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RESEARCH ARTICLE

Effects of epiphytic algae on biomass and physiology of *Myriophyllum spicatum* L. with the increase of nitrogen and phosphorus availability in the water body

Yu-Zhi Song[1,2] · **Jin-Qi Wang**[1] · **Yong-Xia Gao**[2]

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Abstract The disappearance of submerged vascular macrophytes in shallow eutrophic lakes is a common phenomenon in the world. To explore the mechanism of the decline in submerged macrophyte abundance due to the growth of epiphytic algae along a nutrient gradient in eutrophic water, a 2 × 3 factorial experiment was performed over 4 weeks with the submerged macrophyte (*Myriophyllum spicatum* L.) by determining the plant's biomass and some physiological indexes, such as chlorophyll (Chl) content, malondialdehyde (MDA) content, and superoxide dismutase (SOD) activity in the leaves of *M. spicatum* L. on days 7, 14, 21, and 28, which are based on three groups of nitrogen and phosphorus levels in the water body (N-P [mg L^{-1}]: NP1 0.5–0.05, NP2 2.5–0.25, NP3 4.5–0.45) and two levels of epiphytic algae (the epiphytic algae group and the control group). Epiphytic algal biomass was also assayed. The results indicated that epiphytic algal biomass remarkably enhanced in the course of the experiment with elevated levels of nitrogen and phosphorus in the water. Under the same level of nutrient condition, plants' biomass accumulation and Chl content were higher in the control group than that in the epiphytic algae group, respectively, while MDA content and SOD activity in the former were lower than that in the latter. The influences of epiphytic algae on the biomass accumulation and Chl content and MDA content became greater and greater with elevated levels of nutrients. In general, in this experiment, water nutrients promoted the growth of both epiphytic algae and submerged plants, while the growth of epiphytic algae hindered submerged macrophytes' growth by reducing Chl content and promoting peroxidation of membrane lipids in plants.

Keywords *Myriophyllum spicatum* L · Epiphytic algae · Nitrogen and phosphorus concentration · Growth indices · Physiological indices

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✉ Yu-Zhi Song
syz70@nuist.edu.cn

Jin-Qi Wang
w_j_q@sina.com

Yong-Xia Gao
gyx0813@tom.com

[1] Jiangsu Key Laboratory of Agricultural Meteorology, School of Applied Meteorology, Nanjing University of Information Science and Technology, No.219, Ningliu Road, Nanjing, Jiangsu 210044, China

[2] Jiangsu Collaborative Innovation Center on Atmospheric Environment and Equipment Technology (CICAEET), Nanjing University of Information Science and Technology, Nanjing, No.219, Ningliu Road, Nanjing, Jiangsu 210044, China

Introduction

Submerged plants are important in shallow lake ecosystems for keeping stable the macrophyte-dominated clear water states (Jackson 2003; Janssen et al. 2014). Following the process of eutrophication in shallow lakes, water bodies receive excess nutrients, submerged macrophyte abundance, and diversity decline in shallow lakes worldwide (Lauridsen et al. 2003; Sand-Jensen et al. 2000; Qin et al. 2013) and shallow lakes are often exhibiting phytoplankton-dominated turbid water states (Scheffer 1998; Hilt and Gross 2008). Through the reduction of external nutrient loads, a change back from the

turbid, phytoplankton-dominated state to the clear, macrophyte-dominated state is often delayed by stabilizing mechanisms that cause resilience (Roberts et al. 2003; Gulati et al. 2008). Actually, the enrichment of nitrogen and phosphorus in eutrophic water is usually accompanied with the increase of epiphytic algae and planktonic algae (Balls et al. 1989; Romo et al. 2007; Tóth 2013). However, the disappearance of the submerged macrophytes is connected with high planktonic algae population density in eutrophic lakes (Phillips et al. 1978; Xing et al. 2013; He et al. 2014); in the eutrophic lakes, the bloom of epiphytic algae occurring first was observed, then planktonic algae bloom, and the reduction of macrophyte populations is connected with the dense epiphytic algae populations. Therefore, enhanced planktonic algae biomass was considered to be subsequent instead of causative (Phillips et al. 1978; Sand-Jensen et al. 2000; Fong et al. 2000). It has also been repeatedly indicated that the development of submerged macrophytes is hindered by epiphytic algal growth (Sand-Jensen and Søndergaard 1981; Asaeda et al. 2004; Chen et al. 2007), and the shade for epiphytic algae attached on the surface of leaf which was the major factor that suppresses the growth of macrophytes was also taken for granted; even over 80% light intensity decreased through a dense epiphytic community before reaching the chloroplasts (Sand-Jensen and Søndergaard 1981). However, Asaeda et al. (2004) reported that macrophytes could respond to low light-created shade by enhancing Chl content and new shoot number, while they could not produce the same response to the shade created by epiphytic algae. On the contrary, epiphytic algal boom resulted in the decline of leaves' Chl *a* density (Asaeda et al. 2004; Chen et al. 2007; Song et al. 2015). Maybe epiphytic algae induced one or more mechanisms that damaged the photosynthetic organelles of macrophytes and that the chlorophyll content of macrophytes was reduced. The underlying mechanisms behind the disappearance of submerged macrophytes in the eutrophication process needed to be fully understood.

In this paper, *Myriophyllum spicatum* L. was chosen for study. *M. spicatum* L. is a perennial aquatic macrophyte widely distributed all over the world; on the other hand, they could tolerate eutrophic conditions. We assayed biomass accumulation and some physiological indexes, such as Chl content, malondialdehyde content, and activity of superoxide dismutase in the leaves of *M. spicatum* L., under two levels of epiphytic algal loads through laboratory experiments, to verify the hypothesis that epiphytic algal loads have adverse influences on the growth of submerged macrophytes by physiological stress and the effects became greater and greater with the increase of nitrogen and phosphorus concentration in the water.

Materials and methods

Experimental design

Myriophyllum spicatum L. was acquired from East Taihu Lake, and then five terminal apices (terminal 10 cm of shoot) were cultured in each plastic pot (8 cm diameter × 10 cm depth) containing clean sand. Twenty pots with plants were placed in each plastic container (50 cm × 40 cm × 60 cm) containing 40 L tap water and kept in a greenhouse for adaptation.

After 2 weeks of adaptation, a 2 × 3 factorial experiment was conducted with three replicates in a controlled condition, based on two levels of epiphytic algae and three levels of nitrogen and phosphorus concentrations in the water body. Three levels of nitrogen and phosphorus concentrations (N-P, mg L^{-1}) were NP1 0.5–0.05, NP2 2.5–0.25, and NP3 4.5–0.45, according to the levels of nutrients in the eutrophic shallow lakes in China (Qin et al. 2013). Two levels of epiphytic algae were the control group and the epiphytic algae group. In the control group, 24 *Radix swinhoei* (H. Adams) species per container were utilized to control the growth of epiphytic algae according to a preliminary experiment. The added *R. swinhoei* (each about 0.2 g) was obtained from East of Lake Taihu. In the epiphytic algae group, no *R. swinhoei* was added. To prevent the planktonic algal blooms and to hold the initial water column nutrient concentrations in the course of the experiment, 20 L culture solution in each container was renewed once every other day. The total nitrogen and phosphorus in water were assayed daily (The State Environmental Protection Administration 2002), and inorganic nitrogen (NH_4CL and $NaNO_3$(NH_4^+-N/NO_3^--*N* = 1:1)) and inorganic phosphate (NaH_2PO_4) were added as a concentrated solution. To keep the original water volume, evaporated water was supplied with tap water which contains 1.295 ± 0.025 mg L^{-1} of total nitrogen and 0.023 ± 0.001 mg L^{-1} of total phosphorous. As compared with the volume of culture solution in the container, nitrogen and phosphorous added with tap water were negligible. The sampling was conducted on the seventh day after the treatment, and then at 7-day intervals. Three pots with plants were conducted at each sampling time, and instead, three spare pots with five plants were transferred and maintained to the end.

Sample pre-treatment

Some plants were carried out and quickly washed with distilled water at each sampling time. The residual water on plants was absorbed carefully with filter paper, and the fresh weight of leaves was recorded. After that, samples were stored at −80 °C for future assay of physiological indexes. A portion of the remaining plants was conducted to collect epiphytic algae, and a soft brush was utilized to separate epiphytic algae from mature leaves of *M. spicatum* L. in100 mL sterile

distilled water. Separated liquid samples containing the epiphytic algae were not contaminated by the tissues of *M. spicatum* L., and the surface areas were determined for the leaves of *M. spicatum* L. after separation. Five-milliliter separated liquids were preserved immediately in formaldehyde solution (final concentration of 2%) and stored at 4 °C for the identification and enumeration of epiphytic algae later. Fifty-milliliter separated liquids were filtered through 47-mm Whatman GF/C filters and then stored at −20 °C for the assay of chlorophyll *a* (Chl *a*) of epiphytic algae.

Determination of biomass of *M. spicatum* L.

Two pots of plants were conducted from each container on each sampling day, and above-sediment biomass (leaves and stem) was collected and dried to constant weight in an oven at 65 °C.

Determination of biomass and taxonomy of epiphytic algae

Epiphytic biomass was estimated by Chl *a* content of epiphytic algae (Heck et al. 2006). Chl *a* of epiphytic algae was extracted from the prepared frozen filters with 90% acetone and then assayed by the method reported by Loftus and Carpenter (1971). A Nikon Eclipse LV100 POL optical microscope was utilized to identify and enumerate the epiphytic algae (Hu and Wei 2006).

Assay of chlorophyll content in the leaves of *M. spicatum* L.

Frozen leaves of *M. spicatum* L. (0.2 g fresh weight) was ground into a fine powder in liquid nitrogen and extracted with 90% acetone. Following centrifugation (10,000×*g*, 10 min, 4 °C), the supernatant was collected and the absorbance of the supernatant was determined on a spectrophotometer at 645 and 663 nm to estimate chlorophyll contents in the leaves of *M. spicatum* L. (Li and Sun 2000).

Assay of SOD activity and contents of MDA and soluble protein in the leaves of *M. spicatum* L.

Frozen leaves of *M. spicatum* L. (0.5 g) were homogenized thoroughly in 6 mL pre-cooled phosphate-buffered saline solution (PBS, 50 mM, pH 7.8). Following centrifugation (10,000×*g*, 10 min, 4 °C), the supernatant was collected for the assay of malondialdehyde (MDA), superoxide dismutase (SOD), and soluble protein. MDA was assayed by the thiobarbituric acid (TBA) reaction (Li and Sun 2000), SOD activity was assayed by monitoring the inhibition of nitroblue tetrazolium (NBT) reduction according to Beauchamp and Fridovich (1971), and soluble protein was assayed by the method reported by Li and Sun (2000).

Data analysis

Statistical analysis results presented were mean and standard error (SE) and were assayed by utilizing the statistical package SPSS for Windows. A two-way repeated measures ANOVA following Bonferroni test was performed with epiphytic algae and nutrient as the major factors and sampling time as the within-subject factor for multiple comparison tests, and significance levels were taken at $p < 0.05$. To examine the differences among nutrient treatment at the same sampling, a one-way ANOVA was performed and remarkable differences ($p < 0.05$) were determined by Turkey's HSD test. Student's test was also conducted to analyze the remarkable difference in the treatments of epiphytic algae.

Results

Biomass of *M. spicatum* L.

Under the same nutritional conditions, the biomass of *M. spicatum* L. was lower in the epiphytic algae group than that in the control group (Fig. 1: NP1–NP3). More specifically, there was no remarkable change in the biomass of *M. spicatum* L. ($p > 0.05$) between the epiphytic algae group and the control group in the nutrient treatments of NP1 (Fig. 1: NP1). While in the nutrient treatments of NP2 and NP3, there was a remarkable difference ($p < 0.05$) in the biomass of *M. spicatum* L. between the control group and the epiphytic algae group, respectively, except for the first sampling data (Fig. 1: NP2–NP3). This indicated that the effects of epiphytic algae on the biomass of *M. spicatum* L. were enhanced with elevated levels of nitrogen and phosphorus concentrations in the water body (Fig. 1), and at the end of experiment, the biomass of *M. spicatum* L. in the epiphytic algae group decreased by 13.4, 13.6, and 35.6% compared with the control group in the nutrient treatments of NP1, NP2, and NP3, respectively. The results of repeated-measures ANOVA indicated that there were statistical differences ($p < 0.001$) in the biomass of *M. spicatum* L. with elevated levels of nitrogen and phosphorus concentrations and epiphytic algae, respectively (Table 1), and there was a remarkable interactive effect of nutrient and epiphytic algae ($p < 0.001$, Table 1).

Chlorophyll content in the leaves of *M. spicatum* L.

The effects of the epiphytic algae on the Chl contents in leaves of *M. spicatum* L. were remarkable in the course of the experiment (Fig. 2: NP1–NP3). Under the same nutritional conditions, Chl contents of *M. spicatum* L. in the epiphytic algae

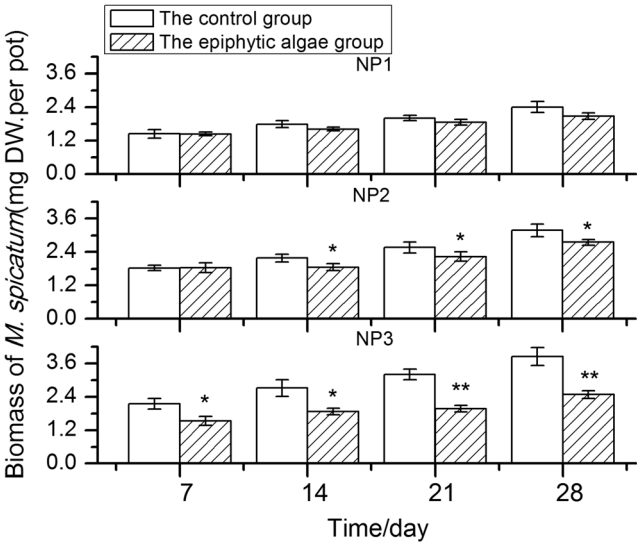

Fig. 1 Biomass of *M. spicatum* L. (mean, SE) at three levels of nitrogen and phosphorus in the water body (N-P (mg L^{-1}): 0.5–0.05 (NP1), 2.5–0.25 (NP2), 4.5–0.45 (NP3)). *Asterisks* indicate a remarkable difference to the control group at the same sampling time by Student's *t* test (*$p < 0.05$; **$p < 0.01$)

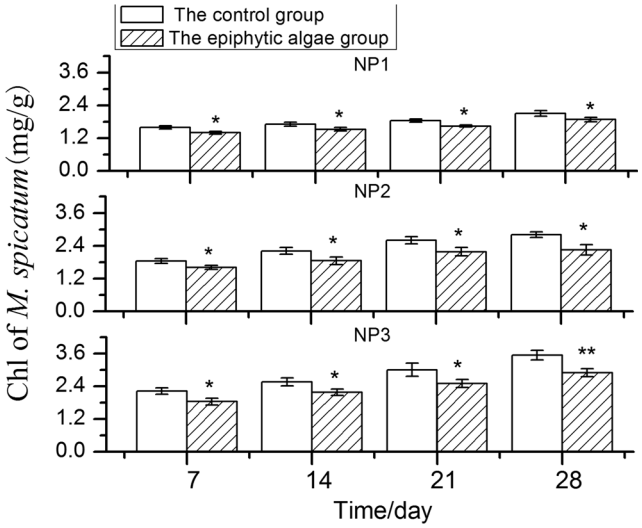

Fig. 2 Chl contents of *M. spicatum* L. at three levels of nitrogen and phosphorus in the water body (NP1, NP2, and NP3 as in Fig. 1). *Asterisks* indicate a remarkable difference to the control group at the same sampling time by Student's *t* test (*$p < 0.05$; **$p < 0.01$)

group were remarkably lower ($p < 0.05$) than that in the control group at the same sampling time (Fig. 2), especially in the nutrient treatments of NP3 (Fig. 2: NP3). This indicated that Chl contents of *M. spicatum* L. were remarkably reduced by the epiphytic algae and the reduction in Chl contents of *M. spicatum* enhanced with elevated levels of nitrogen and phosphorus concentrations. The repeated-measures ANOVA also indicated that there were statistical changes ($p < 0.001$, Table 2) in the Chl contents of *M. spicatum* L. with elevated levels of nitrogen and phosphorus concentrations and epiphytic algae, respectively, and there was a remarkable interactive effect ($p < 0.01$) between levels of nutrient and epiphytic algae (Table 2).

MDA contents in the leaves of *M. spicatum* L.

The influences of epiphytic algae on MDA contents of *M. spicatum* L. were obvious in the experiment (Fig. 3). Specifically, there was no remarkable difference ($p > 0.05$) in MDA contents of *M. spicatum* L. between the epiphytic algae group and the control group in the nutrient treatments of NP1 (Fig. 3: NP1). While in the nutrient treatments of NP2 and NP3, they were remarkably higher ($p < 0.01$) in MDA contents of *M. spicatum* L. in the epiphytic algae group than that in the control group, respectively, except for the first sampling day (Fig. 3: NP2–NP3). At the end of the experiment, MDA contents of *M. spicatum* L. in the epiphytic algae group enhanced by 17.2, 71.3, and 98.2% by contrast with that in the control group. This indicated that the influences of epiphytic algae on MDA contents of *M. spicatum* L. were enhanced with elevated levels of nitrogen and phosphorus concentrations in the water (Fig. 3: NP1–NP3). The repeated-measures ANOVA illustrated that the change of MDA contents was remarkable ($p < 0.01$, Table 3) with the change of epiphytic algae and nutrients and culturing time, respectively, and there was also an interactive effect between levels of nutrient and epiphytic algae ($p < 0.01$, Table 3).

Table 1 The effects of nutrient and epiphytic algae on biomass of *M. spicatum* L. by repeated measures ANOVA

Factors and interactions	*F* value	*p* value
Nutrient	52.628	<0.001*
Epiphytic algae	84.578	<0.001*
Time	182.086	<0.001*
Nutrient × epiphytic algae	25.306	<0.001*
Nutrient × time	3.812	<0.005*
Epiphytic algae × time	8.636	<0.001*
Nutrient × epiphytic algae × time	1.054	0.408

*$p < 0.01$

Table 2 The effects of nutrient and epiphytic algae on chlorophyll contents in leaves of *M. spicatum* L. by repeated measures ANOVA

Factors and interactions	*F* value	*p* value
Nutrient	318.923	<0.001*
Epiphytic algae	157.432	<0.001*
Time	144.808	<0.001*
Nutrient × epiphytic algae	8.199	0.005*
Nutrient × time	9.751	<0.001*
Epiphytic algae × time	2.288	0.095
Nutrient × epiphytic algae × time	0.825	0.825

*$p < 0.01$

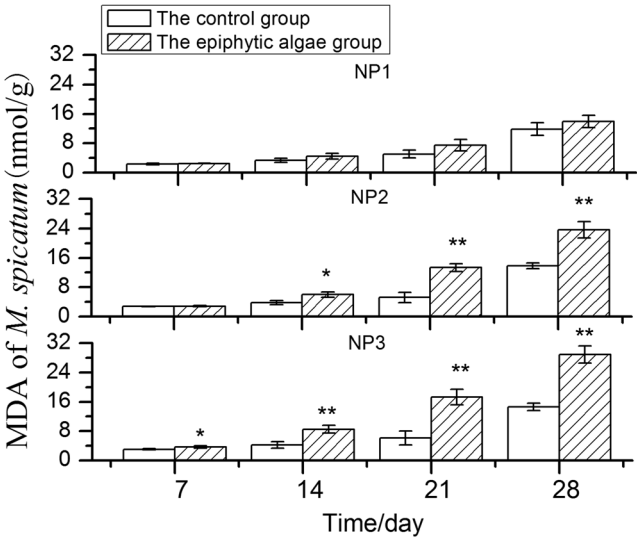

Fig. 3 MDA content in the leaves of *M. spicatum* L. at three levels of nitrogen and phosphorus in the water body (NP1, NP2, and NP3 as in Fig. 1). *Asterisks* indicate a remarkable difference to the control group at the same sampling time by Student's *t* test (*$p < 0.05$; **$p < 0.01$)

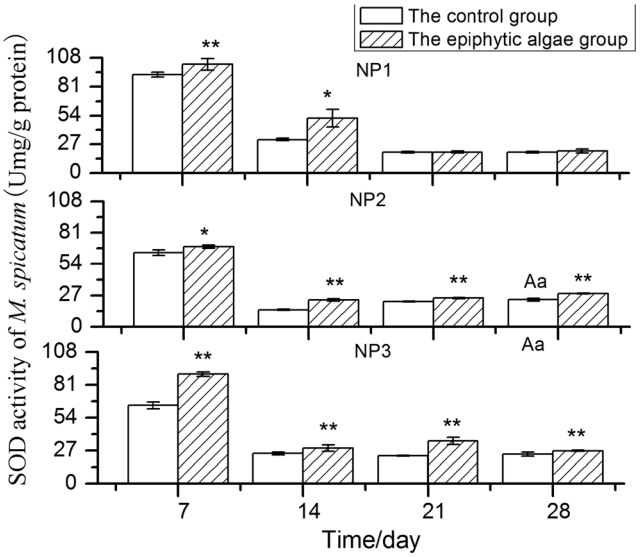

Fig. 4 SOD activity in the leaves of *M. spicatum* L. at three levels of nitrogen and phosphorus in the water body (NP1, NP2, and NP3 as in Fig. 1). *Asterisks* indicate a remarkable difference to the control group at the same sampling time by Student's *t* test (*$p < 0.05$; **$p < 0.01$)

SOD activity in leaves of *M. spicatum* L.

SOD activity of *M. spicatum* L. was also influenced by epiphytic algae (Fig. 4). SOD activity of *M. spicatum* L. was high at the beginning of the experiment then declined remarkably after culturing for 7 days. In the nutrient treatments of NP1, at the first sampling time, SOD activity of *M. spicatum* L. in the epiphytic algae group was remarkably higher than that in the control group ($p < 0.05$), while no remarkable difference ($p > 0.05$) was found on the last sampling day (Fig. 4: NP1). In treatments of NP2 and NP3, SOD activity of *M. spicatum* L. in the epiphytic algae group was remarkably high ($p < 0.01$) over the whole experiment period, as compared with that in the control group, respectively (Fig. 4: NP2–NP3). The results of repeated-measures ANOVA indicated that variation of SOD activity was remarkable ($p < 0.01$) with increasing water nutrients and epiphytic algae, and there was a remarkable interactive effect of nutrients and epiphytic algae ($p < 0.05$, Table 4).

Discussion

The effects of nitrogen and phosphorus on epiphytic algae

During this experiment, epiphytic biomass enhanced with elevated levels of nitrogen and phosphorus concentrations in the water body and was in the order NP3 >NP2 >NP1 (Fig. 5), and the epiphytic biomass enhanced at the end of the experiment in comparison with that at the beginning in all nutrient treatments (Fig. 5). The epiphytic biomass enhanced remarkably with elevated levels of nitrogen and phosphorus and the culture time ($p < 0.01$, Table 5), and there were remarkable

Table 3 The effects of nutrient and epiphytic algae on MDA contents in leaves of *M. spicatum* L. by repeated measures ANOVA

Factors and interactions	*F* value	*p* value
Nutrient	53.645	<0.001*
Epiphytic algae	180.026	<0.001*
Time	611.949	<0.001*
Nutrient × epiphytic algae	25.985	<0.001*
Nutrient × time	15.144	<0.001*
Epiphytic algae × time	54.762	<0.001*
Nutrient × epiphytic algae × time	8.805	0.001*

*$p < 0.01$

Table 4 The effects of nutrient and epiphytic algae on SOD activity in leaves of *M. spicatum* L. by repeated measures ANOVA

Factors and interactions	*F* value	*p* value
Nutrient	58.279	<0.001**
Epiphytic algae	98.326	<0.001**
Time	3008.313	<0.001**
Nutrient × epiphytic algae	4.119	0.043*
Nutrient × time	127.963	<0.001**
Epiphytic algae × time	24.136	<0.001**
Nutrient × epiphytic algae × time	21.134	<0.001**

*$p < 0.05$; **$p < 0.01$

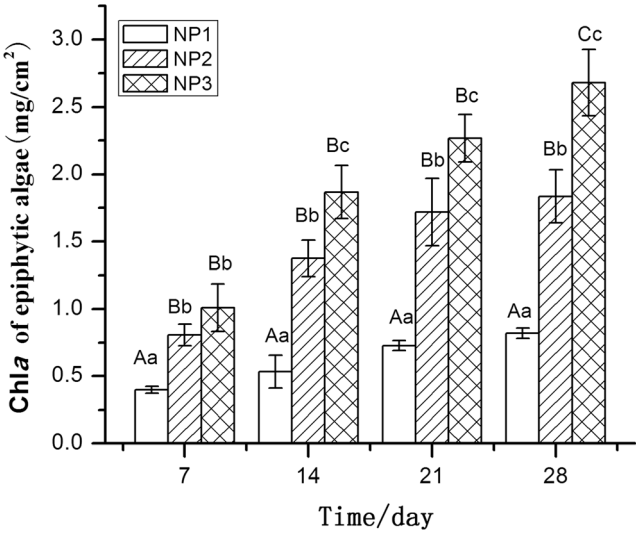

Fig. 5 Biomass of epiphytic algae attached on *M. spicatum* L. (mean, SE) at three levels of nitrogen and phosphorus concentrations in the water body. *Different letters above bars* mean remarkable differences among the treatments of nutrients (NP1, NP2, and NP3 as in Fig. 1), *small letters* mean remarkable difference (one-way ANOVA, $p < 0.05$), *capital letters* mean highly remarkable difference (one-way ANOVA, $p < 0.01$), and *equal letters* mean no differences

interactive effects between water nutrient levels and the culturing time ($p < 0.01$, Table 5).

The effects of nitrogen and phosphorus in the water body on *M. spicatum* L.

It is known that elevated levels of nitrogen and phosphorus enhance the synthesis of Chl and promote photosynthesis of plant in a proper range; while exceeding that range, nitrogen and phosphorus cause stress to plants and affect their normal physiological activities, as do other stresses, such as salinity stress and environmental pollutant stress (Jampeetong and Brix 2009; Zhang et al. 2010; Cai et al. 2012). In the control group, at the end of this experiment, biomasses and Chl contents of *M. spicatum* L. enhanced as the levels of nitrogen and phosphorus concentrations rose from NP1 to NP3, respectively (Figs. 1 and 2), and there were remarkable differences in biomass and Chl content of *M. spicatum* L., respectively, among the nutrient treatments (one-way ANVOA, $p < 0.05$). Chlorophylls are the most abundant pigments responsible for absorption and conversion of light energy, and the rate of photosynthesis is proportional to the chlorophyll contents in plants (Sudhakar et al. 2009). Biomass is the product of photosynthesis. Our results showed that biomass of *M. spicatum* L. increased with increasing Chl contents of *M. spicatum* L. (Fig. 1: NP1–NP3). As one of the products of membrane lipid peroxidation, MDA contents in plants are considered to be a reliable indicator in detecting the effects of environmental stress on plants via lipid peroxidation (Misra and Gupta 2006). In the control group, MDA contents of *M. spicatum* L. enhanced with elevated levels of nitrogen and phosphorus in the water (Fig. 3: NP1–NP3), which indicated that elevated levels of nitrogen and phosphorus induced oxidative stress in *M. spicatum* L. leading to the generation of potent reactive oxygen species (Panda and Khan 2004; Kamara and Pflugmacher 2007). As SOD is the main antioxidant enzyme and acts on scavenging reactive oxygen species, oxidative stress in plants could induce the increases of SOD activity (Panda and Khan 2004; Kamara and Pflugmacher 2007). In this study, SOD activity of *M. spicatum* L. was remarkably higher at the first sampling time than that at the end of the experiment under three nutrient treatments; this phenomenon may be an adaptive response of *M. spicatum* L. to the new environment. At the end of the experiment, SOD activity of *M. spicatum* L. enhanced with elevated levels of nitrogen and phosphorus in the water (Fig. 4: NP1–NP3), while no remarkable increases of SOD activity were shown at the last sampling time (one-way ANVOA, $p > 0.05$), and MDA content showed the same variation law. According to the results of this experiment, we could speculate that elevated levels of nitrogen and phosphorus made stress on *M. spicatum* L. in this experiment, while *M. spicatum* L. could acclimate to the environment by regulating physiological functions. So the growth of *M. spicatum* L. in this experiment was promoted by elevated levels of nitrogen and phosphorus.

Table 5 The effects of nutrient on biomass of epiphytic algae attached on *M. spicatum* L. by repeated measures ANOVA

Factors and interactions	*F* value	*p* value
Nutrient	235.395	<0.001*
Time	69.754	<0.002*
Nutrient × time	8.119	<0.003*

*$p < 0.01$

The effects of epiphytic algae on *M. spicatum* L.

It was apparent that epiphytic algae increased the level of lipid hydroperoxide in this experiment. MDAs of *M. spicatum* L. were remarkably higher in the epiphytic algae group than that in the control group ($p < 0.05$) (Fig. 3), which indicated that epiphytic algae induced oxidative stress in *M. spicatum* L. SOD may be induced by the high-level production of active oxygen species for a stress of epiphytic algae, which have influenced the cell membrane lipid peroxidation degree of leaf tissues, further influencing Chl contents of plants and then biomass (Asaeda et al. 2004; Panda and Khan 2004; Kamara and Pflugmacher 2007). In the nutritional treatments of NP2 and NP3, Chl contents and biomass of *M. spicatum* L. in the epiphytic algae group were remarkably lower than that in the control group at the end of this experiment (*t* test, $p < 0.05$) (Figs.1 and 2). The present results agree with earlier

researches which were performed on submerged macrophytes and indicated that photosynthesis rates were reduced by epiphytic algae (Phillips et al. 1978; Asaeda et al. 2004). For example, Asaeda et al. (2004) reported that the decrease in net photosynthesis rates of *Potamogeton perfoliatus* was about 20–33% for the epiphytic algae attached on its surface. In this experiment, the above-sediment dry weight of *M. spicatum* L. in the epiphytic algae group was reduced by 13.4% in the nutrient treatments of NP1 and up to 35.6% in the nutrient treatments of NP3 as compared with their control group, respectively, and MDA contents of *M. spicatum* L. in the epiphytic algae group enhanced by 17.2, 71.3, and 98.2% by contrast with that in the control group in the nutrient treatments of NP1–NP3, respectively. These indicated that the inhibition of photosynthesis of macrophytes enhanced with the growth and accumulation of epiphytic algae in this experiment. Actually, the response of aquatic macrophyte growth to the epiphytic algae is a complex phenomenon. In this experiment, there was a remarkable difference in the above-sediment dry weight of *M. spicatum* L. with elevated level of nutrients (one-way ANVOA, $p < 0.05$) in the epiphytic algae group and was in the order of NP2 > NP3 > NP1 at the end of the experiment (Fig. 1), while epiphytic algal biomass enhanced with elevated levels of nitrogen and phosphorus concentrations (Fig. 3). At the same time, Chl contents and MDA contents of *M. spicatum* L. also enhanced with elevated levels of nitrogen and phosphorus concentrations in the epiphytic algae group. SOD activities of *M. spicatum* L. changed significantly with the increases in nitrogen and phosphorus concentrations, and they were in the order of NP2 > NP3 > NP1 at the end of the experiment in the epiphytic algae group. These results might be caused by the interactions of the negative influence created by the epiphytic algae and the positive impact made by increasing nutrient (Tables 1, 2, 3, and 4), and the epiphytic algae interacted greatly with the leaf surface of its host (Asaeda et al. 2004). In general, all those demonstrated that epiphytic algae created stress on its host, which results in the increases of the cell membrane lipid peroxidation, further reducing Chl contents and the products of photosynthesis.

Conclusion

1. Elevated levels of nitrogen and phosphorus concentrations in the water body promote the epiphytic algal *M. spicatum* L. and the growth of *M. spicatum L.* in the control of epiphytic algae.
2. Epiphytic algae inhibited the growth of *M. spicatum L.* by influencing indexes of physiology. The effects of epiphytic algae on the growth and physiological indexes of *M. spicatum* L. enhanced with elevated levels of nitrogen and phosphorus concentrations in the water body.

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