



# Phosphorus characteristics and microbial community in the sediment-water-algal system during algal growth

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## Abstract

Phosphorus (P) characteristics in eutrophic lakes change during algal growth. Furthermore, algae have a significant relationship with the microbial communities of lake sediments. This study addressed the influence of algal growth and soluble reactive phosphorus (SRP) concentrations on P characteristics within the sediment-water-algal (SWA) system. Results indicated that the SWA system simulating a high algal bloom level (SWA-HAB) had a correspondingly high SRP concentration ( $258.9 \mu\text{g L}^{-1}$ ), and that algal growth promoted a high SRP concentration in the overlying water. The high SRP concentration in overlying water could support algal growth, resulting in a high chlorophyll a (Chl-a) concentration ( $285.23 \text{ mg L}^{-1}$ ). During algal growth, the P release flux was high in sediments from the high-SRP SWA system, with the highest P release flux measured at  $0.982 \text{ mg m}^{-2} \text{ day}^{-1}$ . Furthermore, microbial community abundance had a significant relationship with Chl-a concentrations in overlying water ( $p < 0.05$ ) and increases with algae growth.

**Keywords** Algal bloom · Phosphorus · Sediment · Overlying water · Pore water · Microbial community

## Introduction

Algal blooms, which are common in lakes of developing countries, are one of the main indicators of eutrophication. Phosphorus (P), one of the key nutrients of concern, is an important limiting factor for algal growth in these aquatic ecosystems (Schindler 1974; Huang et al. 2016b; Conley et al. 2009) and is known for promoting eutrophication and

algal blooms (Lewis et al. 2011); therefore, P reduction in lake systems is paramount for minimizing algal blooms (Li et al. 2016). P in sediments can exist in different chemical forms (such as calcium, iron, and aluminum salts or organic species) and can influence P concentrations in the overlying and pore water. Furthermore, sedimentary P plays an important role in many structural and biochemical components responsible for plant and algal cell growth (Zhou et al. 2005; Dorich et al. 1984). In eutrophic lakes, sediments may act as a P source or sink for the overlying water, via pore water, thereby providing this essential nutrient for algae growth if sediments act as a P source; therefore, understanding the P characteristics in lake systems is important.

Sediment, water, and algae are the major components of lake ecosystems, and most nutrient migration or transformation occurs within the sediment-water-algae (SWA) system. As the limiting nutrient responsible for promoting eutrophication, changes in the P concentration of sediment, water, and algae may cause algal growth or death. A previous study revealed that algal growth is one of the driving forces of P release in sediment (Cao et al. 2016). Moreover, migration or transformation of P can influence primary production in lake ecosystem. In addition, P characteristics in the SWA system might affect the P threshold of algal bloom (Lewis et al. 2011; Scheffer et al. 2001; Rietkerk et al. 2004); therefore, the

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extent of algal blooms and the soluble reactive phosphorus (SRP) concentration in the overlying water significantly influences algal growth. These factors can also highly influence the sedimentary microbial community, which can significantly impact the biogeochemical environment, thereby further influencing algal growth (Haukka et al. 2006; Van der Gucht et al. 2001; Huang et al. 2017).

In this study, sediments and overlying water from a eutrophic plateau lake (Dianchi Lake, China) were collected for simulation experiments. The objective of this study was to illustrate how P concentration changes under different algal bloom levels and different SRP concentrations, and to identify the microbial community of the sediment during algal growth.

## Materials and methods

### Site description and sample collection

The sampling site (24° 50' N, 102° 45' E) was located in Dianchi Lake, Yunnan Province, China. The sediment and overlying water samples were collected in July 2015 from an area that had little algae in the water. The composite surface (10 cm) sediments and overlying water were collected using a sediment and water sampler, respectively. The sediment samples were used for the simulation experiments and were collected within 5-m radius of the sampling site. The sampling procedure was repeated several times ( $n > 5$ ), and the final sediment samples were composed of those subsamples. After sampling, the water and sediment samples were stored on ice and transferred to the laboratory within 24 h.

### Simulation experiments

Water and sediment samples collected from Dianchi Lake were used in the simulation experimental reactor (Fig. 1). The SWA system was built using the simulation experimental reactor and consisted of sediment (5 cm), overlying water (20 cm), and *Microcystis aeruginosa* (*M. aeruginosa*, the dominant algae in Dianchi Lake). The depth ratio of sediment to water (1:4) reflects the native conditions in Dianchi Lake. *M. aeruginosa* (FACHB-905) was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China), and was batch cultivated in BG11 medium (Huang et al. 2016a). All the simulation reactors were made from polymethyl methacrylate and were incubated at  $25 \pm 2$  °C under incandescent light at 20,000 lux with the photoperiods of 12:12 (Jiang et al. 2010).

Three SWA systems were set up to simulate high, medium, and low algal bloom levels. Chlorophyll a (Chl-a) was used to adjust the algal bloom levels by using initial Chl-a concentrations of  $173.83 \mu\text{g L}^{-1}$  (SWA-HAB),  $132.09 \mu\text{g L}^{-1}$  (SWA-MAB), and  $55.51 \mu\text{g L}^{-1}$  (SWA-LAB). The initial soluble

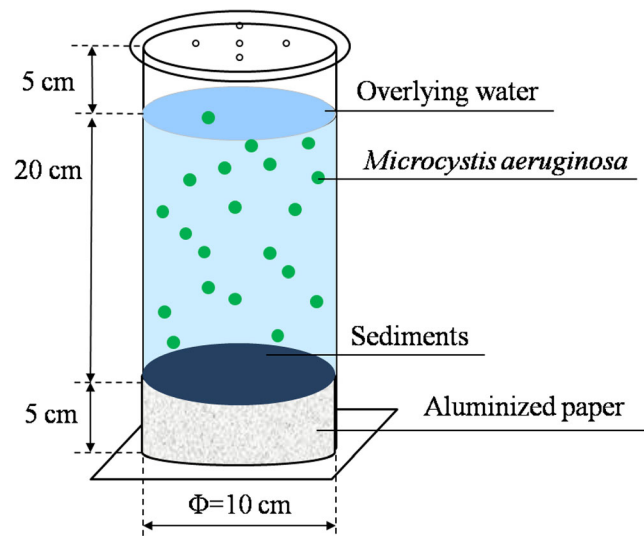


Fig. 1 Picture of simulated reactor for the sediment-water-algae system

reactive phosphorus (SRP) concentration was  $0.03 \text{ mg L}^{-1}$ . In addition, three SWA systems were set up with different initial SRP concentrations:  $0.60 \text{ mg L}^{-1}$  (SWA-HT),  $0.24 \text{ mg L}^{-1}$  (SWA-MT), and  $0.03 \text{ mg L}^{-1}$  (SWA-LT). The initial Chl-a concentration was  $55.51 \mu\text{g L}^{-1}$ . The growth of *M. aeruginosa* followed an S-shaped curved, and exponential growth started after 5 days. Exponential growth continued for approximately 10 days, after which it stabilized (Huang et al. 2016a). Each experimental period lasted for 20 days.

During the simulation experiments, the overlying water and pore water samples were collected ( $n = 3$ ) with a special sampling apparatus (Rhizons MOM) from the water column (1 cm above and 1 cm below the solid/liquid interface) (Cao et al. 2016). Water samples were collected daily for the first 5 days, after which they were collected every second day. Sediment samples were collected using a modified pipetting device on day 12 (samples HT1, MT1, and LT1) and day 20 (samples HT2, MT2, and LT2), and were stored at  $-80$  °C for DNA extraction. The original sediment from Dianchi Lake was designated as S0. Three groups of parallel experiments were set up.

### Analytical methods

The SRP and total dissolved phosphorus (TDP) in the overlying and pore water were determined by the molybdenum blue method (Murphy and Riley 1962) after filtering the solutions with the membrane filter ( $50 \text{ mm} \times 0.45 \mu\text{m}$ , Nylon66). In addition, the intracellular P concentration of a clean, centrifuged algae sample was analyzed using the molybdenum blue method after digestion with  $\text{K}_2\text{S}_2\text{O}_8$  (Cao et al. 2016). The Chl-a concentration was measured according to centrifugation ( $8000g$  for 4 min) and incubation (24 h in a darkened room at 4 °C), and the absorbance of the supernatant

was measured at 665 and 649 nm (Huang et al. 2016a; Yang et al. 2013). The  $P$  release flux was calculated as per Eqs. (1) and (2):

$$M_n = V(C_n - C_0) + \sum_{j=2}^N V_n(C_{j-1} - C_{a(j-1)}) \quad (1)$$

$$F = \frac{M_n}{At} \quad (2)$$

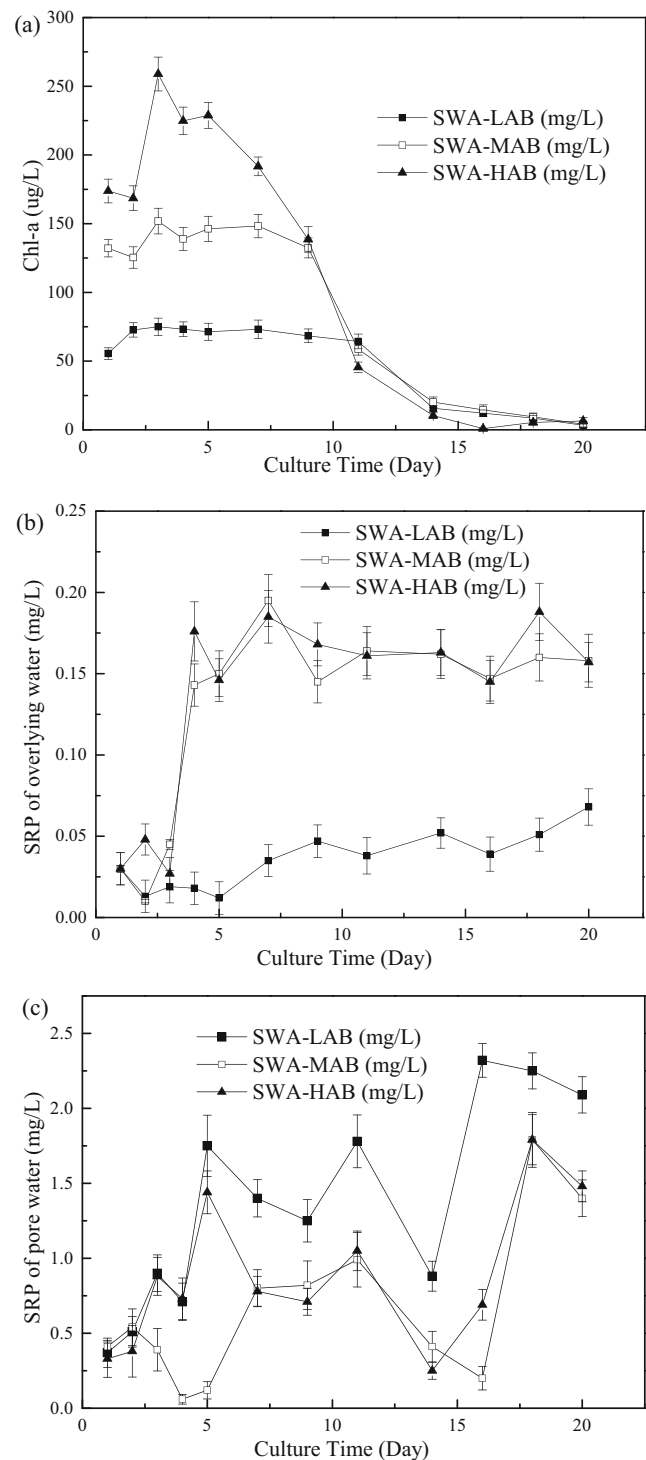
where  $M_n$  is the amount of  $P$  (mg) released with in the experimental period;  $V$  is the volume of the overlying water (L);  $C_n$  is the  $P$  concentration of overlying water at the  $n$ th time ( $\text{mg L}^{-1}$ );  $C_0$  is the initial  $P$  concentration in overlying water ( $\text{mg L}^{-1}$ );  $V_n$  is the volume of the collected overlying water each time (L);  $N$  is the time of sampling;  $C_{(j-1)}$  is the  $P$  concentration of overlying water after sampling at the  $j$ th time ( $\text{mg L}^{-1}$ );  $C_{a(j-1)}$  is the  $P$  concentration of overlying water after adding water at the  $j$ th time ( $\text{mg L}^{-1}$ );  $F$  is the  $P$  release flux of the sediment ( $\text{mg m}^{-2} \text{day}^{-1}$ );  $A$  is the cross-sectional area of the sediment ( $\text{m}^2$ ); and  $t$  is the experimental time (day).

High throughput sequencing and bioinformatic analysis were used to analyze the microbial community in the sediment during algal growth. Total genomic DNA was extracted from samples (250 mg) using a Power Soil DNA Isolation Kit (Mbio Laboratories Inc., San Diego, CA, USA), according to the manufacturer's protocol. The DNA concentration and quality were determined using a NanoDrop spectrophotometer. DNA was diluted to  $10 \text{ ng } \mu\text{L}^{-1}$  with sterile ultrapure water and stored at  $-80^\circ\text{C}$  for further analysis. The V3-V4 region of the bacterial 16S rRNA gene was amplified using a two-step PCR protocol. The first primer set contained the Illumina 5' overhang adapter sequences, allowing for two-step amplicon library creation following manufacturers' protocol.

## Results

### Influence of sediment on P concentration under different algal bloom levels

The influence of three SWA systems (SWA-HAB, SWA-MAB, SWA-LAB) on Chl-*a* and SRP concentrations in overlying and pore water in the simulation experimental reactors was investigated over the 20-day experimental period (Fig. 2). Of the three SWA systems, the initial Chl-*a* concentration of SWA-HAB was the highest ( $173.8 \mu\text{g L}^{-1}$ ). The Chl-*a* concentrations for SWA-HAB, SWA-MAB, and SWA-LAB initially increased, reaching the maximum values (258.9, 132.1, and  $75 \mu\text{g L}^{-1}$ , respectively) on the 3rd day (Fig. 2a). As the simulation progressed, Chl-*a* concentrations declined rapidly, reaching 10.4, 20.2, and  $15.7 \mu\text{g L}^{-1}$ , respectively, on the 14th day. The changes of SRP concentrations in the overlying (Fig. 2b) and pore water (Fig. 2c) were also investigated for the



**Fig. 2** Changes of Chl-*a* concentrations, SRP concentrations in overlying, and SRP of pore water under different algal bloom levels

three systems. During the first 3 days, the SRP concentrations in the overlying water of SWA-MAB and SWA-LAB rose and then declined, while SWA-HAB showed an opposite trend. In general, the SRP concentrations in SWA-MAB and SWA-HAB overlying water rose to a similar degree and displayed a similar trend. In addition, the changes in pore water SRP

concentrations of the three SWA systems fluctuated but slightly increased overall. During the early stage, the SRP concentration of SWA-MAB was similar to that of SWA-LAB; however, during the later stage, the concentration was similar to that of SWA-HAB.

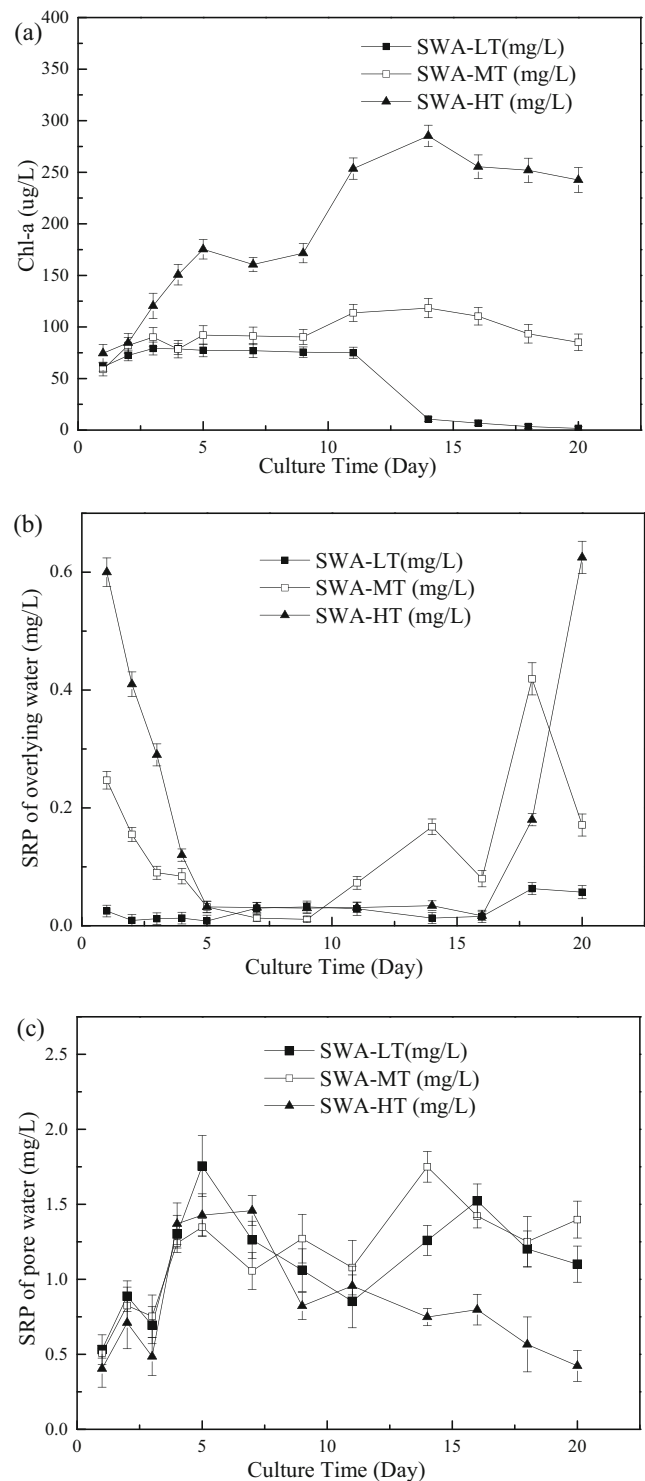
**Influence of initial SRP concentrations in SWA systems during algal growth**

The initial SRP concentration in the SWA system can influence algal growth, thus, the initial SRP concentration in overlying or pore water was investigated with respect to Chl-a concentrations and algal growth. The Chl-a concentration of the three SWA systems had a similar increasing trend in the beginning; however, after 2 days, the algal growth rate of SWA-HT was notably higher (Fig. 3a). The Chl-a concentration increased from 160.62 to 171.63 mg L<sup>-1</sup> after 5 days, reaching its highest value of 285.23 mg L<sup>-1</sup> on the 14th day. The Chl-a concentration in the SWA-MT system increased rapidly between days 9 and 14, reaching a maximum value of 118.31 mg L<sup>-1</sup>, after which the concentration declined slowly and then was maintained at 85.13 mg L<sup>-1</sup>.

Changes in SRP concentration within the three SWA systems in overlying (Fig. 3b) and pore water (Fig. 3c) were then investigated. The SRP concentration of SWA-MT and the SWA-HT in overlying water rapidly decreased in the beginning; however, the SRP concentration of the two systems began to rise on day 9, reaching their highest values (0.419 and 0.625 mg L<sup>-1</sup>) on the 18th and 20th day, respectively. Few fluctuations in SRP concentration were observed for the SWA-LT system. The pore water SRP concentration of the three systems showed a similar trend but a different fluctuation range. The SRP concentration of SWA-LT and SWA-HT had a higher fluctuation range and reached maximum values (1.755 and 1.458 mg L<sup>-1</sup>) on the 5th day; however, there was no obvious fluctuation in SRP concentration for SWA-MT.

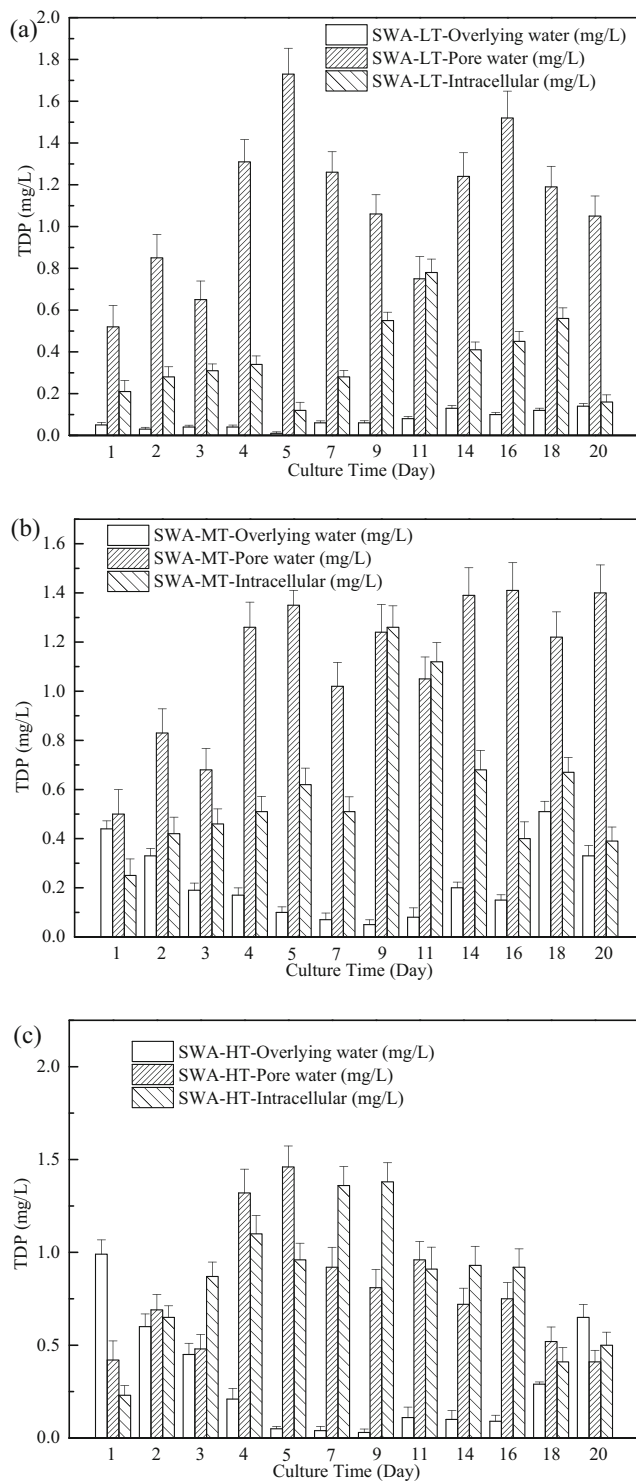
**Changes of TDP concentrations in SWA systems during algal growth**

Figure 4 shows the changes in TDP concentration for the three systems in overlying water, pore water, and algal cell. The TDP concentration of SWA-LT in overlying water, pore water, and algal cells was highly variable. The pore water had the highest TDP (0.52–1.73 mg L<sup>-1</sup>), while intracellular P was relatively low (0.16–0.78 mg L<sup>-1</sup>). TDP in overlying water was the lowest, with a maximum of 0.13 mg L<sup>-1</sup>. During the culture period, the TDP concentration in overlying water increased slightly but remained relatively steady. The TDP concentration of the SWA-MT and SWA-HT gradually increased over time and peaked (1.26 and 1.38 mg L<sup>-1</sup>, respectively) during algal blooms but decreased gradually as the blooms progressed. The SWA-MT intracellular P was higher than that



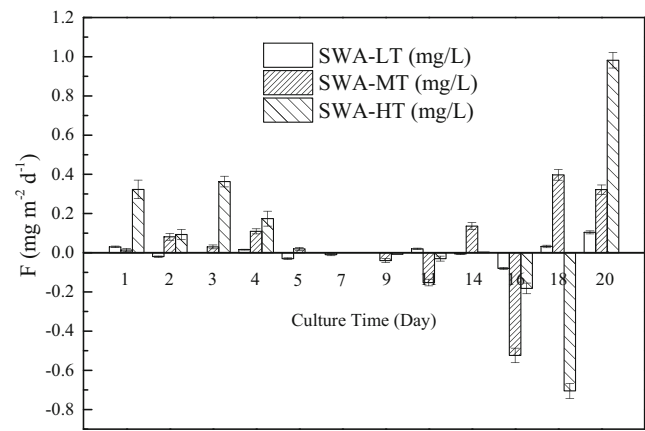
**Fig. 3** Changes of Chl-a concentrations, SRP concentrations in overlying, and SRP of pore water during algae growth under different initial P concentrations

of the pore water between days 9 and 11, while the SWA-HT intracellular P was higher than that of the pore water during the entire culture period. In addition, the TDP in the overlying water of SWA-MT and SWA-HT initially increased but then declined as the experiment progressed (Fig. 4b, c).



**Fig. 4** Changes of intracellular P concentrations and TDP concentrations in overlying and pore water under different initial P concentrations during algae growth.

Figure 5 shows the SRP release flux of sediments. The SRP release flux in SWA-HT was high during both the early and late stages of the algal bloom, with the highest value reaching  $0.982 \text{ mg m}^{-2} \text{ day}^{-1}$ , following SWA-MT of  $0.397 \text{ mg m}^{-2} \text{ day}^{-1}$ .



**Fig. 5** P release fluxes from sediments in the sediment-water-algae system under different initial P concentrations

day<sup>-1</sup>. For the SWA-HT system, the highest SRP release flux reached  $0.104 \text{ mg m}^{-2} \text{ day}^{-1}$ .

### Microbial community of the sediment during algal growth

Among the filtered sequences, a total of 48 phyla were identified in the sediment samples, and the phylum level distributions of bacterial communities in the sediments are shown in Fig. 6. The relative abundance of microbes in the sediment decreased as the algae grew. Sediments collected on the 12th day (HT1, MT1, and LT1) had a higher relative abundance than those collected on the 20th day. *Proteobacteria* was the most abundant phylum across all samples; however, HT1, MT1, and LT1 had a relatively higher abundance (> 60%) than those from the other period. The relative abundance of *Nitrospirae* (4.71–9.16%), *Firmicutes* (2.15–8.59%), and *Chloroflexi* (9.31–16.58%) in the sediments collected on the 20th day (HT2, MT2, and LT2), and the original sediments (S0) was greater than other sediment samples. *Cyanobacteria* (8.92–12.16%) in the sediments collected on the 12th day had higher abundance than other sediment samples. *Bacteroidetes*, *Planctomycetes*, and *Actinobacteria* were the other highly abundant phyla in the sediment samples from different periods. In addition, correlation analysis indicates that the microbial community abundance has a significant relationship with Chl-a concentration in overlying water ( $p < 0.05$ ), and increases with algal growth.

### Discussion

P release in sediments maybe driven by the massive growth of algal blooms. Moreover, a greater transformation and variation exist for different P morphologies between the algae, overlying water, pore water, and sediment. Previous studies indicated that P migration affected the level of primary production in water bodies, which was related to the threshold of nutrients in algal

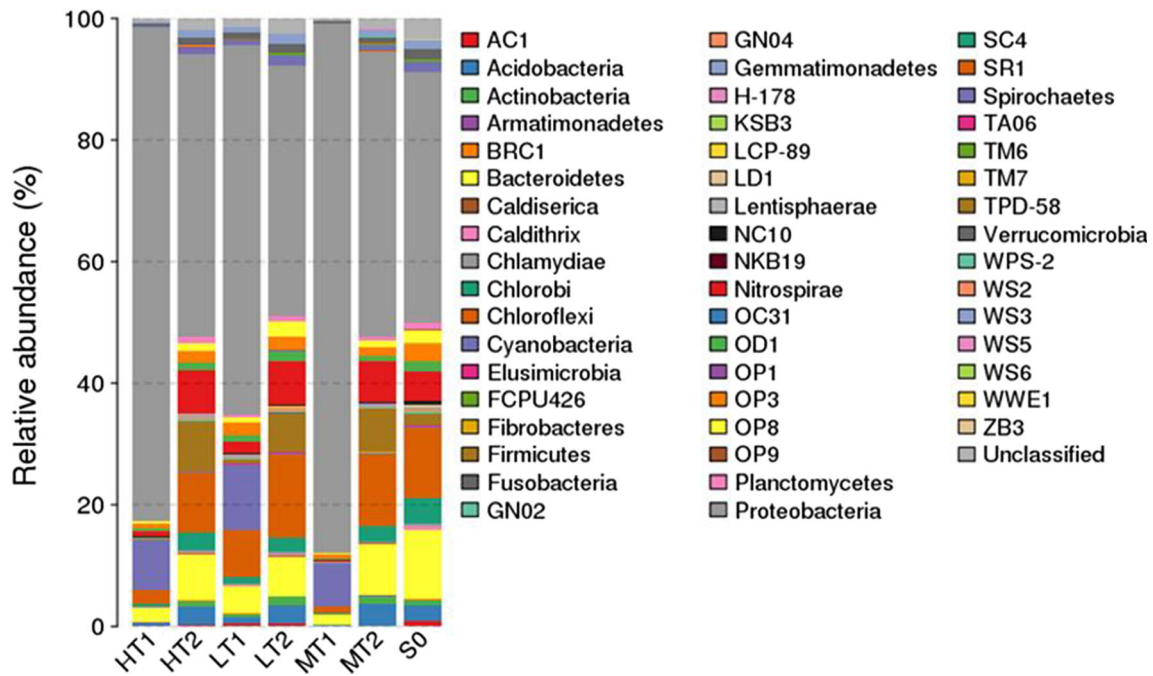


Fig. 6 Relative abundances of different phyla in the sediment samples during the algal growth

blooms, and that the algal bloom and P concentration in overlying water was affected by changes in the SWA system (Scheffer et al. 2001; Lewis et al. 2011; Xu et al. 2015). Thus, this study sought to identify the effect of algal blooms on P concentrations in overlying water and sediments and identified the influence of the different P concentrations in overlying water on the SWA system during the algal growth.

Results indicated that the SWA-HAB system had the highest SRP concentration and that the Chl-a concentration peaked on the 3th day, which is similar to previous research (Cao et al. 2016). Compared with the average algal growth cycle of 30 days, the algal growth cycle in this study was relatively short and declined rapidly, which was likely related to the initial Chl-a concentration. For instance, a higher algal density in the SWA system resulted in a shorter algal bloom cycle. Furthermore, changes in SRP concentrations of overlying waters in the different SWA systems were diverse. During the first 3 days of algal growth, the SRP concentration in the overlying water of SWA-MAB first decreased and then increased, while the SRP of SWA-HAB showed an opposite trend. Overall, the SRP concentration of overlying water in SWA-HAB and SWA-MAB had a similar trend. Algal growth led to an increase in SRP concentration in overlying water (Bai et al. 2009); however, when the bloom reached a higher level, the SRP concentration in the overlying water varied little. Furthermore, the concentration of SRP in pore water of the three SWA systems had a greater fluctuation and roses lightly, which might be caused by a different degree of SRP migration during algal growth in SWA system (Jiang et al. 2006).

The SRP concentration in the overlying water had an effect on the P concentration in each part of SWA system during

algal growth. Results indicated that the Chl-a concentration of the SWA-HT was associated with the fastest growth rate, which showed an increasing trend during days 2 to 5 of the algal bloom, with a second growth period after the 9th day. In contrast, there was only one obvious algal growth trend in the SWA-MT system, as well as the second growth period of algae in SWA-HT system. These results demonstrated that higher P concentrations in overlying water supported algal growth and that the concentration of SRP in the overlying and pore water changes due to different initial P concentrations. Under variable initial P concentrations, the SRP concentration in the three systems showed a similar trend in the pore water, which could be explained by the fact that the pore water in sediments plays an intermediate role in the migration of SRP within the SWA system and was unaffected by the change in P concentration in the overlying water (Zhu et al. 2013). In the SWA-LT system, the SRP concentration in pore water increased the most and had the highest P, while the SRP concentration in the overlying water of the system was lower. This indicated that the sediments in SWA-LT might contribute to the ecological stability of the water body, making the occurrence of algal blooms difficult by not supporting algal survival and reproduction within the system. The change of SRP concentration in overlying water resulted in a gradual decrease in SRP of the sedimentary pore water. The P capacity stored in the pore water was as follows: SWA-LT > SWA-MT > SWA-HT. The ecological stability, with respect to P in lake ecosystem, was as follows: SWA-LT > SWA-MT > SWA-HT.

In addition, the TDP concentration in overlying water, pore water, and algal cells was highly variable within the SWA-LT system. The highest accumulation of TDP was in the pore

water, which also proved that the pore water contained more P. During algal growth, the change in TDP concentration of SWA-MT and SWA-HT indicated that the ecological stability of P within these systems was weakened, and that the SWA-HT pore water supplied more P to the algal cells via the overlying water. The increased P concentration in the overlying water weakened the P contribution of sediment in the lake system.

Microbial communities of the sediment during algal growth were highly variable and growth of these microbes could inhibit the proliferation of algae. During the algal bloom, the abundance of the microbial community tended to increase slightly because of the death and degradation of algal cells. Especially on the 12th day, the algae entered the stage of rapid growth, and the number of algae cells increased in overlying water and sediments, which caused *Cyanobacteria* had the relatively high abundance (Wu et al. 2007; Shao et al. 2011). On the 20th day, large amounts of algae died and degraded onto the sediment surface, and the nutrient contents such as phosphorus or nitrogen in sediments changed (Huang et al. 2017). The transformation of nitrogen formation occurred frequently on the sediment surface, which caused the abundance of *Nitrospirae* on the 20th day was higher than other period (Bruckner et al. 2013). The algal death also caused the increase of organics on the sediment surface, and as a photoautotrophic microbe, *Chloroflexi* possibly participated in the degradation of organics during the process of algal death. Therefore, the relative abundance of *Chloroflexi* in the sediments collected on the 20th day was higher than other sediments. Furthermore, abundance of the microbial communities showed a significant negative correlation with Chl-a concentrations, which also indicates that microbes in the sediment possess ecological plasticity during algal growth and that the abundance of the microbial community might increase with algal growth.

## Conclusions

In the SWA system, higher SRP concentrations in overlying water promoted algal growth, and few influences of algal bloom level on SRP concentration are increasing. Under different initial SRP concentrations, P release flux from sediments showed irregular variation, with the amplitude of fluctuation being higher during the earlier and later period of algal bloom. A competitive relationship existed between algae and microbes in sediment, and microbial community abundance had a significant negative relationship with Chl-a concentrations. The sedimentary microbes possessed ecological plasticity during algal growth, and the abundance of the microbial community could increase with algal growth.

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