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RECENT STUDY ON PLANT-SOIL INTERACTIONS IN CHINA - PART I

Influence of outbreak of macroalgal blooms on phosphorus release from the sediments in Swan Lake Wetland, China

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Abstract
Macroalgal blooms have occurred worldwide frequently in coastal areas in recent decades, which dramatically modify phosphorus (P) cycle in water column and the sediments. Rongcheng Swan Lake Wetland, a coastal wetland in China, is suffering from extensive macroalgal blooms. In order to verify the influence of macroalgal growth on sediment P release, the sediments and filamentous *Chaetomorpha* spp. were incubated in the laboratory to investigate the changes of water quality parameters, P levels in overlying water, and sediments during the growth period. In addition, algal biomass and tissue P concentration were determined. In general, *Chaetomorpha* biomasses were much higher in high P treatments than in low P treatments. Compared with algae + low P water treatment, the addition of sediments increased the algal growth rate and P accumulation amount. During the algal growth, water pH increased greatly, which showed significant correlation with algal biomass in treatments with high P ($P < 0.05$). P fractions in the sediments showed that Fe/Al–P and organic P concentrations declined during the algal growth, and great changes were observed in algae + low P water + sediment treatment for both. As a whole, the sediments can supply P for *Chaetomorpha* growth when water P level was low, and the probable mechanism was the release of Fe/Al–P at high pH condition induced by intensive *Chaetomorpha* blooms.

Keywords: *Macroalgal blooms,* Chaetomorpha *growth, sediments, phosphorus release, Swan Lake Wetland*

Introduction

In recent decades, large macroalgal blooms have occurred throughout the world with an increase in frequency and magnitude (Morand & Briand 1996; Gubelit & Berezina 2010; Ye et al. 2011; Pulina et al. 2012). Since 2007, China has also been subjected to the excessive growth of green macroalgae in Yellow Sea (Liu et al. 2009; Ye et al. 2011; Shao et al. 2012). Macroalgal blooms are vast accumulations of fast-growing filamentous macroalgae in shallow coastal zone, and water eutrophication is generally considered one of the main reasons (Valiela et al. 1997; Anderson et al. 2002; Raven & Taylor 2003; Fenu et al. 2012; Manolaki & Papastergiadou 2012). High nutrient loads, notably nitrogen (N) and phosphorus (P), are the primary factor in the expansion of macroalgal blooms (Menéndez et al. 2002; Dell'uomo & Torrisi 2011; Minggagud & Yang 2013). Bloom-forming macroalgae can efficiently remove nutrients from the water column and exhibit nutrient uptake and storage greatly in excess of their physiological needs (Pecktol et al. 1994; Valiela et al. 1997; Ceschin et al. 2010). When macroalgal blooms break out, the huge uptake coupled with high growth rate result in nutrient depletion in water column. On the other hand, the macroalgal mats constitute an important sink of nutrients in the coastal ecosystem, and the P accumulated can be rapidly recycled into the water during the decomposition phase (Paalme et al. 2002; Nedzarek & Rakusa-Suszczewski 2004; Lan et al. 2012). As a whole, large macroalgal blooms can significantly influence nutrient cycle in the coastal ecosystem. P is a key element of eutrophication processes in many aquatic ecosystems. Due to the frequent outbreak of macroalgal blooms worldwide, P biogeochemical cycle in the sediment–water–algae system is becoming a concern.

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The excessive growth of filamentous algae can destroy P concentration equilibrium at the sediment–water interface, which thus causes modification of P dynamics between the water and sediments (Lavery & McComb 1991; Gomez et al. 1998; Corzo et al. 2009; García-Robledo & Corzo 2011; Zhang et al. 2012). It has been recognized that nutrient exchange between the sediments and macroalgae occurred, and the macroalgae could derive nutrients stored in the sediments to maintain growth in many estuaries (Liere et al. 1982; Gomez et al. 1998; Boyle et al. 2004). For example, Boyle et al. (2004) investigated seasonal variations of nutrients in water column and sediments in a eutrophic southern California estuary, and found that the decreases in sediment nutrients were concurrent with increases in macroalgal cover. Several laboratory studies have demonstrated the ability of macroalgal mats to intercept nutrients releasing from sediments during periods of low water nutrients (Kamer et al. 2004; García-Robledo & Corzo 2011). Kamer et al. (2004) studied the importance of water column and sediments as sources of nutrients to support the growth of *Enteromorpha intestinalis*, and stated that the water column was a primary source of nutrients to the algae when water column nutrient supply was high, and the sediments supplemented nutrient supply to the algae when water column nutrient levels were low. Therefore, the knowledge of P cycle in the macroalgae–sediment–water system is necessary to the control and prediction of macroalgal blooms. Previous studies mainly focused on the contribution of sediments to macroalgal growth as a nutrient source, but the information regarding the effects of macroalgal growth on P release from the sediments is scarce. Liere et al. (1982) earlier inferred that macroalgal growth gave rise to a steeper soluble reactive P (SRP) gradient, which thus stimulated sediment P release. However, it is not clear how macroalgal blooms affect the P exchange at sediment–water interface during the growth phase.

Rongcheng Swan Lake Wetland is an important coastal wetland in eastern Shandong Peninsula, China. Swan Lake is a shallow lagoon connected to Rongcheng Bay of Yellow Sea by a narrow mouth. During the past 3 years, massive filamentous macroalgae (*Chaetomorpha linum* is the dominant species) bloomed in the western and northwestern lake strongly affected by excessive nutrient inputs and others. In this study, surface sediments and living *Chaetomorpha* obtained from algae-dominated region were incubated in the laboratory to investigate the changes of water quality parameters, P levels in overlying water, and sediments during the algal growth, as well as algal biomass and P accumulation amount. The aims of this study were to verify the importance of the sediments as a nutrient source for *Chaetomorpha* growth and to determine the influence of macroalgal growth on P release from the sediments.

Materials and methods

Experimental materials

All materials were collected from Rongcheng Swan Lake, China. In May 2012, surface sediments (0–10 cm) were sampled from the northwestern lake (37°21.447′ N, 122°34.301′ E), at which severe *Chaetomorpha* blooms break out in recent few years (Figure 1). After homogenization, all the samples were kept in the portable refrigerator and delivered to the laboratory immediately. Fresh sediment samples

Figure 1. Location of the study area.

were stored at 2°C for the experiment. Sediment pH value was 6.97.

Filamentous *Chaetomorpha linum* was sampled from the same lake region as sediments. The freshly collected algae were cleaned from macroscopic epiphytes and mud, and then incubated in filtered lake water for 2 days in the laboratory. Thereafter, the algae samples with good health were selected for the growth experiment. The initial tissue P concentration was $1.50 \pm 0.05\,mg\,g^{-1}$, and the water content was 89.65%.

Water samples were taken from the eastern lake where the water quality was good. After being transported to the laboratory, the collected lake water was filtered immediately. To avoid the influence of microbial activity, the filtered water was sterilized by boiling for 10 min. Water pH value was 8.14 and salinity was 31.85 psu. Concentrations of total P (TP) and SRP in water were 0.013 and $0.002\,mg\,L^{-1}$, respectively.

Experiment design

Two initial P concentrations in water were set in the experiment: the addition and no addition of nutrients, which represent high P and low P concentrations in the field, respectively. The high P concentration ($30\,\mu mol\,L^{-1}$) was obtained by the addition of KH_2PO_4, while the N was added as KNO_3 ($480\,\mu mol\,L^{-1}$). In this study, there were five treatments: algae + low P water (ALW), algae + high P water (AHW), algae + low P water + sediment (ALWS), algae + high P water + sediment (AHWS), and low P water + sediment (LWS). Each treatment was performed in triplicate.

The incubation experiments were performed in 5.0-L glass beakers of 14 cm in diameter and 24 cm in height. At first, 500 g of fresh sediment was evenly put into the bottom of container, then 3.0 L of sterile lake water was added into each beaker slowly to avoid the suspension of sediments, and, after 24 h, 10.0 g wet weight (i.e., 1.0 g dry weight) of living *Chaetomorpha* was placed. Before weighing the algae, water was gently squeezed out to get comparable biomasses in all the containers. For the treatment with sediments, the bottom of the container was side-wrapped in black plastic to avoid light. This experiment was conducted under natural light and temperature conditions (about 20°C). Throughout the incubation period, all treatments were kept aerated with air continuously. The incubation lasted 30 days.

During the experimental period, water samples were collected at different time intervals with a pipette for TP, SRP, and chemical oxygen demand (COD) analyses, and the same volume of lake water was added to the container after sampling. The water quality parameters, including pH, dissolved oxygen (DO), and electrical conductivity (EC), were detected *in situ* using a Multi-Parameter Water Quality Instrument (YSI6600). TP and SRP were determined every day during the first 2 weeks and every other day thereafter, while pH was measured every 3 days, and DO, COD, and EC every 5 days. Algal biomass was determined by wet weighing every 3 days, and the growing algae were put back into the container after measuring. Relative growth rates (RGRs) at different time intervals were calculated as RGR ($g\,FW\,d^{-1}$) = $(\ln W_2 - \ln W_1)/(t_2 - t_1)$, in which W_1 is the fresh biomass (g) on day t_1 and W_2 is the fresh biomass (g) on day t_2. At the start and end of incubation, TP and P fractions in sediments and P concentration in algal tissue were analyzed.

Analytical methods

For water sample, the TP concentration was determined through the molybdenum blue colorimetric method after digestion with $K_2S_2O_8$ + NaOH to orthophosphate (Chinese State Environment Protection Bureau [CSEPB] 2002). After filtering through a 0.45-μm membrane, the SRP concentration was analyzed using the molybdenum blue method. COD was measured by the titration method with alkaline potassium permanganate.

For algal sample, the biomass was expressed as wet weight by weighing. The water content was determined by drying at 60°C for 24 h. The tissue P concentration was analyzed using the molybdenum blue method after digestion with $H_2SO_4 + H_2O_2$ of dried algae sample.

For the sediments, the TP concentration was determined using the molybdenum blue method after digestion with $H_2SO_4 + HClO_4$. P fractions were carried out using following method, i.e., according to which sediment P was classified into NaOH-extractable P (Fe/Al–P, P bound to Al, Fe, and Mn oxides and hydroxides), HCl-extractable P (Ca–P, P associated with apatite), inorganic P (IP), and organic P (OP) (Ruban et al. 2001). Fe/Al–P was extracted by 1.0 M NaOH; Ca–P was extracted by 1.0 M HCl. In a separated extraction, IP was extracted by 1.0 M HCl and the residual was treated at 450°C to analyze OP. The P concentration in the filtrate was analyzed using the molybdenum blue method.

Statistical analysis

All statistical analyses were performed using the SPSS 17.0 software package. The correlation analyses were performed using Pearson correlation. The differences in the measured parameters between various treatments were tested by one-way analysis of

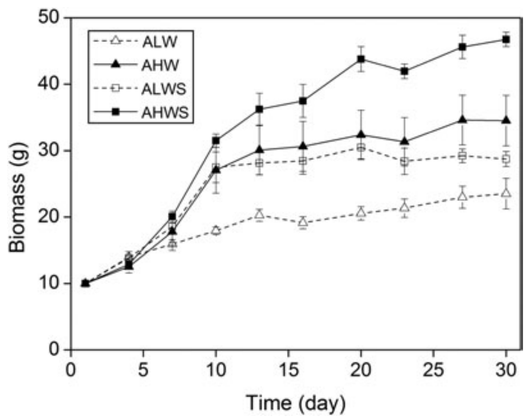

Figure 2. The change of *Chaetomorpha* biomass.

variance (ANOVA) followed by Tukey's test post hoc analysis when appropriate. A two-way ANOVA was conducted to compare the effects of sediments, P level in water, and their interaction with algal biomass and P accumulation amount.

Results

The growth status of Chaetomorpha

Algal biomass. Figure 2 shows that *Chaetomorpha* biomasses were much higher in high P treatments than in low P treatments through the 30-day period. During the first 7 days, there was no great difference among different treatments, after that the difference increased with time. At the end of the experiment, the biomass in AHWS treatment was significantly higher than that in low P treatments ($P < 0.05$). A two-way ANOVA revealed that the water P level ($F = 24.899$, $P = 0.001$) and the sediments ($F = 8.988$, $P = 0.017$) both had a significant effect on algal biomass.

For all the treatments, *Chaetomorpha* grew rapidly during the first 12 days, at which the growth rates ranged from 0.0589 to 0.1073 g FW d^{-1}; then the algae grew slowly with the exception of AHWS treatment; and, after 20 days, the growth rate became very low (Table I). For low P treatments, algae color became yellow and some dead algae occurred at the late stage.

P accumulation amount in algae. Compared with initial concentration (1.50 mg g^{-1}), tissue P concentrations in different treatments all declined after the 30-day incubation, especially for low P treatments (Table II). The accumulation amount in algae was significantly higher in high P treatments than in low P treatments (Table II), and the water P level showed a significant effect on P accumulation amount ($F = 174.76$, $P = 0.000$). However, at the same initial P concentration, there was no great difference between treatments with and without sediments ($P > 0.05$).

The changes of water quality parameters

pH change. The pH value in overlying water showed a visible increase during the incubation, especially for high P treatments (Figure 3(A)). For example, the pH value in AHW treatment increased by about 1.3 units compared with the initial value. There were significant differences between treatments with high P and low P concentrations ($P < 0.05$). At the same P concentration, the great difference between treatments with and without sediments may be related to the buffering capacity of sediments against water pH.

DO change. The highest DO concentration was observed in AHW treatment throughout the incubation period (Figure 3(B)). The similar changing tendency with time was observed in different treatments with algae. The DO concentration increased with time during the first 12 days, then declined, and thereafter changed slightly after

Table I. The growth rate of *Chaetomorpha* at different time intervals (g FW d^{-1}).

Time (days)	ALW	AHW	ALWS	AHWS
0–12	0.0589 ± 0.0015	0.0917 ± 0.0024	0.0862 ± 0.0023	0.1073 ± 0.0032
13–20	0.0017 ± 0.0007	0.0093 ± 0.0003	0.0099 ± 0.0004	0.0236 ± 0.0018
21–30	0.0136 ± 0.0006	0.0064 ± 0.0007	− 0.0059 ± 0.0006	0.0066 ± 0.0009

Table II. Tissue P concentration and P accumulation amount in *Chaetomorpha*.

Treatment	ALW	AHW	ALWS	AHWS
P concentration (mg g^{-1})	0.51 ± 0.08 c	1.36 ± 0.11 a	0.60 ± 0.15 c	1.04 ± 0.03 b
P accumulation amount (mg)	1.16 ± 0.18 b	5.28 ± 0.43 a	1.76 ± 0.47 b	5.23 ± 0.14 a

Note: Different lower case alphabets represent the significance at $P < 0.05$.

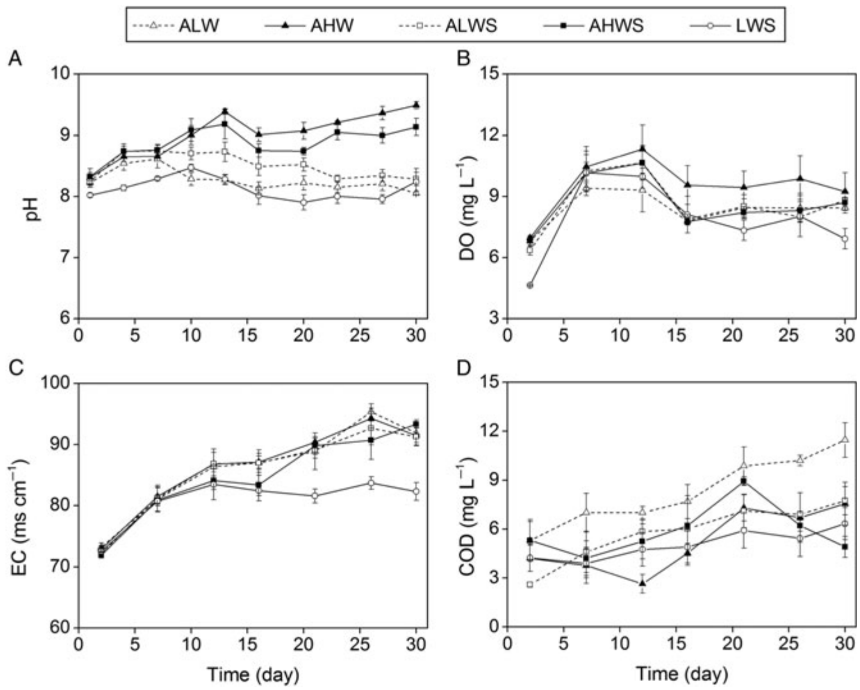

Figure 3. The changes of water quality parameters during the incubation. (A) pH, (B) DO, (C) EC, and (D) COD.

16 days. Compared with treatments with algae, the DO concentration in LWS treatment was lower, which indicated that the *Chaetomorpha* growth led to the DO increase with the exception of aeration in water.

EC and COD changes. In treatments with algae, water EC value increased during the incubation (Figure 3(C)). After 16 days, EC values were much higher in treatments with algae than those without algae, which may be caused by algal death in low P condition. There was no great difference in COD concentration among different treatments ($P > 0.05$), and relatively high concentrations were observed in ALW treatment (Figure 3(D)). For treatments with algae, the COD concentration had an increasing trend with time, especially at the late stage.

Dynamic changes of TP and SRP in overlying water

TP change. TP concentrations in different treatments varied from 0.01 to 0.92 mg L^{-1} during the 30-day period, and the changing tendency with time was different among the treatments (Figure 4(A)). For the two treatments with high P, TP concentrations decreased rapidly with time during the first 10 days, especially for AHW treatment, and then remained stable. However, for low P treatments, TP concentrations were very low (< 0.07 mg L^{-1}) and changed

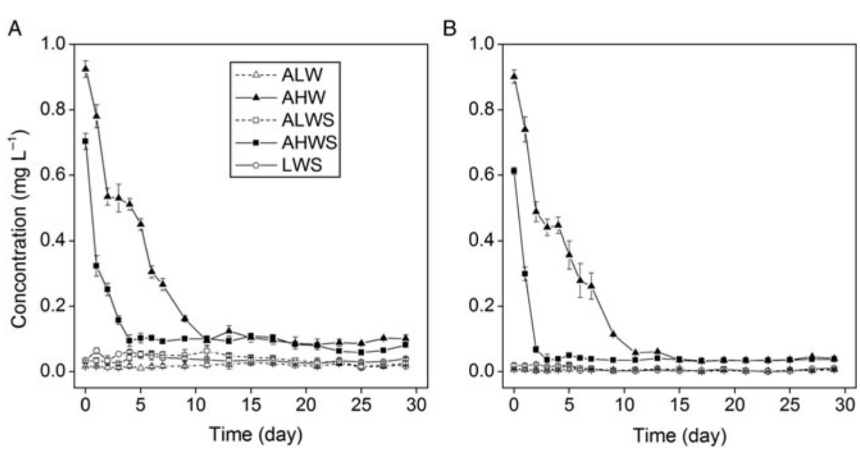

Figure 4. The changes of TP (A) and SRP (B) in overlying water during the incubation.

slightly during the whole period. At the late stage, TP concentrations in all the treatments remained at low levels due to the uptake of the algae.

At low P condition, TP concentrations in ALWS were slightly higher than those in ALW treatment during the 0- to 23-day period. While at high P condition, TP concentrations in the treatment with sediments were significantly lower than those without sediments during the early period, which may be due to P adsorption by the sediments.

SRP change. SRP concentrations in overlying water varied widely through the 30-day incubation, whose rank order of different treatments was similar with TP (Figure 4(B)). For AHWS treatment, SRP concentrations decreased rapidly during the first 3 days and then changed slightly. After 15 days, there was no great difference between the two treatments with high P. For low P treatments, the SRP concentration was very low during the whole period. Compared with TP, SRP showed relatively small difference between the same P treatments at the late stage.

P fractions in the sediments

Ca–P concentration was relatively high in the studied sediments, followed by Fe/Al–P, the lowest being OP (Figure 5). Ca–P showed small change before and after the experiment. During the incubation period, water pH was alkaline, which was unsuitable to the Ca–P release. Fe/Al–P concentrations in different treatments ranged from 117.07 to 127.80 mg kg^{-1}. Compared with the initial value, Fe/Al–P concentration in the treatments with algae declined after the incubation, especially for ALWS treatment (decreased by 8.40%). OP concentrations in the ALWS and AHWS treatments decreased by 6.16 and 3.92 mg kg^{-1}, respectively. For Fe/Al–P and OP, great changes were observed in ALWS treatment. These results showed that Fe/Al–P and OP were mobile during *Chaetomorpha* growth.

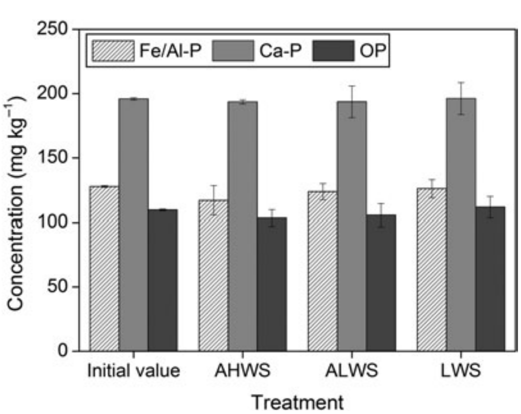

Figure 5. Concentrations of various P forms in sediments before and after the incubation.

Discussion

The influence of Chaetomorpha *growth on water quality parameters*

Previous researches have reported that water pH increased significantly as a result of intensive photosynthesis of primary producers and the release of OH^- during the assimilation of nitrate (Seitzinger 1991; Rydin et al. 2002; Xie et al. 2003). Niemistö et al. (2011) made a comparison between the day and night experiments, and found that water pH also can stay at a high level at night during strong phytoplankton blooms. In this study, *Chaetomorpha* growth resulted in a large increase in water pH during the 30-day incubation, and there were significant differences between the treatments with high P and low P concentrations ($P < 0.05$). In the two treatments with high P, pH showed close relationship with algal biomass ($P < 0.05$); while, for low P treatments, no significant correlation was observed (Table III). This indicated that large *Chaetomorpha* blooms could cause a visible increase in water pH. During the first 12 days, the algal growth rate was fast, consequently DO concentration increased with time. However, there was a poor correlation between DO and biomass ($P > 0.05$) probably due to the continuous aeration with air during the incubation. For pH and DO, great changes were found at high P treatments. Compared

Table III. Relationships between algal biomass with water pH, COD, DO, and EC in different treatments.

	Parameters	Equation	r	P
ALW treatment				
Biomass	pH	$y = -0.023x + 8.692$	0.547	0.102
	COD	$y = 0.566x - 2.660$	0.901	0.006
	DO	$y = 0.088x + 6.672$	0.346	0.447
	EC	$y = 2.012x + 47.054$	0.964	0.000
AHW treatment				
Biomass	pH	$y = 0.038x + 8.019$	0.929	0.000
	COD	$y = 0.125x + 1.813$	0.567	0.184
	DO	$y = 0.074x + 7.512$	0.482	0.273
	EC	$y = 0.794x + 64.526$	0.965	0.000
ALWS treatment				
Biomass	pH	$y = -0.003x + 8.570$	0.090	0.806
	COD	$y = 0.255x - 0.644$	0.936	0.002
	DO	$y = 0.065x + 6.994$	0.285	0.536
	EC	$y = 1.033x + 59.567$	0.944	0.001
AHWS treatment				
Biomass	pH	$y = 0.013x + 8.441$	0.690	0.027
	COD	$y = 0.054x + 3.986$	0.468	0.289
	DO	$y = 0.011x + 8.286$	0.107	0.819
	EC	$y = 0.521x + 66.802$	0.948	0.000

with the treatments without sediments, the correlation coefficients in the treatments with sediments were relatively small, which may be attributed to the buffering capacity and microbial consumption of sediments (Gao et al. 2004; Zhang et al. 2013).

The influence of water SRP concentration on Chaetomorpha *growth*

The bloom-forming macroalgae, such as *Ulva*, *Chaetomorpha*, and *Cladophora*, are characterized by rapid nutrient uptake and growth rates (Peckol et al. 1994; Villares et al. 1999; Anderson et al. 2002; Raven & Taylor 2003; Tel-or & Forni 2011). In this study, the water P level and the addition of sediments both had a significant effect on *Chaetomorpha* biomass ($P < 0.05$). During the first 12 days, *Chaetomorpha* grew well and the biomass increased rapidly with time; after that, the algae grew slowly and the biomass remained stable (except in AHWS treatment) (Figure 3). For AHW treatment, algal biomass showed significant correlation with water SRP concentration ($P < 0.05$). In AHW treatment, *Chaetomorpha* grew fast at the early stage, and meanwhile SRP concentration decreased rapidly; after 12 days, the algae grew slowly at low SRP condition (Figure 6). While, for AHWS treatment, the SRP concentration decreased sharply during the first 3 days, which may be a result of P adsorption by the sediments at high P condition as well as the uptake of algae. In low P treatments, the SRP concentration was very low, and algal biomass changed slightly with time at the late stage. As a whole, algal biomasses were much higher in high P treatments than in low P treatments. These results indicated that filamentous algae *Chaetomorpha* could not grow at low P conditions for a long time, but had high uptake ability for P at high P conditions.

In previous studies, tissue nutrient concentration showed different relationships with algal growth rate

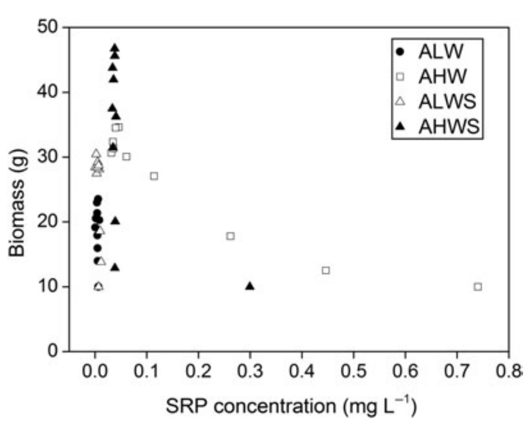

Figure 6. Relationships between algal biomass and SRP concentration during the 30-day incubation.

(Peckol et al. 1994; Villares et al. 1999; Menéndez et al. 2002). For example, Villares et al. (1999) found a significant relationship between tissue P levels and growth rate of *Ulva* sp. in the Rias Bajas (northwest Spain). However, no close relationship was observed between tissue N and RGR for both *Cladophora vagabunda* and *Gracilaria tikvahiae* (Peckol et al. 1994). In this study, the tissue P concentration had no significant correlation with the RGR ($P > 0.05$), which may be a result of the algae immediately allocating nutrients to the formation of new tissue, rather than storing the compounds.

The influence of Chaetomorpha *growth on sediment P release*

At the same P concentration, there were great differences in RGR between treatments with and without sediments, especially for the low P level (Table II), which indicated that the sediments supplied nutrients for *Chaetomorpha* growth. This was also confirmed by the concentration changes of various P forms in sediments before and after the incubation (Figure 5). Sediment P release mainly depends on P fractions in sediments, P concentration gradients at the interface, and environmental conditions (Gomez et al. 1998; Shao et al. 2012; Zhang et al. 2012). Fe/Al–P and OP have been reported to be mobile and bioactive for algae in several researches (Gao et al. 2005; Zhu et al. 2013). Similar to these findings, Fe/Al–P and OP concentrations declined during the algal growth in our study, and great concentration change was observed in low P treatment for both. The OP decrease may be caused by mineralization of the organic matter by bacterial activity, as Gomez et al. (1998) observed by seasonal investigation in a Mediterranean lagoon.

Water pH has an important role in controlling the P release. High pH value in the water can induce P release from aerobic sediments by OH^- exchange with PO_4^{3-} on the surfaces of metal oxides–hydroxides (Seitzinger 1991; Steinberg 2011). Xie et al. (2003) stated that cyanobacterial bloom in freshwater systems induced sediment P release in summer, which was mediated by high pH caused by intense algal photosynthesis and depressed concentrations of nitrate N. Similar results were observed in the Lake Hiidenvesi subjected to strong phytoplankton blooms (Niemistö et al. 2011). In this study, the water column was under oxidation condition during the incubation (Figure 3(B)). The Fe/Al–P decline in sediments may be contributed to water pH increase induced by *Chaetomorpha* growth, the maximum reaching 9.49, in which Fe/Al–P was easy to release from sediment by ligand-exchange reactions.

In conclusion, the P cycle at the sediment–water interface in Swan Lake was influenced by both pH and P concentration gradients during the *Chaetomorpha* growth period. At high water P condition, the sediments can adsorb P in water and act as a P link. When water P level was low, *Chaetomorpha* could not keep alive for a long time, at which the sediments can supply P for macroalgal growth, such as the release of Fe/Al–P at high pH condition. In Swan Lake, mobile P concentration (the sum of Fe/Al–P and OP) was high, which may be one of the reasons why massive amounts of *Chaetomorpha* occurred throughout the year. The water column, sediments, and macroalgal tissue are three nutrient pools, and P transfer mechanism among them during the outbreak of macroalgal blooms needs further study.

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