, $P < 0.05$; Fig. 6). In contrast, within N_{add} (B, D and F), the BCC and the ACC were significantly higher in treatments F and B than in the other four treatments respectively. Acidimicrobia, Corynebacteriales, Cytophagaceae and some unassigned bacteria were significantly different in treatment C. In treatment E, the genera Ferruginibacter and hgcI clade were the main biomarkers. In addition, the Rhodopirellula of Planctomycetes was enriched in treatment D.
Fig. 4. Relative abundance of bacteria taxa at the phylum level for the experimental days 0, 1, 5, 13, 21, and the treatments A to E. Treatments: A, undisturbed, no nutrient addition; B, undisturbed, nutrient addition; C, continuously disturbed, no nutrient addition; D, continuously disturbed, nutrient addition; E, intermittently disturbed, nutrient addition; F, intermittently disturbed, nutrient addition.

Fig. 5. LEfSe results showing the taxonomic differences of bacterial communities in six treatments. LEfSe = Linear discriminant analysis (LDA) effect size. Different colored circles represent different treatments and these bacteria were significantly enriched. Yellow circles represent the taxa with non-significant differences. All detected taxa were assigned to phyla (outermost), classes, orders, families, and genera (innermost) using linear discriminant analysis (LDA) with LDA > 2.0 and $P < 0.05$. The diameters of the circles are proportional to relative abundance. Treatments: A, undisturbed, no nutrient addition; B, undisturbed, nutrient addition; C, continuously disturbed, no nutrient addition; D, continuously disturbed, nutrient addition; E, intermittently disturbed, nutrient addition; F, intermittently disturbed, nutrient addition.
Erd (AvgCC) was higher, in the six networks than in those of their treatments (Table 3), indicating more interactions among the species in coefficients (edges were captured in those networks (Fig. 7; Table 3). The average different treatments were examined to determine their ecological in

3.5. Co-occurrence networks of bacterial communities

The co-occurrence patterns of bacterial communities among different treatments were examined to determine their ecological interactions. Based on correlation analysis, different quantities of nodes and edges were captured in those networks (Fig. 7; Table 3). The average path length (APL) was lower, and the average clustering coefficient (AvgCC) was higher, in the six networks than in those of their Erdős–Rényi random networks. Accordingly, the small-world co-efficients (σ) > 1, suggested that these networks have small-world properties (Tang et al., 2020; Zhang et al., 2021).

In N_no-add treatments, the co-occurrence networks had a higher graph density (GD) and a higher average degree (AD) than did those in N_add treatments (Table 3), indicating more interactions among the species in N_no-add than in N_add. In addition, the co-occurrence patterns of those bacterial communities were mostly positively structured in all treatments, revealing ecological mutualistic relationships or cooperation and niche overlap in the bacterial communities. Notably, the proportion of positive correlations among edges in N_add treatments (B: 85.0%; D: 91.9%; F: 74.7%) were significantly higher than those in N_no-add treatments (A: 60.9%; C: 68.7%; E: 61.2%).

Keystone species were identified in these networks, according to the nodes with a high value of degrees and a low value of betweenness centrality (Berry and Widder, 2014; Zhang et al., 2020). Cyanobacteria were identified as keystone species in treatment A, while Bacteroidetes and Verrucomicrobia were the keystone species in treatment E. In treatment A, our results revealed that Proteobacteria were significantly positively correlated with Microcystis spp. and with Synechococcus sp. (|r| ≥ 0.8, P < 0.01; similarly hereinafter), respectively, yet significantly negatively correlated with Anabaenopsis arnoldii. In addition, the Spearman’s correlation between Verrucomicrobia and Cyclotella spp. showed significantly negative correlations, however, Verrucomicrobia were significantly positively correlated with Anabaenopsis arnoldii. In treatment C, Proteobacteria, Actinobacteria, Verrucomicrobia, Bacteroidetes, and Synechococcus sp. formed complex interactions, and had a modular network structure. In treatment E however, the algae Microcystis spp., Cyclotella spp., and Aulacoseira granulata formed a small-network by their interaction with bacteria.

4. Discussion

4.1. Responses of bacterial diversity to wind-wave disturbance and nutrient addition

In our study, the bacterial diversity in disturbed mesocosms without nutrient addition (C: continuously disturbed; E: intermittently disturbed) increased significantly compared to undisturbed controls (A), while bacterial diversity under the synergistic effects of disturbance and nutrients (B, D, and F) decreased significantly compared to no nutrient addition conditions (A, C, and E) (Fig. 2). There are several possible explanations for this result. First, hydrodynamic disturbance changes the distribution of nutrients in the water, which was beneficial for nutrient adsorption and competition of bacteria, and the appropriate disturbance intensity was most conducive to the chemotaxis of bacteria (Taylor and Stocker, 2012). Second, disturbance reduces the predation pressure of bacteria, which was conducive to the accumulation of bacterial biomass and promotes the growth of bacteria (Peters et al., 2002; Zhou and Qin, 2018). Third, appropriate disturbance promotes phytoplankton to secrete dissolved organic matter (DOM), which is beneficial to the maintenance of bacterial diversity (Benavides et al., 2013).

However, our result is contrary to the findings of Zhou et al. (2017), who investigated bacterial diversity in Lake Taihu with a field simulation experiment, and found that bacterial diversity was not significantly different among low, medium and high disturbances. This contrast may be attributed to the different disturbance patterns and time scales in the two studies. In our study, we simulated disturbance as continuous (wave pump used every day) or intermittent (pump used on days 1, 5, 9, 13, 17, and 21), whereas Zhou et al. (2017) studied four intensities of disturbance: calm water (ε: 0), low (ε: 1.12 × 10⁻⁶ m² s⁻³), medium (ε: 2.95 × 10⁻⁷ m² s⁻³) and high disturbance (ε: 1.48 × 10⁻⁴ m² s⁻³); furthermore, our study was conducted for 21 days, whereas Zhou et al. (2017) conducted their field simulation for 9 days.

We further found that bacterial diversity was not significantly different between continuously disturbed and intermittently disturbed treatments (P > 0.05; Fig. 2). This result is consistent with previous research findings (Shade et al., 2010; Shade et al., 2012; Li et al., 2015; Zhou et al., 2017). Shade et al. (2010) reported that bacterial diversity was resistant to these disturbances, even after intense mixing incidents such as typhoons. In addition, although wind-wave disturbance was more intense in Lake Taihu, bacterial diversity maintained high similarity across locations (Shade et al., 2012; Li et al., 2015; Tang et al., 2017).

Bacterial diversity in shallow eutrophic lakes is often influenced by the synergy of disturbance and nutrients (Cai et al., 2016). In our present study, we added excessive nutrients to simulate hyper-eutrophication that is experienced in Lake Taihu during cyanobacteria bloom or ephemeral hypoxia events. Our result showed that although disturbance was beneficial for increasing bacterial diversity, addition of excess nutrients decreased bacterial diversity, indicating that nutrients have a greater impact on bacterial diversity than does disturbance. It is well known that nutrients play a vital role in bacterial growth (Kolmonen et al., 2011). Excess nutrients are one of the most important factors affecting bacterial diversity in aquatic ecosystems, although other factors such as disturbance also have potential effects (Liu et al., 2021). For the last two decades, many studies have revealed that the maximum bacterial diversity generally occurred at intermediate levels of nutrients or productivity (Kassen et al., 2006; Zvirgmaier et al., 2015; Tang et al.,
Fig. 7. Co-occurrence networks for bacterial-algal (A, C, E) and bacterial (B, D, F) communities in six treatments. A connection represents a significant abs(r) > 0.8 (P < 0.01). Different colored circles represent different bacterial taxa. The size of each node is proportional to the number of connections (i.e., degrees), and the nodes are colored according to different phyla. Red and green edges indicate positive and negative correlations, respectively. A, C, and E, no nutrient addition; B, D, and F, nutrient addition.
In our present study, ANOSIM revealed that the BCCs were significantly addition could alter BCC (Burton and Johnston, 2010; Chi et al., 2021).

4.2. Changes of BCC in response to wind-wave disturbance and nutrient addition, the negative correlation between nutrient increase and bacterial diversity decrease may be due to indirect effects. Addition of inorganic nitrogen and phosphorus (eutrophication) leads to a higher material diversity decrease may be due to indirect effects. Addition of nutrients were not only dissolved but also given in an excessive amount. Therefore growth of the planktonic algae should not have been restricted, and could be independent of the nutrients produced by bacterial mineralization in the process of degrading organic matter; this may be main reason for the insignificant relationship between the algal and bacterial communities. Thus, our results supported our second hypothesis that BCC under conditions of disturbance and nutrient addition exhibit different variations over time.

4.3. Differences in bacterial co-occurrence patterns in response to wind-wave disturbance and nutrient addition

For two decades, many researches have revealed correlations among bacterial communities (Pinhasi et al., 2004; Liu et al., 2014; Liu et al., 2019a; Xia et al., 2020). However, the co-occurrence patterns of bacterial communities under conditions of simultaneous disturbance and nutrient input have rarely been investigated. Our results revealed that the proportion of positive correlations among edges in $N_{add}$ were significantly higher than that in $N_{no-add}$ (Table 3). This result agrees with Zhou et al. (2021), who found that the proportion of positive correlations was increased substantially with increasing nutrient levels. In addition, Chi et al. (2021) investigated the co-occurrence pattern of soil microorganisms, and found more positive correlations under nitrogen and phosphorus stress. Overall, we suggested that nutrient input may increase the proportion of niche overlap, and enhance positive interactions among bacteria. Higher availability of nutrients may also reduce competitive pressure among members of the bacterial community (Zhou et al., 2021). This result was also supported by finding that nutrient addition could alleviate bacterial competition, reducing the negative correlation among bacteria (Chi et al., 2021). Thus, adjustment of bacterial co-occurrence patterns is a strategy to respond to environmental changes such as nutrient input.

Our results revealed that positive correlations play a dominant role
in the co-occurrence networks of bacteria and algae in $N_{\text{no-add}}$ conditions (Table 3; Fig. 7), indicating that algae indirectly affect the co-occurrence patterns of bacterial communities. This result was consistent with research by Xia et al. (2020), who studied bacteria and algal community compositions and correlations in a plateau lake using co-occurrence network analysis, and revealed that positive correlations dominated in the bacterial and algal co-occurrence networks. The positive correlations between bacteria and algae (e.g., *Proteobacteria* and *Microcystis* spp., *Synechococcus* and *Anabaenopsis arnoldii*) may illustrate that bacteria-algae can thrive under specific disturbance conditions, and that functional interdependencies exist among them. Generally, algae, as primary producers, provide DOM for bacteria. In addition, bacteria can provide nutrients (such as nitrogen) for algae, and protect these algae by producing antibiotics and competition, so as to further safeguard the symbiosomal function (Pantos et al., 2015). In summary, with the indirect effect of disturbance, specific algae interact with specific bacteria, thereby shaping changes in the bacterial community structure.

Furthermore, we also found negative correlations (e.g., *Proteobacteria* and *Anabaenopsis arnoldii*; *Verrucomicrobia* and *Cyclotella* spp.) between bacteria and algae from co-occurrence network in $N_{\text{no-add}}$. There are several possible explanations for this result. First, heterotrophic bacteria obtain nitrogen more efficiently from organic compounds (such as leucine) than do algae, and heterotrophic bacteria compete for nutrients (Xia et al., 2020). Second, studies have reported that healthy macroalgae can release specific chemicals (Jha et al., 2013), which can affect bacterial colonization on their surfaces, inhibiting bacterial overgrowth (Kouzuma and Watanabe, 2015). Third, algae excrete toxic chemicals that killed bacteria, and different bacteria taxa can produce algicidal metabolites (Amin et al., 2012; Kouzuma and Watanabe, 2015). Thus, our third hypothesis was supported.

Our study has provided new insight into bacterial communities’ responses to simultaneous disturbance and nutrient addition. However, there are some limitations which should be considered. First, the tank experiment may have a marginal effect. Second, we used only a single level of nutrient addition, to simulate the hyper-eutrophication of Lake Taihu during cyanobacterial bloom or ephemeral hypoxia events. Further work is required to reveal the synergistic effects of wind-wave disturbance and different nutrient gradients on lake bacterial communities in different seasons.

5. Conclusions

In the present mesocosm study, we investigated the bacterial communities of Lake Taihu under conditions of disturbance and nutrient addition. Bacterial diversity in disturbed mesocosms increased significantly compared to undisturbed controls, while bacterial diversity under the synergistic effects of disturbances and nutrients decreased significantly compared to no nutrient addition conditions. Our results revealed that under conditions of disturbance and nutrient addition showed different temporal variations. Furthermore, the continuously disturbed and intermittently disturbed conditions did not have significantly different effects on bacterial diversity and BCCs. The changes in BCCs among no nutrient addition conditions may be indirectly influenced by algae, affecting the succession of bacterial communities. With the indirect effect of disturbance, specific algae interact with specific bacteria, thereby shaping changes in the BCC. In addition, nutrient input increases the proportion of niche overlap, and enhances positive interactions among bacteria.

CRediT authorship contribution statement

**Zhen Shen:** Investigation, Data curation, Formal analysis, Software, Writing – original draft. **Guijuan Xie:** Investigation, Formal analysis, Methodology. **Wei Tian:** Investigation, Software, Formal analysis, Supervision. **Keqiang Shao:** Project administration, Resources, Funding acquisition. **Guijun Yang:** Validation, Visualization, Supervision. **Xiangming Tang:** Conceptualization, Investigation, Software, Writing – review & editing, 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.

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**Supplementary materials**

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2022.100168.

**References**

Amin, S.A., Parker, M.S., Armbrust, E.V., 2012. Interactions between diatoms and bacteria. Microbiol. Mol. Biol. Rev. 76 (3), 667–684. https://doi.org/10.1128/MMBR.00007-12.

Anderson, D.M., Gilbert, P.M., Burkholder, J.M., 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries 25 (4), 704–726. https://doi.org/10.1080/02727700290084721.

Barberán, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. The ISME J. 6 (2), 304–315. https://doi.org/10.1038/ismej.2011.191.

Benavides, M., Agawin, N.S., Aristegui, J., Peene, J., Stal, L.J., 2013. Dissolved organic nitrogen and carbon release by a marine unicellular diazotrophic cyanobacterium. Aquat. Microb. Ecol. 69 (1), 69–80. https://doi.org/10.3389/ame.01621.

Berry, D., Widdier, S., 2014. Deciphering microbial interactions and detecting keystone species with co-occurrence networks. Front. Microbiol. 5, 219. https://doi.org/10.3389/fmicb.2014.00219.

Burton, G.A., Johnston, E.L., 2010. Assessing contaminated sediments in the context of multiple stressors. Environ. Toxicol. Chem. 29 (12), 2625–2643. https://doi.org/10.1002/etc.332.

Cai, J., Bai, C.R., Tang, X.M., Dai, J.Y., Gong, Y., Hu, Y., Shao, K.Q., Zhou, L., Gao, G., 2018. Characterization of bacterial and microbial eukaryotic communities associated with an ephemeral hypoxia event in Taihu Lake, a shallow eutrophic Chinese lake. Environ. Sci. Pollut. Res. 25 (31), 31543–31557. https://doi.org/10.1007/s11356-018-2987-x.

Cai, Y.J., Yu, Y.J., Liu, J.S., Dai, X.L., Xu, H., Lu, Y., Gong, Z.J., 2016. Macronutrient gradient community structure in a large shallow lake: Distinguishing the effect of eutrophication and wind-wave disturbance. Limnoloxia 59, 1–9. https://doi.org/10.1016/j.limno.2016.03.006.

Cao, Y.Y., Zhao, D.Y., Zeng, J., Huang, R., He, F., 2021. Biogeographical patterns of abundant and rare bacterial and microeukaryotic subcommunities in connected freshwater lake zones subjected to different levels of nutrient loading. J. Appl. Microbiol. 130 (1), 123–132. https://doi.org/10.1111/jam.14720.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7 (5), 335–336. https://doi.org/10.1038/nmeth.f.303.

Chen, J., Wu, J.L., Liu, M., Li, L.Q., Zhang, W.J., Wang, D.S., Ma, T., 2021. Bacterial community structure in the surface sediments of different habitats of Baiyangdian Lake, Northern China: effects of nutrient conditions. J. Soils Sed. 21 (4), 1866–1874. https://doi.org/10.1007/s13668-021-02901-6.

Chen, S.N., Yan, M.M., Huang, T.L., Zhang, H., Liu, K.W., Huang, X., Li, N., Miao, Y.T., Sekar, R., 2020. Disentangling the drivers of Microcystis community composition: Metabolic profile and co-occurrence of bacterial community. Sci. Total Environ. 739, 140062. https://doi.org/10.1016/j.scitotenv.2020.140062.

Chi, Z.F., Ju, S.J., Li, H., Li, J.L., Wu, H.T., Yan, B.X., 2021. Deciphering epiphytic bacterial community and function potential in a Chinese delta under exogenous nutrient input and salinity stress. Catena 201, 105212. https://doi.org/10.1016/j.catena.2021.105212.

Davidson, T.A., Audet, J., Swehling, J.C., Lauridsen, T.L., Sorensgaard, M., Landkildehus, F., Larsen, S.E., Jeppesen, E., 2015. Eutrophication effects on greenhouse gas fluxes from shallow-lake mesocosms override those of climate warming. Global Change Biol. 21 (12), 4449–4463. https://doi.org/10.1111/gcb.13962.
Z. Shen et al.

Ju, W., Li, J., Yu, W., Zhang, R., 2016. iGraph: an incremental data processing system for large-scale data. Bioinformatics 32 (1), 1-7. https://doi.org/10.1093/bioinformatics/btw644.

Gettel, N.J., McCabe, D.J., 2002. Species co-occurrence: a meta-analysis of JM Diamond’s assembly rules model. Ecology 83 (8), 2091–2096. https://doi.org/10.1890/01-1693.

Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14, 313–334. https://doi.org/10.1016/j.hal.2011.10.027.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12 (6), 1-18. https://doi.org/10.1186/gb-2011-12-6-n60.

Shade, A., Chiu, C.Y., McMahon, K., 2010. Differential bacterial diversity promotes emergent community robustness to lake mixing: an epiphenomenon to hypolimnion transport experiment. Environ. Microbiol. 12 (2), 455–466. https://doi.org/10.1111/j.1462-2920.2009.01927.x.

Shade, A., Read, J.S., Youngblut, N.D., Fierer, N., Knight, R., Krutz, T.K., Lott, N.R., Roden, E.E., Stanley, E.H., Stombaugh, J., 2012. Lake microbial communities are resilient after a whole-ecosystem disturbance. ISME J. 6 (12), 2153–2167. https://doi.org/10.1038/ismej.2012.56.

Shao, K.Q., Gao, X., Yang, J.Y., Cai, H., Wu, Y., Zhang, L., Qin, B.Q., 2013. Low resilience of the particle-associated bacterial community in response to frequent wind-wave disturbance in freshwater macrophytes. Microbes Environ. 28 (4), 450–456. https://doi.org/10.1093/mee/mts016.

Shao, K.Q., Gao, G., Yang, X., Yang, Y.F., Chu, H.Y., 2021. Co-occurrence network to explore the effects of bio-augmentation on the microbial community in the yellow river estuary. Microbiome 2 (1), 1. https://doi.org/10.1186/s40168-020-00240-1.

Shao, K.Q., Gao, G., Yang, Y.F., Chu, H.Y., 2020. Strong linkages between dissolved organic carbon and bacterial community composition in large shallow eutrophic Lake Taihu: High overlap between free-living and particle-associated assemblages. Limnol. Oceanogr. 62 (4), 1366–1382. https://doi.org/10.1002/lno.10502.

Shao, T.X., Gao, G., Qiu, B., Zhou, Y., Shi, Y., Liu, X., Yang, Y.F., Chu, H.Y., 2021. Co-occurrence network to explore the effects of bio-augmentation on the microbial community in the yellow river estuary. Microbiome 2 (1), 1. https://doi.org/10.1186/s40168-020-00240-1.

Shao, T.X., Gao, G., Qiu, B., Zhou, Y., Shi, Y., Liu, X., Yang, Y.F., Chu, H.Y., 2021. Co-occurrence network to explore the effects of bio-augmentation on the microbial community in the yellow river estuary. Microbiome 2 (1), 1. https://doi.org/10.1186/s40168-020-00240-1.

Shao, T.X., Gao, G., Qiu, B., Zhou, Y., Shi, Y., Liu, X., Yang, Y.F., Chu, H.Y., 2021. Co-occurrence network to explore the effects of bio-augmentation on the microbial community in the yellow river estuary. Microbiome 2 (1), 1. https://doi.org/10.1186/s40168-020-00240-1.

Shao, T.X., Gao, G., Qiu, B., Zhou, Y., Shi, Y., Liu, X., Yang, Y.F., Chu, H.Y., 2021. Co-occurrence network to explore the effects of bio-augmentation on the microbial community in the yellow river estuary. Microbiome 2 (1), 1. https://doi.org/10.1186/s40168-020-00240-1.

Shao, T.X., Gao, G., Qiu, B., Zhou, Y., Shi, Y., Liu, X., Yang, Y.F., Chu, H.Y., 2021. Co-occurrence network to explore the effects of bio-augmentation on the microbial community in the yellow river estuary. Microbiome 2 (1), 1. https://doi.org/10.1186/s40168-020-00240-1.

Shao, T.X., Gao, G., Qiu, B., Zhou, Y., Shi, Y., Liu, X., Yang, Y.F., Chu, H.Y., 2021. Co-occurrence network to explore the effects of bio-augmentation on the microbial community in the yellow river estuary. Microbiome 2 (1), 1. https://doi.org/10.1186/s40168-020-00240-1.

Shao, T.X., Gao, G., Qiu, B., Zhou, Y., Shi, Y., Liu, X., Yang, Y.F., Chu, H.Y., 2021. Co-occurrence network to explore the effects of bio-augmentation on the microbial community in the yellow river estuary. Microbiome 2 (1), 1. https://doi.org/10.1186/s40168-020-00240-1.
eutrophic lake. Sci. Rep. 7 (1), 16850. https://doi.org/10.1038/s41598-017-17242-z.

Zhou, J., Richlen, M.L., Sehein, T.R., Kulis, D.M., Anderson, D.M., Cai, Z., 2018a. Microbial community structure and associations during a marine dinoflagellate bloom. Front. Microbiol. 9, 1201. https://doi.org/10.3389/fmicb.2018.01201.

Zhou, J.Z., Ning, D.L., 2017. Stochastic community assembly: does it matter in microbial ecology? Microbiol. Mol. Biol. Rev. 81 (4), e00002–17. https://doi.org/10.1128/MMBR.00002-17.

Zhou, L., Zhou, Y.Q., Tang, X.M., Zhang, Y.L., Zhu, G.W., Székely, A.J., Jeppesen, E., 2021. Eutrophication alters bacterial co-occurrence networks and increases the importance of chromophoric dissolved organic matter composition. Limnol. Oceanogr. 66 (6), 2319–2332. https://doi.org/10.1002/lno.11756.

Zhou, Y.Q., Davidson, T.A., Yao, X.L., Zhang, Y.L., Jeppesen, E., Garcia de Souza, J., Wu, H.W., Shi, K., Qin, B.Q., 2018b. How autochthonous dissolved organic matter responds to eutrophication and climate warming: evidence from a cross-continental data analysis and experiments. Earth-Sci. Rev. 185, 928–937. https://doi.org/10.1016/j.earscirev.2018.08.013.

Zhu, G.W., Qin, B.Q., Gao, G., 2005. Direct evidence of phosphorus outbreak release from sediment to overlying water in a large shallow lake caused by strong wind wave disturbance. Chin. Sci. Bull. 50 (6), 577–582. https://doi.org/10.1007/BF02897483.

Zwirglmaier, K., Keiz, K., Engel, M., Geist, J., Raeder, U., 2015. Seasonal and spatial patterns of microbial diversity along a trophic gradient in the interconnected lakes of the Österseen Lake District, Bavaria. Front. Microbiol. 6, 1168. https://doi.org/10.3389/fmicb.2015.01168.