

Higher resistance of a microcystin (MC)-producing cyanobacterium, *Microcystis* to the submerged macrophyte *Myriophyllum spicatum*

Yunni Gao

Henan Normal University

Hui Yang

Henan Normal University

Longfei Li

Henan Normal University

Xiaofei Gao

Henan Normal University

Mei Li

Henan Normal University

Jing Dong

Henan Normal University

Man Zhang

Henan Normal University

Jingxiao Zhang

Henan Normal University

Xuejun Li (✉ xjli@htu.cn)

Henan Normal University

Zhiying Lu

UAB College of Arts and Sciences: The University of Alabama at Birmingham College of Arts and Sciences

Michele A Burford

Australian Rivers Institute Nathan campus: Griffith University Australian Rivers Institute

Research Article

Keywords: *Microcystis*, *Myriophyllum spicatum*, microcystin, bacterioplankton, physiochemical parameters

Posted Date: January 13th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2390290/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on April 14th, 2023. See the published version at <https://doi.org/10.1007/s11356-023-26654-6>.

Abstract

Microcystin (MC) -producing and non-MC-producing *Microcystis* strains typically coexist in *Microcystis*-dominated blooms. However, the interaction between submerged plants and *Microcystis* at strain level is not clear. This study aimed to assess the effects of a submerged macrophyte *Myriophyllum spicatum* on one MC-producing versus one non-MC-producing strains of the cyanobacterium *Microcystis* using plant-*Microcystis* co-culture experiments. The impacts of *Microcystis* on *M. spicatum* were also examined. It showed that the MC-producing *Microcystis* strain had a higher resistance to negative impacts by the cocultured submerged plant *M. spicatum* than the non-MC-producing strain. By contrast, the plant *M. spicatum* was impacted more by the MC-producing *Microcystis* than the non-MC-producer. The associated bacterioplankton community was affected more by the MC-producing *Microcystis* than the cocultured *M. spicatum*. The MC cell quotas were significantly higher in the coculture treatment (the PM+ treatment, $p < 0.05$), indicating that the production and release of MCs might be a key factor responsible for the reduced impact of *M. spicatum*. The higher concentrations of dissolved organic and reducing inorganic compounds might eventually exacerbate the recovering capacity of co-existing submerged plants. Overall, this study indicated that the capacity to produce MCs, as well as the density of *Microcystis* should be taken into account when attempting to re-establish submerged vegetation to undertake remediation works.

1 Introduction

Submerged macrophytes and phytoplankton are key primary producers in lakes. Increased nutrient loading and global climate change shift many lakes from a clear state dominated by submerged plants into a turbid state dominated by phytoplankton. Then, phytoplankton blooms, including cyanobacteria blooms, may occur frequently, leading to severe ecological and economic damage (Scheffer et al. 2001; Huisman et al. 2018). The re-establishment of the submerged vegetation community in lakes has been proposed to be an important strategy for controlling cyanobacterial blooms and managing eutrophicated shallow waterbodies broadly (Pan et al. 2011; Bai et al. 2020). Macrophytes have been shown to selectively inhibit cyanobacteria on multiple scales (Zhu et al., 2010; Švanys et al., 2014). The bloom-forming cyanobacterial genus *Microcystis* is more sensitive to the coexisting submerged plant *M. spicatum* than green algae (Körner and Nicklisch, 2002; Jeong et al., 2021). However, dense cyanobacterial blooms have been shown to impair growth of submerged macrophytes by releasing various primary and secondary metabolites (Zhang et al., 2021). Whether submerged macrophytes or cyanobacteria are the winners when grown together may depend on various biotic and abiotic factors, many of which have not been thoroughly elucidated. This situation hinders the effective implementation of submerged vegetation restoration practices.

The cyanobacterial genus *Microcystis* is an ubiquitous non-nitrogen-fixing bloom-former found throughout freshwater waterbodies in tropical, subtropical and temperate zones (Xiao et al. 2018). It can produce monocyclic heptapeptides toxins, namely, microcystins (MCs), which have been consistently reported to inhibit the photosynthesis of aquatic plants over a range of exposure concentrations (Rastogi

et al., 2014; Zhang et al., 2022). Strains vary in their MC-producing capacity, and non-MC-producing strains typically coexist in *Microcystis*-dominated blooms (Bormans et al., 2020; Sidelev et al., 2020; Liu et al., 2021). Different strains may dominate in different seasons or at different stages of bloom formation in the same or different waterbodies (Kardinaal et al., 2007; Alexova et al., 2016) driven by environmental variation (Davis et al., 2009; Van De Waal et al., 2011; Lei et al., 2015; Alexova et al., 2016; Suominen et al., 2017). The composition of *Microcystis*-dominated blooms is therefore an important factor determining the population toxicity and impact on other aquatic organisms, including submerged macrophytes.

A previous study found that several MC-producing *Microcystis* strains are more sensitive to exposed isolated macrophyte allelochemicals than non-MC-producing strains (Gao et al., 2020). However, understanding the response of MC-producing and non-MC-producing strains to submerged macrophytes remains a puzzle. We hypothesised that MC-producing *Microcystis* may have a higher resistance to co-existing macrophytes rather than non-MC-producing *Microcystis* strains.

Bacterioplankton, a fundamental component of aquatic ecosystems, plays a key role in the transformation of nutrients and may be closely linked to primary producers (Liu et al., 2015; Seymour et al., 2017). In the large, shallow subtropical Taihu Lake of China, the bacterioplankton community composition has been shown to vary between the macrophyte- and phytoplankton-dominated states (Wu et al. 2007). Submerged macrophytes or phytoplankton community succession have been reported separately to participate in structuring bacterioplankton community composition (Niu et al., 2011; Zeng et al., 2012). The substances released or extracted from *Microcystis* cells have been shown to alter the epiphytic bacterial composition on the surfaces of the submerged macrophytes (Jiang et al., 2019; Zhang et al., 2021). Our recent work find planktonic and epiphytic bacteria community responded differently to the *Microcystis* cell extracts (Gao et al., 2022). However, the response of the bacterioplankton community to the interaction between submerged macrophytes and cyanobacteria is unclear. We hypothesized that the winner of the competition between submerged macrophytes and cyanobacteria plays the major role in affecting the bacterioplankton community structure and function.

The submerged macrophyte *M. spicatum* is widespread in freshwater and low-salinity coastal areas around the world (Liu et al., 2018). It has been reported to be a plant species with strong allelopathic effects against cyanobacterium *Microcystis* (Körner and Nicklisch, 2002; Jeong et al., 2021). Hence, in the present study, we selected a MC-producing and a non-MC-producing *Microcystis* strain for one-on-one coexistence experiments with the submerged macrophyte *M. spicatum* to test the above two hypotheses. The responses of both *Microcystis* strains under coculture conditions were observed to determine whether their interaction outcome is related to strains with or without MC-production capacity. The responses of the bacterioplankton community and the changes of dissolved carbon, nitrogen and phosphorus were also examined during the interaction between *Microcystis* and *M. spicatum*.

2 Materials And Methods

2.1 *M. spicatum* plants and *Microcystis*

Fresh *M. spicatum* plants were obtained from Honghu Lake (N29.827°, E113.476°) in Hubei Province, China, and cultivated in aquaria with 10 cm-deep sediments in a greenhouse until they grew new roots. Uniform plant shoots were selected and cleaned for subsequent preculture.

One MC-producing (FACHB 915) and one non-MC-producing (FACHB 1005) *Microcystis* strain were obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences. The prepared plant shoots and *Microcystis* cells were precultured separately in 1/10 diluted BG11 medium (Gao et al., 2022)) under climate-controlled conditions at $22 \pm 3^\circ\text{C}$ and light exposure at $25 \mu\text{mol photon (PAR) m}^{-2} \text{ s}^{-1}$ with 12 h light/12 h dark cycles in the laboratory before the experiments. The *Microcystis* cells in exponential growth phase were used for experiments.

2.2 Experimental design

Five experimental treatments were prepared, namely: the P, PM+, PM-, M+ and M- treatments. The monoculture control of the plant *M. spicatum* without the addition of *Microcystis* cells was the P treatment, while the monoculture controls of the MC-producing and non-MC-producing *Microcystis* strains without plants were set as the M+ and M- treatments, respectively. For the coculture treatments with *M. spicatum* (the PM+ and PM- treatments), plant apical tissues (12 cm long) were secured with sterile glass beads in beakers at the density of $2.0 \pm 0.05 \text{ g fresh weight per litre}$. Then, the prepared MC-producing or non-MC-producing *Microcystis* cells were added to the corresponding beakers at the final concentration of $5.0 \pm 0.2 \times 10^9 \text{ cells L}^{-1}$. Six replicates were conducted for the P, PM- and PM+ treatments, and three replicates were conducted for the M- and M+ treatments. All the beakers were covered with breathable sealing membranes and cultured under the same conditions mentioned above.

Subsamples were collected on days 0, 3, 6, 9, 12, 15 and 18 to measure changes in growth and physiology of both *Microcystis* strains, as well as extracellular and intracellular MCs concentrations of the MC-producing strain. At the end of the experiment, the culture media was collected for bacterioplankton community analysis. Chemical parameters were measured on day 0 and day 18. Dissolved carbon and ammonia nitrogen concentrations were measured every two days. The morphological changes in the plants were recorded by using photographs.

2.3 Growth and physiology measurement of *Microcystis*

A 5 mL sample of well-mixed *Microcystis* was collected to determine optical density at a wavelength of 680 nm by using a spectrophotometer (SPECORD210 Plus, Jena, Germany), and Fv/Fm photosynthetic yield values were measured with an AquaPen-C 100 fluorometer (Photon Systems Instruments, Drasov, Czech Republic) in accordance with the manufacturer's instructions. Another fresh homogeneous subsample was collected for malondialdehyde (MDA) measurement by using assay kits from Nanjing Jiancheng Company, China. The increase in MDA concentrations reflects membrane lipid peroxidation

due to the overproduction of reactive oxygen species and the damage to antioxidant systems in *Microcystis* cells (Lu et al. 2016).

2.4 Quantification of chemical parameters and MCs

The *Microcystis* culture solution was filtered through a 0.45 µm filter membrane, and the filtrate was collected for subsequent nutrient analyses. The dissolved total nitrogen (TDN), dissolved total phosphorus (TDP), phosphate (PO₄-P), nitrate (NO₃-N), nitrite (NO₂-N) and ammonia (NH₄-N) were measured by using colorimetric methods in accordance with the Chinese National Standards for Water Quality (The National Environmental Protection Agency of China, 2002). Dissolved total carbon (TDC), dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) were determined by using a total organic carbon analyser (Multi N/C 3100, Jena, Germany) (The National Environmental Protection Agency of China, 2002).

The *Microcystis* culture solution was centrifuged at 10 000 rpm for 15 min. The supernatant was collected and diluted 20 times for the determination of extracellular MC concentrations. The cell pellets were disrupted by freezing and thawing five times in liquid nitrogen and centrifuged at 10 000 rpm for 15 min. The supernatant was collected and diluted 20 times for the determination of intracellular MC concentrations. The concentration of MC in solutions was determined with a MC-LR ELISA kit (Institute of Hydrobiology, CAS). It has sensitivity of 0.1 µg L⁻¹.

2.5 Bacterioplankton community composition

A total of 200 mL of *Microcystis* culture solution was filtered through 0.22 µm acetate fibre filters and frozen at - 80 °C for further DNA extraction and analysis. DNA extraction and high-throughput sequencing were carried out by Majorbio BioPharm Technology Co. Ltd. (Shanghai, China). DNA was extracted by using the Fast DNATM SPIN Kit. The V3–V4 hypervariable region of 16S rRNA was amplified by using a barcoded universal primer set, which included 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTW TCTAAT-3'). High-throughput sequencing was conducted by using the Illumina MiSeq platform.

2.6 Data analysis

Microsoft Excel 2019 and IBM SPSS Statistics 26.0 were used for data analysis. Data is presented as means ± standard deviations. One-way ANOVA was performed to compare the differences in *Microcystis* performance and chemical parameters amongst experimental treatments at the same sampling point at the $p < 0.05$ level.

Bioinformatics analysis was conducted by using a Majorbio Cloud Platform (www.majorbio.com) to analyse the bacterioplankton community composition and to reveal correlations between physiochemical parameters and bacterioplankton community structure. Nonmetric multidimensional scaling (NMDS) was used to illustrate the differences amongst different treatments.

3 Results

3.1 Responses of the *Microcystis* strains and *M. spicatum*

Under the P treatment, *M. spicatum* plant shoots grew well during the experiment in the absence of *Microcystis*, with the plant biomass increasing by 11% for 18 days. Under the monoculture treatments, the cell concentrations of MC-producing (the M + treatment) and non-MC-producing *Microcystis* strains (the M – treatment) increased during the experiment (Fig. S1). The average growth rates of the monocultured MC-producing and non-MC-producing strains from day 0 to day 18 were 0.011 and 0.036 day⁻¹, respectively. Under the PM + treatment, the leaves of *M. spicatum* fell off on day 9, and almost all plant shoots had fractured and decayed on day 15. Under the PM – treatment, some of the plant shoots continued to grow even on day 15.

The cell concentrations of *Microcystis* expressed as OD₆₈₀ values under the PM + and PM – treatments were only 62.7% and 42.7% of those under the M + and M – treatments on day 9 and continued to decrease to 16.6% and 5.0%, respectively, on day 18 (Fig. 2A). The photosynthetic activity, expressed as the Fv/Fm ratios, of the non-MC-producing *Microcystis* strain reduced more significantly under the PM – treatment than those of the MC-producing strain under the PM + treatment from day 9 ($p < 0.01$). The percentage of the control was 56.7% on day 9 and decreased to 48.3% on day 18 under the PM – treatment. The Fv/Fm values of the MC-producing *Microcystis* strain under the PM + treatment reduced to 74.6% and 73.0% of those of the control on days 9 and 18, respectively (Fig. 2B).

The MDA concentrations of both *Microcystis* strains co-cultured with *M. spicatum* were significantly higher than those of the monoculture controls from day 12 ($p < 0.05$). The MDA concentrations under the PM – and PM + treatments were 162.2% and 156.5% of the corresponding controls, respectively, on day 12. At most sampling points, the MDA concentrations were significantly higher under the PM – treatment than under the PM + treatment ($p < 0.05$, Fig. 2C).

3.2 Changes in intracellular and extracellular microcystin concentrations

The cell quotas of intracellular and extracellular MC concentrations were significantly higher under the PM + treatment than under the M + treatment from day 12 ($p < 0.05$). Under the M + and PM + treatments, the average cell quotas of intracellular MC concentrations were 17.84 and 36.82 fg cell⁻¹, respectively, and those of extracellular MC concentrations were 14.13 and 40.54 fg cell⁻¹, respectively, at the end of the experiment (Fig. 2).

3.3 Responses of bacterioplankton

A total of 482 operational taxonomic units (OTUs) were classified at a 97% similarity threshold from 656 915 raw sequence reads of the 15 bacterioplankton samples. The diversity indices of the

bacterioplankton community (Shannon index) ranged between 1.75 and 3.59 (Fig. 3). The Shannon value was the highest under the PM – treatment but was lowest under the M – treatment, indicating that the coexistence of the plant *M. spicatum* markedly increased the bacterioplankton diversity under the treatments. The richness estimator of the bacterioplankton community (Chao1 index) ranged from 117.17 to 175.62. The highest value was observed under the P treatment, and the lowest value was found under the PM + treatment, indicating that the coexistence of the MC-producing *Microcystis* decreased the bacterioplankton richness.

Proteobacteria was the most dominant phylum in all samples with relative abundance ranging from 73% under the PM + treatment to 86% under the P treatment (Fig. S2). The highest relative abundance of Bacteroidetes was 12.1% under the PM – treatment, but the lowest was under the M – treatment. The relative abundance of Actinobacteriota under the M – treatment and the relative abundance of Firmicutes under the M + treatment was the highest. Gemmatimonadetes was found only under the M + and PM + treatments with the relative abundances of 5% and 14%, respectively.

The distribution of the 30 most dominant bacterial genera under the five experimental treatments is illustrated in Fig. 4. In the P treatment, *Acinetobacter* (Proteobacteria) was the most dominant genus, accounting for 63.2% of the bacterial abundance. In the M – treatment, *Phreatobacter* (Proteobacteria) accounted for 65.5% of the bacterial abundance. They were less dominant under the PM – treatment, where the relative abundances of *Acinetobacter* and *Phreatobacter* were 0.3% and 3.0%, respectively. More than 13 genera, including *Chryseobacterium* (Bacteroidetes) and *Novosphingobium* (Proteobacteria), had higher relative abundances, and at least six genera, including *Phreatobacter* and *Rhodococcus* (Actinobacteriota), had considerably lower relative abundances under the P and PM – treatments than under the M – treatment. More than 13 genera showed higher relative abundances, and 8 genera showed lower relative abundances under the M + and PM + treatments than under the P treatment.

NMDS was applied by using unweighted UniFrac distance to determine the differences amongst the treatments (Fig. 5). The first axis revealed that the bacterioplankton communities under the P and PM – treatments were different from those under the M – treatment. The second axis demonstrated the similarity between bacterioplankton compositions under the M + and PM + treatments, which were significantly different from those under the P treatment ($p < 0.05$).

PICRUSt prediction revealed a significantly higher abundance of clusters of orthologous groups associated with energy production and conversion; lipid transport and metabolism; inorganic ion transport and metabolism; secondary metabolite biosynthesis; transport and catabolism, and other unknown functions under the M – treatment than under the PM – and P treatments (Fig. 6, $P < 0.05$). The abundances of clusters of orthologous groups associated with amino acid transport and metabolism; carbohydrate transport and metabolism; transcription; inorganic ion transport and metabolism, function unknown and signal transduction mechanisms were significantly higher under the PM + and M + treatments than under the P treatment ($P < 0.05$).

3.4 Changes in the dissolved carbon, nitrogen and phosphorus

The dissolved carbon concentrations during the experiment ranged from 5.64 mg L^{-1} to 65.41 mg L^{-1} for DOC, and $1.44\text{--}30.07 \text{ mg L}^{-1}$ for DIC. The DOC concentrations under PM-, PM + and M + treatments demonstrated an obvious increasing trend during the experiment (Table 1, Fig. S3). At the end of the experiment, the P treatment had the lowest DOC concentrations, whereas the PM + and M + treatments had the highest DOC concentrations. The DIC concentrations under the coculture treatments were higher than those under the corresponding monoculture treatments at most sampling times. The increment in DIC concentrations under the PM + and PM - treatments was larger than that under the M+, M - and P treatments.

The DON and $\text{NO}_3\text{-N}$ concentrations ranged from 29.97 mg L^{-1} to 32.68 mg L^{-1} , and from 12.25 mg L^{-1} to 21.89 mg L^{-1} during the experiment. The DON concentrations decreased by a range of 19.4% – 90.7% under all treatments during the experiment (Table 1). At the end of the experiment, the DON concentration under the P treatment was the lowest, whereas those under the PM - and PM + treatments were the highest. The $\text{NO}_3\text{-N}$ concentrations increased by 78.5% under the P treatment, but decreased by 32.5–68.5% under the other four treatments during the experiment.

The $\text{NO}_2\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations under the P treatment kept a level less than 0.03 mg L^{-1} for $\text{NO}_2\text{-N}$ and 0.3 mg L^{-1} for $\text{NH}_4\text{-N}$ during the experiment (Table 1, Fig. S3). However, their concentrations increased obviously especially under the PM + and PM - treatments. At the end of the experiment, the $\text{NO}_2\text{-N}$ concentrations increased to 3.16 and 1.14 mg L^{-1} under the PM + and PM - treatments. The $\text{NH}_4\text{-N}$ concentrations under the PM + and PM - treatments increased to 2.45 mg L^{-1} and 1.68 mg L^{-1} , respectively, on day 18.

The DOP and $\text{PO}_4\text{-P}$ concentrations decreased by varying degrees under all the treatments during the experiment (Table 1). On day 18, the DOP concentrations ranged from 0.01 mg L^{-1} for the M + treatment to 0.78 mg L^{-1} for the PM + treatment. The $\text{PO}_4\text{-P}$ concentrations ranged from 0.05 mg L^{-1} for the M- treatment to 0.78 mg L^{-1} for the PM- treatment.

Table 1

Concentrations of dissolved nitrogen and phosphorus in the culture solution of the treatments at the end of the experiment and the increasing (+) or decreasing (-) ratio compared to the concentrations in the beginning. Different lowercase letters indicate significant differences amongst groups (one-way ANOVA, $p < 0.05$).

Group		DOC	DIC	DON	NO ₃ -N	NO ₂ -N	NH ₄ -N	DOP	PO ₄ -P
M-	Concentrations (mg L ⁻¹)	34.84 ± 0.11 ^d	10.73 ± 0.55 ^d	3.84 ± 0.13 ^c	10.11 ± 0.34 ^d	0.44 ± 0.01 ^c	0.79 ± 0.05 ^d	0.05 ± 0.02 ^c	0.05 ± 0.00 ^e
	Ratio (%)	+ 52.5	+ 200.9	-52.9	-53.3	-18.5	+ 39.6	-78.9	-94.4
PM-	Concentrations (mg L ⁻¹)	44.44 ± 4.11 ^c	24.93 ± 1.50 ^b	5.98 ± 0.70 ^a	14.73 ± 0.35 ^b	1.14 ± 0.02 ^b	1.68 ± 0.04 ^c	0.03 ± 0.01 ^c	0.78 ± 0.01 ^a
	Ratio (%)	+ 93.7	+ 593.9	-19.4	-32.5	+ 115.1	+ 182.3	-93.3	-13.3
P	Concentrations (mg L ⁻¹)	21.22 ± 0.55 ^e	11.04 ± 1.45 ^d	1.89 ± 0.48 ^e	21.86 ± 0.67 ^a	0.03 ± 0.01 ^e	0.29 ± 0.04 ^e	0.30 ± 0.01 ^b	0.21 ± 0.01 ^c
	Ratio (%)	+ 276.1	+ 665.8	-90.7	+ 78.5	+	+ 178.6	-64.1	-72.0
PM+	Concentrations (mg L ⁻¹)	58.22 ± 1.66 ^b	30.07 ± 2.66 ^a	4.76 ± 0.38 ^b	6.89 ± 0.79 ^e	3.16 ± 0.02 ^a	2.45 ± 0.05 ^a	0.78 ± 0.10 ^a	0.32 ± 0.01 ^b
	Ratio (%)	+ 76.1	+ 833.9	-37.8	-68.5	+ 953.3	+ 392.9	-8.5	-41.8
M+	Concentrations (mg L ⁻¹)	65.41 ± 3.18 ^a	17.04 ± 1.20 ^c	3.10 ± 0.17 ^d	12.51 ± 0.28 ^c	0.13 ± 0.00 ^d	2.16 ± 0.08 ^b	0.01 ± 0.01 ^c	0.10 ± 0.01 ^d
	Ratio (%)	+ 97.6	+ 462.4	-52.1	-42.9	-55.2	+ 317.5	-99.0	-78.3

4 Discussion

4.1 Higher resistance of the MC-producing *Microcystis* strain

The present study found a considerable difference in the responses of MC-producing versus non-MC-producing *Microcystis* strains by using one-on-one coexistence experiments with the submerged macrophyte *M. spicatum* under controlled laboratory conditions. The MC-producing *Microcystis* strain

showed a higher resistance to the cocultured submerged plant *M. spicatum* than the non-MC-producing strain. By contrast, *M. spicatum* was damaged seriously and rapidly in the presence of MC-producing *Microcystis* cells instead of non-MC-producing cells. In contrast to this study, a previous mesocosm study found that coexisting MC- and non-MC-producing *M. aeruginosa* subpopulations were both inhibited by *M. spicatum* (Švanys et al. 2014). Consistent with our study, the morphology and physiological status of the aquatic macrophyte *Egeria densa* showed greater impact to exposure to MC-producing *Microcystis* cells than non-MC-producing cells (Amorim et al. 2017). This might be related to the ecotoxicity of MCs to submerged macrophytes (Jiang et al., 2011; Rastogi et al., 2014; Li et al., 2020a).

Pure MCs at low concentrations have been shown to significantly affect the physiological structure and function of the submerged macrophyte *Vallisneria spiralis* (Jiang et al. 2011). We observed significant stimulation of MC production and MC release by MC-producing *Microcystis* during coculture with the plant *M. spicatum* in our study. Additionally, the elevated production of toxic compounds by *Microcystis* has been shown to defend this species against the grazer *Daphnia* and *Planktothrix*, another cyanobacterium (Sadler and von Elert, 2014; Briand et al., 2017). Herein, MC production and release might be a defensive strategy for the MC-producing strain to enhance its resistance against *M. spicatum*. Therefore, higher MC cell quotas combined with a high abundance of MC-producing *Microcystis* subpopulations may play an important role in affecting the survival and restoration efficiency of submerged macrophytes. This situation should be taken into account when undertaking aquatic vegetation restoration projects in eutrophic waterbodies.

This study had higher initial cell concentrations of *Microcystis* in coexistence experiments compared with previous studies (Zhu et al., 2010; Švanys et al., 2014). This may cause light shading of *M. spicatum* from the beginning of the experiment. However, the rapid disappearance of non-MC-producing *Microcystis* cells starting on day 9 was observed in the present study. The concentrations of dissolved carbon, nitrogen and phosphorus during the experiment were considerably higher than those in the typical eutrophic lakes (Ye et al., 2011; Xu et al., 2017), and were sufficient to avoid imposing nutritional restrictions on plants and *Microcystis* in the coculture systems. Hence, we deduced that the stronger allelopathic effects from *M. spicatum* must be the main reason for the out-competition of the non-MC-producing *Microcystis* by *M. spicatum*.

When exposed to pure allelochemicals or algicides, MC-producing *Microcystis* strains have been shown to be more sensitive than non-MC-producing strains in some studies. In mono- and coculture systems, the toxigenic *M. aeruginosa* strain was more sensitive to pyrogallol, a typical allelochemical derived from *M. spicatum*, than the nontoxigenic strains (Gao et al., 2020). The MC-producing strain was more sensitive to hydrogen peroxide treatment than non-MC-producing strains (Latour et al. 2022). This contrasts to the present study where plants and *Microcystis* cells are in contact and influence each other directly in plant-*Microcystis* co-existing conditions. Therefore, there is value in future coculture experiments simulating natural conditions in order to understand the interactions between submerged macrophytes and cyanobacteria.

4.2 The responses of abiotic microenvironment and bacterioplankton

The forms and concentrations of dissolved carbon, nitrogen and phosphorus changed in association with the interaction between *Microcystis* and *M. spicatum*. The significantly higher concentrations of DOC and $\text{NH}_4\text{-N}$ under the PM + and M + treatments than those under the P, M-, PM - treatments at the end of the experiment indicated that the presence of the MC-producing *Microcystis* enhanced the accumulation of dissolved organic matter and reducing substances. $\text{NH}_4\text{-N}$ content has been widely found to be significantly positively correlated with total microcystin concentrations (Wei et al. 2022). The highest $\text{NH}_4\text{-N}$ concentrations were accompanied by an obvious increase in intra- and extracellular MC concentrations under the PM + treatment during the experiment.

The DIC, DON, $\text{NO}_2\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations in the PM + and PM - treatments were much higher than those in the corresponding mono-culture treatments. These results were indicative of the quicker release and accumulation of organic and reducing inorganic substances during the decline of *Microcystis* cells or plant shoots in the coculture systems than in the monoculture systems. Dead or living *Microcystis* cells passively or actively release organic carbon into the environment (Chen et al. 2018). Organic carbon concentrations increase during the decomposition of aquatic plants (Zhang et al. 2018). The decline in *Microcystis* cells might result in the generation of a high amount of $\text{NH}_4\text{-N}$, whose toxicity plays key roles in the deterioration of submerged macrophytes (Li et al. 2020b). The partial loss of *M. spicatum* under the PM - treatment might be due to the increasing amount of $\text{NH}_4\text{-N}$ during the decline of non-MC-producing *Microcystis*. The increase in organic matter and reducing inorganic substances, including $\text{NO}_2\text{-N}$ and $\text{NH}_4\text{-N}$, might have eventually exacerbated the lose-lose situations of co-existing plants and *Microcystis*. However, further studies on an expanded scale of time and space at multi-strain levels are needed to find the threshold at which the submerged macrophytes could survive and dominate to guide the practice of submerged vegetation restoration in *Microcystis*-dominated waterbodies.

The structure and predicted function of bacterioplankton community in different treatments is closely related to the competing allelopathic activities of *M. spicatum* and different *Microcystis* strains. The effective suppression of the plant *M. spicatum* by MC-producing *Microcystis* in the PM + treatment might accompany with stronger effects of MC-producing *Microcystis* on the bacterioplankton community than *M. spicatum*. Meanwhile, the obvious inhibition of non-MC-producing *Microcystis* by *M. spicatum* in the PM - treatment was suggestive of the more rapid responses of bacterioplankton to the allelopathic activity of *M. spicatum* than non-MC-producing *Microcystis*.

Rhodobacter, one genus that was dominant only under the PM + and M + treatments, is found in free-living bacterial communities in freshwater aquatic environments (Moody and Williamson, 2013) and has been reported to include strains with the abilities to degrade organic pollutants (Bai et al., 2020). Interestingly, *Terrimicrobium*, an anaerobic bacterium was in the highest abundance under the PM + treatment wherein $\text{NH}_4\text{-N}$ concentrations were the highest (Qiu et al. 2017).

The genera with higher abundance under the P treatment than under the M + and PM + treatments indicated that these genera preferred oxidising environments with high nitrogen concentrations. *Acinetobacter* is a genus of denitrifying bacteria belonging to Gammaproteobacteria (Zhang et al., 2021). Its high abundance might be related to the higher NO₃-N concentrations in the P treatment. Some strains isolated from this genus execute strong cyanolytic activity (Osman et al. 2017). *Chryseobacterium*, which had higher abundance under the P and PM – treatments than under the M + and PM + treatments, was also isolated from Lake Taihu; this genus has the ability to produce algicidal compounds (Guo et al. 2015).

The six bacterioplankton genera, including *Phreatobacter* (Proteobacteria) and *Rhodococcus* (Actinobacteriota), with higher relative abundances under the M – treatment than under the P and PM – treatments. *Phreatobacter* has been reported to dominate in drinking water distribution systems with low chlorine concentrations (Perrin et al. 2019). *Rhodococcus*, an aerobic bacterium, has often been reported to use hydrocarbons as its only source of carbon and energy. This genus includes strains that can enhance the growth of *Microcystis* and degrade cyanotoxins (Berg et al., 2009; Rohrbacher and St-Arnaud, 2016; Kumar et al., 2019).

5 Conclusions

This study demonstrated a higher resistance of the MC-producing *Microcystis* to the cocultured submerged macrophyte *M. spicatum* than that of the non-MC-producing strain. It was accompanied by more serious damage on *M. spicatum* by the MC-producing strain than the non-MC-producing strain. Significant stimulation of MC production and release during coculture with the plant *M. spicatum* (the PM + treatment) might account for the higher resistance of the MC-producing *Microcystis*. Meanwhile, the structure and predicted function of bacterioplankton communities at genus level responded more rapidly to the MC-producing *Microcystis* under the PM + treatment than *M. spicatum*. The increased dissolved organic and reducing inorganic compounds released by living and decaying *Microcystis* cells might eventually exacerbate the recovering capacity of co-existing submerged plants.

Therefore, the results of this study suggested that substantial differences can be found between cyanobacterial strains in terms of the competition between macrophytes and cyanobacteria. MC-producing capability and population abundance of MC-producing *Microcystis* were important factors to affect the reestablishment of submerged vegetation. This situation should be considered when undertaking remediation works.

Declarations

Funding

The work was funded by the National Program for the introduction of high-end foreign experts (G2021026024L), National Natural Science Foundation of China (No. U1904124), major public welfare

projects in Henan Province (201300311300), and Breeding Project of Henan Normal University (HNU2021PL05).

Authors Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Yunni Gao, Hui Yang, Longfei Li, and Xiaofei Gao. The first draft of the manuscript was written by Yunni Gao and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethical Approval

Not applicable

Consent to Participate

Not applicable

Consent to Publish

Not applicable

Availability of data and materials

Not applicable

References

1. Alexova R, Dang TC, Fujii M, et al (2016) Specific global responses to N and Fe nutrition in toxic and non-toxic *Microcystis aeruginosa*. Environ Microbiol 18:401–413. <https://doi.org/10.1111/1462-2920.12958>
2. Amorim CA, Ulisses C, Moura AN (2017) Biometric and physiological responses of *Egeria densa* Planch. cultivated with toxic and non-toxic strains of *Microcystis*. Aquat Toxicol 191:201–208. <https://doi.org/10.1016/j.aquatox.2017.08.012>
3. Bai G, Zhang Y, Yan P, et al (2020) Spatial and seasonal variation of water parameters, sediment properties, and submerged macrophytes after ecological restoration in a long-term (6 year) study in Hangzhou west lake in China: Submerged macrophyte distribution influenced by environmental variables. Water Res 186:116379. <https://doi.org/10.1016/j.watres.2020.116379>
4. Bai L, Zhang Q, Wang C, et al (2019) Effects of natural dissolved organic matter on the complexation and biodegradation of 17A-ethinylestradiol in freshwater lakes. Environ Pollut 246:782–789.

<https://doi.org/10.1016/j.envpol.2018.12.098>

5. Berg KA, Lyra C, Sivonen K, et al (2009) High diversity of cultivable heterotrophic bacteria in association with cyanobacterial water blooms. *ISME J* 3:314–325.
<https://doi.org/10.1038/ismej.2008.110>
6. Bormans M, Savar V, Legrand B, et al (2020) Cyanobacteria and cyanotoxins in estuarine water and sediment. *Aquatic Ecology* 54(2): 625-640. <https://doi.org/10.1007/s10452-020-09764-y>
7. Briand E, Reubrech S, Mondegue F, et al (2017) Chemically mediated interactions between *Microcystis* and *Planktothrix*. Impact on their physiology and metabolic profiles. *Phycotox2017 Conférence Annu Gdr Phycotox - GIS Cyano 2017* 14-16 mars 2017, Gif sur Yvette, Fr 00:
<https://doi.org/10.1111/1462-2920.14490>
8. Chen X, Huang Y, Chen G, et al (2018) The secretion of organics by living *Microcystis* under the dark/anoxic condition and its enhancing effect on nitrate removal. *Chemosphere* 196:280–287.
<https://doi.org/10.1016/j.chemosphere.2017.12.197>
9. Davis TW, Berry DL, Boyer GL, Gobler CJ (2009) The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. *Harmful Algae* 8:715–725. <https://doi.org/10.1016/j.hal.2009.02.004>
10. Gao Y, Lu J, Orr PT, et al (2020) Enhanced resistance of co-existing toxigenic and non-toxic *Microcystis aeruginosa* to pyrogallol compared with monostrains. *Toxicon* 176:.
<https://doi.org/10.1016/j.toxicon.2020.01.013>
11. Gao Y, Yang H, Gao X, et al (2022a) Ecological damage of submerged macrophyte *Myriophyllum spicatum* by cell extracts from microcystin (MC) - and non-. 40:1732–1749
12. Gao Y, Yang H, Gao X, et al (2022b) Ecological damage of submerged macrophyte *Myriophyllum spicatum* by cell extracts from microcystin (MC)- and non-MC-producing cyanobacteria, *Microcystis*. *J Oceanol Limnol* 40:1732–1749. <https://doi.org/10.1007/s00343-022-1449-y>
13. Guo X, Liu X, Pan J, Yang H (2015) Synergistic algicidal effect and mechanism of two diketopiperazines produced by *Chryseobacterium* sp. strain GLY-1106 on the harmful bloom-forming *Microcystis aeruginosa*. *Sci Rep* 5:1–12. <https://doi.org/10.1038/srep14720>
14. Huisman J, Codd GA, Paerl HW, et al (2018) Cyanobacterial blooms. *Nat Rev Microbiol* 16:471–483.
<https://doi.org/10.1038/s41579-018-0040-1>
15. Jeong S, Yang D, Joo S, Park S (2021) Allelopathic inhibition effects of *Myriophyllum spicatum* on growths of bloom-forming cyanobacteria and other phytoplankton species in coexistence experiments. *J Plant Biol* 64:501–510. <https://doi.org/10.1007/s12374-021-09322-5>
16. Jiang J, Gu X, Song R, et al (2011) Microcystin-LR induced oxidative stress and ultrastructural alterations in mesophyll cells of submerged macrophyte *Vallisneria natans* (Lour.) Hara. *J Hazard Mater* 190:188–196. <https://doi.org/10.1016/j.jhazmat.2011.03.023>
17. Jiang M, Zhou Y, Ji X, et al (2019) Responses of leaf-associated biofilms on the submerged macrophyte *Vallisneria natans* during harmful algal blooms. *Environ Pollut* 246:819–826.
<https://doi.org/10.1016/j.envpol.2018.12.081>

18. Kardinaal WEA, Tonk L, Janse I, et al (2007) Competition for light between toxic and nontoxic strains of the harmful cyanobacterium *Microcystis*. Appl Environ Microbiol 73:2939–2946.
<https://doi.org/10.1128/AEM.02892-06>
19. Körner S, Nicklisch A (2002) Allelopathic growth inhibition of selected phytoplankton species by submerged macrophyte. J Phycol 871:862–871. <https://doi.org/10.1046/j.1529-8817.2002.t01-1-02001>
20. Kumar P, Hegde K, Brar SK, et al (2019) Potential of biological approaches for cyanotoxin removal from drinking water: A review. Ecotoxicol Environ Saf 172:488–503.
<https://doi.org/10.1016/J.ECOENV.2019.01.066>
21. Latour D, Perrière F, Purdie D (2022) Higher sensitivity to hydrogen peroxide and light stress conditions of the microcystin producer *Microcystis aeruginosa* sp PCC7806 compared to non-producer strains. Harmful Algae 114:102219. <https://doi.org/10.1016/j.hal.2022.102219>
22. Lei L, Li C, Peng L, Han BP (2015) Competition between toxic and non-toxic *Microcystis aeruginosa* and its ecological implication. Ecotoxicology 24:1411–1418. <https://doi.org/10.1007/s10646-015-1456-2>
23. Li Q, Gu P, Zhang C, et al (2020a) Combined toxic effects of anatoxin-a and microcystin-LR on submerged macrophytes and biofilms. J Hazard Mater 389:122053.
<https://doi.org/10.1016/j.jhazmat.2020.122053>
24. Li Q, Gu P, Zhang H, et al (2020b) Response of submerged macrophytes and leaf biofilms to the decline phase of *Microcystis aeruginosa*: Antioxidant response, ultrastructure, microbial properties, and potential mechanism. Sci Total Environ 699:. <https://doi.org/10.1016/j.scitotenv.2019.134325>
25. Liu L, Yang J, Yu Z, Wilkinson DM (2015) The biogeography of abundant and rare bacterioplankton in the lakes and reservoirs of China. ISME J 9:2068–2077. <https://doi.org/10.1038/ismej.2015.29>
26. Liu Q, Sun B, Huo Y, et al (2018) Nutrient bioextraction and microalgae growth inhibition using submerged macrophyte *Myriophyllum spicatum* in a low salinity area of East China Sea. Mar Pollut Bull 127:67–72. <https://doi.org/10.1016/j.marpolbul.2017.11.031>
27. Liu Y, Chen Y, Fang H, et al (2021) Relationship between morphospecies and microcystin-producing genotypes of *Microcystis* species in Chinese freshwaters. J Oceanol Limnol. 39: 1926-1937.
<https://doi.org/10.1007/s00343-020-0276-2>
28. Lu Z, Zhang Y, Gao Y, et al (2016) Effects of pyrogalllic acid on *Microcystis aeruginosa*: oxidative stress related toxicity. Ecotoxicol Environ Saf 132:413–419.
<https://doi.org/10.1016/j.ecoenv.2016.06.039>
29. Moody RG, Williamson MP (2013) Structure and function of a bacterial Fasciclin I Domain Protein elucidates function of related cell adhesion proteins such as TGFBIp and periostin. FEBS Open Bio 3:71–77. <https://doi.org/10.1016/j.fob.2013.01.001>
30. NEPAC (The National Environmental Protection Agency of China) (2002) Standard methods for the examination of water and wastewater. 4th edn (in Chinese).

31. Niu Y, Shen H, Chen J, et al (2011) Phytoplankton community succession shaping bacterioplankton community composition in Lake Taihu, China. *Water Res* 45:4169–82.
<https://doi.org/10.1016/j.watres.2011.05.022>
32. Osman OA, Beier S, Grabherr M, Bertilsson S (2017) Interactions of freshwater cyanobacteria with bacterial antagonists. *Appl Environ Microbiol* 83:1-18. <https://doi.org/10.1128/AEM.02634-16>
33. Pan G, Yang B, Wang D, et al (2011) In-lake algal bloom removal and submerged vegetation restoration using modified local soils. *Ecol Eng* 37:302–308.
<https://doi.org/10.1016/j.ecoleng.2010.11.019>
34. Perrin Y, Bouchon D, Delafont V, et al (2019) Microbiome of drinking water: A full-scale spatio-temporal study to monitor water quality in the Paris distribution system. *Water Res* 149:375–385.
<https://doi.org/10.1016/j.watres.2018.11.013>
35. Qiu Y L, Turlousse DM, Matsuura N, et al (2017) Draft genome sequence of *Terrimicrobium saccharophilum* NM-5 T , a facultative anaerobic soil bacterium of the class spartobacteria.
<https://doi.org/10.1128/genomeA>
36. Rastogi RP, Sinha RP, Incharoensakdi A (2014) The cyanotoxin-microcystins: Current overview. *Rev Environ Sci Biotechnol* 13:215–249. <https://doi.org/10.1007/s11157-014-9334-6>
37. Rohrbacher F, St-Arnaud M (2016) Root Exudation: The ecological driver of hydrocarbon rhizoremediation. *Agronomy* 6:19. <https://doi.org/10.3390/agronomy6010019>
38. Sadler T, von Elert E (2014) Physiological interaction of *Daphnia* and *Microcystis* with regard to cyanobacterial secondary metabolites. *Aquat Toxicol* 156:96–105.
<https://doi.org/10.1016/j.aquatox.2014.08.003>
39. Scheffer M, Carpenter S, Foley JA, et al (2001) Catastrophic shifts in ecosystems. *Nature* 413:591–596. <https://doi.org/10.1038/35098000>
40. Seymour JR, Amin SA, Raina J-B, Stocker R (2017) Zooming in on the phycosphere: the ecological interface for phytoplankton-bacteria relationships. *Nat Microbiol* 2: 17065.
<https://doi.org/https://doi.org/10.1038/nmicrobiol.2017.65>
41. Sidelev S, Zubishina A, Chernova E (2020) Distribution of microcystin-producing genes in *Microcystis* colonies from some Russian freshwaters: Is there any correlation with morphospecies and colony size? *Toxicon* 184:136–142. <https://doi.org/10.1016/j.toxicon.2020.06.005>
42. Suominen S, Brauer VS, Rantala-Ylinen A, et al (2017) Competition between a toxic and a non-toxic *Microcystis* strain under constant and pulsed nitrogen and phosphorus supply. *Aquat Ecol* 51:117–130. <https://doi.org/10.1007/s10452-016-9603-2>
43. Švanys A, Paškauskas R, Hilt S (2014) Effects of the allelopathically active macrophyte *Myriophyllum spicatum* on a natural phytoplankton community: a mesocosm study. *Hydrobiologia* 737:57–66. <https://doi.org/10.1007/s10750-013-1782-4>
44. Van De Waal DB, Verspagen JMH, Finke JF, et al (2011) Reversal in competitive dominance of a toxic versus non-toxic cyanobacterium in response to rising CO₂. *ISME J* 5:1438–1450.
<https://doi.org/10.1038/ismej.2011.28>

45. Wei H, Jia Y, Wang Z (2022) Microcystin pollution in lakes and reservoirs: A nationwide meta-analysis and assessment in China. *Environ Pollut* 309:119791. <https://doi.org/10.1016/j.envpol.2022.119791>
46. Wu QL, Zwart G, Wu J, et al (2007) Submersed macrophytes play a key role in structuring bacterioplankton community composition in the large, shallow, subtropical Taihu Lake, China. *Environ Microbiol* 9:2765–2774. <https://doi.org/10.1111/j.1462-2920.2007.01388.x>
47. Xiao M, Li M, Reynolds CS (2018) Colony formation in the cyanobacterium *Microcystis*. *Biol Rev* 93:1399–1420. <https://doi.org/10.1111/brv.12401>
48. Xu H, Paerl HW, Zhu G, et al (2017) Long-term nutrient trends and harmful cyanobacterial bloom potential in hypertrophic Lake Taihu, China. *Hydrobiologia* 787:229–242. <https://doi.org/10.1007/s10750-016-2967-4>
49. Ye L, Shi X, Wu X, et al (2011) Dynamics of dissolved organic carbon after a cyanobacterial bloom in hypereutrophic Lake Taihu (China). *Limnologica* 41:382–388. <https://doi.org/10.1016/j.limno.2011.06.001>
50. 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* 78:177–184. <https://doi.org/10.1128/AEM.05117-11>
51. Zhang L, Huang S, Peng X, et al (2021a) Potential ecological implication of *Cladophora oligoclora* decomposition: Characteristics of nutrient migration, transformation, and response of bacterial community structure. *Water Res* 190:116741. <https://doi.org/10.1016/j.watres.2020.116741>
52. Zhang L, Zhang S, Lv X, et al (2018) Dissolved organic matter release in overlying water and bacterial community shifts in biofilm during the decomposition of *Myriophyllum verticillatum*. *Sci Total Environ* 633:929–937. <https://doi.org/10.1016/j.scitotenv.2018.03.275>
53. Zhang W, Gu P, Zheng X, et al (2021b) Ecological damage of submerged macrophytes by fresh cyanobacteria (FC) and cyanobacterial decomposition solution (CDS). *J Hazard Mater* 401:123372. <https://doi.org/10.1016/j.jhazmat.2020.123372>
54. Zhang Y, Vo Duy S, Munoz G, Sauvé S (2022) Phytotoxic effects of microcystins, anatoxin-a and cylindrospermopsin to aquatic plants: A meta-analysis. *Sci Total Environ* 810:. <https://doi.org/10.1016/j.scitotenv.2021.152104>
55. Zhu J, Liu B, Wang J, et al (2010) Study on the mechanism of allelopathic influence on cyanobacteria and chlorophytes by submerged macrophyte (*Myriophyllum spicatum*) and its secretion. *Aquat Toxicol* 98:196–203. <https://doi.org/10.1016/j.aquatox.2010.02.011>

Figures

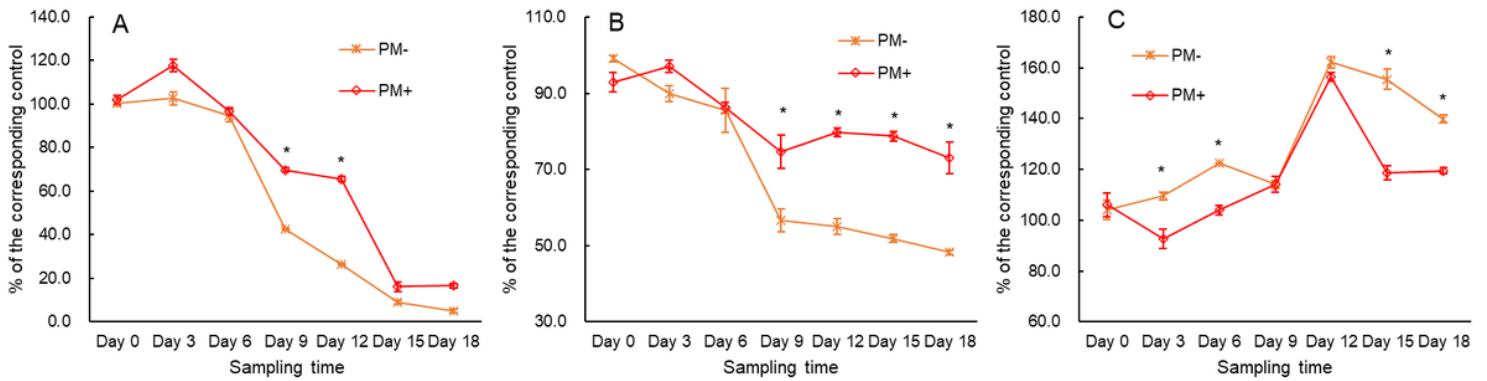


Figure 1

Growth and physiological responses of MC-producing and non-MC-producing *Microcystis* strains under the coculture treatments with *M. spicatum* (PM+, PM-) compared with the corresponding monoculture controls (M+, M-). Notes: A, OD₆₈₀; B, Fv/Fm values; C, MDA concentrations. * indicates significant differences between treatments (one-way ANOVA, $p < 0.05$).

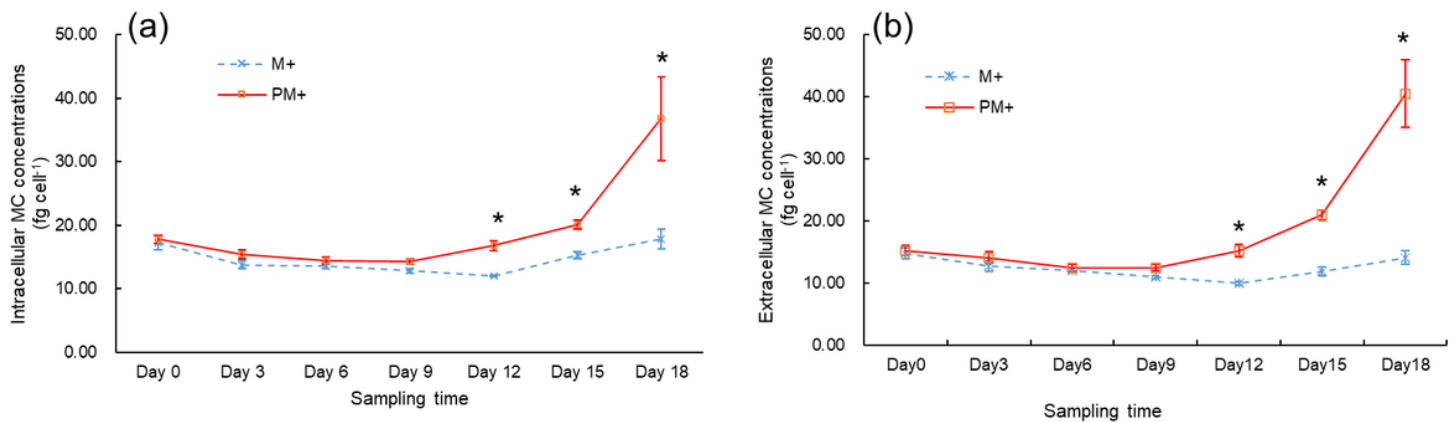


Figure 2

Intracellular (a) and extracellular (b) MC concentrations of MC-producing *Microcystis* strains under the M+ and PM+ treatments. * indicates significant differences between treatments (one-way ANOVA, $p < 0.05$).

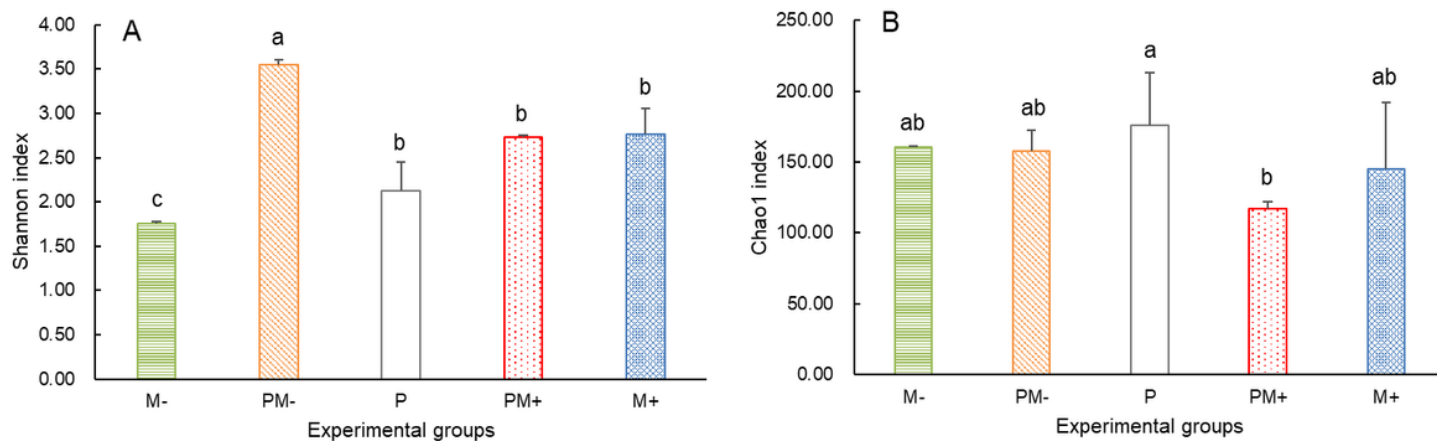


Figure 3

Shannon (A) and Chao1 index (B) of the bacterioplankton community under the experimental treatments. Different lowercase letters indicate significant differences amongst experimental groups (one-way ANOVA, $p < 0.05$).

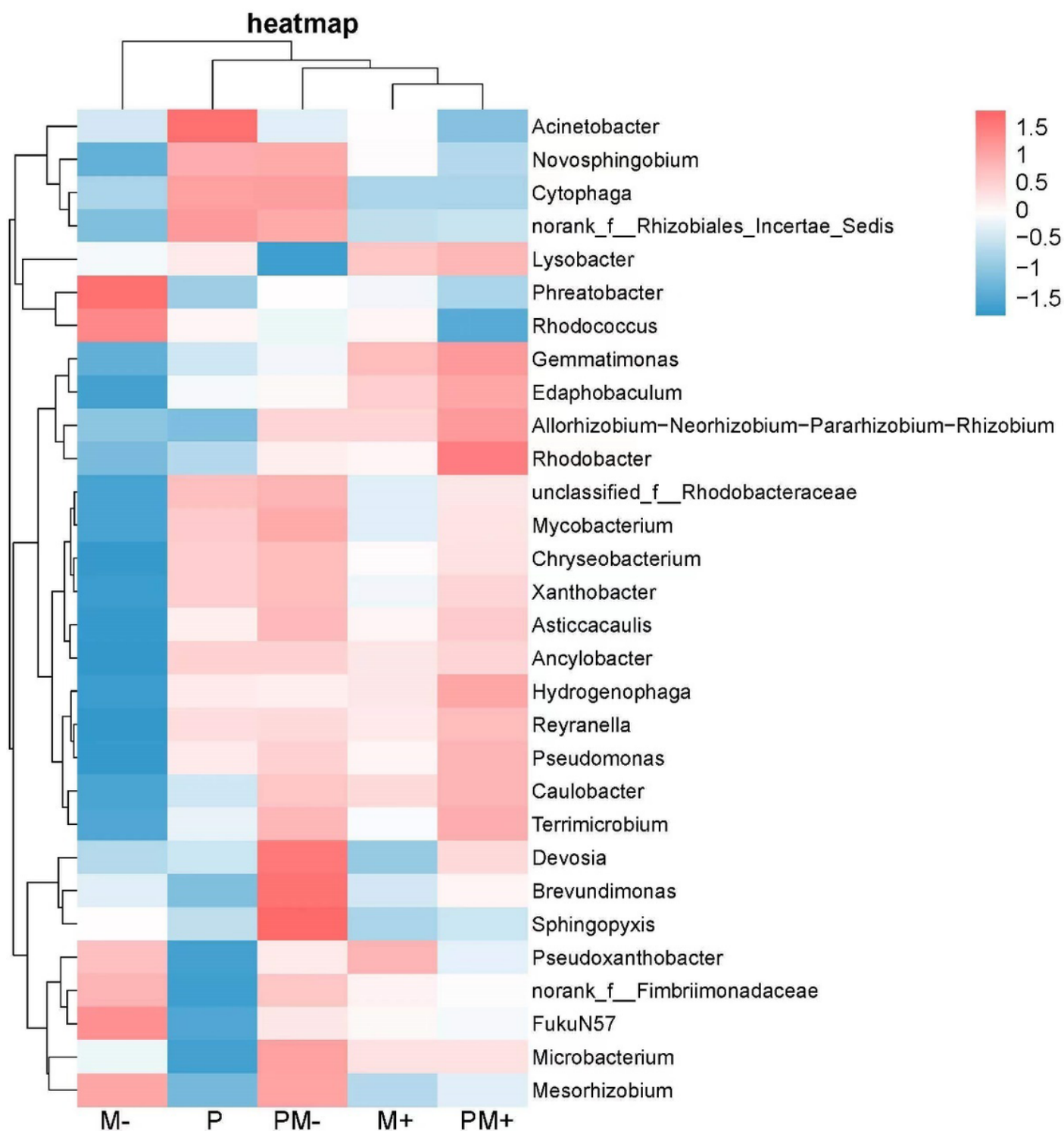


Figure 4

Hierarchical cluster heatmap of the 30 most dominant genera of bacterioplankton under the experimental treatments.

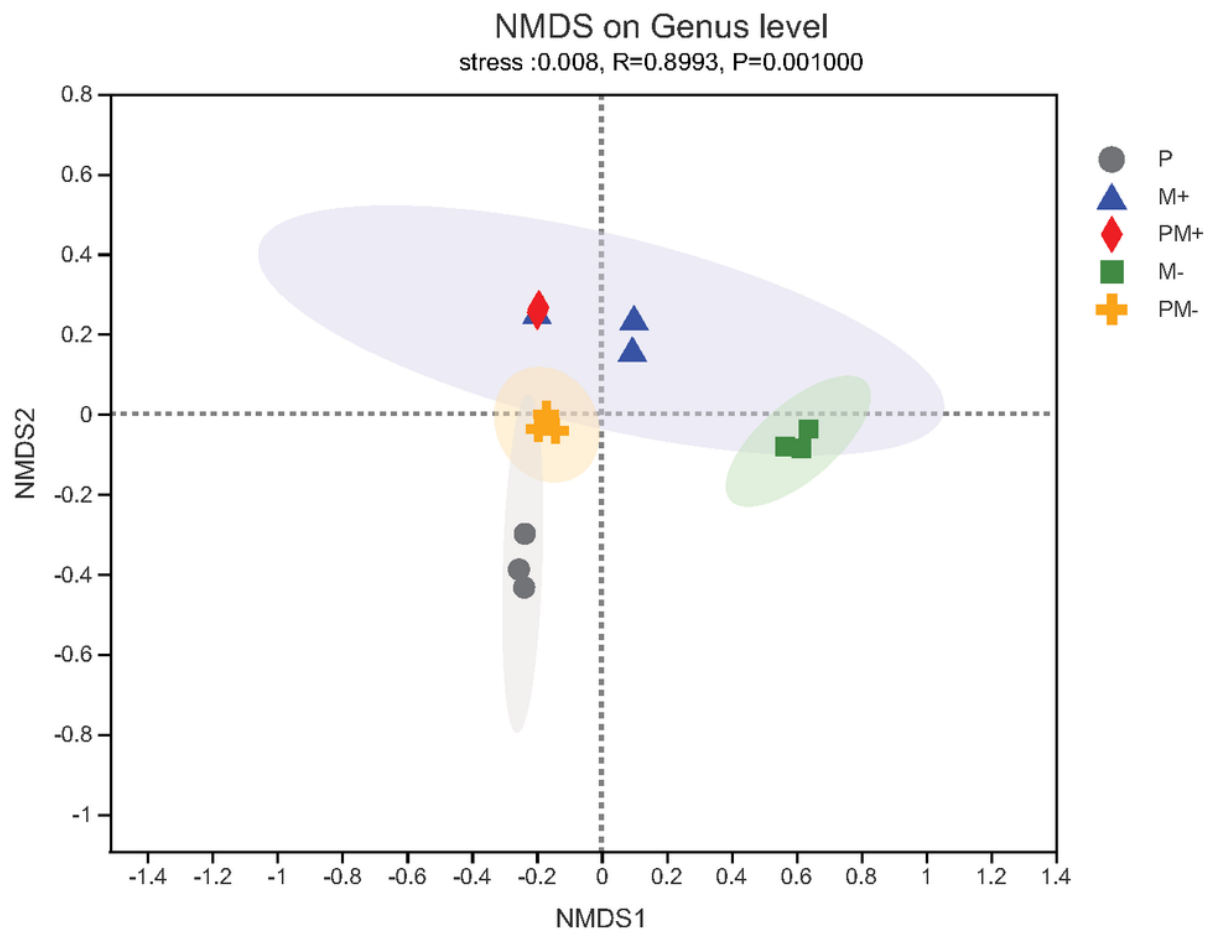


Figure 5

NMDS diagram showing the differences in bacterioplankton composition at the genus level amongst experimental treatments. Ellipses represent 95% confidence interval.

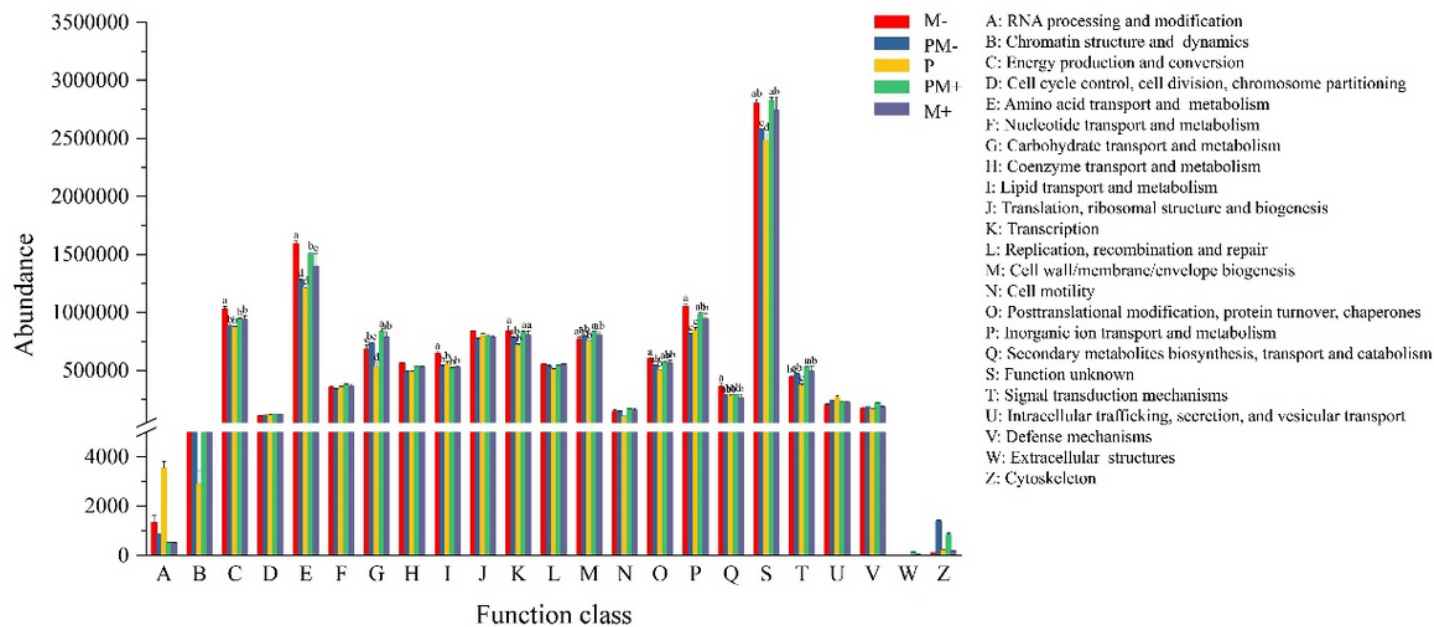


Figure 6

PICRUSt clusters of the orthologous groups of bacterioplankton and their abundances under experimental treatments. Different lowercase letters indicate significant differences amongst groups (one-way ANOVA, $p < 0.05$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementarymaterialsofYunnimanuscript.docx](#)