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Increasing phytoplankton-available phosphorus and inhibition of macrophyte on phytoplankton bloom

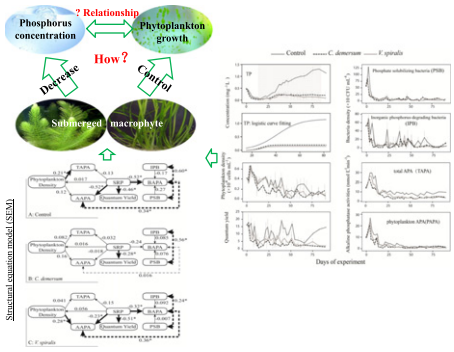
Yanran Dai, Juan Wu, Xiaohang Ma, Fei Zhong, Naxin Cui, Shuiping Cheng *

Key Laboratory of Yangtze River Water Environment, Ministry of Education, Tongji University, Shanghai 200092, China

HIGHLIGHTS

- Phytoplankton has its strategies to increasing available P in the water column.
- Facilitating the total APA level in water and cooperating with bacteria is an essential strategy.
- *C. demersum* and *V. spiralis* both had prominent performance on regulating the phytoplankton growth.
- *C. demersum* held more potential on controlling algal density and inhibiting quantum yield.

GRAPHICAL ABSTRACT



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ABSTRACT

We assembled mesocosms to address the coherent mechanisms that an increasing phosphorus (P) concentration in water columns coupled with the phytoplankton bloom and identify the performance gap of regulating phytoplankton growth between two macrophyte species, *Ceratophyllum demersum* L. and *Vallisneria spiralis* L. Intense alkaline phosphatase activities (APA) were observed in the unplanted control, with their predominant part, phytoplankton APA (accounting for up to 44.7% of the total APA), and another large share, bacterial APA. These correspond with the large average concentration of total phosphorus (TP), total dissolved phosphorus (TDP) and soluble reactive (SRP) as well as high phytoplankton density in the water column. The consistency among P concentrations, phytoplankton density and APA, together with the positive impact of phytoplankton density on total APA revealed by the structural equation modelling (SEM), indicates that facilitated APA levels in water is an essential strategy for phytoplankton to enhance the available P. Furthermore, a positive interaction between phytoplankton APA and bacteria APA was detected, suggesting a potential collaboration between phytoplankton and bacteria to boost available P content in the water column. Both macrophyte species had a prominent performance on regulating phytoplankton proliferation. The phytoplankton density and quantum yield in *C. demersum* systems were all significantly lower (33.8% and 24.0%) than those in *V. spiralis* systems. Additionally, a greater decoupling effect of *C. demersum* on the relationship between P, APA, phytoplankton density, bacteria dynamic and quantum yield was revealed by SEM. These results imply that the preferred tactic of different species could lead to the performance gap.

* Corresponding author at: 1239 Siping Road, Shanghai 200092, China.
E-mail address: shpcheng@tongji.edu.cn (S. Cheng).

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1. Introduction

Excessive nutrients from point and diffuse pollution trigger phytoplankton blooms in numerous freshwater systems (rivers, lakes and reservoirs), which create hypoxic "dead zones" in the water body and taint drinking water (Bennett et al., 2001; Carpenter, 2008). So far, a wealth of effort has been devoted to disclosing the relationships between phytoplankton blooms and nutrients, and finding efficient ways to mitigate eutrophication (Trimbee and Prepas, 1987; Ahlvik et al., 2014). However, we are still plagued by the problem.

Phosphorus (P) as a common limiting nutrient plays a more important role than nitrogen (N) in the proliferation of bloom-forming 'nuisance' algae (Correll, 1998; Carpenter, 2008). Unlike N having high losses to the atmosphere related to denitrification, P is widely observed in the more efficient recycling in waters (Nixon et al., 1981). Using a 37-year whole-lake nutrient manipulation experiment in one Canadian lake, Schindler et al. (2008) found P inputs to directly control phytoplankton blooms, and the blooms were even more exacerbated when N inputs were decreased without simultaneously dwindling P inputs. This emphasized the importance of controlling P concentration for preventing algal blooms. In practice, despite considerable attempts to control the external P input, the internal loading of P in waters has become another stumbling block, not least in the anoxic hypolimnion of eutrophic lakes (Cyr et al., 2009; Spears et al., 2012), and the relevant manipulating approach is lacking. In addition, phytoplankton can excrete extracellular alkaline phosphatase enzyme (APA) to hydrolyse organic P source for supplying PO_4^{3-} for uptake (Young et al., 2010). Harke et al. (2012) identified the expression of the genes involved in the hydrolysis of phosphomonoesters (*phoX*) and high affinity P-transport (*pstS* and *sphX*) in *Microcystis aeruginosa* were regulated by external dissolved inorganic phosphorus (DIP) concentration. Some bacteria can also decompose the unavailable organic P into DIP, and thereby contribute to phytoplankton blooms (Zhao et al., 2012).

Since the earliest studies (Yount, 1964; Scheffield, 1967), evidence has amounted to state that macrophytes have a strong negative impact on phytoplankton biomass increase and are important for maintaining a clear-water state (De Backer et al., 2012; Dai et al., 2014). Despite it has been documented that macrophyte can inhibit algal growth directly by competing for resources (nutrient and light), excreting allelopathic substances (harmful to algal growth) and altering hydraulic conditions (lower turbulence intensity) (Van den Berg et al., 1998; Mulderij et al., 2007). Macrophyte provides shelter for zooplankton and juvenile fish as well as habitat for macroinvertebrates, which could facilitate the lessening of phytoplankton species richness through grazing (Muylaert et al., 2010). Furthermore, they can reduce sediment resuspension and reinforce sedimentation, which can contribute to controlling the release of internal P loading (Horppila and Nurminen, 2003; Schulz et al., 2003). In recent decades, macrophytes have been widely used for the ecological restoration to maintain a 'clear' state, and their efficiencies in controlling nutrients (especially P) dynamics in different waters were examined and optimized (Shilton et al., 2012; Moore et al., 2016; Zhang et al., 2016). Yet, our knowledge about how macrophyte could inhibit phytoplankton bloom to some extent is still limited, particularly as regards how they may interfere with the phytoplankton phosphorus-acquisition process.

In the present study, we set up several mesocosms with two widespread submerged macrophyte species in China, *Ceratophyllum demersum* L. and *Vallisneria spiralis* L. They grow fast and can easily develop into dense stands. Both of them yielded good results in improving water quality despite their morphological distinction (Dai et al., 2012; Qiu et al., 2001). With these mesocosms, we aim to address the mechanisms of phytoplankton to enhance the available phosphorus in water columns, as well as achieve a greater understanding of how macrophyte regulate the phytoplankton growth and identify the performance gap between different macrophyte species.

2. Materials and methods

2.1. Experimental mesocosms

A total of 9 PVC tanks (length × width × height: 0.6 m × 0.5 m × 0.8 m) were used for simulation of the lake system and comparative analyses. A 10-cm layer of sediment was placed in each tank, and then the tank was filled with water. This process caused a large amount of sediment resuspension, but based on the observation, it took roughly 3–4 days for most of the suspended particles to settle down in the systems. Sediment used in these mesocosms was collected from the top 0–10 cm of sediment in a eutrophic landscape river flowing through Tongji University, Shanghai. The whole sediment was thoroughly mixed in an open container prior to the experiments. Sediment content of total phosphorus (TP), organic phosphorus (OP), inorganic phosphorus (IP), total nitrogen (TN) and organic matter (OM) was 1.15 ± 0.04, 0.91 ± 0.02, 0.24 ± 0.03, 1.50 ± 0.06 and 55.63 ± 2.07 g/kg on dry weight (DW) basis, respectively. The overlying water was also directly taken from the same river at 0.5 m depth. The concentration of TP, total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), particulate phosphorus (PP), TN, ammonia nitrogen (NH_4^+-N), chemical oxygen demand (COD) was 0.487 ± 0.001, 0.41 ± 0.00, 0.38 ± 0.00, 0.07 ± 0.03, 4.92 ± 0.19, 4.14 ± 0.06 and 14.37 ± 0.90 mg/L, respectively.

Submerged macrophyte (*C. demersum* and *V. spiralis*) were both collected in June 2014 from Donghu Lake in Wuhan. *C. demersum*, a free-floating submerged species, has fluffy, filamentous, bright-green leaves; *V. spiralis*, a rooted submerged species has narrow, linear leaves. They were pre-incubated for about 4 weeks in a bigger tank with the same water and sediment as the mimic systems. After removing the adherent water on plants with a line wedge of bibulous paper, these two macrophyte species were evenly planted in three tanks (about 0.50 kg fresh weight per square meter; *C. demersum*: ~25 plants; *V. spiralis*: ~20 plants) immediately after filling water, respectively. This experiment manipulated three experimental treatments (planted with *C. demersum*; planted with *V. spiralis*; unplanted control), with three replicates for each. All the mesocosms were exposed to natural sunlight in an open room with the transparent roof. During the experimental period, the air temperature ranged from 20.0 °C to 38.0 °C. An appropriate amount of tap water was added periodically (2–4 days) to maintain the initial water level.

2.2. Sampling procedure

The study was carried out from 7 July to 4 October (90 days) in 2014. Overlying water samples (0.2 m) were collected every 2–8 days, with a relatively intensive sampling frequency in the first half of the experiment. All the samples taken to the laboratory were analysed or preserved immediately.

2.3. Chemical analysis

TN, NH_4^+-N, and TP of water samples were analysed according to Standard Methods in Environment Monitoring of China (National Bureau of Environment Protection, 2002). COD was measured using a spectrophotometer (DR/2800, Hach Co., Loveland, CO, USA). A fraction of each water sample was filtered with a 0.45 μm pore size hydrophilic Polyether sulfone (PES) membrane, and then determined for TDP using the same measurement as that for TP. SRP was determined according to Murphy and Riley (1962). After the estimation of TP, TDP and SRP, it was then possible to calculate the dissolved organic phosphorus, $DOP = TDP - SRP$, and the particulate phosphorus, $PP = TP - TDP$.

Sediment samples were all naturally air-dried and sieved with a standard 100-mesh sieve. P fractions were determined using the SMT protocol (Ruban et al., 1999). The TP concentration in sediments was determined by the ascorbic acid method after igniting the sediment at