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Responses of alkaline phosphatase activity to wind-driven waves in a large, shallow lake: Implications for phosphorus availability and algal blooms

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ABSTRACT

Phosphorus is a vital nutrient for algal growth, thus, a better understanding of phosphorus availability is essential to mitigate harmful algal blooms in lakes. Wind waves are a ubiquitous characteristic of lake ecosystems. However, its effects on the cycling of organic phosphorus and its usage by phytoplankton remain poorly elucidated in shallow eutrophic lakes. A mesocosm experiment was carried out to investigate the responses of alkaline phosphatase activity fractions to wind waves in large, shallow, eutrophic Lake Taihu. Results showed that wind-driven waves induced the release of alkaline phosphatase and phosphorus from the sediment, and dramatically enhanced phytoplanktonic alkaline phosphatase activity. However, compared to the calm conditions, bacterial and dissolved alkaline phosphatase activity decreased in wind-wave conditions. Consistently, the gene copies of *Microcystis phoX* increased but bacterial *phoX* decreased under wind-wave conditions. The ecological effects of these waves on phosphorus and phytoplankton likely accelerated the biogeochemical cycling of phosphorus and promoted phytoplankton production in Lake Taihu. This study provides an improved current understanding of phosphorus availability and the phosphorus strategies of plankton in shallow, eutrophic lakes.

Introduction

Eutrophication and harmful algal blooms (HABs) have become worldwide concerns, both become an area of active research looking for their causes and potential control strategies (Paerl et al., 2016; Schindler et al., 2016). Nutrient availability is an important factor to control the development of HABs (Brookes and Carey, 2011). Particularly, phosphorus (P) as the main contributor leads to anthropogenic eutrophication of lake systems (Schindler et al., 2016). Knowledge of the underlying mechanism influencing its biogeochemical cycling is necessary to address a number of problems associated with eutrophication in freshwater systems. Although researches mainly focused on dissolved labile P, growing evidence indicated that organic P (Po), regarded as a "reserve",

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was an important driver of lake productivity (Boyer et al., 2006). A better understanding of Po utilization is needed to possibly control eutrophication and HABs in lakes (Ren et al., 2017).

One of the mechanisms to generate P is to release it from Po by enzymatic cleavage. For this, alkaline phosphatase (AP) has been regarded as the most important enzyme that hydrolyzes a variety of Po compounds into orthophosphate to compensate for P deficiency (Chrost and Overbeck, 1987). AP is adaptative and its synthesis and activity are strongly affected by various environmental and biological factors, such as nutrient (nitrogen, P, and their ratio), light, hydrodynamics, atmospheric particulate matter, bacteria, phytoplankton, and macrophytes (Liu et al., 2012; Zheng et al., 2017; Feng et al., 2018; Ma et al., 2018; Vrba et al., 2018; Xu et al., 2018; Brinkmann et al., 2019; Song et al., 2019). AP activity (APA) is known to be common in water and sediment in lake ecosystems, which is generally regulated by dissolved inorganic P concentration and widely used as a bioassay for P limitation (Taylor and Lean, 2018; Ma et al., 2019). Measurement of APA can then provide insights into the rates of organic matter decomposition and P remineralization (Jackson et al., 2013). Trophic status and hydrological conditions have great impact on the sediments APA in lakes (Zhang et al., 2007). Large shallow lakes are frequently exposed to wind-driven waves, which are important disturbances for lake ecosystems (Guasto et al., 2012; Prairie et al., 2012). Wind-driven sediment resuspension has significant effects on P behavior in shallow lake systems (Huang et al., 2016). The remineralization of Po from suspended particulates serving as the main source of internal P loading for phytoplankton growth in Lake Taihu (Chao et al., 2017). Our previous study indicated that turbulence can accelerate the biogeochemical cycle of P and played an important role in P strategies of plankton in Lake Taihu (Zhou et al., 2016a). However, although wind waves are widespread occurrence, its effects on APA are poorly elucidated in shallow eutrophic lakes. This limits our current understanding on the role of wind-wave processes in P cycling and its influence on the outbreak of algal blooms in shallow, eutrophic lakes (Prentice et al., 2015).

China is currently experiencing the challenges of lake eutrophication, with Lake Taihu being one of the most serious observed (Qin et al., 2019). In 2007, Lake Taihu has experienced a horrible *Microcystis* bloom event, resulting in a drinking water shortage for over 4 million residents in Wuxi (Qin et al., 2010). The massive amount of stored Po in the sediments represent an important bioavailable source of P, and with its resuspension, significantly delay the recovery from eutrophication (Ni et al., 2016). Wind waves significantly enhanced the sediment resuspension as well as P release from the bottom sediment, which was one important factor controlling HABs in Lake Taihu (Huang et al., 2016). Our previous studies showed that the flux of annual total P released by sediments was 21,000 tons (2–6 times of P external input; Qin et al., 2006), and 88% of internal P release occurred under conditions accompanied by wind waves in Lake Taihu (Qin et al., 2004). Also, 34.1% of dissolved organic phosphorus (DOP) could be hydrolyzed, and more than 70% of enzymatically hydrolyzed DOP came from AP-hydrolyzed DOP, verifying that AP had a major role in the hydrolysis of DOP to fulfill the P requirements of phytoplankton (Ma et al., 2019). The shortest mineralization time of enzymatically hydrolysable phosphorus was calculated as 69 min in Lake Taihu (Jiang et al., 2019). Moreover, organically-bound P is an important portion of the sediment phosphorus in Lake Taihu, which induces APA and may lead to the release of bioavailable phosphates from the organic sediments (Zhou et al., 2008). Interestingly, intense wave actions, such as typhoon, can promote intense phytoplankton primary production, stimulating the harmful *Microcystis* blooms in Lake Taihu (Zhu et al., 2014). However, the role of wind-driven waves on the cycling of P remains unclear in shallow eutrophic lakes.

In our previous study, we examined the effects of turbulence on the APA of phytoplankton and bacteria without sediments in Lake Taihu (Zhou et al., 2016a). To further understand P availability and strategies used for its utilization by plankton in natural field conditions, this study simulated the in situ conditions to further explored the APA fractionations under wind-wave conditions. Also, this allowed us to verify the effects of wind waves on the cycling of P as well as the strategies used by plankton. Understanding of the biogeochemical cycles controlling P availability will provide insights on how to possibly regulate P release from sediments and thereby reduce risks of any HABs occurrence in shallow eutrophic lakes.

1. Materials and methods

1.1. *Study site*

Lake Taihu is China's third-largest freshwater lake with a total surface area of 2230 km^2, which is a typical shallow eutrophic lake with an average depth of 1.9 m, a maximum depth of 2.6 m (Qin et al., 2007). It is a key source of drinking water for more than 10 million people (Qin et al., 2010). This system is strongly influenced by wind-driven waves. From 1957 to 2015, ESE winds with an average wind speed of 3.5 $\pm$ 0.2 m/sec prevailed over Lake Taihu from April to August, and NNW winds with an average wind speed of 3.4 $\pm$ 0.2 m/sec dominated in other months (Wu et al., 2019). According to field observations in Lake Taihu, wind speed over 3 m/sec generally induced sediment resuspension, and its frequency was about 88% (Qin et al., 2006). The Meiliang Bay locates in the north region of Lake Taihu, which is one of the most eutrophic regions in the lake (Qin et al., 2007). The mesocosms were placed in the Taihu Laboratory for Lake Ecosystem Research, located at the coast of Meiliang Bay (31°41′835″N, 120°22′044″E).

1.2. *Experimental setup*

The experiment was carried out from July 13th to July 22th 2016 in a total of twelve tanks made by plexiglass that has maximal capacities of 126 L (Appendix A **Fig. S1**, detailed in our previous study (Zhou et al., 2016a)). The sediment was used a Petersen grab sampler to collect the upper 10 cm sediment in Meiliang Bay (31°26′07″N, 120°11′18″E). The characteristics of sediment were summarized in Appendix A Tables S1 and S2. The collected sediment was thoroughly mixed to homogenize and then placed at the bottom of each tank. The lake water was then gently pumped into each tank with a rubber tube. Each tank has 0.5 cm thick sediment and 96 L lake water. Finally, all tanks were floated and fixed in an outside artificial pond (10 $\times$ 10 $\times$ 2 m), which was filled with lake water, to simulate near-natural field conditions. All tanks stood for 3 days before starting the experiment.

In Lake Taihu, the corresponding energy dissipation rates (ε) significantly varied from 6.01 $\times$ 10^{-8} to 2.39 $\times$ 10^{-4} m^2 s^{-3} (Zhou et al., 2016a). Submerged wave-maker pumps (WP, Jiebao, China; Appendix A **Fig. S1**) were fixed under the water surface with magnets in each tank to create hydrodynamic turbulence similar to wind-induced waves in Lake Taihu. In this study, the ε in the wind-wave treatments were 1.12 $\times$ 10^{-6} (low), 2.95 $\times$ 10^{-5} (medium), and 1.48 $\times$ 10^{-4} m^2 s^{-3} (high), corresponding Reynolds numbers (Re) values were 5500, 16,371, and 92,620. A treatment without wind waves (calm), was considered as the control. Each treatment was conducted in triplicate.

Table 1 – Summary of environmental characteristics measured in the control (calm) and three wind-wave treatments (low, medium, and high) during the experiment.

Parameter	Control	Low	Medium	High
WT (°C)	29.5 ± 1.4	29.5 ± 1.4	29.6 ± 1. 4	29.7 ± 1.4
pH	9.1 ± 0.1	**8.8 ± 0.2****	**8.7 ± 0.2*****	**8.7 ± 0.2*****
DO (mg/L)	9.6 ± 2.1	8.7 ± 1.0	8.7 ± 1.6	8.4 ± 1.3
SS (mg/L)	39.3 ± 12.4	50.0 ± 28.6	140.3 ± 60.0	**457.1 ± 200.3*****
TN (mg/L)	1.51 ± 0.28	1.98 ± 0.19	**2.56 ± 0.40*****	**3.82 ± 0.83*****
TDN (mg/L)	1.15 ± 0.17	1.05 ± 0.19	1.07 ± 0.19	1.17 ± 0.12
PN (mg/L)	0.95 ± 0.17	1.43 ± 0.20	**2.07 ± 0.47*****	**3.39 ± 0.86*****
NH_4^+ (mg/L)	0.12 ± 0.04	0.14 ± 0.03	**0.16 ± 0.04***	0.10 ± 0.03
NO_3^- (mg/L)	0.046 ± 0.031	0.040 ± 0.035	0.048 ± 0.036	0.062 ± 0.039
NO_2^- (mg/L)	0.002 ± 0.003	0.002 ± 0.003	0.003 ± 0.003	0.002 ± 0.003

WT: water temperature; DO: dissolved oxygen; SS: Suspended solids; TN: total nitrogen; TDN: total dissolved nitrogen; PN: particulate nitrogen; NH_4^+: ammonium; NO_3^-: nitrate; NO_2^-: nitrite. Bold values and asterisks indicate significant difference between the wind-wave treatments and the control were determined by ANOVA: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

1.3. *Sampling and analysis*

Dissolved oxygen (DO), pH, and water temperature (WT) were measured with 6600 multi-sensor sonde (Yellow Springs Instruments, California, USA). Water samples were collected daily by collecting 1 L of vertically integrated water using a tube sampler, which was analyzed including total nitrogen (TN), total phosphorus (TP), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), nitrite (NO_2^--N), ammonium (NH_4^+-N), nitrate (NO_3^--N), Suspended solids (SS), and chlorophyll *a* (Chl *a*), following the methods described by Zhu et al. (2014). Particulate nitrogen (PN) and particulate phosphorus (PP) were obtained by subtracting TDN/P from TN/P. Dissolved organic phosphorus (DOP) was obtained by subtracting SRP from TDP.

1.4. *Size fractionation APA*

To investigate the APA associated with algal and bacterial particles, each sample was divided into two subsets according to their particle sizes. Specifically, a 100 mL water sample was orderly filtered through 2 µm and 0.2 µm pore-size polycarbonate filter (Millipore, Ireland). The filter was then shredded and stored in a 2 mL sterile centrifuge tube at −80 °C until used for DNA extraction. The difference between the APA measured in the unfiltered water and the two filtrates were used to calculate the enzymatic activity associated with different size fractions. These measurements from the unfiltered water and 0.2 µm filtrate were hereafter referred to as the total APA (TAPA) and dissolved APA (DAPA), respectively. The activity associated with the 0.2–2.0 µm fractions were mainly assumed to be bacterial APA (BAPA), while those from > 2.0 µm were that of the phytoplanktonic APA (PAPA) (Nedoma et al., 2006). APA was determined spectrophotometrically as the release of *p*-nitrophenol from the model substrate *p*-nitrophenyl phosphate (pNPP), which were detailed in Gao et al. (2006). All samples were measured in triplicate.

1.5. *DNA extraction and qPCR*

Gene expression of phosphatase genes from cyanobacteria and other bacteria was explored using the *phoX* gene (Lin et al., 2018). DNA was extracted from the filters using the FastDNA Power-Max Soil DNA Isolation Kit (MP Biomedical, USA) following the manufacturer's protocol. The DNA subsequently served as templates for quantitative real-time polymerase chain reaction (PCR) amplification, which was used to estimate the expression of bacterial *phoX* (F) 5′-GARGAGAACWTCCACGGYTA-3′ / (R) 5′-GATCTCGATGATRTGRCCRAAG-3) (Sebastian and Ammerman, 2009) and *Microcystis phoX* (5′-TGATTAGCCTGGCGAAAGAT-3′ / (R) 5′-CTGCAGGGTGCCAATAATTT-3′) (Harke et al., 2012). The qPCR program was detailed in Harke et al. (2012). Each PCR run has three negative controls without the DNA template.

1.6. *Statistical analysis*

One-way analysis of variance (ANOVA) was used to analyze the differences between and among the treatments. Post Hoc Multiple Comparisons of the treatment means were performed by Tukey's least significant difference procedure. Pearson's correlation coefficient was used to explore the relationships between APA fractions and nutrients. Statistical analyses were performed in SPSS 22.0 statistical package for personal computers, with the level of significance set at $P < 0.05$ for all tests.

2. Results

2.1. *Environmental characteristics*

WT ranged from 27.3 to 32.4 °C with the mean of DO was 8.9 ± 1.6 mg/L during the experiment ($P > 0.05$, Table 1). pH was significantly smaller in the treatments (low, medium, and high) than in the control (calm, $P < 0.05$). The nitrogen concentrations varied significantly during the experiment. The average TN and PN were significantly smaller in the control than in the treatments ($P < 0.05$), while there were no significant differences for TDN, NO_2^-, and NO_3^- among treatments.

The concentrations of TP and PP increased in the treatments, especially the medium and high treatments, which were significant differences with the control ($P < 0.01$, Fig. 1**a and b**). The PP accounted for the most of TP, which increased with the intensities of wind waves (control, 76.8% ± 7.5%; low, 83.0% ± 5.1%; medium, 88.0% ± 7.4%; high, 95.6% ± 0.8%). However, the TDP concentrations were lowest in the high while it fast increased in the low and medium after 4 days ($P > 0.05$, Fig. 1**c**). Similar to TDP, DOP was lowest in the high with a significant difference with the control ($P < 0.01$, Fig. 1**d**). However, in contrast to PP, the proportions of DOP to TDP decreased with the increase of wind-wave intensity (control, 82.8% ± 5.0%; low, 71.5% ± 8.8%; medium, 66.0% ± 10.8%; high, 57.5% ±

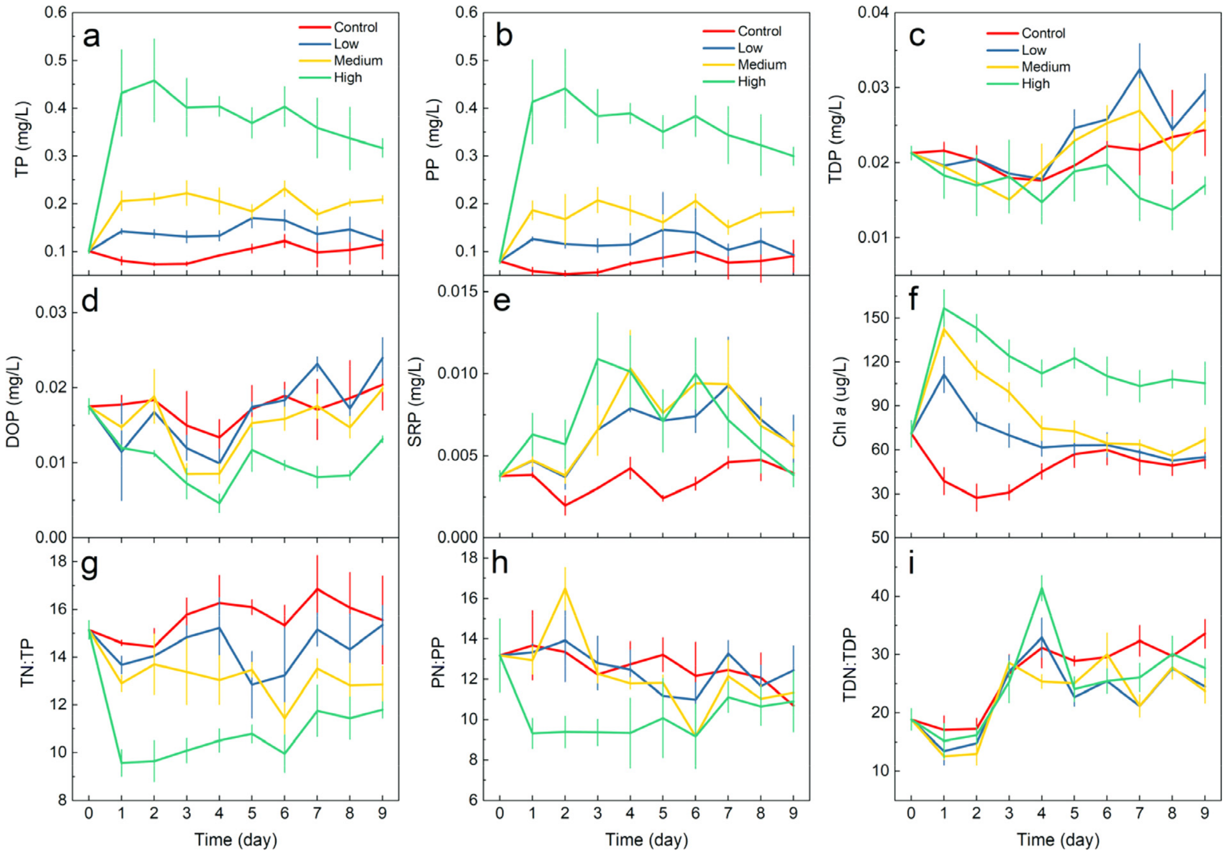

Fig. 1 – Variations of phosphorus (TP, a; PP, b; TDP, c; DOP, d; SRP, e), Chl *a* (f), and the mass ratio of nitrogen and phosphorus (TN:TP, g; PN:TP, h; TDN:TDP, i) in the control (calm) and three wind-wave treatments (low, medium, and high) during the experiment.

16.6%). The SRP concentration increased and was significantly higher in the treatments than in the control ($P < 0.05$, Fig. 1e).

It was observed that Chl *a* rapidly increased and reached a maximum value at day 1, followed by a gradual decrease in the treatments in the succeeding days (Fig. 1f). In contrast, for the control, Chl *a* concentration was first observed to decrease before day 2 and then increased during the experiment (Fig. 1f). The 9-day average of Chl *a* in the calm (48.6 ± 13.4 µg/L) was significantly lower compared to low (68.6 ± 16.9 µg/L, $P > 0.05$), medium (82.6 ± 27.4 µg/L, $P < 0.05$), and high (115.7 ± 23.3 µg/L, $P < 0.05$). Moreover, the stoichiometric ratios between N and P were uniformly higher in the control than in the wind-wave treatments (Fig. 1g, h, and i). The ratios of TN:TP and PN:PP quickly deceased in the high with a significant difference with the control ($P < 0.05$), while the TDN:TDP first decreased and then increased after 2 days for all treatments ($P > 0.05$).

2.2. APA fractions

The variability of size-fractionated APA was observed in the different wind-wave treatments. TAPA increased in the control during the experiment but was still generally lower than the treatments (Fig. 2a). Compared to the control, TAPA dramatically increased on day 1 and then fluctuated afterward in the treatments (Fig. 2a, $P > 0.05$). Similar to TAPA, PAPA rapidly increased and was significantly higher in the treatments than in the control (Fig. 2b, $P < 0.05$). However, in the treatments, BAPA and DAPA were significantly lower than the control ($P < 0.05$) and generally decreased with the increase of wind-wave intensity (Fig. 2c and d). During the experiment, PAPA dominated TAPA by comprising 75.5% ± 15.8% of the total fractions (Fig. 3). PAPA dominated as the wind-wave intensity increased, with significant difference between the treatments (low, 74.3% ± 6.6%; medium, 78.0% ± 7.3%; high, 91.7% ± 4.2%) and control (54.0% ± 12.4%, $P < 0.05$, Fig. 3). The remaining fractions of TAPA were shared by BAPA (18.7% ± 13.7%) and DAPA (6.8% ± 2.8%), both of which varied little during the experiment (Fig. 3).

The gene *phoX* in the treatments were significantly upregulated compared to the control during the experiment. The average copy of *Microcystis phoX* was higher in the treatments (low, 3.6 ± 1.8 fold enrichment; medium, 4.2 ± 1.6 fold enrichment; high, 2.4 ± 0.7 fold enrichment) compared to the control (Fig. 4). However, similar to BAPA, *bacterial phoX* was downregulated with the increase of wind-wave intensity, and there were no significant changes among the treatments ($P > 0.05$, Fig. 4).

2.3. The relationships between apa fractions and nutrients

Correlations between APA fractions and nutrients were summarized in Table 2. TN, TP, PP, TN:TP, and Chl *a* were significantly positively correlated with APA fractions except for TAPA ($P < 0.05$). Meanwhile, TDP and DOP were only positively correlated with BAPA ($P < 0.05$), while SRP had significant negative correlations with BAPA and DAPA ($P < 0.05$). Moreover, PN:PP ratios were negatively correlated with TAPA and PAPA ($P < 0.05$) but no apparent pattern was observed between TDN:TDP ratios and all APA fractions ($P > 0.05$).

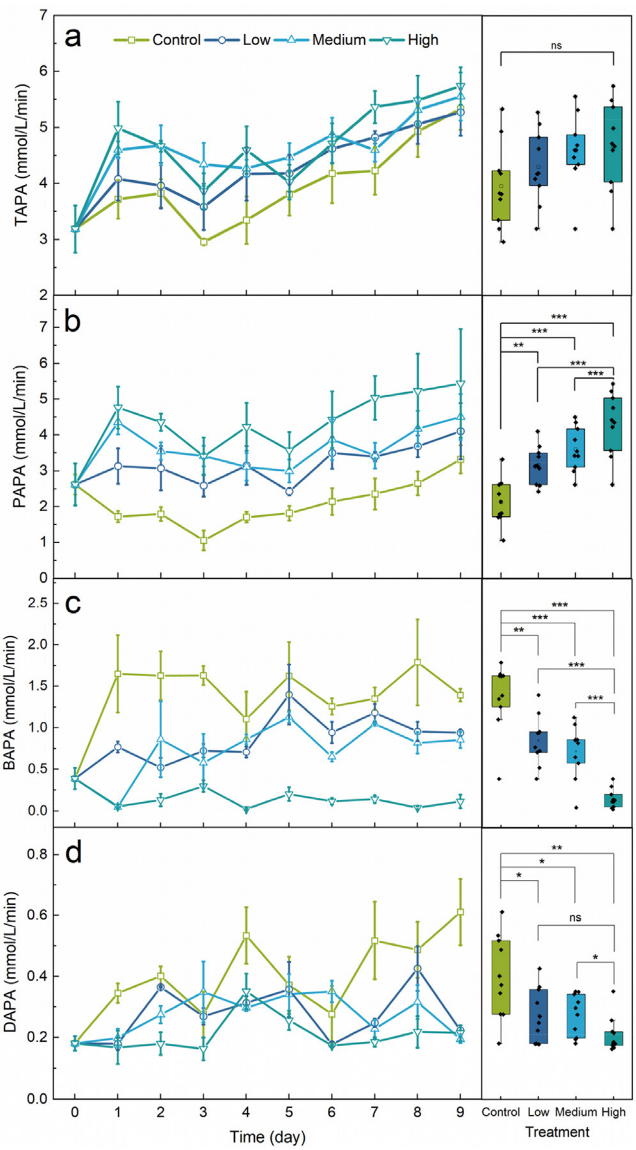

Fig. 2 – Variations of alkaline phosphatase activity (APA) fractions in the calm condition and three wind-wave treatments during the experiment, including total APA (TAPA, a), phytoplanktonic APA (PAPA, b), bacterial APA (BAPA, c), and dissolved APA (DAPA, d). Control (black square), low (circle), medium (triangle), and high (inverted triangle).

Table 2 – Summary of Pearson's correlation values of alkaline phosphatase activity (APA) fractions including total (TAPA), phytoplanktonic (PAPA), bacterial (BAPA), and dissolved (DAPA) with nutrient concentrations during the experiment. The sample number was 112.

	TAPA	PAPA	BAPA	DAPA
TN	**0.202***	**0.546*****	**-0.625*****	**-0.427*****
TP	0.158	**0.485*****	**-0.597*****	**-0.386*****
TDP	-0.109	-0.071	**0.345*****	0.082
PP	0.147	**0.482*****	**-0.612*****	**-0.386*****
DOP	0.125	-0.105	**0.428*****	0.150
SRP	-0.024	0.143	**-0.278****	**-0.228***
TN:TP	-0.038	**-0.389*****	**0.630*****	**0.386*****
TDN:TDP	0.095	0.054	0.042	0.091
PN:PP	**-0.212***	**-0.277****	0.159	0.034
Chl *a*	0.161	**0.508*****	**-0.620*****	**-0.439*****

TN: total nitrogen; TP: total phosphorus; TDP: total dissolved phosphorus; PP: particulate phosphorus; DOP: dissolved organic phosphorus; SRP: soluble reactive phosphorus; TN:TP: the mass ratio of TN and TP; TDN:TDP: the mass ratio of TDN and TDP; PN:PP: the mass ratio of PN and PP; Chl *a*: chlorophyll *a*. Bold values and asterisks indicate that the correlation is significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

3. Discussion

The frequent and intense wind waves usually result in the release of sediment nutrient in large shallow lakes (Cardoso and Marques, 2009). Remineralization of Po in resuspended particulates is considered to be an important source for algae production in lakes (Chao et al., 2017). Despite size fractionation by filtration is never completely absolute (i.e., overlapping size) (Yuan et al., 2017), different APA fractions have been considered as good indicators of phytoplanktonic and bacterial P stress (Labry et al., 2005). In this study, the results showed that the predominance of APA from algal fraction was further promoted under wind-wave conditions (Fig. 2). In contrast, BAPA and DAPA decreased when exposed to wind-wave stress (Fig. 2). This was consistent with the gene copies observed for *Microcystis phoX* and bacterial *phoX* (Fig. 4). Wind-driven waves could possibly induce the release of sediment AP and Po, which then influence and regulate planktonic APA. These accelerated the biogeochemical cycle of P and promoted primary production in Lake Taihu. This information provides a further understanding of P transformation and phytoplankton growth in shallow eutrophic lakes.

The hydrodynamic processes associated with wind waves are recognized to be a key factor that impacts P dynamics, algal growth, and its bloom persistence in shallow eutrophic lakes (Huang et al., 2015; Søndergaard et al., 2003; Zhu et al., 2014). In our previous study without sediment, we found that wind-induced turbulence decreased BAPA but enhanced PAPA in Lake Taihu, and TAPA was dominated by PAPA (66%–93%) (Zhou et al., 2016a). Similarly, with the presence of the sediments, PAPA significantly increased but BAPA decreased in the wind-wave treatments compared to the control in this study (Fig. 2, $P < 0.05$). It has been widely recognized that hydrodynamic turbulence could increase the diffusion rate of nutrients in the cell surface (Guasto et al., 2012), enhancing the uptake of nutrients (Bergstedt et al., 2004; Honzo and Wuest, 2008; Prairie et al., 2012). However, in the process of P uptake, the competitive success of bacteria versus phytoplankton at low SRP condition is greatly higher because of their surface to volume ratio advantage, especially under turbulent conditions (Drakare, 2002; Honzo and Wuest, 2008; Løvdal et al., 2007). It could be deduced that bacteria experience less stress with P depletion than phytoplankton under wind-wave conditions. In Lake Taihu, there is significant correlation between bacterial *phoX* abundances and BAPA (Dai et al., 2015). In this study, the variations of PAPA and BAPA were consistent with the gene levels of phytoplanktonic and bacterial *phoX* under different conditions (Fig. 4). This also corroborates previous observations of Xu et al. (2008), where phytoplankton dominated at high levels of SRP, while bacteria were more advantageous under low SRP concentrations.

Phytoplankton communities were generally dominated by *Microcystis* during the experiment (except in the high treat-

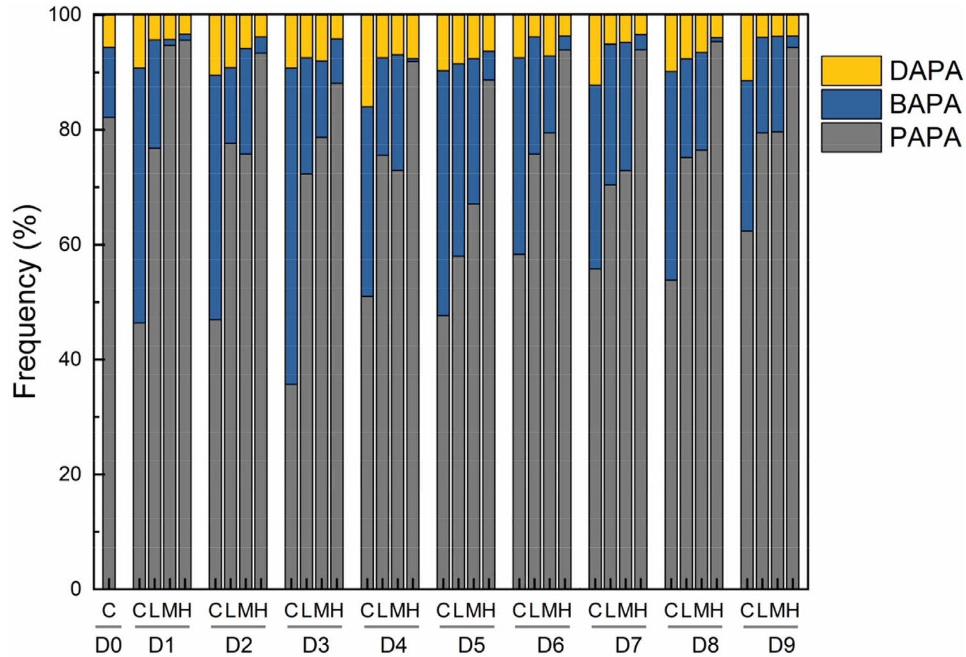

Fig. 3 – The proportional composition of APA fractionations (phytoplanktonic APA, PAPA; bacterial APA, BAPA; dissolved APA, DAPA) in the calm conditions and three wind-wave treatments during the experiment. D5 is the sample collected on day 5, and C, L, M, and H represent the calm, low, medium, and high treatments, respectively.

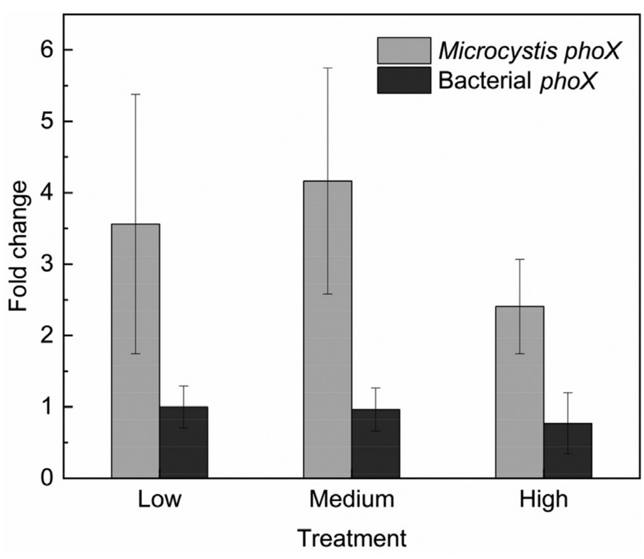

Fig. 4 – Mean fold change in gene copies for *Microcystis phoX* and bacterial *phoX* in the three wind-wave treatments (low, medium, and high) relative to the control (calm) during the experiment.

ment after day 6), which was confirmed to be capable of synthesizing AP (Yuan et al., 2017). Studies showed that *Microcystis* developed many adaptive strategies to efficiently utilize P to cope with P-limitation (Shen and Song, 2007; Yue et al., 2014). It can take up and store P as polyphosphates, allowing it to survive and dominate for a long time even under P-deficient conditions (Otten et al., 2012). Due to the accelerated growth of *Microcystis*, P consumption increased while the cellular P quota significantly decreased under turbulent conditions, which then elevated APA expression (Zheng et al., 2017). Therefore, wind-driven waves likely enhanced the expression of phytoplanktonic *phoX* and PAPA but decreased the expression of bacterial *phoX* and BAPA in this study. It is widely known that Cyanobacteria, Chlorophyte, and Bacillariophyta can excrete AP in Lake Taihu (Ma et al., 2019). However, *Microcystis phoX* only reflected the APA of *Microcystis*, which not included the APA of other phytoplankton. In the high treatments, turbulence quickly shifted the competitive balance from cyanobacteria to sinking diatom (Zhou et al., 2016b), and wind waves induced the resuspension and release of algal APA (including *Microcystis* and other phytoplankton) from the sediment. These may be one important reason to explain the inconsistence between *Microcystis phoX* and PAPA in the medium and high treatments (**Figs 2 and 4**).

Several studies reported that AP was extensive in lake water and sediments (Torres et al., 2016; Zhou et al., 2008). Intense wind waves could induce sediment resuspension, resulting in the release of AP and benthic algae from the sediments (Zhang et al., 2007; Zhu et al., 2014). This cascade ultimately caused a dramatic increase in Chl *a*, TAPA, and PAPA at the start of wind waves (**Figs. 2 and 3**). Although the PAPA increased, BAPA decreased under wind-wave conditions (**Fig. 2**), indicating that APA in the sediment may be dominated by PAPA. Moreover, the release of inorganic P from the sediments temporarily alleviated P deficiency under wind-wave conditions, causing the PAPA to fluctuate during the experiment (**Fig. 2**). Phytoplanktonic AP is thought to be the main consumers of DOP, which promotes the conversion of bio-available P to SRP (Chao et al., 2017; Wang et al., 2018). In this study, the concentration of Po in the sediment was 0.52 g/kg, which accounted for 51.5% of TP (Appendix A **Table S1**). Moreover, DOP was highly bioavailable, comprising around 19.7% of the total organic P in the sediments (Appendix

A **Table S2**). Previous reports showed that around 34.1% of DOP can be hydrolyzed into SRP by AP in Lake Taihu (Ma et al., 2019). Under wind-wave conditions, algae synthesized more phosphatase and accelerated the regeneration of the bioavailable Po from resuspended sediment. This may partially explain why the concentration of DOP was higher in the control than in the treatments, while it was opposite for SRP (**Fig. 1**). The expression of *phoX* also revealed that *Microcystis* may be efficiently exploiting organic sources of P to support phytoplankton growth (Harke et al., 2012), which was consistent with the observation that tropical cyclones stimulated harmful *Microcystis* blooms in Lake Taihu (Zhu et al., 2014). Moreover, it is generally believed that a bit of hydrodynamic turbulence could benefit phytoplankton, while stronger ones inhibit cyanobacterial growth and prevent the formation of blooms (Huang et al., 2015; Zhou et al., 2016b; Zheng et al., 2017). Indeed, due to resuspension of the particle, the low transparency of the water column prevents the entry of light, and highly turbulent conditions in the high treatment were not suitable for phytoplankton growth. The enhanced phytoplankton biomass in the wind-wave treatments may be associated with the resuspension of algae rather than sedimentation (Appendix A **Fig. S2**). Consequently, this suggested that wind-induced waves accelerated the biogeochemical cycling of P and supported phytoplankton to overcome P limitation. Our results further indicate that wind-driven waves are an important driver of P biogeochemical cycling in shallow eutrophic lake ecosystems.

4. Conclusion

Wind-driven waves can be a key process affecting P dynamics and algal blooms in shallow eutrophic lakes, which significantly induced the AP, DOP, and benthic algae release from sediment and subsequently affected P behavior and algal growth. During cyanobacterial bloom periods, wind-waves dramatically increased the phytoplanktonic APA but decreased bacterial and dissolved APA in Lake Taihu. Consistently, the gene copy of *Microcystis phoX* was higher in the wind-wave treatments (above 2.4 times) than in the control, which was opposite for bacterial *phoX*. The dramatic increase in phytoplanktonic APA and *Microcystis phoX* likely hydrolyzed the bioavailable organic P released from the resuspended sediments, providing available P sources for further reproduction of phytoplankton in Lake Taihu. This study suggests that wind-driven waves likely accelerate the biogeochemical cycling of P and change the P strategies of plankton during cyanobacterial bloom periods in shallow eutrophic lakes, which seem to promote the productivity of phytoplankton and their subsequent blooms.

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Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2020.06.022.

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