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Short communication

Elimination of cyanobacteria and cyanobacterial microcystin with increase in heterogeneity

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ABSTRACT

Cyanobacterial blooms and their corresponding toxins have resulted in the reduction of heterogeneity within aquatic ecosystems, and *vice versa*. Yet much remain to be known whether high heterogeneity can lead to effective elimination of cyanobacteria and microcystin (MC). In this work, by introducing biota (macrophyte and bivalve) and abiota (artificial substrate) to increase heterogeneity, cyanobacterial and MC elimination were enhanced. With increasing heterogeneity, the mean removal efficiencies of cyanobacteria increased from 58.86% to 79.11%. Correspondingly, the mean MC removal efficiencies enhanced from 42.38% to 74.16%. The biofilm was shown to be the most efficient for removing cyanobacteria and MC, whereas the bivalve was less efficient for cyanobacterial removal than the macrophyte and had no effect on MC removal. Furthermore, crustaceans were significantly enriched in all the heterogeneous systems; the introduced bivalve increased respiratory activity in the substrate biofilm. Conclusively, increasing heterogeneity appears to be a not only viable solution for improving the quality of water in protected sources but also potential criterion for restoring and managing aquatic ecosystems.

1. Introduction

Cyanobacterial blooms increase the turbidity of aquatic ecosystems and deplete oxygen as the cyanobacteria die, which diminishes physical habitat heterogeneity and simplifies aquatic disturbance regimes (Daily, 1997). They further smother aquatic plants, thereby suppressing the functional habitats of invertebrates and fish, and ultimately homogenizing species pools and simplifying the ecosystem's structure (Huisman et al., 2005; Rahel, 2000). These reductions in complexity within an aquatic ecosystem can also exacerbate cyanobacterial blooms, deteriorating ecological service in an ecosystem used for recreation and drinking water supplies (Dodds et al., 2009; Brookes and Carey, 2011). Moreover, a few cyanobacteria produce toxic secondary metabolites, particularly microcystin (MC), which pose a risk to human health when surface waters are used as a drinking water source and for recreational purposes (Falconer, 2005).

Various strategies to mitigate cyanobacterial cells and dissolved toxins have been proposed. Because of ecological compatibility and low cost, techniques based on organisms that metabolize cyanobacteria and MC have been widely adopted. One group (Nimptsch et al., 2008; Wu et al., 2010) of these methods employs the biotransformation process of aquatic macrophytes (and periphyton); another (Bourne et al., 2006; Ji et al., 2009) uses microorganisms (and biofilm) grown on the surface of a substrate in bioreactors; third group (Vaughn et al., 2004; Ibelings et al., 2007) depends on cyanobacteria predation by fish and filtration by mussels. However, these studies exploit only a single species to degrade cyanobacteria and MC, which simply implants organisms rather than remediating the ecosystem (José et al., 2009). The introduction of a single species may also lead to homogenization of biota, in turn, accelerating cyanobacterial bloom and MC release (Vitousek et al., 1997). In addition, because of discrepancies in experimental designs and scales, very few studies can quantify the relative effects of aquatic macrophytes, microorganisms, and aquatic animals in removing cyanobacteria and MC.

Heterogeneity, including physical habitat heterogeneity (abiota) and species diversity (biota), strongly influences water and nutrient dynamics, trophic interactions, and disturbance regimes to ultimately maintain ecosystem functions such as primary production and decomposition (Gao et al., 2000; Hector and Bagchi, 2007). Thus, with the aforementioned linkage between cyanobacterial bloom and reduction of heterogeneity in mind, it was hypothesized that as heterogeneity increases, ecological

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integrity becomes organized to control the growth of cyanobacteria and its by-product, MC. In this study, mesocosm experiments with heterogeneous systems were designed to verify the supposition that increasing heterogeneity reduces cyanobacteria and MC and determine the relative effects of biota and abiota in these removal processes.

2. Materials and methods

2.1. Site description

The study site was located in Meiliang Bay a severely eutrophic region of 125 km^2 area in the northern part of Lake Taihu, China, which serves as an important water resource for drinking water, irrigation, aquaculture, and industrial activities in proximal cities. In the warm season, a dense toxic scum of cyanobacterial bloom occurs frequently every year in the surface water of the bay (Pu et al., 1998). During the study period, cyanobacteria, particularly *Microcystis aeruginosa*, comprised a large proportion of the phytoplankton community. The physicochemical parameters of the raw water are listed in Table 1.

Mesocosms were created outdoors in four rectangular concrete flow-through tanks having identical inner dimensions, 3.6 m long, 2.0 m wide, and 1.8 m deep. Lake water, at 1.0 m deep and 5.0 m away from the shore, was continuously drawn at a flow rate of 308 L h^{-1} by a drawlift which connected a suction pipe with an effuser. Water from the effuser was even distributed into four parallel tanks at a flow rate of 77 L h^{-1} by four regulations composed of flowmeter and tap.

2.2. Manipulation of heterogeneity

Three different heterogeneous systems were separately launched into the three tanks, floating on the water *via* a buoyant framework. The density of the system, which is the ratio of system volume to tank volume, was 8.49%. The surface area of each heterogeneous system was 1 m^2, equal to a 13.6% coverage rate (surface area of the heterogeneous system/water surface area of the experimental tank) for each tank. The fourth tank was untreated and was used as the control to indicate unmanipulated homogeneous conditions.

The three systems employing two or three components of macrophyte, bivalve, and artificial substrate, vertically arranged from upper to lower, were manipulated as follows:

System I: bivalve + artificial substrate;

System II: macrophyte + artificial substrate;

System III: macrophyte + bivalve + artificial substrate.

Specifically, in terms of their autochthonous and water purifying functions, floating-leaved *Ipomoea aquatica* and freshwater clam *Corbicula fluminea* were applied as aquatic macrophyte and bivalve, respectively. *I. aquatica* (1.5 kg; wet) obtained from a greenhouse was transplanted to systems II and III after being uprooted, and was carefully rinsed with tap water. *C. fluminea* (4.0 kg; wet; approximately 400 individuals) obtained from a local market was almost equivalent to 10 g dry (0.97 g dry weight), and these were stocked in six separate cages fastened to systems I and III. A semisoft assembly medium as artificial substrate was installed into these systems for microbe assemblage and biofilm growth within variable habitats. There were 81 clusters of semisoft assembly medium in each system. Each cluster contained eight plastic ring skeletons with a spacing of 7 cm. Polypropylene rayon fibers were affixed to the plastic ring skeletons.

2.3. Analyses of chlorophyll a and microcystin

Every five days, water sample was collected by a siphon sampler. At the outflow side of the tank, one end of the cylindrical sampler with 2.0 m length and 0.10 m diameter was vertically dipped into water till 1.8 m depth. Then a rubber stopper was plugged into the other end of the sampler to generate siphon. The sampler was quickly drown out from the water surface and was transferred into a container to mix water siphoned by the sampler. Water sample taken from the container was analyzed for chlorophyll a (Chl-*a*) and MC. Because of the dominance of cyanobacteria in the algal community (Table 1), Chl-*a* was employed to evaluate the biomass of cyanobacteria, which was determined by a spectrophotometer after filtration through glass fiber filters and extraction with 96% ethanol. MC-LR was selected to evaluate the amount of MC removed owing to its high occurrence frequency and carcinogenic potential in the water source (WHO, 1998); it was analyzed by high-performance liquid chromatography (HPLC). Water samples fixed with glacial acetic acid at 5% (v/v) and 90% aqueous methanol mixture were filtered through glass fiber filters (0.45 μm); then loaded to Supelco C18 SPE cartridges at a flow rate of approximately 5 mL min^{-1}. The cartridges were then rinsed with 40 mL of deionized water, followed by 20 mL of 10% (v/v) methanol in water and 20 mL of 20% (v/v) methanol in water. The MC was eluted three times from the C18 SPE cartridge by 5 mL of 0.1% (v/v) trifluoroacetic acid (TFA) in methanol. The resulting extraction was evaporated to dryness under a gentle stream of air at 45 °C after filtration with a 0.45 μm pinhead filter. The dry residue was reconstituted in 400 μL methanol with 0.1% (v/v) TFA and then stored at −20 °C until HPLC analysis. MC-LR was quantitatively analyzed by HPLC (Agilent 1100, USA) on the basis of a calibration curve constructed by analyzing commercially available MC-LR standards (Wako Pure Chemical Industries, Ltd., Japan). The purity of MC-LR fractions was assessed by quantifying the closely retained impurities by fitting them to the MC-LR calibration curve (Ramanan et al., 2000).

The bivalves (*C. fluminea*) were collected from heterogeneous systems I and III every 45 days to quantify their accumulated MC. The collected bivalves were immediately frozen at −40 °C until MC analysis. Freeze-dried bivalves were removed from their shells and dissected to obtain visceral mass. The tissue was ground with a mortar and pestle and extracted three times using a butanol–methanol–water solution (1:4:15, v/v) for 24 h with stirring. After the extract was centrifuged at 18,000 rpm for 1 h at 4 °C, the

Table 1
Raw water quality during experiment period systems.

Item	Min	Max	Mean	Standard deviation
Water temperature (°C)	14.3	30.1	25.1	4.55
pH	7.6	8.1	7.8	0.17
TN (mg/L)	2.45	14.20	5.15	3.61
$NH4^+$–N (mg/L)	0.40	6.85	2.16	2.05
TP (mg/L)	0.32	1.56	0.97	0.37
TOC (mg/L)	7.13	28.00	12.96	6.56
Proportion of cyanobacterial cells (%)	35.8	92.2	83.5	18. 6