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Environmental effects by introducing *Potamogeton crispus* to recover a eutrophic Lake

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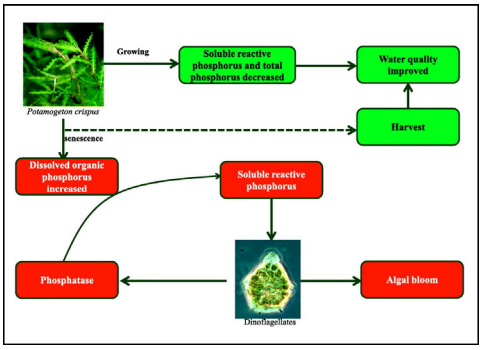
HIGHLIGHTS

- Effect of recovering vegetation is ambiguous and underlying mechanism is unclear.
- Comparative study was performed to detect the effects from biochemical point of view.
- Senescence and decay of submerged macrophyte resulted in organic phosphorus release.
- Dinoflagellate bloom occurred by efficiently hydrolyzing DOP via phosphatase.
- P form and P using strategy of algae jointly shape the phytoplankton community.

GRAPHICAL ABSTRACT



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ABSTRACT

Re-establishing submerged vegetation is considered an important tool to restore shallow eutrophic lakes. A whole year comparative field study was performed in a eutrophic lake and its connected pond with *Potamogeton crispus* in order to determine the effects of the growth and senescence of submerged macrophytes on structure of phytoplankton. *P. crispus* improved the water quality at the growing season in terms of improving transparency, decreasing total phosphorus, soluble reactive phosphorus (SRP) and chlorophyll *a* concentrations and slowering turnover rate of dissolved organic phosphorus (DOP). Meanwhile, dominant species shift from Chlorophyta to Diatom. Notably, senescence and decomposition of *P. crispus* in late spring resulted in an abrupt increase of DOP, providing a suitable growing environment for Euglena and dinoflagellates and a *Peridiniopsis* bloom occurred owing to their advantage in utilizing DOP. *Peridiniopsis* excreted phosphatase as evidence by simultaneously in situ enzyme labelled fluorescence (ELF) labelling and main alkaline phosphatase activity contributed by large particles, suggesting that the dominance of dinoflagellate with low SRP is enabled by its ability to efficiently hydrolyze DOP. Under the scenario of worldwide application of re-establishing submerged vegetation, our results provide the evidence of the negative environmental effects that occurred when transplanting *P. crispus* to recover a eutrophic lake.

1. Introduction

One of the most serious problems caused by eutrophication of shallow lakes is the disappearance of submerged macrophytes (a clear, vegetation-dominated state) and the switch to a turbid, phytoplankton-dominated state (Hilt et al., 2006). From the ecological point of view, shallow lake

Abbreviations: P, phosphorus; SRP, soluble reactive phosphorus; DOP, dissolved organic phosphorus; DTP, dissolved total phosphorus; TP, total phosphorus; APA, alkaline phosphatase activity; ELF, enzyme labelled fluorescence; ELFP, ELF phosphate; ELFA, ELF alcohol; Chl *a*, chlorophyll *a*; DOM, dissolved organic matter.

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ecosystems can shift between the two stable states (Mcgowan et al., 2005). Recovering or re-establishing stable submerged vegetation is considered an important tool to restore shallow eutrophic lakes as their presence promotes clear water and biodiversity through several feedbacks (Scheffer et al., 1993). A low water turbidity is maintained through direct and indirect effects of the submerged vegetation, e.g. nutrient competition, allelopathy, improved possibilities for zooplankton to avoid fish predation (resulting in increased zooplankton grazing on phytoplankton) and decreased sediment resuspension (Blindow et al., 1998; Weisner et al., 1997; Schriver et al., 1995; Hargeby et al., 1994; Moss et al., 1994; Scheffer, 1990; Timms and Moss, 1984). While natural restoration of submerged vegetation in the lake needs a long time, perturbative recovery or reestablishment of submerged vegetation in shallow lakes was applied worldwide in recent decades. *Potamogeton crispus* L. is a submerged macrophyte that is widely distributed throughout China and is often used in ecological restoration for eutrophic sites (Xie et al., 2015). Most restoration accomplishments of eutrophic lake have been attributed to the success of aquatic macro-vegetation (Hilt, 2006; Körner and Dugdale, 2003; Boedeltje et al., 2001; Qiu et al., 2001; Hosper, 1998). However, the effect on phytoplankton of artificial introduction of submerged vegetation was sometimes negative (Ayala et al., 2007). The relationship between phytoplankton and whole system submerged macrophyte biomass could be positive (Rooney and Kalff, 2000). Therefore, the relationships between the phytoplankton and submerged macrophyte are not well known. It is essential that scientific research provides the evidence to underpin the cost-effective and efficient decision making that is required for the successful management of eutrophication in lakes.

Modifying phosphorus (P) status in lakes is one of the important mechanism by which submerged macrophytes may exert their influence on phytoplankton. The relationship between chlorophyll *a* (Chl *a*) and the limiting nutrient (mostly P) significantly differed between lakes with and without submerged vegetation (Takamura et al., 2003). Previous studies always focused on the effect of submerged macrophyte during the growing season (Zhou et al., 2000). In the decomposition period of submerged macrophytes, the decaying macrophytes could be an internal P source for the lake and considerable quantities of P release into the water column. The amount of P released in the decomposition process of macrophytes in the lake is so great that if the above-ground biomass is removed, phosphate leached to the overlying water can be reduced to less than half within 10 years (Asaeda et al., 2000). The quantity and quality of P released by macrophyte may influence phytoplankton in terms of density and composition. However, little is known since long time series that monitor biological change through the transition of the two states are scarce.

P is always considered to be a key element limiting phytoplankton in lakes. When ambient available P is scarce, cyanobacteria and algae can produce extracellular alkaline phosphatase (AP) to hydrolyze organic P for compensation of P deficiency (Gillor et al., 2002; Spijkerman and Coesel, 1998; Feuillade et al., 1990). Despite the importance of extracellular phosphatase excreted by phytoplankton, concrete evidence of its detection has been difficult to obtain. This is because traditional methods predominately rely on inferring the biochemical enzyme activity through size fractionation. Enzyme labelled fluoresce (ELF) technology made it possible to detect extracellular phosphatase in situ.

Differences in the presence and localization of phosphatases in different algal populations and seasonal variations of algal phosphatase activity could be detected (Rengefors et al., 2001; Telford et al., 2001; Dyhrman and Palenik, 1999; González-Gil et al., 1998). The extracellular phosphatase response of phytoplankton to the decaying of submerged macrophyte is largely unknown.

The aim of this work was to answer the following two questions: (1) how the growth and decay of submerged macrophytes affected phosphorus composition, P regeneration efficiency and therefore shaped the structure of phytoplankton in the water column, and (2) was transplanting submerged macrophyte an effective method to restore a lake from eutrophication at high P level? Firstly, we compared P and phytoplankton in terms of concentration (density) and composition between an urban shallow eutrophic lake and its connected pond with transplanted submerged vegetation for a whole year. Meanwhile, we determined the extracellular phosphatase kinetics and phosphatase excreted by phytoplankton in situ using ELF method. The P turnover time was calculated in parallel. Finally, P uptake strategy of phytoplankton and its connection to P cycling triggered by decaying of submerged maccrophytes was discussed and the effect of artificial transplanting submerged macrophyte to restore lake from eutrophication was estimated.

2. Material and methods

2.1. Study sites and sampling

A small pond (300 m^2) was successfully introduced submerged macrophyte (*P. crispus*) before sampling. It was adjacent and connected to Lake Nanhu (30°27′58″-30°29′46″N; 114°20′33″-114°23′25″E), which is a hypertrophic urban lake and lacking the macrophyte coverage. Three couples of corresponding sampling sites were selected in the pond and lake (Fig. 1). Mixed samples from the upper layer (0–1 m) were collected with a Friedinger sampler at the sampling sites monthly or weekly dependent on aiming in 2007. Two litres of the sample were taken for chemical, plankton and enzyme analysis. Phytoplankton samples (1 L) for quantitative analysis were preserved with Lugol's solution and concentrated to 30 mL after one-week sedimentation. Phytoplankton species were identified according to Hu et al. (1980).

2.2. Phosphatase activity

2.2.1. Spectrophotometrical phosphatase detection

Extracellular alkaline phosphatase activity (APA) in the water samples was determined using a modified procedure by Boon (1989) and Gage and Gorham (1985). Triplicates of 5 mL water samples were supplied with Tris-HCl buffer (pH = 8.5, final concentration 13 mmol L^{-1}), Na_3N (final concentration 5 mmol L^{-1}), and *p*-nitrophenyl phosphate (*p*NPP, final concentration 0.3 mmol L^{-1}), and the samples were incubated at 37 °C for 4 **h**. Absorbancy of *p*-nitrophenol was measured spectrophotometrically at 410 nm and corrected for blank. APA was determined in unfiltered sample (APA_T) and in the filtrates through 3.0 μm and 0.45 μm membrane filters ($APA_{<3.0\mu m}$ and $APA_{<0.45\mu m}$, respectively). The contribution of APA to the coarser ($APA_{>3.0\mu m}$) and finer ($APA_{0.45-3.0\mu m}$) fractions were calculated as follows: $APA_{>3.0\mu m} = APA_T - APA_{<3.0\mu m}$, $APA_{0.45-3.0\mu m} = APA_{<3.0\mu m} - APA_{<0.45\mu m}$ (Chróst et al., 1984). Kinetics of total APA was determined using a method adapted from Berman (1970). Substrate was added at eight final concentrations ranging from 0.01 mmol L^{-1} to 1.8 mmol L^{-1}. Maximum velocity (V_{max}) and Michaelis constant (K_m) values were estimated by fitting the linearized Michaelis-Menten equation (Dick and Tabatabai, 1984), and the Lineweave-Burk plot was used (Zhou et al., 2001). Turnover times (T) of DOP were estimated as K_m/V_{max} (**Nedoma and Vrba,** 2006).

2.2.2. Microscopic phosphatase detection

ELF® 97 phosphate (ELFP, Molecular Probes) was used for microscopic detection of extracellular APA in the phytoplankton according to the protocol by Štrojsová et al. (2003). The incubation started by adding the ELFP solution (final concentration 27 μmol L^{-1}) and samples were incubated at 25 °C for different time periods (2–4 h). Each incubation was terminated by transferring the sample to a filter holder (diameter 7 or 10 mm, depending on phytoplankton density) with a membrane filter (Millipore; 0.45 μm pore size) and its immediate filtering over mild vacuum (<20 kPa). The filter with retained plankton was placed on a microscope slide and embedded it with the anti-fading reagent Citifluor AF1 (Citifluor, London, UK).