Article

Interactions between Phosphorus Enrichment and Nitrification Accelerate Relative Nitrogen Deficiency during Cyanobacterial Blooms in a Large Shallow Eutrophic Lake

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ABSTRACT: Regime shifts between nitrogen (N) and phosphorus (P) limitation, which trigger cyanobacterial succession, occur in shallow eutrophic lakes seasonally. However, the underlying mechanism is not yet fully illustrated. We provide a novel insight to address this from interactions between sediment P and nitrification through monthly field investigations including 204 samples and microcosm experiments in Lake Chaohu. Total N to P mass ratios (TN/TP) varied significantly across seasons especially during algal bloom in summer, with the average value being 26.1 in June and descending to 7.8 in September gradually, triggering dominant cyanobacterial succession from *Microcystis* to *Dolichospermum*. The regulation effect of sediment N/P on water column TN/TP was stronger in summer than in other seasons. Iron-bound P and alkaline phosphatase activity in sediment, rather than

ammonium, contributed to the higher part of nitrification. Furthermore, our microcosm experiments confirmed that soluble active P and enzymatic hydrolysis of organic P, accumulating during algal bloom, fueled nitrifiers and nitrification in sediments. These processes promoted lake N removal and led to relative N deficiency in turn. Our results highlight that N and P cycles do not exist independently but rather interact with each other during lake eutrophication, supporting the dual N and P reduction program to mitigate eutrophication in shallow eutrophic lakes.

KEYWORDS: lake eutrophication, N and P limitation, nitrification, sediment P, N deficiency
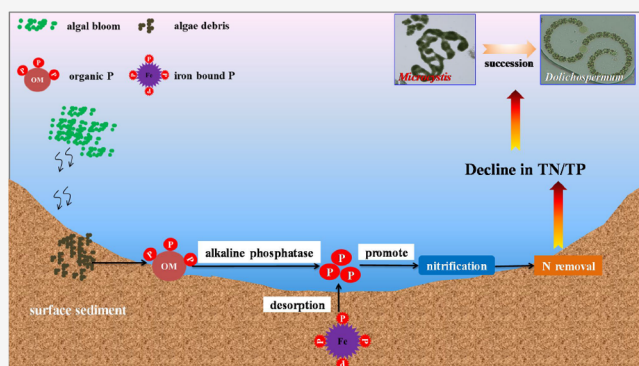
1. INTRODUCTION

Both absolute levels of nitrogen (N) and phosphorous (P) and their ratios are the main driving factors affecting phytoplankton growth and succession in freshwater lakes.[1,2] One of the central concerns regarding lake ecosystems is whether P or N determines their primary productivity, giving rise to controversy over whether N or P reduction programs should be proposed to mitigate eutrophication. P has largely ever been considered as the solely limiting nutrient.[3−5] However, increasing studies showed that phytoplankton communities in lakes are N limited or co-limited, especially in summer[6−8] and in eutrophic lakes.[9−11] The seasonal nutrient limitation shifts indicated by N/P ratios could significantly trigger cyanobacterial succession (N2-fixing/non-N2-fixing cyanobacteria) in many eutrophic lakes.[12−14]

Lake Chaohu is the fifth largest shallow freshwater lake in China, in which nutrient limitation shift from P to N limitation occurred during summer algal bloom, followed by cyanobacterial succession from a non-N2-fixing taxon (*Microcystis*) to N2-fixing taxon (*Dolichospermum*).[15] However, it is not yet well elucidated whether interactions between sediment nutrient biogeochemical cycles can drive this transition in a water

column, which is important in predicting species occurrence and developing bloom management strategies. Generally, the over-proliferating algae carrying large amounts of N and P are deposited into the sediment, leading to the enrichment of nutrients. The settled organic N is transformed into ammonium through ammonification. Nitrification converts ammonium to nitrite or nitrate in aerobic surface sediments under the mediation of microorganisms, such as ammonia-oxidizing archaea (AOA)[16] and bacteria (AOB).[17] It plays a vital role in the N cycle of lake sediments, since it supplies substrates for N removal by denitrifying microorganisms or anaerobic ammonia oxidation in anoxic subsurface sediments. The synergistic action of these processes can help achieve effective N removal.[18]

Decomposition of algal organic matters can enhance the accumulation of sediment inorganic P fractions, including iron-bound P $(Fe(OOH) \sim P)$ and calcium-bound P $(CaCO_3 \sim P)$, and organic P (OP) in sediments,[19,20] including enzymatic hydrolyzable OP.[21] However, endogenous P release from $Fe(OOH) \sim P$ under anoxic conditions could be a tremendous P source in eutrophic lakes.[22,23] Furthermore, sedimentary organic matter in sediments could stimulate organic P-mineralizing bacteria (OPB) to produce extracellular alkaline phosphatase to hydrolyze OP.[24] Both regeneration pathways of P promote algal blooms, forming a feedback loop between sediment P flux and algal blooms. Microorganisms generally have higher P requirements than eukaryotes due to the greater need for P-rich RNA to synthesize proteins that support rapid growth.[25] However, previous work has not explored the effects of P enrichment on AOA and AOB as well as their function, which may accelerate N deficiency and regulate the shifts of N and P limitation during lake eutrophication.

Herein, we performed monthly field investigations from a whole year and microcosm experiments in Lake Chaohu to understand in detail interactions between P enrichment and nitrification and their regulation on N and P limitation in water columns. The following are the objectives of this study: (1) to illustrate how P enrichment in sediments affects ammonia-oxidizers in eutrophic lakes and the concomitant effects on nitrification; (2) to clarify the role of nitrification enhancement in sediments in the N and P limitation transitions and the cyanobacterial succession in eutrophic lakes.

2. MATERIALS AND METHODS

2.1. Site Description and Sample Collection.
Lake Chaohu (31°25′–31°42′N, 117°16′–117°51′ E) is the fifth largest freshwater lake in China. It has a surface area of 760 km^2 and a mean depth of 3.06 m[26] and can generally be divided into two regions from Zhongmiao Temple to Qitouzui Cape. Spatial heterogeneity of the eutrophication status exists in the lake, with west Lake Chaohu (WLC) being more eutrophic than east Lake Chaohu (ELC).[27] *Microcystis* blooms always occurred in late spring and summer seasons and *Dolichospermum* dominated in autumn and winter in Lake Chaohu.

Water and sediment cores from 17 sites along a trophic gradient were collected monthly from April, 2013, to March, 2014. These samples included 7 sites in ELC and 10 sites in WLC (Figure 1). Water samples were used to measure total phosphorus (TP) and total nitrogen (TN) concentrations. Composite sediment samples were stored at 4 °C for measuring potential nitrification rates (PNRs), alkaline phosphatase activity (APA), phosphorus fractionations, and other sediment physiochemical parameters including SRP and dissolved inorganic nitrogen (DIN; DIN = NH_4^+ + NO_2^- + NO_3^-) in interstitial water. Sediment samples from 11 sites of April, June, September, and February were preserved at −80 °C for subsequent DNA extraction and molecular analyses for AOA and AOB. In addition, 500 mL of water samples was collected for phytoplankton analysis at each site in June, Autumn, and November, 2013.

2.2. Phytoplankton Analyses.
Phytoplankton analysis was performed at each site in June, Autumn, and November, 2013. Samples were fixed with Lugol's solution. Samples were counted directly using an Olympus microscope at 400× total
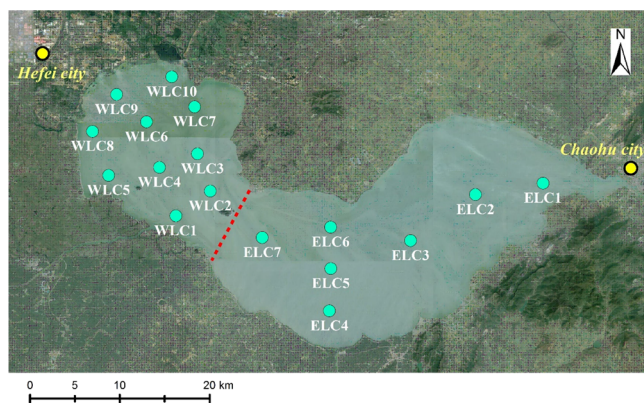

Figure 1. Sampling sites in Lake Chaohu. ELC and WLC represent the east (ELC) and west (WLC) part of Lake Chaohu, respectively.

magnification. Then, the abundance of each species at each site was calculated based on the results of identification and counts.

2.3. Determination of Environmental Parameters and Potential Nitrification Rates (PNRs).
The different N and P compounds in water samples were analyzed following standard methods for the examination of water and wastewater.[28] Water TN and TP were both determined by a persulfate method, while TN was digested under alkaline conditions. Filter samples of interstitial water were prepared through 0.45 μm membrane filters for determination of SRP, NH_4^+, NO_2^-, and NO_3^-. Then, SRP was determined by a vanadomolybdophosphoric acid colorimetric method. NH_4^+ was determined by a phenate method through formation of indophenol, an intensely blue compound, which is formed by the reaction of ammonia, hypochlorite, and phenol catalyzed by sodium nitroprusside. NO_2^- was determined through formation of a reddish purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulfanilamide with N-(1-naphthyl)-ethylenediamine dihydrochlorid. NO_3^- was determined by an ultraviolet spectrophotometric screening method under 220 and 275 nm.

Sediment P fractionations, consisting of iron-bound P $(Fe(OOH) \sim P)$, calcium-bound P $(CaCO_3 \sim P)$, acid-soluble organic P (ASOP), and hot NaOH-extractable organic P (P_{alk}), were extracted sequentially and according to Golterman.[29] APA was analyzed spectrophotometrically as p-nitrophenol resulting from the cleavage of phosphate from p-nitrophenylphosphate.[30]

PNRs were measured using the shaken-slurry method.[31] Briefly, 5.0 g of fresh sediment was weighed into 250 mL sterile Erlenmeyer flasks containing 100 mL of phosphate buffer (1 mM, pH 7.4) and 0.5 mL of $(NH_4)_2SO_4$ (0.25 M). The flasks were incubated on an orbital shaker (180 rpm) at 25 °C for 24 h. Subsamples (5 mL) of the slurry were taken at 1, 4, 10, 16, and 24 h after the incubation onset to analyze NO_3^- and NO_2^-, as described above. PNRs were then calculated as the nitrate and nitrite production per unit time per gram dry sediment.

2.4. Molecular Analyses.

(1) DNA isolation. DNA was extracted from 0.3 g of fresh sediment samples using a Power Soil DNA kit (Mo Bio Laboratories, Carlsbad, CA) following the manufacturer's instructions.

(2) qPCR. DNA concentrations of each sample were first measured by light absorption at 260 nm using an Ultramicro spectrophotometer (NanoDrop 8000, Ther-

mo). AOA and AOB abundances were then evaluated by targeting *amoA* gene sequences using triplicate qPCR reactions using an ABI STEPONE PLUS thermal cycler (Applied Biosystems, Foster City, CA). The primers used for archaeal *amoA* genes were Arch-*amoA*F/Arch-*amoA*R,[32] and *amoA*-1F/*amoA*-2R were used for bacterial *amoA* genes.[33] Each reaction was performed in a 50 μL volume containing about 10 ng of DNA, 0.4 mg/mL of bovine serum albumin, 0.4 mM concentration of each primer, 1 μL of ROX reference dye (50×), and 25 μL of SYBR Premix EX Taq (Takara Shuzo, Shiga, Japan). PCR products were confirmed by melt curve analysis and agarose gel electrophoresis. A known copy number of linearized plasmids containing an *amoA* gene clone was used as a standard reference for the AOA and AOB results. The amplification efficiencies for archaeal and bacterial *amoA* genes were 89−92% ($R^2 > 0.98$) and 86−89% ($R^2 > 0.99$), respectively.

2.5. Microcosm Experiments To Detect Endogenous P Effects on Nitrification. *2.5.1. Microcosm Experiment To Evaluate Effects of SRP on Nitrification.* A sensitive bioassay method was designed to assess the microbial availability of phosphate for heterotrophic growth,[34] finding a linear relationship between heterotrophic cell growth and P concentration added. Based on this, a developed bioassay method was applied to full-scale nitrifying filters to evaluate P limitation on AOB growth.[35] In this study, the developed method was adapted to assess the effects of SRP on ammonia-oxidizers in Lake Chaohu sediments. Detailed adaptation methods for the application toward ammonia-oxidizers are shown in the Supporting Information.

2.5.2. Microcosm Experiment To Evaluate Effects of Organic P Hydrolysis on Nitrification. To test the hypothesis that sediment organic P can stimulate nitrification through enzymolysis via alkaline phosphatase excreted by OPB in Lake Chaohu, further microcosm experiment was conducted. The dominative OPB in sediment of WLC, which was affiliated to *Bacillus* sp., was isolated, enriched, and then transplanted to the sediment cores of ELC and WLC, respectively. After incubation for 20 days, sediment APA, PNRs, and the abundances of AOA and AOB were measured. Additional methodological details for these experiments are provided in the Supporting Information.

2.6. Statistical Analyses. Multiple linear regression (MLR) was performed to analyze the explanation of different N and P (including APA, Fe(OOH) ∼ P, CaCO₃ ∼ P, ASOP, P_{alk} content in sediments, SPR and NH_4^+ concentrations of interstitial water) on sediment PNRs. Subsequently, a hierarchical partitioning was used to estimate the relative importance of each explanatory variable based on their contribution to adjust R^2 generated by MLR. Hierarchical partitioning can calculate the variable importance from all subset models, based on an unordered assessment of importance. All the analyses were performed with the R packages "vegan" and "rdacca.hp".[36]

Pearson's correlation tests were used to identify different relationships among the $\log(x)$ transformed data. Transformation was conducted to ensure that the distribution of the data was minimally skewed and to achieve a fit close to a normal distribution. To evaluate differences between group means at the 95% confidence interval in the microcosm experiments, independent-sample t-tests (two-tailed) and one-

way ANOVA were used. Graphing was conducted using R 4.0.3. The statistical significance level was set at α = 0.05 ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Spatio-Temporal Variations of N and P Limitation in Lake Chaohu. The stoichiometric ratios of TN/TP determine which element is limiting prime productivity in lakes, with N limiting when the mass N/P ratio is less than 9, P limiting when the ratio is greater than 22.6, and N and P co-limiting when the ratio is intermediate.[37] Mass ratios of TN/TP in the water column of Lake Chaohu ranged from 3.3 to 59.9 in our dataset including 204 samples from 12 months (Figure 2), indicating significant nutrient limitation shifts. It is
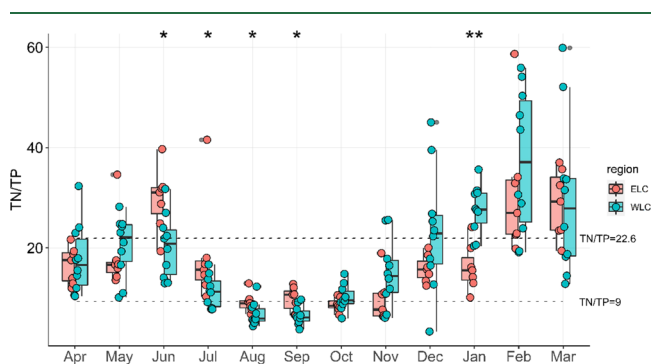

Figure 2. TN/TP in the water column of Lake Chaohu from different months. For each box, the horizontal line shows the median. Significant levels are indicated by "*" and "**" with $P < 0.05$ and $P < 0.01$, respectively.

worth noting that TN/TP had a significant downward trend during summer (from June to August) (averaged 26.1 in June) and reached the lowest value of the year in August and September (7.7 and 7.8 in average, respectively), which was below the N limitation threshold value (Figure 2). The same trends were found in either subsection of Lake Chaohu. Ratios of TN/TP in June were 29.7 and 20.3 in ELC and WLC, respectively, and then declined to 9.1 and 6.8 in August (Figure 2). In addition, *Microcystis* abundance was significantly higher than that of *Dolichospermum* in June and August, while *Dolichospermum* dominated over *Microcystis* in November after the summer blooms (Figure S2), indicating the succession from non-N₂-fixing cyanobacteria (*Microcystis*) to N₂-fixing cyanobacteria (*Dolichospermum*) during blooms in Lake Chaohu. *Microcystis* and *Dolichospermum* were the most common taxa of bloom-forming cyanobacteria in eutrophic lakes, and seasonal successions between them occurred. The factors controlling their successions can be attributed to external environmental factors (e.g., temperature; nutrients)[13] and interaction between these two species (e.g., P using strategy; allelopathic interactions).[38,39] Relative N deficiency can usually contribute to the dominance of *Dolichospermum* with N₂-fixing function.[12−14,40]

No significant differences of *Microcystis* abundances between ELC and WLC were recorded in June and November. However, in August, *Microcystis* abundances in WLC were significantly higher than in ELC. The significant difference of *Dolichospermum* abundances between ELC and WLC was only recorded in June with those in ELC higher than those in WLC (Figure S3). Unlike other seasons, the TN/TP ratio in WLC with a higher trophic status was significantly lower than that in
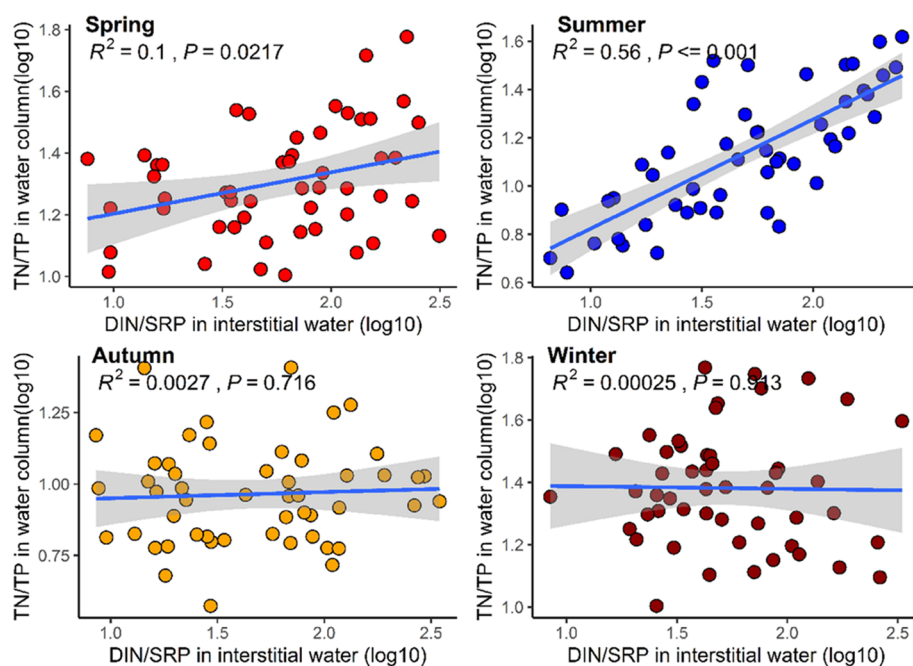
Figure 3. TN/TP in the water column vs DIN/SRP in interstitial water of Lake Chaohu from different seasons. The shaded area represents a 95% confidence interval.

ELC in all months of summer. It is consistent with other studies demonstrating stoichiometric imbalance of N and P in shallow eutrophic lakes with TN/TP ratios decreasing across eutrophication gradients.[9,11,12] Generally, internal nutrient cycling, other than external cycling, decided the nutrient limitations in eutrophic lakes.[41,42] In our study, DIN/SRP ratios in interstitial water were significantly and positively correlated with TN/TP ratios in the water column only in summer and spring, and the correlation was more powerful in summer apparently (Figure 3). So, nutrient cycling in sediments plays an even more important role in regulating patterns of nutrient limitation in water columns during warmer seasons or algal blooms.[42] This may be explained in two ways. On the one hand, *Microcystis* blooms generally occurred in summer whereas *Dolichospermum* dominated in other seasons in Lake Chaohu.[43] N_2-fixing, which can affect N/P ratios in water columns, could be negligible in summer. On the other hand, sediment activities of P release and N loss were more powerful during the algal blooms driven by the algal degradation, which can contribute to regime shift of nutrient limitations in water columns.[44,45] When TN/TP dropped to N limiting threshold, *Dolichospermum*, considered dominant when N was limited or TN/TP was low, reached an abundance peak in November according to another research which was carried out in the same year of our study.[46] Afterward, TN/TP in the water column increased gradually as N fixation increased by *Dolichospermum* (Figure 2). Thus, relative N deficiency and shifts from P limitation to N limitation in Lake Chaohu may be regulated by internal nutrient cycling in sediments during the summer bloom, triggering the cyanobacterial succession from the non-N_2-fixing taxon (*Microcystis*) and N_2-fixing taxon (*Dolichospermum*).

3.2. Regulation of Sediment Nitrification on Variations of N and P Limitation. The mechanisms underlying relative N deficiency during summer bloom in eutrophic lakes are not yet fully well-understood. P is a limiting factor on microbial growth. Its accumulation in environments may

contribute to the enhancement of varied N cycling function, such as organic N metabolism,[47,48] N_2-fixation,[49] and denitrification.[50] However, direct coupling between sediment nitrification function and P enrichment has been given little research attention in illustrating the stoichiometric imbalance of N and P in eutrophic lakes. In this study, sediment PNRs were higher in WLC than in ELC across seasons and reached maximum levels in summer (Figure 4). In addition, PNRs were significantly and negatively correlated with DIN/SRP in interstitial water, especially in summer (Figure 4), which could regulate the TN/TP in the water column further. Another seasonal field investigation shows that both denitrification and anaerobic ammonia oxidation (Anammox) activities were higher in summer and WLC than those of other seasons and ELC, respectively, in Lake Chaohu.[43] Thus, pronounced sediment nitrification may couple denitrification or Anammox by supplying substrates and then stimulate N removal. Nitrification was strengthened during eutrophication and contributed to the N deficiency in turn in Lake Chaohu.

Ammonia-oxidation, mediated by both AOA and AOB, plays a major role in N removal in eutrophic lakes. AOA and AOB were simultaneously pervasive within various habitats, while niche differentiation generally partitions their distributions. In this study, AOA abundance, AOB abundance, and ratios of AOA/AOB ranged from 3.8×10^5 to 6.5×10^9 copies/dry sediment, 9.8×10^5 to 8.5×10^9 copies/dry sediment, and 3.3×10^{-4} to 3.7×10^3, respectively (Figure S4). Ratios of AOA/AOB were significantly higher in ELC than in WLC, and AOB dominated over AOA in WLC in spring and summer seasons (Figure S4). Our study is consistent with other reports which documented divergent patterns of AOA and AOB abundances in response to trophic gradients, with AOB dominating over AOA as the eutrophication status gradually increased.[51−53] In addition, PNRs were significantly and negatively related to AOA abundances but positively associated with AOB abundances (Figure 5). Thus, AOB played a more important role in nitrification during eutrophication in Lake Chaohu.
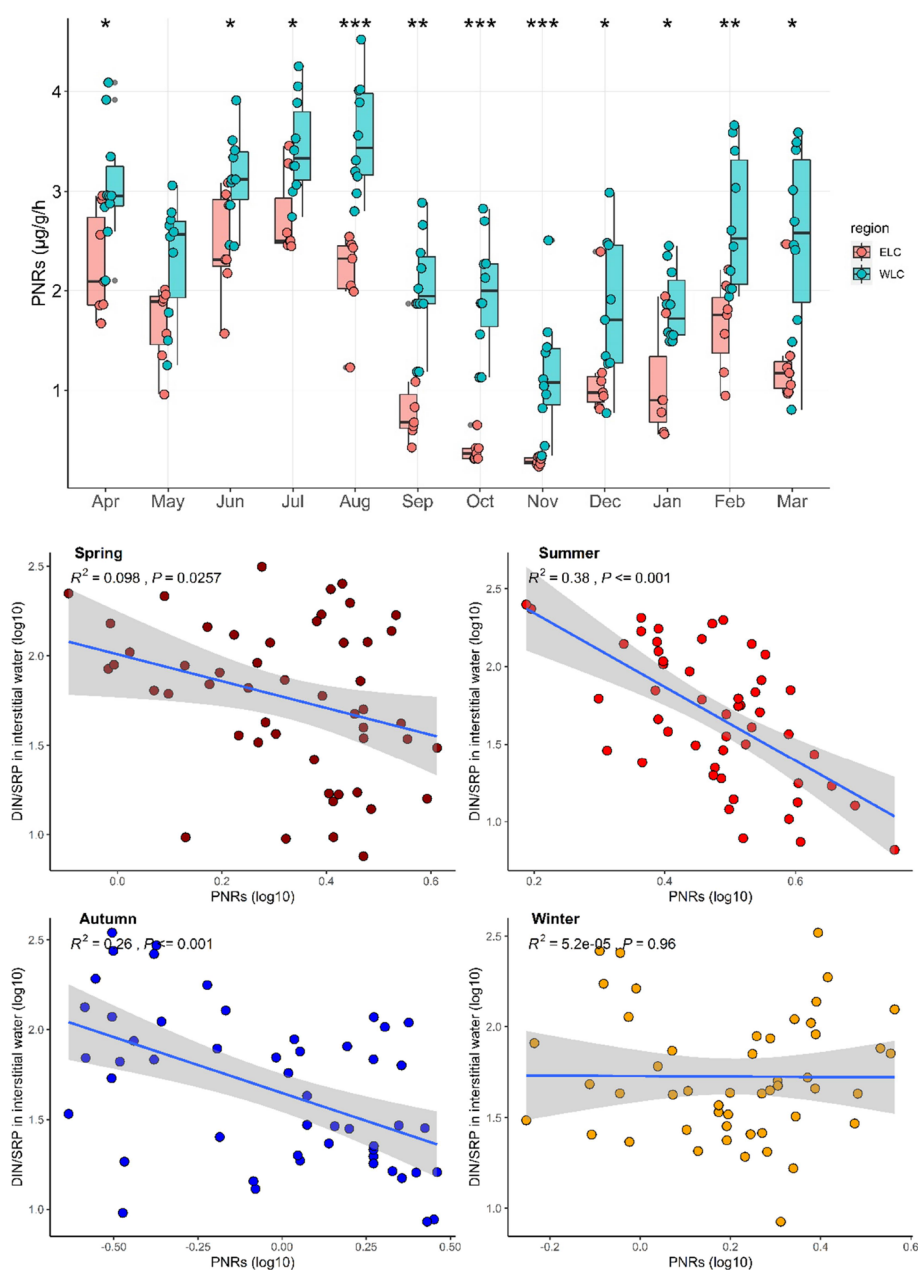
Figure 4. Sediment PNRs in WLC and ELC (top panel). Significant levels are indicated by "*", "***", and "****" with $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. Sediment PNRs vs DIN/SRP in interstitial water of Lake Chaohu from different seasons (bottom panel). The shaded area represents a 95% confidence interval.

NH_4^+ enrichment during eutrophication stimulated nitrification activities in Lake Chaohu sediments, while the responses of AOA and AOB to NH_4^+ contrasted. PNRs and AOB abundances in sediment were significantly and positively correlated with NH_4^+ concentrations in interstitial water, while AOA abundances had a significantly negative relationships with NH_4^+ concentrations (Figure 5). Generally, ammonia oxidation kinetics determined the niche differentiation of AOA and AOB with AOA having higher substrate affinities,[27,54,55] although some specific cultured species were reported to have a wide range of substrate affinities. The specific substrate affinity of AOA exceeded that of AOB by more than 200-fold,[56] suggesting that AOA are better adapted to habitats with low ammonium availability. Moreover, AOB are more tolerant to ammonium than AOA.[54] Thus, NH_4^+

enrichment, released from the degradation of organic matter in sediments, could be partly responsible for the shifts from the importance of AOA to AOB in nitrification during Lake Chaohu nitrification.

3.3. Contribution of Interactions between Endogenous P and Nitrification to N Deficiency. Internal P loading from the sediment can trigger a positive feedback of lake eutrophication, while its effects on nitrification and nitrification-associated microorganisms have rarely been reported. $Fe(OOH) \sim P$ is the dominant inorganic P in Lake Chaohu especially in WLC, which accounted for 45.8−57% of the total P fraction in ELC and for 67−75% in WLC across seasons (Figure S5). In addition to inorganic P, the accumulated OP in sediments is also considered to be a ticking time bomb that jeopardizes lake water quality during
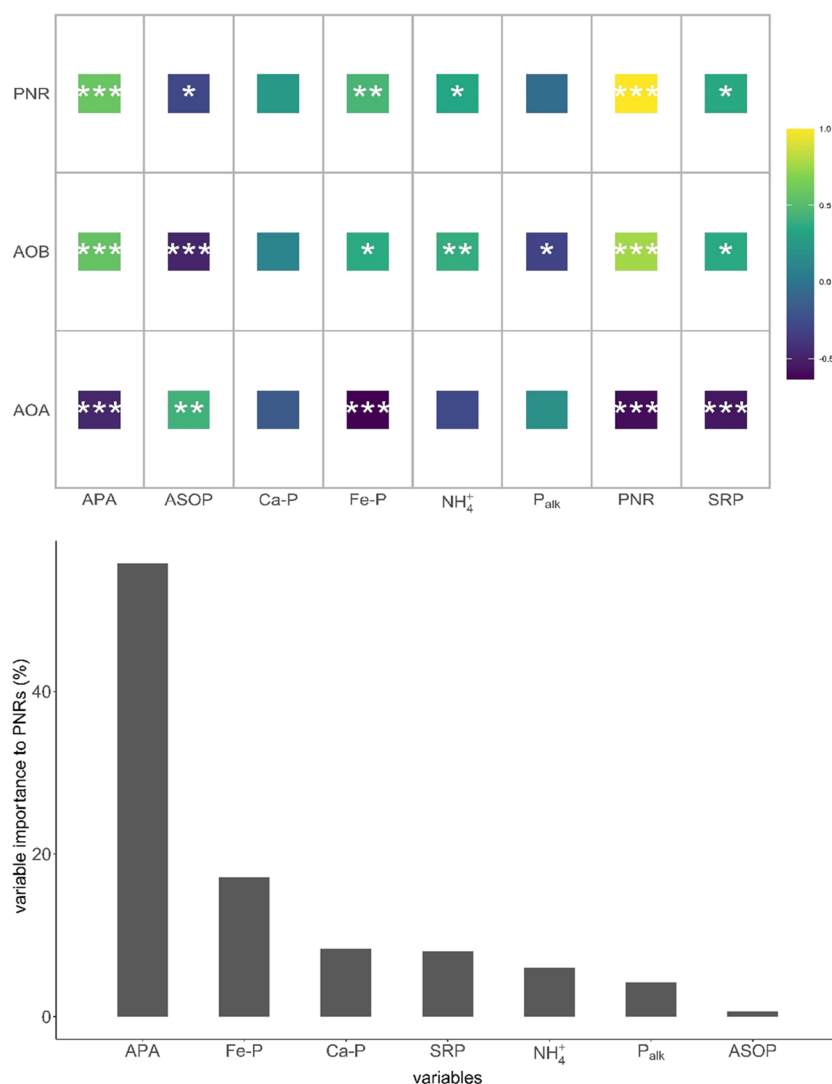
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Figure 5. Heatmap showing correlations between environmental variables and nitrifiers' abundance as well as PNRs (top panel). Significant levels are indicated by "*", "**", and "****" with $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. Individual importance (%) of the environmental variables to explain PNRs based on hierarchical partitioning of adjustR^2 generated by a multiple linear regression model (bottom panel).

eutrophication.[57] The enhanced lake eutrophication level will significantly increase the content of enzymatic hydrolyzable OP in sediments.[20] In our study, sediment APA was significantly higher in WLC than in ELC from all months and peaked during summer (Figure S6). It was demonstrated that APA in sediments were influenced by the perishing of algae, with APA being enhanced during algal blooms in eutrophic lakes.[58]

AOB abundances and PNRs were significantly and positively correlated with SRP concentrations in interstitial water and Fe(OOH) ∼ P content and APA in sediments (Figure 5). Based on hierarchical partitioning of adjustR^2 generated by MLR, the variable among NH_4^+, SRP, APA, Fe(OOH) ∼ P, $CaCO_3$ ∼ P, ASOP, and P_{alk}, which contributed to the highest part of PNRs in the year scale, was APA (55.9%), followed by Fe(OOH) ∼ P (17.1%) (Figure 5). Thus, P enrichment, both inorganically and organically, may directly and considerably fuel ammonia-oxidizers and then improve nitrification function. Our microcosm experiments confirmed this further. In the first microcosm experiment, we used sediments of Lake Chaohu to purify and culture ammonia-oxidizers and detected the variations of their abundance and nitrification activities as

we added different amounts of SRP. Results showed that PNRs and AOB and AOA abundances significantly increased as SRP concentrations increased (Figure 6), revealing the positive effect of P on nitrification activity as well as ammonia-oxidizers. Second, OPB, affiliated to *Bacillus* sp., were isolated and enriched from the sediment of Lake Chaohu. Microcosm experiments were carried out to determine if or not OP hydrolysis by OPB can accelerate nitrification. In the treatments with *Bacillus* sp., PNRs were 2.55 and 4.02 μg/g/h of ELC and WLC sediments, respectively, compared to 2.01 and 3.21 μg/g/h for the controls. APA significantly increased from 135.2 and 297.1 μg/g/h in the controls to 237.9 and 375.2 μg/g/h in the *Bacillus* sp. treatments of ELC and WLC sediments, respectively. In addition, AOB abundances were also significantly higher in *Bacillus* sp. treatments than in the controls, while no significant differences in AOA abundances were observed between treatments (Figure 7).

Sediment nitrification plays an important role in N loss in the lake ecosystem since it can provide substrates to both denitrification and annamox, converting reactive N to gaseous forms. The promoting effect of sediment P enrichment on nitrification in Lake Chaohu may be attributed to microbial P
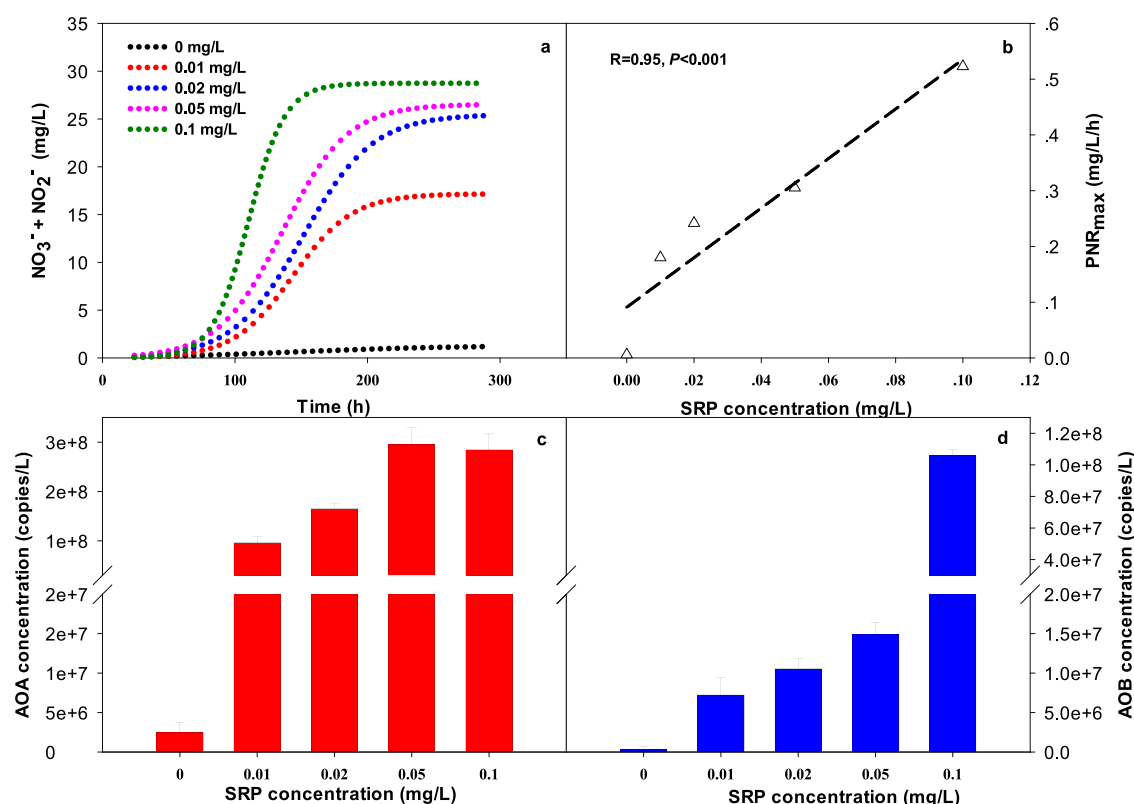
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Figure 6. Effects of SRP on nitrification via incubation bioassays. Monod kinetics for $NO_3^- + NO_2^-$ production (a). Relationship between PNR_{max} and added concentrations of SRP (b). AOA abundances (c) and AOB abundances (d) at the end of incubation bioassays. Bars show means of replicate measurements with standard errors.
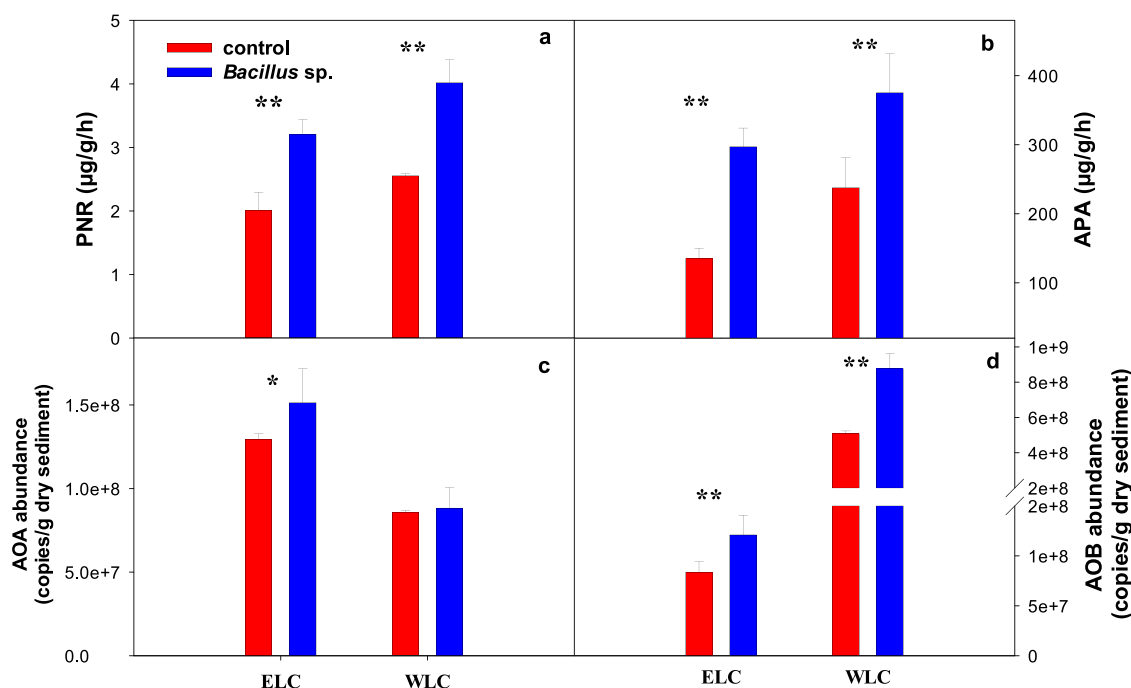

Figure 7. Comparisons of PNRs (a), APA (b), AOA abundances (c), and AOB abundances (d) between treatments in the microcosm experiment to evaluate effects of organic P hydrolysis on nitrification. *Bacillus* sp. represents treatment added with OPB isolated and enriched from sediment of Lake Chaohu. Significant levels are indicated by "*", "**", and "****" with $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. Bars show means of replicate measurements with standard errors.

limitation, indicated by high DIN/SRP ratios in interstitial waters. This is in conformity with the principle of microbial nutrient stoichiometry; that is, stoichiometry of microbial biomass in relation to ambient nutrient availability determines microbial nutrient limitation, and microbial metabolism of one nutrient can also be limited by another.[59] P promotion on

nitrification[60,61] as well as denitrification[50,60,61] was reported in a wide range of ecosystems. In addition, though P-rich wastewater was considered to be the inhibitor to anammox activity of granules or flocs, lower-level P (155 mg/L), which is far higher than in the lake ecosystem, could stimulate its activity.[62] In conclusion, P released from the sediment during eutrophication may contribute to N loss from the lake ecosystem through facilitating coupled N transformation processes including nitrification−denitrification and nitrification-annamox.

Under anoxic/anaerobic conditions, $Fe(OOH) \sim P$ had an extremely high P release potential in the sediment of shallow lakes.[63] Degradation of organic matter originating from recent phytoplankton blooms in many shallow lakes was thought to decrease the redox potential in the surface sediment and contribute to the release of P adsorbed on $Fe(OOH) \sim P$.[64] Nitrification is an oxygen-consuming process that consumes 2 mol of oxygen per mol of ammonium oxidized. Oxygen consumption by nitrification accounted for 27−30% of the total oxygen consumption in sediments of Lake Erie.[65] Nitrification promoted by P accumulation may contribute to oxygen depletion in surface sediments and then lead to P release to water columns, which can contribute to relative N deficiency further. Thus, N and P cycles in eutrophic lakes' sediment do not exist independently but rather interact with each other. Namely, P enrichment during cyanobacterial blooms can fuel nitrification activities in sediments, and pronounced nitrification can facilitate release of available phosphate from sediments to water columns in turn. These processes further accelerate relative nitrogen deficiency. So, it is far from enough to mitigate lake eutrophication by controlling P or N sorely, while dual control of N and P is recommended for lake management.

In summary, our results confirmed relative importance of N and P in limiting productivity transited in shallow eutrophic lakes during algal bloom. The average TN/TP mass ratio of Lake Chaohu was 26.1 (P limited) in June and then dropped to 7.7 and 7.8 (N limited) in August and September, respectively. Interactions between sediment P and nitrification may drive these shifts. Monthly field investigations across a year and microcosm experiments showed that sediment P enrichment, both inorganically and organically, can fuel nitrifiers and nitrification activity, leading to relative N deficiency. These processes in sediments may trigger the succession from non-N_2-fixing cyanobacteria to N_2-fixing cyanobacteria after summer blooms. Our results provide novel insights that N and P cycles do not exist independently but rather interact with each other dynamically during lake eutrophication, supporting the dual N and P reduction program to mitigate eutrophication in shallow eutrophic lakes.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c07599.

> Detail methods for microcosm experiments to detect endogenous P effects on nitrification (Figure S1), schematic diagram microcosm experiment to evaluate effects of organic P hydrolysis on nitrification (Figure S2), *Microcystis* and *Dolichospermum* abundance in water columns of Lake Chaohu from June, August, and November (Figure S3), *Microcystis* abundance (top

panel) and *Dolichospermum* abundance (bottom panel) in water columns of ELC and WLC from June, August, and November, "*" above the column indicating a significant difference between ELC and WLC with $P <$ 0.05 (Figure S4), AOA abundance (top panel), AOB (middle panel) abundance, and ratios of AOA/AOB (log 10) (bottom panel) in sediments of Lake Chaohu from different seasons, "*" or "ns" above the column indicating a significant difference between ELC and WLC with $P < 0.05$ or $P \geq 0.05$, respectively (Figure S5), content of different P fractions in sediment of east and west Chaohu, $Fe(OOH) \sim P$, $CaCO_3 \sim P$, ASOP, and Palk representing iron-bound P, calcium-bound P, acid-soluble organic P, and hot NaOH-extractable organic P, respectively, bars indicating means with standard errors (Figure S6), APA in sediment of ELC and WLC from different months, significant levels indicated by "*", "**", and "****" with $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively (PDF)

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Notes

The authors declare no competing financial interest.

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