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Growth and photosynthesis responses of microcystin (MC)- and non-MC-producing *Microcystis* strains during co-culture with the submerged macrophyte *Myriophyllum spicatum*

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ABSTRACT

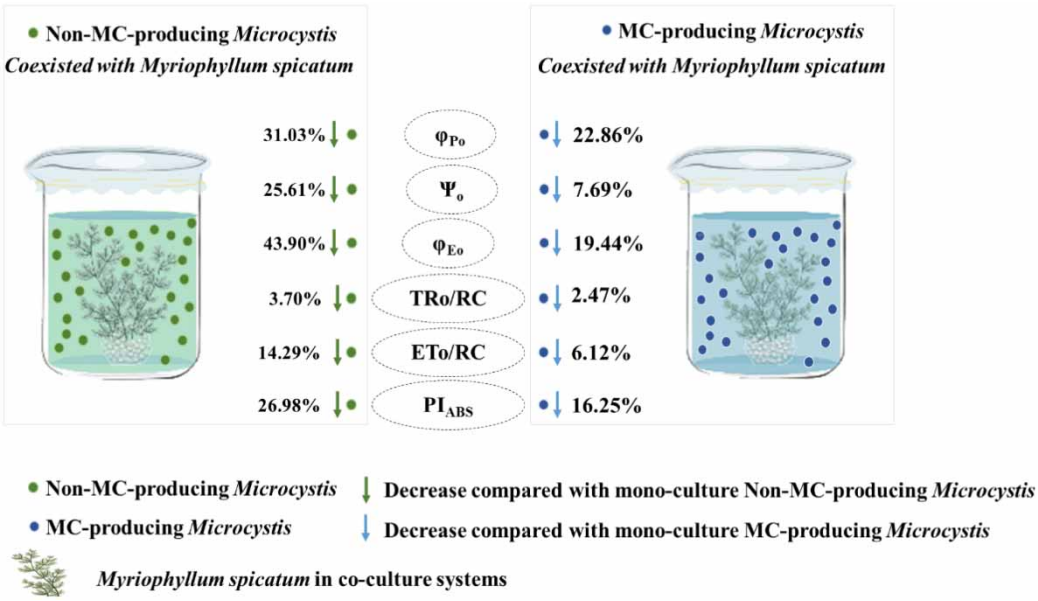
The growth and photosynthetic responses of microcystin (MC)- and non-MC-producing *Microcystis* to the submerged macrophyte *Myriophyllum spicatum* were investigated under plant-*Microcystis* co-culture conditions (PMC+, PMC−), in comparison with their corresponding mono-culture controls (SMC+, SMC-). The OJIP chlorophyll *a* fluorescence transient was recorded and analyzed using JIP-test parameters. In comparison with the corresponding mono-culture controls, the quantum efficiencies of the electron transport chain expressed as parameters Ψ_o and φEo decreased by more than 25% in the PMC− group, much higher than those in the PMC+ group ($p < 0.05$). The decreasing extent of the electron transport flux ratio ETo/RC was significantly larger in the PMC− group than in the PMC+ group ($p < 0.05$). The performance index (PI_{ABS}) decreased by 26.98% in the PMC− group and by 16.25% in the PMC+ group. These results indicated that the non-MC-producing *Microcystis* was more sensitive than the MC-producing strain when co-cultured with *M. spicatum*, and the efficiency and energy flux of electron transport might be the main targets of Photosystem II. Future research should focus on the intraspecific composition and toxigenic levels of *Microcystis* populations interacting with submerged macrophytes to guide the use of submerged vegetation restoration.

Key words: chlorophyll *a* fluorescence transient, JIP-test, *Microcystis* strains, *Myriophyllum spicatum*, photosystem II

HIGHLIGHTS

- Plant-*Microcystis* co-culture systems were established to compare the responses of two *Microcystis* strains to *Myriophyllum spicatum*.
- The OJIP chlorophyll *a* fluorescence transient measurement was used to quantify the photosynthetic responses of *Microcystis*.
- Quantum efficiencies and energy fluxes for the electron transport chain of PS II were more sensitive in the non-MC-producing *Microcystis* than in the MC-producing strain.

GRAPHICAL ABSTRACT



INTRODUCTION

The frequency and intensity of cyanobacterial blooms, especially *Microcystis* blooms, are increasing worldwide because of nutrient enrichment and global warming (Paerl *et al.* 2016). *Microcystis* are the most common bloom-dominated species in eutrophic waters, and they are distributed from temperate to tropical regions worldwide (Xiao *et al.* 2018). *Microcystis*-dominated blooms are composed of microcystin (MC)- and non-MC-producing populations (Liu *et al.* 2021). Their composition proportion varies depending on the waterbody, season, and bloom formation stage, driven by environmental factors such as light, CO_2 level, and phosphorus concentrations (Lei *et al.* 2015). The intraspecific composition also affects the accurate assessment and effective elimination of cyanobacterial blooms. However, the responses of MC- and non-MC-producing *Microcystis* strains to biotic and abiotic stresses in aquatic ecosystems remain poorly understood to date.

Submerged plants are crucial primary producers that maintain the integrity and stability of shallow aquatic ecosystems. They can significantly reduce turbidity by preventing sediment re-suspension (Bai *et al.* 2020). In addition, these plants maintain the good biological structure of aquatic ecosystems and provide a good living environment for other aquatic organisms (Wood *et al.* 2017). They absorb excess nutrients from water (Ferreira *et al.* 2018) and exert allelopathic inhibition on algal growth (Hilt *et al.* 2006). Submerged plants inhibit cyanobacteria by releasing allelochemicals, which can destroy the structure of cyanobacteria cells and effectively inhibit the occurrence of cyanobacterial blooms (Yu *et al.* 2019), without obvious effects on eukaryotic phytoplankton (Zhu *et al.* 2010).

The reestablishment of submerged vegetation is an important strategy to restore the structure and function of eutrophic aquatic ecosystems (Bai *et al.* 2020). The underlying physiological mechanisms include affecting photosynthesis, breaking cell membranes, reducing enzyme activities, and disturbing ultrastructure and gene expression (Gao *et al.* 2017). However, few studies investigated the intraspecific responses of cyanobacteria such as *Microcystis* to the stress from submerged macrophytes in the same ecological niche. The responses of MC- and non-MC-producing *Microcystis* strains to plant allelochemicals including tannic acid and pyrogallol were reported but with inconsistent results for the two allelochemicals (Švanys *et al.* 2016; Gao *et al.* 2020a). It is well-known that submerged macrophytes could produce and release various allelochemicals simultaneously to affect target organisms (Nakai *et al.* 2012). Whether MC-producing or non-MC-producing strains are more sensitive to the allelopathy from submerged macrophytes is not clear. The co-existence status of plants and cyanobacteria in eutrophic waterbodies must be simulated to verify the abovementioned phenomenon in plant-cyanobacterial co-culture systems. Plant-cyanobacteria co-culture systems were previously used to explore the inhibition effects and physiological mechanisms of submerged plants on different cyanobacterial species (Körner & Nicklisch 2002). However, such

systems have been less used to investigate the strain responses to the stress from submerged macrophytes simulating conditions in actual shallow waterbodies.

Photosynthesis is the most basic and important physiological and ecological characteristic of phytoplankton. Chlorophyll fluorescence parameters can accurately reflect the photosynthesis of *Microcystis aeruginosa* (Campbell *et al.* 1998). Chlorophyll fluorescence transients and subsequent JIP tests have been widely used to reflect the structure and function of photosystem II (PSII) in phototrophic phytoplankton (Ni *et al.* 2012). The current study aimed to investigate the responses of MC- and non-MC-producing *Microcystis* strains to the stress from the submerged macrophyte *M. spicatum*, with strong allelopathic inhibition activity (Nakai *et al.* 1999), under co-culture conditions. The cell concentrations of *Microcystis* were set to be higher than those in previous studies to mimic the dense *Microcystis* blooms (Liu *et al.* 2016). The PSII performance dynamics of both *Microcystis* strains during 12-day experiments were measured.

MATERIALS AND METHODS

Cultivation of plants and *Microcystis*

Fresh *M. spicatum* plants were obtained from the Honghu Lake (N29.827°, E113.476°) in Hubei Province, and cultivated in an aquarium with 10 cm sediments in our greenhouse until new roots grew. Well-grown and uniform plant tissues were selected and cleaned for subsequent preculture in 1/10 diluted BG11 medium (Table 1) under climate-controlled conditions at 22 ± 3 °C, light at 2,000 lux with 12 h light/12 h dark cycles in the lab before the experiments. Our preliminary experiment proved that 1/10 diluted BG11 medium was suitable for the growth of *M. spicatum*.

One MC-producing strain (FACHB 915) and one non-MC-producing *Microcystis* strain (FACHB 1005) were obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB), the Chinese Academy of Sciences. Their MC-producing ability was also identified by FACHB. *Microcystis* was precultured in 1/10 diluted BG11 medium under the same conditions as *M. spicatum*, and the cells in the exponential phase after 5-day culture were prepared for the experiments.

Experimental design

Two co-culture experimental groups (PMC−, PMC+), two *Microcystis* mono-culture groups (SMC-, SMC+) and one *M. spicatum* mono-culture group (P) were established to compare the responses of two *Microcystis* strains to *M. spicatum*. Sterilized 1/10 diluted BG11 medium was prepared at 1 L per beaker. Plant apical tissues of *M. spicatum* with 12 cm length were cultivated at 2.0 ± 0.05 g fresh weight per litre in the beakers of the P, PMC− and PMC+ groups in sextuplicate. The prepared MC- and non-MC-producing *Microcystis* cells were then added to the beakers of the PMC+ and PMC− groups, respectively, at a final cell concentration of $4.50 \pm 0.06 \times 10^6$ cells mL^{-1} in the range of dense bloom populations in eutrophic waters (Liu *et al.* 2016). The same amount of *Microcystis* cells was added into the beakers of the SMC+ and SMC- groups in triplicate as the *Microcystis* mono-culture control. All the beakers were covered with breathable sealing membranes, and cultured under the conditions described above for 12 days. The subsamples were collected every 3 days to measure the growth and photosynthesis parameters of both *Microcystis* strains.

Table 1 | The composition of 1/10 diluted BG11 medium

Ingredient	Concentration (mg L^{-1})	Ingredient	Concentration (μg L^{-1})
$NaNO_3$	0.15	H_3BO_3	0.28
K_2HPO_4	0.02	$MnCl_2{\cdot}4H2O$	0.18
$MgSO_4{\cdot}7H_2O$	0.0375	$ZnSO_4{\cdot}7H_2O$	0.022
$CaCl_2{\cdot}2H_2O$	0.018	$Na_2MoO_4{\cdot}2H_2O$	0.039
Citric acid	0.003	$CuSO_4{\cdot}5H_2O$	0.008
Ferric ammonium citrate	0.003	$Co(NO_3)_2{\cdot}6H_2O$	0.005
$EDTANa_2$	0.0005		
Na_2CO_3	0.01		

Growth and photosynthesis measurement

Fresh *Microcystis* fluid samples were collected, and 1% Lugo fluid was added to fix the samples. A 0.1 mL fixed sample was placed into the phytoplankton counting box (0.1 mL) to be counted under a biological microscope (E100, Nikon Eclipse). The inhibition rate of the co-cultured plants on both *Microcystis* strains was calculated based on changes in cell density by using the following equation:

$$\text{Inhibition ratio } (\%) = 100 \times (\text{Pc} - \text{Pt})/\text{Pc}$$

where Pt is the cell density for the tested strains in the co-culture groups, and Pc is that in the corresponding mono-culture control on the same day.

Fresh *Microcystis* fluid samples were dark-adapted for 20 min and then subjected to an AquaPen-C 100 fluorometer (Photon Systems Instruments, Drasov, Czech Republic) to record O-J-I-P chlorophyll *a* fluorescence transient in accordance with the manufacturer's instructions. The JIP-test parameters were automatically extracted to assess the PS II performance of MC- and non-MC-producing *Microcystis* strains in different experimental groups (Table 2). The fluorescence yields at 50 μs, 2 ms, and 50 ms were denoted as F_o, F_j, and F_i, respectively (Appenroth *et al.* 2001). V_j represents the relative variable fluorescence intensity at the J step, φ_{Po} represents the maximum quantum yield for primary photochemistry, Ψo represents the probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A (at $t = 0$) and φEo represents the quantum yield for electron transport (at $t = 0$). The specific energy flux ratios in the electron transport chain included parameters ABS/RC, TRo/RC, ETo/RC, and DIo/RC, representing the energy fluxes ratio, the trapped energy flux ratio, the electron transport flux ratio, and the dissipated energy flux ratio per reaction centre, respectively (Wang *et al.* 2021). PI_{ABS} represents the performance index on an absorption basis (Ni *et al.* 2012).

Data analysis

Microsoft Excel 2019 and Origin 2021b were used for data analysis in the study. Data are presented as the means ± standard deviations of samples. After determining the normality using Shapiro-Wilks test and homogeneity using Bartlett's test, one-way ANOVA was performed among the experimental groups, and multiple comparisons were performed using the least significance difference test. Significant difference was considered at $p < 0.05$. Difference significances between groups on the same day are represented by uppercase letters (*i.e.*, A, B, C, D) and difference significances within groups at different time-points are represented by lowercase letters (*i.e.*, a, b, c, d, e).

RESULTS

Growth responses of *Microcystis*

The cell density of both *Microcystis* strains co-cultured with the plant *M. spicatum* was significantly lower than that in the corresponding mono-culture controls from day 9 ($p < 0.05$), when the cell density in the PMC− treatment dropped to a lower level than the initial value (Figure 1(a)). The corresponding inhibition ratio of the non-MC-producing *Microcystis* strains

Table 2 | The formulae of the extracted JIP test parameters in the study

Parameter	Formulae
V_j	$V_j = (F_j - F_o)/(F_m - F_o)$
φ_{Po}	$\varphi_{Po} = TRo/ABS = 1 - F_o/F_m$
Ψ_o	$\Psi_o = ETo/TRo = (F_m - F_j)/(F_m - F_o)$
φ_{Eo}	$\varphi_{Eo} = ETo/ABS = (F_m - F_j)/F_m$
ABS/RC	$ABS/RC = Mo \cdot (1/V_j) \cdot (1/\varphi_{Po})$
TRo/RC	$TRo/RC = Mo \cdot (1/V_j)$
ETo/RC	$ETo/RC = Mo \cdot (1/V_j) \cdot \Psi_o$
DIo/RC	$DIo/RC = (ABS/RC) - (TRo/RC)$
PI_{ABS}	$PI = (RC/ABS) \cdot [\varphi_{Po}/(1 - \varphi_{Po})] \cdot [\Psi_o/(1 - \Psi_o)]$

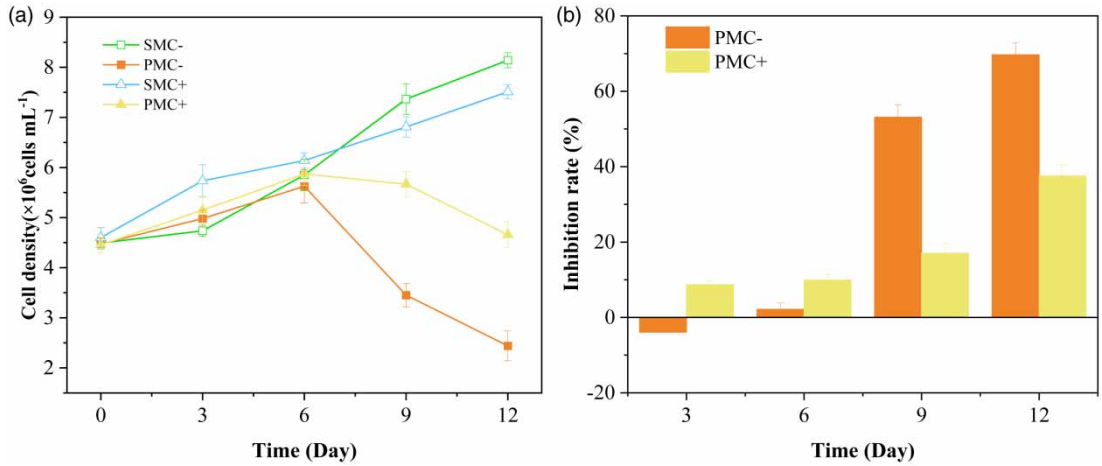


Figure 1 | The cell density (a) of MC- and non-MC-producing *Microcystis* in mono- and co-culture groups, and the corresponding inhibition rate by *M. spicatum* (b).

was significantly higher than that of the MC-producing strains from day 9. The inhibition rate reached 69.70% for the non-MC-producing *Microcystis* strains and 37.50% for the MC-producing strain on day 12 (Figure 1(b)).

Fast chlorophyll-a fluorescence responses of *Microcystis*

The OJIP-curves of both strains in the co-culture groups were beneath those in the corresponding mono-culture groups. Fluorescence no longer increased when it reached the J step, and the O-J-I-P rise changed to the O-J (J = I = P) rise in the PMC− group on day 12 (Figure 2). The F_j values increased in all treatments, but were lower in the co-culture groups than in the mono-culture groups on day 12. In the PMC− group, the F_j value decreased by 25.13% compared with the SMC- group. In the PMC+ group, the value decreased by 22.81% based on that in the SMC+ group. By contrast, the relative variable fluorescence intensity (V_j) increased over time, from 0.21 to 0.39 in the PMC− group and from 0.19 to 0.28 in the PMC+ group (Table 3, $p < 0.05$). The V_j values in the PMC− and PMC+ groups were 2.3-fold and 1.3-fold those in the SMC- and SMC+ groups, respectively. The F_i values increased over time in the four groups. However, the F_i values in the co-culture groups were always lower than those in the corresponding mono-culture groups. The decreasing ratio was 26.14% for the PMC− group and 36.14% for the PMC+ group in comparison with the SMC- and SMC+ groups, respectively.

PSII behavior of *Microcystis*

The φ_{Po} values, the maximum quantum yield for primary photochemistry, were not different among all experimental groups in the beginning ($p > 0.05$). But they differed significantly in the end of the experiment through 12-day changes at varying degrees ($p < 0.05$). For non-MC-producing *Microcystis*, the φ_{Po} in the PMC− group began to drop significantly from day 3,

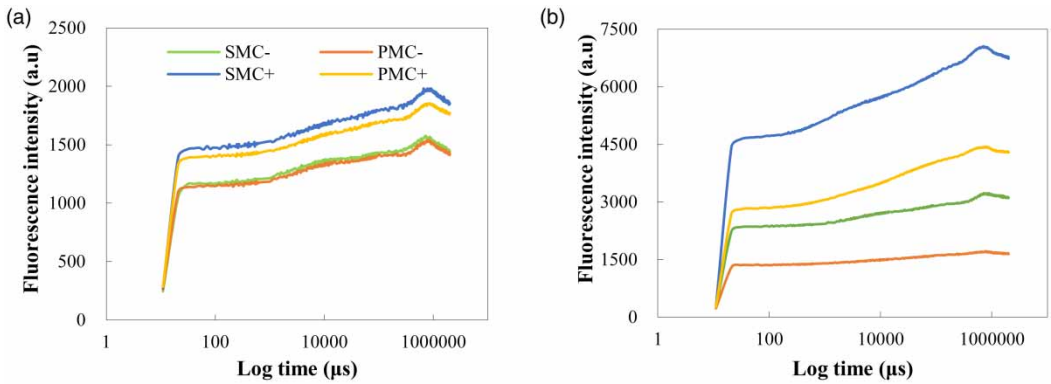


Figure 2 | Fast chlorophyll fluorescence introduction curves of *Microcystis* cells in various experimental groups on day 0 (a) and day 12 (b).

Table 3 | F_j, F_i and V_j values of MC- and non-MC-producing *Microcystis* in mono- and co-culture groups

		Treatments			
Parameters	**Time**	**SMC-**	**PMC−**	**SMC +**	**PMC +**
F_j	0d	$1{,}216.33 \pm 1.53^{Ce}$	$1{,}197.83 \pm 9.15^{De}$	$1{,}671.00 \pm 5.57^{Ae}$	$1{,}564.00 \pm 30.79^{Be}$
	3d	$1{,}415.33 \pm 6.66^{Bd}$	$1{,}365.67 \pm 48.94^{Bd}$	$2{,}108.50 \pm 58.13^{Ad}$	$2{,}047.60 \pm 13.54^{Ad}$
	6d	$1{,}647.67 \pm 7.51^{Cc}$	$1{,}614.00 \pm 79.80^{Cb}$	$2{,}697.00 \pm 9.54^{Ac}$	$2{,}959.40 \pm 49.74^{Bc}$
	9d	$1{,}772.67 \pm 10.60^{Cb}$	$1{,}710.50 \pm 11.26^{Da}$	$3{,}169.00 \pm 8.74^{Bb}$	$3{,}478.60 \pm 33.92^{Aa}$
	12d	$2{,}089.67 \pm 9.81^{Ca}$	$1{,}564.50 \pm 6.22^{Dc}$	$4{,}261.50 \pm 10.02^{Aa}$	$3{,}289.60 \pm 117.40^{Bb}$
F_i	0d	$1{,}247.67 \pm 5.51^{Be}$	$1{,}236.83 \pm 4.54^{Bd}$	$1{,}744.00 \pm 62.45^{Ae}$	$1{,}705.00 \pm 55.79^{Ae}$
	3d	$1{,}696.00 \pm 8.66^{Cd}$	$1{,}562.00 \pm 38.43^{Bc}$	$2{,}754.67 \pm 7.64^{Ad}$	$2{,}428.00 \pm 36.14^{Bd}$
	6d	$1{,}874.33 \pm 6.66^{Bc}$	$1{,}680.33 \pm 28.86^{Cb}$	$3{,}187.00 \pm 2.00^{Ac}$	$2{,}734.20 \pm 90.53^{Bc}$
	9d	$2{,}109.00 \pm 4.58^{Bb}$	$1{,}880.33 \pm 29.15^{Ca}$	$3{,}685.33 \pm 7.02^{Ab}$	$3{,}699.60 \pm 37.47^{Aa}$
	12d	$2{,}287.67 \pm 15.04^{Ca}$	$1{,}689.7 \pm 29.30^{Db}$	$5{,}451.67 \pm 10.60^{Aa}$	$3{,}481.40 \pm 74.34^{Bb}$
V_j	0d	0.24 ± 0.02^{Aa}	0.21 ± 0.02^{Ac}	0.21 ± 0.04^{Aa}	0.19 ± 0.03^{Bc}
	3d	0.23 ± 0.01^{ABa}	0.24 ± 0.02^{Ab}	0.19 ± 0.02^{Ca}	0.21 ± 0.02^{BCbc}
	6d	0.20 ± 0.03^{ABab}	0.23 ± 0.02^{Abc}	0.20 ± 0.01^{ABa}	0.19 ± 0.02^{Bc}
	9d	0.18 ± 0.03^{Cb}	0.36 ± 0.03^{Aa}	0.21 ± 0.01^{BCa}	0.24 ± 0.02^{Bb}
	12d	0.17 ± 0.02^{Db}	0.39 ± 0.00^{Aa}	0.22 ± 0.01^{Ca}	0.28 ± 0.01^{Ba}

Difference significances between and within groups are represented by uppercase and lowercase letters, respectively, at $p < 0.05$ levels.

and it decreased by 31.03% compared with that in the SMC- group on day 12. For MC-producing *Microcystis*, the φ_{Po} in the PMC+ group began to drop significantly from the 6th day, and it decreased by 22.86% compared with that in the SMC+ group on day 12. A stable state without significant changes in Ψ_o during the experiment was observed in the SMC- and SMC+ groups. However, the Ψ_o in the co-culture groups decreased. In the PMC− group, the value dropped significantly from 0.79 to 0.61, with a decreasing ratio of 25.61% compared with that in the SMC- group. The Ψ_o decreased from 0.81 to 0.72 in the PMC+ group, with a decreasing ratio of 7.69% compared with that in the SMC+ group. The quantum yield for electron transport (φ_{Eo}) increased from 0.24 on day 0 to 0.41 for the SMC- group and 0.36 for SMC+ group on day 12, which were significantly higher than in the PMC− and PMC+ groups (Table 4, $p < 0.05$). The decreasing ratio was 43.90% for the PMC− group and 19.44% for the PMC+ group.

The ABS/RC values maintained stable status with a small fluctuation in the SMC- and SMC+ groups, but the values decreased significantly from 2.87 on day 0 to 2.66 on day 12 in the PMC− group. The ABS/RC value in the PMC+ group

Table 4 | Φ_{po}, Ψ_o and φ_{Eo} values of MC- and non-MC-producing *Microcystis* in mono- and co-culture groups

		Treatments			
Parameters	**Time**	**SMC-**	**PMC−**	**SMC +**	**PMC +**
φ_{Po}	0d	0.33 ± 0.00^{Ab}	0.32 ± 0.00^{Aa}	0.32 ± 0.01^{Abc}	0.32 ± 0.01^{Aa}
	3d	0.34 ± 0.02^{Aab}	0.31 ± 0.01^{Ba}	0.33 ± 0.01^{ABb}	0.31 ± 0.01^{Ba}
	6d	0.34 ± 0.00^{Aa}	0.28 ± 0.01^{Bb}	0.33 ± 0.00^{Cb}	0.29 ± 0.01^{Bb}
	9d	0.35 ± 0.00^{Aa}	0.20 ± 0.01^{Cc}	0.34 ± 0.01^{Aa}	0.25 ± 0.01^{Bc}
	12d	0.29 ± 0.01^{Bc}	0.20 ± 0.01^{Dd}	0.35 ± 0.01^{Aa}	0.27 ± 0.01^{Cb}
Ψ_o	0d	0.76 ± 0.02^{Ba}	0.79 ± 0.02^{Aba}	0.79 ± 0.04^{ABa}	0.81 ± 0.02^{Aa}
	3d	0.77 ± 0.01^{BCa}	0.76 ± 0.02^{Cb}	0.81 ± 0.02^{Aa}	0.79 ± 0.02^{ABa}
	6d	0.80 ± 0.03^{ABa}	0.77 ± 0.02^{Bab}	0.80 ± 0.01^{ABa}	0.81 ± 0.02^{Aa}
	9d	0.82 ± 0.03^{Aa}	0.63 ± 0.03^{Cc}	0.79 ± 0.01^{ABa}	0.76 ± 0.02^{Bb}
	12d	0.83 ± 0.02^{Aa}	0.61 ± 0.00^{Dcd}	0.78 ± 0.02^{Ba}	0.72 ± 0.01^{Cc}
φ_{Eo}	0d	0.24 ± 0.02^{Ae}	0.26 ± 0.01^{Ac}	0.24 ± 0.02^{Ad}	0.25 ± 0.01^{Ae}
	3d	0.27 ± 0.01^{Bd}	0.29 ± 0.01^{Ab}	0.27 ± 0.01^{Bc}	0.28 ± 0.01^{ABd}
	6d	0.31 ± 0.02^{Ac}	0.31 ± 0.01^{Aa}	0.32 ± 0.01^{Ab}	0.32 ± 0.02^{Ab}
	9d	0.35 ± 0.01^{Ab}	0.27 ± 0.01^{Bc}	0.35 ± 0.01^{Aa}	0.34 ± 0.01^{Aa}
	12d	0.41 ± 0.02^{Aa}	0.23 ± 0.02^{Dd}	0.36 ± 0.01^{Ba}	0.29 ± 0.01^{Cc}

Difference significances between and within groups are represented by uppercase and lowercase letters, respectively, at $p < 0.05$ levels.

did not decrease significantly during the experiment, but was significantly higher than that in the SMC+ group on days 6 and 9 ($p < 0.05$). The ABS/RC value for MC-producing *Microcystis* was significantly higher than that for the non-MC-producing strain (Figure 3(a), $p < 0.05$). The TRo/RC values in the SMC- and SMC+ groups slightly changed during the experiment, whereas they decreased from 1.62 on day 0 to 1.56 in the PMC− group and 1.58 in the PMC+ group on day 12 (Figure 3(b)). No significant differences were observed between the PMC− and PMC+ groups ($p > 0.05$). The ETo/RC values gradually decreased in all treatments. The largest decline occurred in the PMC− group, followed by the PMC+ group, and the smallest drop appeared in the SMC+ group. The ETo/RC values in the PMC− and PMC+ groups were 85.71% and 93.85% of those in the SMC- and SMC+ groups on day 12, respectively (Figure 3(c)). The DIo/RC values in the SMC- and SMC+ groups showed no significant changes during the experiment ($p > 0.05$). However, the DIo/RC values in the PMC− group decreased significantly from 1.18 on day 0 to 1.10 on day 12, whereas those in the PMC+ group increased from 1.71 on day 0 to 1.75 on day 12 (Figure 3(d)).

The performance index (PI_{ABS}) values increased from 0.48 on day 0 to 0.63 on day 12 in the SMC- group, and from 0.56 to 0.80 in the SMC+ group. However, the PI_{ABS} values in the PMC− group decreased significantly from 0.49 to 0.46. Although the PI values in the PMC+ group increased from 0.56 to 0.67, the increasing degree was obviously smaller than that in the SMC+ group (Table 5). The PI_{ABS} values in the PMC− and PMC+ groups decreased by 26.98% and 16.25%, respectively, in comparison with those in the corresponding mono-culture controls.

DISCUSSION

The different responses of MC- and non-MC-producing *Microcystis* to co-existing *M. spicatum* were proven from the growth and photosynthesis aspects of *Microcystis* in the present study. Previous studies found that cyanobacteria such as *Microcystis*

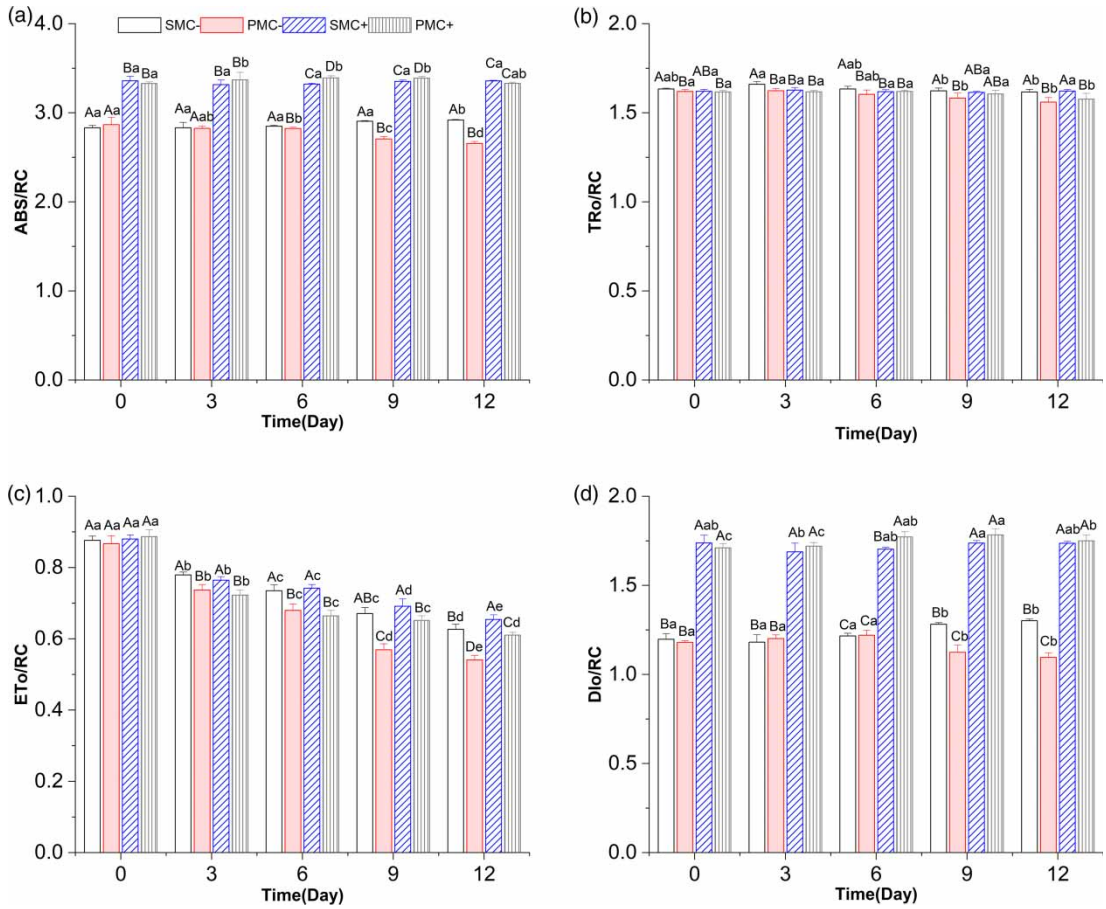


Figure 3 | ABS/RC (a), TRo/RC (b), ETo/RC (c), and DIo/RC (d) values of MC- and non-MC-producing *Microcystis* in mono- and co-culture groups (differences between and within groups are represented by uppercase and lowercase letters, respectively, $p < 0.05$).

Table 5 | Changes in PI_{ABS} values of MC- and non-MC-producing *Microcystis* cultivated with *M. spicatum* during the experiment (differences between and within groups are represented by uppercase and lowercase letters, respectively, $p < 0.05$)

Parameters	Time	Treatments			
		SMC-	PMC−	SMC+	PMC+
PI_{ABS}	0d	0.48 ± 0.02^{Bde}	0.49 ± 0.01^{Ba}	0.56 ± 0.02^{Ae}	0.56 ± 0.02^{Ad}
	3d	0.54 ± 0.02^{Bd}	0.52 ± 0.01^{Bc}	0.65 ± 0.02^{Ad}	0.63 ± 0.02^{Ac}
	6d	0.60 ± 0.01^{Ccd}	0.55 ± 0.01^{Da}	0.72 ± 0.02^{Ac}	0.68 ± 0.02^{Bb}
	9d	0.62 ± 0.01^{Cb}	0.50 ± 0.02^{Db}	0.76 ± 0.02^{Ab}	0.73 ± 0.02^{Ba}
	12d	0.63 ± 0.01^{Ca}	0.46 ± 0.02^{Dd}	0.80 ± 0.01^{Aa}	0.67 ± 0.01^{Bb}

were more sensitive to submerged plants than green algae (Jasser 1995; Hilt *et al.* 2006). In addition, MC- and non-MC-producing *Microcystis* strains showed different responses to nutrients and light (Davis *et al.* 2009; Lei *et al.* 2015). This study demonstrated that non-MC-producing *Microcystis* strains were more easily inhibited by the co-existence of submerged plants. This finding was consistent with the studies using the plant allelochemicals tannic acids and 12 *Microcystis* strains worldwide (Švanys *et al.* 2016), but contrary to the previous investigation using pyrogallol and three *Microcystis* strains isolated in Australia, where the toxigenic *M. aeruginosa* strain was more sensitive to pyrogallol than non-toxigenic strains (Gao *et al.* 2020a). The plant-*Microcystis* co-culture systems used in the current study reflected the interaction environments of cyanobacteria and submerged plants in actual waterbodies. The interaction and battle between the plants and *Microcystis* under co-culture conditions were close to the actual situation in eutrophic water bodies, which was not considered in the exposure experiments using pure plant allelochemicals. Submerged macrophytes could release various allelochemicals to inhibit *Microcystis* under co-culture conditions, while *Microcystis* strains release allelochemicals to affect plants simultaneously. The MC-producing *Microcystis* strain could release MCs. MCs have been widely reported to consistently affect photosynthesis of aquatic macrophytes (Zhang *et al.* 2022). We indeed observed more damage of submerged macrophyte *M. spicatum* co-existing with the MC-producing *Microcystis* strain than with the non-MC-producing strain in the present study. This might be the reason why MC-producing *Microcystis* strain demonstrated more resistance to the stress from *M. spicatum* than the non-MC-producing strain. Different responses of MC- and non-MC-producing *Microcystis* strains to co-existing *M. spicatum* indicated that attention should be paid to the intraspecies composition and toxigenic levels of cyanobacteria when using restoration measures with submerged vegetation to prevent and control cyanobacteria blooms in shallow eutrophic waterbodies.

The photosynthetic pigments (*e.g.*, chl *a*), photosynthetic oxygen evolution and maximum quantum yield of PSII (φ_{Po}) are frequently used as parameters to investigate the photosynthesis activity of cyanobacteria under the stress from plants under co-existing conditions (Körner & Nicklisch 2002; Zhu *et al.* 2010). Chlorophyll fluorescence transients (OJIP curves) and subsequent JIP tests were used to reveal the status and behavior of PSII in MC- and non-MC-producing *Microcystis* strains when co-existing with *M. spicatum* in the present study. The OJIP transients reflected the successive reduction of the electron acceptor pools of PSII (Govindjee 1995). The J step and I step represent an accumulation of $Q_A^- Q_B$ and $Q_A^- Q_B^-$ forms, respectively. Lower F_j and F_i values of the MC- and non-MC-producing *Microcystis* strains when co-existing with *M. spicatum* than in mono-cultures indicated that the electron transfer from the primary bound plastoquinone Q_A^- to the second bound plastoquinone Q_B was blocked by the stress from *M. spicatum*.

All JIP-test parameters indicated more serious damage to the PSII behavior and performance of non-MC-producing *Microcystis* cells than on MC-producing strain from *M. spicatum*. The parameters φ_{Po} and φ_{Eo} represent the maximum quantum yield for primary photochemistry and quantum yield for electron transport, respectively. The parameter Ψ_o reflects the quantum efficiency of electron transfer from Q_A^- to Q_B (Appenroth *et al.* 2001). The effect differences on φ_{Eo} and Ψ_o between the MC- and non-MC-producing *Microcystis* strains were larger than those on φ_{Po}. It further indicated that electron transport efficiency of PSII in the non MC-producing *Microcystis* cells was much more affected by co-existing *M. spicatum* than that in MC-producing cells, and the acceptor side of the electron transport chain might be the specific target. This result is consistent with the effects of salinity stress on PSII in *Ulva lactuca* (Xia *et al.* 2004).

The structure and function of PSII differ among various phototrophs. The fluorescence transients of cyanobacteria *Anabaena* sp., *Planktothrix rubescens*, and *Limnothrix redekei* are different (Strassert & Srivastava 1995). The chlorophyll fluorescence parameters of *M. aeruginosa* and *Chlorella pyrenoidosa* are different (Liu *et al.* 2019). Among the four

parameters to reflect the specific energy fluxes ratios in the electron transport chain, the initial TRo/RC and ETo/RC values for both strains in each experimental group were similar, but the initial ABS/RC and DIo/RC values differed obviously between two strains. This result indicates differences in PSII behavior between *Microcystis* strains. The parameters ABS/RC, TRo/RC, ETo/RC and DIo/RC represent the energy fluxes ratio, the trapped energy flux ratio, the electron transport flux ratio, and the dissipated energy flux ratio per reaction centre, respectively (Wang *et al.* 2021). During the experiment, the inhibition activity of *M. spicatum* on TRo/RC was weak for both strains, but the inhibition on ETo/RC was much higher for the non-MC-producing *Microcystis* strain than for the MC-producing strain. This parameter, together with φ_{Eo} and Ψ_o, provided multiple evidence that the electron transfer of non-MC-producing *Microcystis* was hampered more seriously than that of the MC-producing strain during the co-culture with *M. spicatum*.

The performance index on an absorption basis, PI_{ABS}, is the combination of three independent parameters, namely, RC/ABS, φ_{Po}, and ψ_o. This index is more sensitive than φ_{Po} for *Microcystis* to water soluble substances of *Dendranthema indicum* flowers (Gao *et al.* 2020b). It is also sensitive for other aquatic phototrophs to biotic and abiotic environmental stresses (Wang *et al.* 2021). The inhibition ratio of co-existing *M. spicatum* on PI_{ABS} for non-MC-producing *Microcystis* was 1.7-fold that for MC-producing *Microcystis*, calculated according to the decreasing ratio in comparison with the corresponding mono-culture groups on day 12. However, the φ_{Po} values did not show such big differences between the two strains. The parameter RC/ABS in plant-*Microcystis* co-culture treatments showed an increasing trend in comparison with that in mono-culture controls. Hence, the value of the parameter PI_{ABS} was largely determined by ψ_o in the current study. The higher inhibition ratio of *M. spicatum* on ψ_o and PI_{ABS} suggested the electron transfer chain of the non-MC-producing *Microcystis* was much more sensitive than that of MC-producing *Microcystis* to co-existing *M. spicatum*. Here, one submerged plant was tested; whether the electron transfer chain of the non-MC-producing *Microcystis* is more sensitive to other submerged macrophytes than MC-producing *Microcystis* needs further investigation.

CONCLUSIONS

The growth and photosynthesis of both *Microcystis* strains were inhibited to varying degrees when co-cultured with the submerged plant *M. spicatum*. Chlorophyll a fluorescence transients and subsequent JIP-test parameters demonstrated more serious damage to the efficiency and energy flux of the electron transport of PSII in the non-MC-producing *Microcystis* than in the MC-producing strain. The electron acceptor side of the electron transport chain might be the specific target. These results provided detailed photosynthetic evidence that the strain composition and MC-producing ability of *Microcystis* affected their interactions with submerged macrophytes and the elimination of *Microcystis*-dominated blooms.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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