Testing the effect of the submerged macrophyte *Ceratophyllum demersum* (L.) on heterotrophic bacterioplankton densities under different levels of nitrogen and phosphorus concentrations in shallow lake mesocosms

Deshou Cuna,b, Yanran Dai,a, Yaocheng Fan,a,b, Feihua Wanga and Wei Lianga

aState Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China; bUniversity of Chinese Academy of Sciences, Beijing, China

**ABSTRACT**

To investigate the effect of submerged macrophytes on heterotrophic bacterioplankton communities in response to nutrient enrichment, we simulated mesocosms to test two factors, namely, the presence of *Ceratophyllum demersum* (L.) and the level of nutrients (slight and medium nutrient enrichment) under four possible system combinations for a duration of more than 3 months. The results show that *C. demersum* can affect the temporal dynamics of heterotrophic bacterioplankton density (HBD) and cause it to decrease. However, the effect of *C. demersum* on HBD was more pronounced under medium nutrient enrichment. The mean values of HBD in the treatment and control systems under slight nutrient enrichment were $1.30 \times 10^5$ cells mL$^{-1}$ and $1.34 \times 10^5$ cells mL$^{-1}$, respectively; whereas for medium nutrient enrichment, they were $1.78 \times 10^5$ cells mL$^{-1}$ and $2.65 \times 10^5$ cells mL$^{-1}$, respectively. The total nitrogen (TN) and total phosphorus (TP) concentrations were maintained throughout the experiment, and no significant differences were observed in the pH value, chlorophyll a (Chl. a) concentrations or dissolved organic carbon (DOC) levels between the systems with and without macrophytes, regardless of the nutrient level. Furthermore, linear mixed models revealed that environmental variables had a limited impact on HBD and that *C. demersum* had no significant direct effect on the environmental variables in the systems. A likely explanation is higher predation on bacterioplankton in the mesocosms, although allelopathic effects exerted by *C. demersum* cannot be excluded.

**ARTICLE HISTORY**

Received 3 March 2022
Accepted 24 May 2022

**KEYWORDS**

Bacteria; dissolved organic carbon; macrophyte abundance; nutrient enrichment; temporal variation

**CONTACT**

Yanran Dai yanrandai@hotmail.com; yanrandai@ihb.ac.cn State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China

Supplemental data for this article is available online at https://doi.org/10.1080/02705060.2022.2083709.

© 2022 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Introduction

Shallow lakes, the most abundant types of lakes in the world (Wetzel 2001), are biologically sensitive to changes in the surrounding environment (Elliott et al. 2006). Currently, nutrient overenrichment (especially of N and P), associated with urban, agricultural and industrial development, is one of the most notable human-induced changes to lake environments (Shi et al. 2017). To restore degraded shallow lakes, numerous efforts have been made and a series of measures have been applied (Coops and Doef 1996, Gulati et al. 2008, Jeppesen et al. 2007). During restoration processes, researchers have observed that the clear state of waters in shallow lakes is often associated with macrophytes, especially submerged macrophytes, over a wide range of nutrient concentrations (Moss et al. 1996). This remarkable phenomenon has stimulated intensive research to investigate the importance of submerged macrophytes in shallow lakes. To date, the role of submerged macrophytes in structuring lakes has been well addressed (Jeppesen et al. 2012). Furthermore, many mechanisms for maintaining the clarity of shallow lakes have been uncovered, e.g., enhancing the grazing of zooplankton on algae, reducing sediment resuspension, stabilizing sediment nutrients, sequestering nutrients by direct uptake, supporting the growth of epiphytons, and introducing allelopathic compounds (Blindow et al. 2014, Ferreira et al. 2018, Horppila and Nurminen 2003, van Donk and van de Bund 2002). However, there are still knowledge gaps in understanding how the effects of submerged macrophytes on other aquatic organisms are affected by nutrient enrichment.

In the 1940s, bacteria were recognized as key organisms in lake ecosystem processes that greatly influence lake water quality (Newton et al. 2011). Since then, a number of studies have been conducted to explore the effect of submerged macrophytes on bacterioplankton growth and community compositions (Dai et al. 2019, Wu et al. 2007, Zeng et al. 2012). In addition to the observation that the species composition, structure and function of a microbial community are closely related to the growth and morphology of macrophytes (Levi et al. 2017, Zeng et al. 2012), and the underlying mechanisms have also been addressed. One widely accepted mechanism is relevant to bottom-up control, suggesting that submerged macrophytes can create a great variety of microhabitats and heterogeneous niches by altering the surrounding pH, nutrient availability, etc. (Reitsema et al. 2018, Svany et al. 2014), as well as by releasing dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) (De Kluijver et al. 2015, Juan et al. 2014, Stanley et al. 2012), which are naturally expected to affect microbial growth and abundance (Lindstrom et al. 2005, Warnecke et al. 2005). Another well-recognized mechanism that is relevant to top-down regulation indicates that submerged macrophytes can modify food web interactions and thus affect the microbial community (Özen et al. 2018, Vakkilainen 2005). In the past two decades, due to the development of technologies used for microbial community analyses, e.g., polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), flow cytometry, and high-throughput sequencing (HTS), the role of submerged macrophytes in structuring bacterioplankton communities in shallow lakes of different trophic statuses has received increasing attention (Gasol and Del Giorgio 2000, He et al. 2014, Huss and Wehr 2004, Wu et al. 2007, Zeng et al. 2012). However, most studies have solely described the changes in the microbial communities and their compositions with eutrophication, and how nutrients (N and P) and the presence of macrophytes affect bacterial abundance has not been specifically tested, and thus, it requires further examination.

In this study, we simulated shallow lake mesocosms and chose a widespread species, *Ceratophyllum demersum* (L.), which can adapt to eutrophic conditions (Zhu et al. 2018), to explore whether the effect of *C. demersum* on heterotrophic bacterioplankton density
(HBD) changes with nutrient levels. We specifically focused on heterotrophic bacterioplankton because they play important roles in metabolizing organic carbon and recycling nutrients in aquatic ecosystems (Cotner and Biddanda 2002). Since the dominant regulation mechanisms for bacterial communities in shallow lakes, top-down or bottom-up, have been suggested as being closely related to the trophic status of the lake (Billen et al. 1990, Muylaert et al. 2002, Pace and Cole 1994), we hypothesized that the response of HBD to the growth of C. demersum would be distinct for different water N and P levels, potentially inversely correlated. We also hypothesized that the effect of C. demersum growth on HBD would change with N and P levels mainly through altering the bottom-up control of heterotrophic bacterioplankton growth. This is because bacterial growth is more strongly controlled by bottom-up factors in nutrient-rich systems (Gasol et al. 2002).

Materials and methods

Experimental mesocosms set-up

A total of twenty high-density polyethylene tanks (293 L; 70 cm in top diameter; 57 cm in bottom diameter; 92 cm in height) were used to simulate shallow lakes. These tanks were placed on the roof of a three-story building of the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China (30°32′41.1″N, 114°21′20.6″E). A 10-cm layer of quartz sand (particle size: 2–4 mm) was placed in each tank, and then the tank was filled with 254 L of tap water mixed with eutrophic lake water (taken from Guanqiao Lake, 30°32′5.43″N, 114°22′25.02″E). The initial water depth in each tank was 80 ± 0.20 cm, and the surface area was 0.385 m². An appropriate amount of tap water was added immediately if the water depth dropped below the initial water level due to evaporation. However, we made no adjustments to the water level when it rose above 80 cm due to rainfall. During the experimental period, the photosynthetically active radiation at 20 cm above the water surface at 7:00 a.m. was measured by a digital illuminance metre (TES1559P, Electrical Electronic Corp., Taiwan, China). Each tank was randomly assigned a nutrient enrichment level treatment: slight enrichment (S-E; 0.5 mg/L total nitrogen (TN) and 0.05 mg/L total phosphorus (TP)) or medium enrichment (M-E; 1.5 mg/L TN and 0.15 mg/L TP), which are in accordance with the nutrient standards of the lake (Brown and Simpson 2001). To maintain the nutrient levels, we frequently monitored the N and P concentrations in each tank and added nutrients based on the calculation results for nutrient content differences. N was supplemented with ammonium sulfate ((NH₄)₂SO₄), potassium nitrate (KNO₃) and calcium nitrate (Ca(NO₃)₂) at a 3:1 ratio of nitrate (NO₃⁻N) to ammonium (NH₄-N). In addition, KNO₃ and Ca(NO₃)₂ each provided half of the nitrate N. P was supplemented with ammonium phosphate (NH₄H₂PO₄). In addition, we added trace elements with 1/10 Hoagland’s solution every month (Aravind and Prasad 2004). At each nutrient level, we randomly selected half of the tanks and planted C. demersum in them. Thus, there were four different representative systems: slight nutrient enrichment treatment with macrophytes (S-E treatment), slight nutrient enrichment control without macrophytes (S-E control), medium nutrient enrichment treatment with macrophytes (M-E treatment) and medium nutrient enrichment control without macrophytes (M-E control). Each treatment was replicated 5 times for a total of 20 mesocosms.

C. demersum was collected in early June from East Lake, Wuhan (30°33′9.38″N, 114°20′57.16″E). Then, the plants were planted in a large tank with N-free and P-free 1/
10 Hoagland’s solution. The water depth in this tank was maintained at 80 cm. During the approximately three weeks preculture, the water temperature was within the range of 20 to 29°C, and the photosynthetically active radiation was within the range of 0.007 μmol/(m²-s) to 0.88 μmol/(m²-s). On 1 July 2018, we selected robust plants with identical lengths (approximately 17.02 cm ± 3.36 cm). After removing the water adhering to the plants with a line wedge of bibulous paper, *C. demersum* was evenly planted in 10 tanks (200 g fresh weight in each mesocosm, approximately 0.50 kg per square metre). We observed that all the transplanted plants settled to the bottom of the tanks in the following hours. The experiment lasted until October 9, 2018.

**Sampling procedures**

Water samples were collected from 0.1 m below the water surface every 2–5 days, with a relatively higher sampling frequency during the first few days of the experiment. We first collected water samples for heterotrophic bacterial counts using 10 mL sterilized syringes and 10 mL sterilized EP tubes, and then collected water samples using 500 mL acid-cleaned bottles for chemical analyses. The samples were collected 7:00 a.m., and the plant height and coverage were measured at 5:00 p.m. on the same day. All samples taken to the laboratory were analysed or preserved immediately.

**Heterotrophic bacterioplankton density**

A 750 μL water sample was fixed with 250 μL of 4% paraformaldehyde in phosphate-buff ered saline (PBS, 0.1 M, pH =7.4, 0.22 μm filtered) solution and stored in the dark for 2–4 hours at room temperature. Then, the samples were diluted with sterile pure water (at a ratio of 1:10 to improve detection accuracy) and sonicated for 4-6 minutes (P = 10–100 W) without destroying the cell membrane. Afterwards, they were filtered through a bolting cloth with a pore size of 20 μm to separate the bacterioplankton from the larger particles (Patent CN103926189 B).

To count the density of heterotrophic bacterioplankton, the filtered subsamples were stained with SYBR Green I at a final diluted concentration of 1:10,000 for 20 min in the dark at room temperature (Marie et al. 1997). All bacterioplankton populations were analysed with a CytoFLEX flow cytometer (BD Accuri™ C6 Flow cytometer, USA) and CytExpert software. Following the process described in the referenced patent (CN103926189 B), we also randomly chose 5–10 samples and counted the cells by microscopy to guarantee the accuracy of the flow cytometry results. For epifluorescence microscopy, 0.5 mL of the water samples were diluted with sterile pure water (1:50), and then 5 mL subsamples were incubated with DAPI (2 μg/L final conc.) for 10 min and filtered onto black 0.2 μm Nuclepore filters. The filters were kept frozen until counting on a LEICA DMI 3000B microscope under UV excitation. In line with the findings of Jochem (2000), the cell numbers from flow cytometry corresponded with those from epifluorescence microscopy.

**Macrophyte abundance**

The abundance of *C. demersum* was regularly recorded as the percent volume infested (PVI), which is the proportion of the water column inhabited by the submerged macrophytes (Canfield Jr et al. 1984) and calculated as the product of percentage coverage and plant height divided by water depth. We visually estimated the percentage coverage. Each
plant height was measured with a ruler, and then we calculated the mean value for each tank. The initial macrophyte PVI in our experimental mesocosms was 8.58% ± 0.54%.

**Environmental variables**

The concentrations of TN, TP and chlorophyll $a$ (Chl.$a$) in the water columns were determined according to standard methods (APHA 2005). The TN concentration was determined by ultraviolet spectrophotometry method with alkaline potassium persulfate digestion. To determine the TP concentration, we first digested the water samples with potassium persulfate and then used spectrophotometry method to determine them. We extracted Chl. $a$ from the water samples with 90% acetone, and then determined the Chl. $a$ concentration using spectrophotometry method. The DOC in water samples was measured using an organic carbon analyser (Elementar Vario TOC, Germany) after being filtered through a recombusted (at 850°C) Whatman GF/F filter (0.45 µm mesh size). Before water sampling, the water temperature, pH and dissolved oxygen (DO) were also monitored using YSI Professional Plus handheld multiparameter probes (Yellow Springs Instrument Company, USA) at 0.1 m below the water surface.

**Statistical analyses**

We performed repeated measurements of the analyses of variance (ANOVA) on the HBD, macrophyte abundance and other environmental variables to test the temporal differences between the treatments with and without macrophytes, as well as between the treatments under different nutrient conditions. Significance levels were adjusted using Huynh-Feldt and Greenhouse–Geisser corrections to account for sphericity in the variance: covariance data matrices (von Ende 2001). Post hoc contrasts were conducted with Tukey’s multiple comparisons test ($P < 0.05$). The HBD was log-transformed prior to statistical analyses. To test the difference in TN and TP concentrations in the different systems, one-way ANOVA followed by LSD post hoc multiple comparisons were performed. SPSS 23.0 was used to compile and analyse the data.

We used linear mixed models to explore how HBD (log $x$ transformed) was related to the biotic and abiotic variables in each of the four groups. In each model, we fitted fixed effects for Chl. $a$, TN, TP, DOC, T, pH and DO. The date of sampling was assigned as a random term to account for any temporal autocorrelations between the heterotrophic bacteria. In both treatment groups, PVI was also included as a fixed effect. To detect whether there were any problematic collinearities between explanatory variables, we calculated the variance inflation factor (VIF) for the explanatory variables in each model. All VIFs were less than 10 (Supplementary Figure S1). We performed model selection and model averaging by comparing the Akaike information criterion (AIC) for all the possible subset models. First, the model with the lowest AIC was identified. Then, the subset model was selected if the difference between its AIC and the lowest AIC was less than 2. The selected subset models, including the model with the lowest AIC, were averaged according to Burnham and Anderson (2002). We fitted the linear mixed-effects model using the R package ‘lme4’ (Bates et al. 2014) and conducted model selection and averaging with the R package ‘MuMIn’ (Bartoñ 2019).
Results

Temporal variations in macrophyte abundance

During the first ten days, all the mesocosm systems were in an unstable state, that is, the resuspended particles were in the process of settling; macrophytes, phytoplankton and other biotic communities were in the adaptation phase; and there were especially dramatic fluctuations in HBD (Figure S2). Hence, we excluded the acclimatization period and took July 11 as the actual starting date for our experiments.

During the experimental period, under the two different nutrient conditions, macrophyte PVI showed a similar temporal pattern, peaking in August and presenting a sharp decrease in the following month (Figure 1). However, under M-E conditions, the maximum macrophyte PVI occurred approximately 10 days later than that under S-E conditions. Meanwhile, there was a similar time interval for plant withering and death. Throughout the experiment, significantly higher macrophyte PVI was observed in the M-E systems than in the S-E systems ($P < 0.05$; Table 1). The mean PVI values in the systems under S-E and M-E conditions were 17.6% and 12.4%, respectively.

Temporal variations in heterotrophic bacterioplankton density

Compared with M-E systems, the temporal patterns in HBD between the systems under S-E conditions were relatively similar (Figure 2). There were most ups and downs in HBD in the M-E control system. Meanwhile, HBD in the M-E treatment system was the
most stable, with a clear decreasing trend after August. However, the HBD in both M-E systems was significantly higher than that in the S-E systems ($P < 0.05$; Table 1). In addition, the frequency and magnitude of HBD fluctuations in the M-E treatment system decreased more markedly than those under S-E conditions.

Under S-E conditions, before the macrophyte PVI sharply declined on August 20 (Figure 2), the HBD in the treatment systems was significantly lower than that in the control system (Figure 2A, $P < 0.01$); however, afterwards, significantly higher HBD was occasionally detected in the treatment systems, especially after September 14. The mean values of HBD throughout the experiment in the treatment and control systems under S-E conditions were $1.30 \times 10^5$ cells mL$^{-1}$ and $1.34 \times 10^5$ cells mL$^{-1}$, respectively (Figure 2A; Table 1). There was no significant difference in the mean HBD value between the two S-E systems (Table 1). Under M-E conditions, most of the time, the HBD in the treatment systems was lower than that in the control (Figure 2B). The mean values of HBD in the treatment and control systems were $1.78 \times 10^5$ cells mL$^{-1}$ and $2.65 \times 10^5$ cells mL$^{-1}$, respectively (Table 1). The presence of $C. \text{demersum}$ under M-E conditions was identified as a significant factor in decreasing HBD in the treatment systems throughout the experiment ($P < 0.01$; Table 1).

**Table 1.** The results of the ANOVA on the HBD (heterotrophic bacterioplankton density), PVI (percent volume infested) and other environmental variables. Chl. $a$: chlorophyll $a$, DOC: dissolved organic carbon, WT: water temperature, DO: dissolved oxygen, S-E treatment: slight enrichment treatment system, S-E control: slight enrichment control system, M-E treatment: medium enrichment treatment system, and M-E control: medium enrichment control system.

| Indicator | Nutrient level | Plant | Interaction | S-E treatment | S-E control | M-E treatment | M-E control |
|-----------|----------------|-------|-------------|---------------|------------|---------------|------------|
| PVI       | $P < 0.001$    |       |             | $12.35 \pm 4.88^b$ | $17.19 \pm 4.01^a$ |
| HBD ($1 \times 10^5$ cells mL$^{-1}$) | $P < 0.001$ | $P < 0.001$ | 0.001 | $1.30 \pm 0.85^a$ | $1.34 \pm 0.78^b$ | $1.78 \pm 1.16^b$ | $2.65 \pm 2.20^a$ |
| Chl.$a$ (ug L$^{-1}$) | $P < 0.001$ | 0.35 | 0.443 | $7.20 \pm 1.63^b$ | $7.13 \pm 1.88^b$ | $9.23 \pm 1.88^b$ | $8.50 \pm 2.23^a$ |
| DOC (mg L$^{-1}$) | $P < 0.001$ | 0.053 | 0.442 | $28.31 \pm 3.70$ | $28.24 \pm 3.72$ | $28.45 \pm 3.69$ | $28.29 \pm 3.61$ |
| WT ($^\circ$C) | 0.002 | 0.055 | 0.035 | $7.50 \pm 1.66^b$ | $7.46 \pm 1.71^c$ | $8.08 \pm 3.26^b$ | $8.90 \pm 2.86^a$ |
| DO (mg L$^{-1}$) | 0.002 | 0.658 | 0.261 | $9.77 \pm 0.42^b$ | $9.65 \pm 0.43^b$ | $9.95 \pm 0.43^a$ | $10.00 \pm 0.50^a$ |

Different letters indicate significant differences among the different mesocosm systems ($P < 0.05$).

**Temporal variations in environmental parameters**

Despite the temporal variation in the concentrations of TN and TP, the actual concentrations in the different systems were very close to the setting concentrations (Figure 3). During the experimental period, the photosynthetically active radiation at 20 cm above the water surface at 7:00 a.m. was within the range of 0.007–1.53 $\mu$mol/(m$^2$-s), with a mean value of 0.66 $\mu$mol/(m$^2$-s) (further details can be found in Supplementary Figure S3).

Compared with the S-E systems, the Chl. $a$ concentration showed a higher frequency and magnitude of fluctuations in the M-E systems (Figure 4A). In addition, an overall rise in Chl. $a$ concentration in the two systems under S-E conditions was observed, while there was an irregular temporal pattern in the two M-E systems. No significant differences were found among the mean Chl. $a$ concentration values of the four systems (Table 1).

In general, the DOC concentration in the four different systems showed a similar temporal pattern with distinct fluctuations (Figure 4B). Throughout the experiment, significantly different mean values of DOC concentrations were found among systems under different nutrient levels ($P < 0.05$; Table 1) but not within the same nutrient level.
However, during the plant growth stage, under M-E conditions, the DOC concentration in the treatment system was significantly higher than that in the control system ($P < 0.05$).

During the experiment, the water temperature in all the mesocosms showed the same temporal variations, varying between 21.10 and 33.37°C (Figure 5A). No significant differences were observed among all four systems (Table 1). Compared with the S-E systems, DO and pH in the M-E systems showed a greater degree of variability over time (Figure 320 D. CUN ET AL.)
Figure 3. Boxplots showing the concentrations of total nitrogen (TN, A) and total phosphorus (TP, B) in different systems. S-E treatment: slight enrichment treatment system, S-E control: slight enrichment control system, M-E treatment: medium enrichment treatment system, and M-E control: medium enrichment control system. The different letters above boxes indicate significant differences within treatment; IQR: interquartile range.

Figure 4. Temporal dynamics of chlorophyll a (Chl. a, A) and dissolved organic carbon (DOC, B) in the different mesocosm systems. Positive or negative standard deviations are shown.
In addition, there were greater dissimilarities in the temporal patterns of DO and pH between the two systems under M-E conditions. However, a significant difference was found in the mean value of DO between the two M-E systems (\( P < 0.05 \); Table 1). There was no significant difference between the two S-E systems in either the mean value of DO or pH (Table 1).

**Linear mixed effect analyses**

Compared with the control, both the lowest AIC model and the average model for HBD in the treatment systems had fewer environmental variables selected as the explanatory variables (Figure 6). According to the average model, the potential drivers for HBD in the S-E treatment system included TP, TN and pH, while in its control group, the potential drivers were TP, water temperature, pH and DO; for the M-E systems, in the treatment system, the only potential driver was TP, while in the control system, the potential drivers included TP, TN, and pH. In addition, the S-E treatment system pH showed a significant effect on HBD.

**Discussion**

Previous studies have revealed that submerged macrophytes play an important role in shaping the coexisting bacterial community (Wu et al. 2007, Yin et al. 2020, Zhang et al. 2016). However, most of the previous studies merely profiled bacterial community
compositions in water with and without submerged macrophytes under a single trophic condition. In addition, a large number of studies have reported that bacterial growth and community composition are closely related to nutrient conditions (Degerman et al. 2013, Nguyen et al. 2021, Saida et al. 2000). Hence, there is a need to delve into the possible change in the impact of submerged macrophytes on bacterioplankton resulting from nutrient enrichment. In this study, although the mean values of HBD in both the two treatment systems were lower than those in the control systems, there was a significantly larger decrease in HBD in the system containing higher levels of N and P. The different impacts of the submerged macrophytes on the bacterioplankton could be related to the significantly different macrophyte PVI. In a shallow eutrophic lake, De Kluijver et al. (2015) observed that an increase in submerged macrophytes resulted in a decrease in bacterioplankton biomass. In addition to the magnitude, the frequency of HBD fluctuations decreased more distinctly due to the presence of C. demersum in the system containing higher levels of N and P. These results indicate that the temporal dynamics of HBD could be affected to a greater extent by C. demersum growth in aquatic ecosystems with higher N and P concentrations.

The regulation of bacterial community biomass and productivity by 'bottom-up' effects (i.e., by the resources in available organic matter) and 'top-down' effects (i.e., by the grazing pressures exerted by predators) has drawn considerable attention in the last several decades (Berdjeb et al. 2011, Billen et al. 1990, Jardillier et al. 2004, Shurin et al. 2012). Experiments in aquatic microbial communities have provided empirical evidence for the widely regarded conceptual idea of a simple model of the 'Killing the Winner (KtW)' motif, which postulates that bacterial community diversity is a feature that is essentially controlled in a top-down manner by viruses, while community composition is bottom-up
controlled by competition for limiting nutrients (Pradeep Ram et al. 2020, Storesund et al. 2015, Töpper et al. 2013). To test the second hypothesis that the bottom-up regulation of heterotrophic bacterioplankton was simultaneously affected by *C. demersum* growth and nutrient levels, the temporal variations in several environmental variables that have been documented to be closely related to bacterial growth were investigated (Islam et al. 2019, Rieck et al. 2015, Yannarell and Triplett 2005). Based on mesocosm experiments, Huss and Wehr (2004) concluded that competition for N and suppression of phytoplankton growth (a major source of DOC) were the main mechanisms for the significant negative effects of *Vallisneria americana* on bacterial numbers under ammonium-rich and ammonium-poor conditions, respectively. However, in this study, the use (and depletion) of nutrients by *C. demersum* was apparently not the cause because the N and P concentrations were maintained at the same level in the treatment and control systems throughout the experiment. In contrast with reported findings that showed a strong coupling between phytoplankton growth and bacterioplankton abundance (Coveney and Wetzel 1995, Mikhailov et al. 2019, Wang et al. 2019), in this study, Chl. *a* concentration was not identified as a predictor for HBD. Previous studies have revealed the direct and indirect inhibitory effects of submerged macrophytes (including *C. demersum*) on phytoplankton growth (Dai et al. 2017, Körner and Nicklisch 2002, Vanderstukken et al. 2011). Contrary to expectations, the growth of *C. demersum* under the two different nutrient conditions had no significant effect on the Chl. *a* concentrations. This conflicting finding might be due to the relatively low macrophyte coverage because for cases of low macrophyte abundance, phytoplankton biomass is mainly determined by nutrient concentrations (Lau and Lane 2002). However, in this study, no significant difference was found between the S-E and M-E systems. This could be because N and P were not the limiting factors for phytoplankton growth in the systems.

DOC is the main energy and biomass carbon source for heterotrophic bacterioplankton (Fenchel and Jørgensen 1977). The quantity of DOC has been reported as an important abiotic factor regulating heterotrophic bacterioplankton growth (Roiha et al. 2012, Vrede 2005). In this study, in parallel with DOC concentrations, HBD was significantly higher in both M-E systems than in the S-E systems, but DOC concentration was excluded as a predictor in all the linear models. It has been well documented that submerged macrophytes can release a portion of their organic carbon DOC, which can be consumed by bacterioplankton growth (De Kluijver et al. 2015, Findlay et al. 1992). Significantly higher DOC concentrations in the M-E treatment system during the plant growth stage were observed. Nonetheless, throughout the experiment, there was no significant difference in the mean value of DOC concentrations between the treatment and control systems regardless of the nutrient levels. In addition to DOC, water temperature and pH have also long been hypothesized to govern the growth of heterotrophic bacterioplankton (Apple et al. 2006, Kamjunke et al. 2005, Mayo and Noike 1996, Vrede 2005). In this study, there were no significant differences in the mean values of water temperature and pH between the treatment and control systems. Taken together, these results failed to support the second hypothesis.

In the mesocosms, data was not collected on the abundance of protists, bacterivores (e.g., heterotrophic nanoflagellates and ciliates), or the effect of viruses. Hence, there is no direct evidence to explain why N and P enrichment could lead to the enhanced negative effect of *C. demersum* on heterotrophic bacterioplankton growth. However, a positive relationship with ciliates and PVI was observed by Özen et al. (2018), along with lower bacterial biomasses in macrophyte-dominated lakes. Similarly, by studying protozoan grazing in shallow macrophyte and planktonic lakes, Zingel and Nöges (2008) found that the
highest biomasses of ciliates occurred in macrophyte-dominated sites. These results showed significantly lower DO in the M-E treatment system, which could indicate a higher respiration rate because a higher level of DO release should be expected from macrophytes and phytoplankton. In addition, the linear mixed models reveal the limited impact of environmental variables and a nonsignificant direct effect of PVI on HBD in the systems. Hence, despite a lack of direct evidence, it is reasonable to infer that the interactions of *C. demersum* growth and nutrient enrichment could lead to the enhanced top-down regulation of heterotrophic bacterioplankton growth.

Admittedly, there are limitations to this study. For example, polyethylene tanks were used to mimic shallow lakes, which inevitably affects plant growth and may make the results differ from those in field experiments. Moreover, solely one submerged macrophyte species was investigated in this study, and the experiment lasted only several months. To obtain general conclusions, studies on other species or communities and longer experiments are greatly needed. Most importantly, more attention should be given to the impact of top-down forces in structuring freshwater bacterial growth and communities.

In summary, this study further confirms that submerged macrophytes could have negative effects on HBD and provides empirical evidence that N and P enrichment could modify the effect of *C. demersum* on the temporal dynamics of heterotrophic bacterioplankton. Observations suggest that the negative effect of *C. demersum* on HBD was more closely related to a strengthened top-down effect on the heterotrophic bacterioplankton in the mesocosms. The most likely reason is that inorganic nutrient enrichment increased macrophyte abundance, which could favour ciliates by providing them with more refuge (Özen et al. 2018) and thus enhance the negative effect of *C. demersum* on HBD. However, it should be stressed that targeted experiments are required to verify this mechanism, since in this study, the temporal dynamics of microfaunal predators or allelochemical concentration was not monitored.

**Acknowledgements**

We thank Dr. Yan Wang for helping with HBD measurement. This study was financed by the National Key Research and Development Program of China (2016YFC0503601-01) and the National Natural Science Foundation of China (51609238).

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

This study was financed by the National Key Research and Development Program of China (2016YFC0503601-01) and the National Natural Science Foundation of China (51609238).

**Data availability statement**

Data are available from the authors upon request.
References

APHA. 2005. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.

Apple JK, Del Giorgio PA, Kemp WM. 2006. Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary. Aquat Microb Ecol 43(3):243–254.

Aravind P, Prasad MNV. 2004. Zinc protects chloroplasts and associated photochemical functions in cadmium exposed Ceratophyllum demersum L., a freshwater macrophyte. Plant Sci 166(5):1321–1327.

Bartočín K. 2019. inventor. Package `MuMIn`: Multi-model inference, version 1.43. 6.

Bates D, Mächler M, Bolker B, Walker S. 2014. Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:14065823.

Berdjeb L, Ghiglione J-F, Jacquet S. 2011. Bottom-up versus top-down control of hypo- and epilimnion free-living bacterial community structures in two neighboring freshwater lakes. Appl Environ Microbiol 77(11):3591–3599.

Billen G, Servais P, Becquevort S. 1990. Dynamics of bacterioplankton in oligotrophic and eutrophic aquatic environments: bottom-up or top-down control? Hydrobiologia 207(1):37–42.

Blindow I, Hargeby A, Hilt S. 2014. Facilitation of clear-water conditions in shallow lakes by macrophytes: differences between charophyte and angiosperm dominance. Hydrobiologia 737(1):99–110.

Brown T, Simpson J. 2001. Determining the trophic state of your lake. Watershed Prot Tech Urban Lake Manage 3(4):771–781.

Burnham K, Anderson D. 2002. Model selection and multimodel inference: a practical information-theoretic approach, 2nd edition. Springer-Verlag.

Canfield DE Jr, Shireman JV, Colle DE, Haller WT, Watkins II CE, Maceina MJ. 1984. Prediction of chlorophyll a concentrations in florida lakes: importance of aquatic macrophytes. Can J Fish Aquat Sci 41(3):497–501.

Coops H, Doef RW. 1996. Submerged vegetation development in two shallow, eutrophic lakes. Hydrobiologia 340(1–3):115–120.

Cotner JB, Biddanda BA. 2002. Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems 5(2):105–121.

Coveney MF, Wetzel RG. 1995. Biomass, production, and specific growth rate of bacterioplankton and coupling to phytoplankton in an oligotrophic lake. Limnol Oceanogr 40(7):1187–1200.

Dai Y, Wu J, Zhong F, Cui N, Cheng S. 2017. Increasing phytoplankton-available phosphorus and inhibition of macrophyte on phytoplankton bloom. Sci Total Environ 579:871–880.

De Kluijver A, Ning J, Liu Z, Jeppesen E, Gulati R, Middelburg J. 2015. Macrophytes and periphyton carbon subsidies to bacterioplankton and zooplankton in a shallow eutrophic lake in tropical China. Limnol Oceanogr 60(2):375–385.

Degerman R, Dinasquet J, Riemann L, de Luna SS, Andersson A. 2013. Effect of resource availability on bacterial community responses to increased temperature. Aquat Microb Ecol 68(2):131–142.

Elliott J, Jones I, Thackeray S. 2006. Testing the sensitivity of phytoplankton communities to changes in water temperature and nutrient load, in a temperate lake. Hydrobiologia 559(1):401–411.

Fenchel TM, Jørgensen BB. 1977. Detritus food chains of aquatic ecosystems: the role of bacteria. Adv Microbial Ecol 1:1–58.

Ferreira TF, Crosetti LO, Marques DMM, Cardoso L, Fragoso CR, Jr, van Nes EH. 2018. The structuring role of submerged macrophytes in a large subtropical shallow lake: Clear effects on water chemistry and phytoplankton structure community along a vegetated-pelagic gradient. Limnologica 69:142–154.

Findlay S, Pace ML, Lints D, Howe K. 1992. Bacterial metabolism of organic carbon in the tidal freshwater Hudson Estuary. Mar Ecol Prog Ser 89(2):147–153.

Gasol JM, Del Giorgio PA. 2000. Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Sci Mar 64(2):197–224.

Gasol JM, Pedrós-Alió C, Vaqué D. 2002. Regulation of bacterial assemblages in oligotrophic plankton systems: results from experimental and empirical approaches. Antonie Van Leeuwenhoek 81(1–4):435–452.

Gulati RD, Pires LMD, Van Donk E. 2008. Lake restoration studies: failures, bottlenecks and prospects of new ecotechnological measures. Limnologica 38(3–4):233–247.

He D, Ren L, Wu QL. 2014. Contrasting diversity of epibiotic bacteria and surrounding bacterioplankton of a common submerged macrophyte, Potamogeton crispus, in freshwater lakes. FEMS Microbiol Ecol 90(3):551–562.
Horppila J, Nurminen L. 2003. Effects of submerged macrophytes on sediment resuspension and internal phosphorus loading in Lake Hiidenvesi (southern Finland). Water Res 37(18):4468–4474.

Huss A, Wehr J. 2004. Strong indirect effects of a submerged aquatic macrophyte, Vallisneria americana, on bacterioplankton densities in a mesotrophic lake. Microb Ecol 47(4):305–315.

Islam M, Shafi S, Bandh SA, Shameem N. 2019. Impact of environmental changes and human activities on bacterial diversity of lakes. In: Freshwater Microbiology. Elsevier. pp. 105–136.

Jardillier L, Basset M, Domaizon I, Belan A, Amblard C, Richardot M, Debroas D. 2004. Bottom-up and top-down control of bacterial community composition in the euphotic zone of a reservoir. Aquat Microb Ecol 35(3):259–273.

Jeppesen E, Sondergaard M, Meerhoff M, Lauridsen TL, Jensen JP. 2007. Shallow lake restoration by nutrient loading reduction—some recent findings and challenges ahead. In: Shallow Lakes in a Changing World. Hong Kong. pp. 239–252.

Jeppesen E, Sondergaard M, Sondergaard M, Christoffersen K. 2012. The structuring role of submerged macrophytes in lakes. Springer Science & Business Media.

Jochem FJ. 2000. Bacterioplankton in the western Gulf of Mexico: analysis by epifluorescence microscopy and flow cytometry. Proceedings of the AGU Ocean Sciences Meeting.

Juan M, Casas JJ, Elorrieta MA, Bonachela S, Gallego I, Fuentes-Rodríguez F, Fenoy E. 2014. Can submerged macrophytes be effective for controlling waterborne phytopathogens in irrigation ponds? An experimental approach using microcosms. Hydrobiologia 732(1):183–196.

Kamjunke N, Tittel J, Krumbeck H, Beulker C, Poerschmann J. 2005. High heterotrophic bacterial production in acidic, iron-rich mining lakes. Microb Ecol 49(3):425–433.

Körner S, Nicklisch A. 2002. Allelopathic growth inhibition of selected phytoplankton species by submerged macrophytes. J Phycol 38(5):862–871.

Lau S, Lane S. 2002. Nutrient and grazing factors in relation to phytoplankton level in a eutrophic shallow lake: the effect of low macrophyte abundance. Water Res 36(14):3593–3601.

Levi PS, Starnawski P, Poulsen B, Baattrup Pedersen A, Schramm A, Riis T. - 2017. Microbial community diversity and composition varies with habitat characteristics and biofilm function in macrophyte-rich streams. Oikos 126(3):398–409.

Lindstrom ES, Kamst-Van Agterveld MP, Zwart G. 2005. Distribution of typical freshwater bacterial groups is associated with pH, temperature, and lake water retention time. Appl Environ Microbiol 71(12):8201–8206.

Marie D, Partensky F, Jacquet S, Vaulot D. 1997. Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Appl Environ Microbiol 63(1):186–193.

Mayo AW, Noike T. 1996. Effects of temperature and pH on the growth of heterotrophic bacteria in waste stabilization ponds. Water Res 30(2):447–455.

Mikhailov IS, Bukin YS, Zakharova YR, Usoltseva MV, Galachyants YP, Sakirkov MV, Blinov VV, Likhoshway YV. 2019. Co-occurrence patterns between phytoplankton and bacterioplankton across the pelagic zone of Lake Baikal during spring. J Microbiol 57(4):252–262.

Moss B, Madgwick J, Phillips G. 1996. Guide to the restoration of nutrient-enriched shallow lakes.

Muyaert K, Van der Gucht K, Vloemans N, Meester LD, Gillis M, Vyverman W. 2002. Relationship between Bacterial Community Composition and Bottom-Up versus Top-Down Variables in Four Eutrophic Shallow Lakes. Appl Environ Microbiol 68(10):4740–4750.

Newton RJ, Jones SE, Eiler A, McMahon KD, Bertilsson S. 2011. A Guide to the Natural History of Freshwater Lake Bacteria. Microbiol Mol Biol Rev 75(1):14–49.

Nguyen J, Lara-Gutiérrez J, Stocker R. 2021. Environmental fluctuations and their effects on microbial communities, populations and individuals. Fems Microbiol Rev 45(4):1–2.

Özen A, Tavşanoğlu ÜN, Çakırşoğlu AI, Levi EE, Jeppesen E, Beklioglu M. 2018. Patterns of microbial food webs in Mediterranean shallow lakes with contrasting nutrient levels and predation pressures. Hydrobiology 806(1):13–27.

Pace ML, Cole JJ. 1994. Comparative and experimental approaches to top-down and bottom-up regulation of bacteria. Microb Ecol 28(2):181–193.

Pradeep Ram AS, Keshri J, Sime-Ngando T. 2020. Differential impact of top-down and bottom-up forces in structuring freshwater bacterial communities. FEMS Microbiol Ecol 96(2):fiaa005.

Reitsema RE, Meire P, Schoelynck J. 2018. The future of freshwater macrophytes in a changing world: dissolved organic carbon quantity and quality and its interactions with macrophytes. Front Plant Sci 9: 629.

Rieck A, Herlemann DP, Jürgens K, Grossart H-P. 2015. Particle-associated differ from free-living bacteria in surface waters of the Baltic Sea. Front Microbiol 6:1297.
Roïha T, Tiirola M, Cazzanelli M, Rautio M. 2012. Carbon quantity defines productivity while its quality defines community composition of bacterioplankton in subarctic ponds. Aquat Sci 74(3):513–525.

Saida H, Maekawa T, Satake T, Higashi Y, Seki H. 2000. Gram stain index of a natural bacterial community at a nutrient gradient in the freshwater environment. Environ Pollut 109(2):293–301.

Shi K, Zhang Y, Zhou Y, Liu X, Zhu G, Qin B, Gao G. 2017. Long-term MODIS observations of cyanobacterial dynamics in Lake Taihu: Responses to nutrient enrichment and meteorological factors. Sci Rep 7(1):40326.

Shurin JB, Clasen JL, Greig HS, Kratina P, Thompson PL. 2012. Warming shifts top-down and bottom-up control of pond food web structure and function. Phil Trans R Soc B 367(1605):3008–3017.

Stanley EH, Powers SM, Lottig NR, Buffam I, Crawford JT. 2012. Contemporary changes in dissolved organic carbon (DOC) in human-dominated rivers: is there a role for DOC management? Freshw Biol 57:26–42.

Storesund JE, Erga SR, Ray JL, Thingstad TF, Sandaa R-A. 2015. Top-down and bottom-up control on bacterial diversity in a western Norwegian deep-silled fjord. FEMS Microbiol Ecol 91(7):fi076.

Svanys A, Paškauskas R, Hilt S. 2014. Effects of the allelopathically active macrophyte Myriophyllum spicatum on a natural phytoplankton community: a mesocosm study. Hydrobiologia 737(1):57–66.

Töpper B, Thingstad TF, Sandaa R-A. 2013. Effects of differences in organic supply on bacterial diversity subject to viral lysis. FEMS Microbiol Ecol 83(1):202–213.

Vakkilainen K. 2005. Submerged macrophytes modify food web interactions and stability of lake littoral ecosystems. Lahti: University of Helsinki.

van Donk E, van de Bund WJ. 2002. Impact of submerged macrophytes including charophytes on phytoand zooplankton communities: allelopathy versus other mechanisms. Aquat Bot 72(3-4):261–274.

Vanderstukken M, Mazzeo N, Van Colen W, Declerck SA, Muylaert K. 2011. Biological control of phytoplankton by the subtropical submerged macrophytes Egeria densa and Potamogeton illinoensis: a mesocosm study. Freshw Biol 56(9):1837–1849.

von Ende CN. 2001. Repeated-measures analysis. Des Anal Ecol Exp 8:134–157.

Vrede K. 2005. Nutrient and temperature limitation of bacterioplankton growth in temperate lakes. Microb Ecol 49(2):245–256.

Wang H, Zhu R, Zhang X, Li Y, Ni L, Xie P, Shen H. 2019. Abiotic environmental factors override phytoplankton succession in shaping both free-living and attached bacterial communities in a highland lake. AMB Exp 9(1):1–13.

Warnecke F, Sommaruga R, Sekar R, Hofer JS, Pernthaler J. 2005. Abundances, identity, and growth state of actinobacteria in mountain lakes of different UV transparency. Appl Environ Microbiol 71(9):5551–5559.

Wetzel RG. 2001. Limnology: lake and river ecosystems.

Wu QL, Zwart G, Wu J, Kamst van Agterveld MP, Liu S, Hahn MW. 2007. Submersed macrophytes play a key role in structuring bacterioplankton community composition in the large, shallow, subtropical Taihu Lake, China. Environ Microbiol 9(11):2765–2774.

Yannarell AC, Triplett EW. 2005. Geographic and environmental sources of variation in lake bacterial community composition. Appl Environ Microbiol 71(1):227–239.

Yin X, Lu J, Wang Y, Liu G, Hua Y, Wan X, Zhao J, Zhu D. 2020. The abundance of nirS-type denitrifiers and anammox bacteria in rhizospheres was affected by the organic acids secreted from roots of submerged macrophytes. Chemosphere 240:124903.

Zeng J, Bian Y, Xing P, Wu QL. 2012. Macrophyte species drive the variation of bacterioplankton community composition in a shallow freshwater lake. Appl Environ Microbiol 78(1):177–184.

Zhang S, Pang S, Wang P, Wang C, Guo C, Addo FG, Li Y. 2016. Responses of bacterial community structure and denitrifying bacteria in biofilm to submerged macrophytes and nitrate. Sci Rep 6(1):36178.

Zhu G, Yuan C, Di G, Zhang M, Ni L, Cao T, Fang R, Wu G. 2018. Morphological and biomechanical response to eutrophication and hydrodynamic stresses. Sci Total Environ 622-623:421–435.

Zingel P, Nöges T. 2008. Protozoan grazing in shallow macrophyte-and plankton lakes. Fundam Appl Limnol 171(1):15–25.