SUPPORTING INFORMATION

for

Interactions between phosphorus enrichment and nitrification accelerate relative nitrogen deficiency during cyanobacterial blooms in a large shallow eutrophic lake

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1. Materials and methods for SI

1.1 Microcosm experiment to evaluate effects of SRP on nitrification

In this study, method developed by de Vet et al. was used to assess the effect of SRP on ammonia-oxidizers in sediment of Lake Chaohu.[1] Sediment cores were collected in October 2016 from both ELC and WLC, and then uniformly mixed to provide ammonia-oxidizers.

(1) Culture medium

Inorganic nutrient solution was used as the medium for both the enrichment of the mixed inoculum and the incubation experiments. To ensure that only P of the inorganic nutrients is limiting for microbial growth, nutrients including trace elements were added as follows: 2.14 mM NH_4HCO_3; 1.09 mM $KHCO_3$; 0.992 mM $CaCl_2{\cdot}2H_2O$; 0.44 mM $MgSO_4{\cdot}7H_2O$; 2.40 mM $NaHCO_3$; 0.12 mM Na_2-EDTA; 1mM $FeCl_3{\cdot}6H_2O$; 1 mM $MnCl_2{\cdot}4H_2O$; 1μm $ZnSO_4{\cdot}7H_2O$, 1μm $CoCl_2{\cdot}6H_2O$, 1μm $CuSO_4{\cdot}5H_2O$, 1μm $Na_2MoO_4{\cdot}2H_2O$).

(2) Inoculum

A mixed culture of ammonia-oxidizers originating from mixed sediment was used as inoculum. To be specific, 5 g of mixed sediment samples were added to 200 mL of culture medium described above and then incubated for 3 days at 25 ℃ and 180 rpm in an aerated condition. After the culture, 1 mL of the inoculum was added to another 200 mL of culture medium to operate the next culture cycle. Total 5 culture cycles were conducted to obtain ammonia-oxidizers inoculum.

(3) Experimental setup

According to SRP concentrations in the interstitial water of Lake Chaohu, 5 treatments of incubation experiments were executed under the addition of different amounts of phosphate into 200 mL of culture medium described above with final SRP concentrations 0, 0.01, 0.02, 0.05, and 0.1 mg/L. 1 mL of inoculum obtained was added to each culture medium. Incubations were conducted for 288 hours at 25 ℃ and 180 rpm in an aerated condition. All treatments were run in triplicate.

NO_2^- and NO_3^- concentrations were measured every 24 hours to monitor nitrification activity. Monod equation was used to fit NO_2^- + NO_3^- generation curve, and then maximum slopes of each curve were calculated through MATLAB software to characterize the maximum nitrification rate (PNR_{max}). After the end of the incubation, 50 mL of sample was taken for the DNA extraction using the Power Water DNA kit (Mo Bio Laboratories, Carlsbad, CA) following the manufacturer's instructions. AOA and AOB concentrations targeting *amoA* genes were determined by qPCR.

1.2 Microcosm experiment to evaluate effects of organic P hydrolysis on nitrification

(1) OPB isolation and enrichment

OPB was isolated from sediment of Lake Chaohu, collected in October 2016, using the traditional colony forming unit (CFU) method.[2] Briefly, slurry containing 5 g sediment and 45 mL sterile water was diluted for1000 times. 0.2 mL diluent was added to organic phosphorus medium (glucose, 10 g; lecithin, 0.025 g; $CaCO_3$, 5 g; NaCl, 0.3 g; $(NH_4)_2SO_4$, 0.5 g; $MgSO_4$, 0.3 g; KCl, 0.3 g; $MnSO_4$, 0.03 g; $FeSO_4$, 0.036 g; Agar, 20 g; pH 7.2; sterile water, 1000 mL). Dominated bacterial colonies

were picked up based on unique colony morphology after incubated at 30 °C for 96 h, and then transferred into Luria-Bertan medium for the enrichment. Dominated isolates were screened for phylogenetic analysis of the partial 16S rRNA gene sequences according to Yang et al. .[3]

(2) Experimental setup

The top of 5 cm sediment cores of ELC and WLC collected in October 2016 were placed into PVC columns with a diameter of 10cm, covered by 20 cm filtered and sterilized lake water. OPB enriched from Chaohu sediment, which was affiliated to *Bacillus* sp. according to phylogenetic analysis described above, were inoculated to both ELC and WLC sediments. Treatments without OPB inoculation were as control and all treatments were run in triplicate at 25 ± 1 ℃ in the dark for 20 days. After incubation, sediments of each treatment were used to determine PNRs, APA, AOA and AOB abundances.

2. Supporting Figures

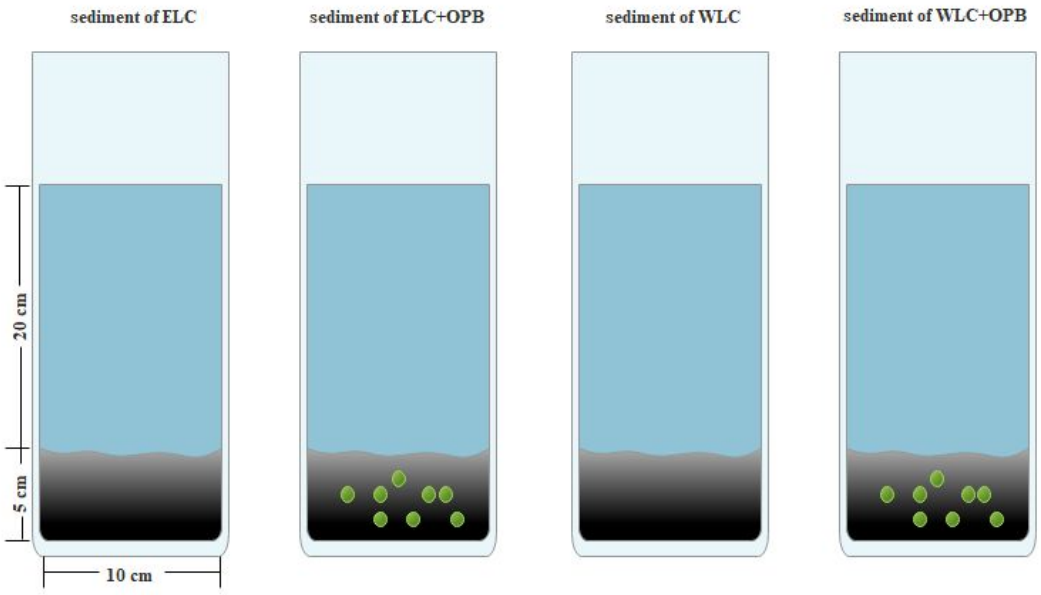

Figure S1. A schematic diagram microcosm experiment to evaluate effects of organic P hydrolysis on nitrification

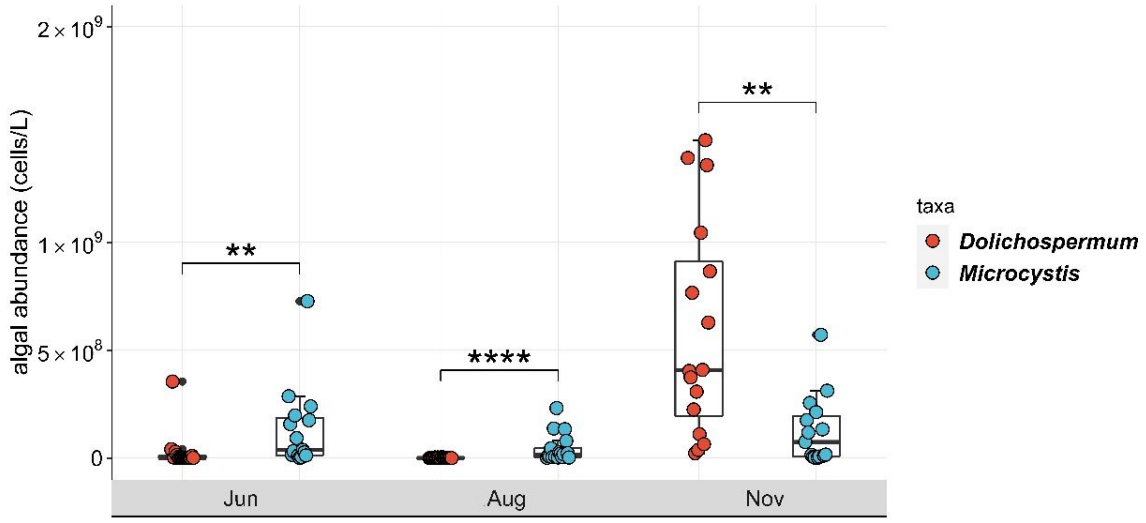

Figure S2. *Microcystis* and *Dolichospermum* abundance in water columns of Lake Chaohu from June, August and November.

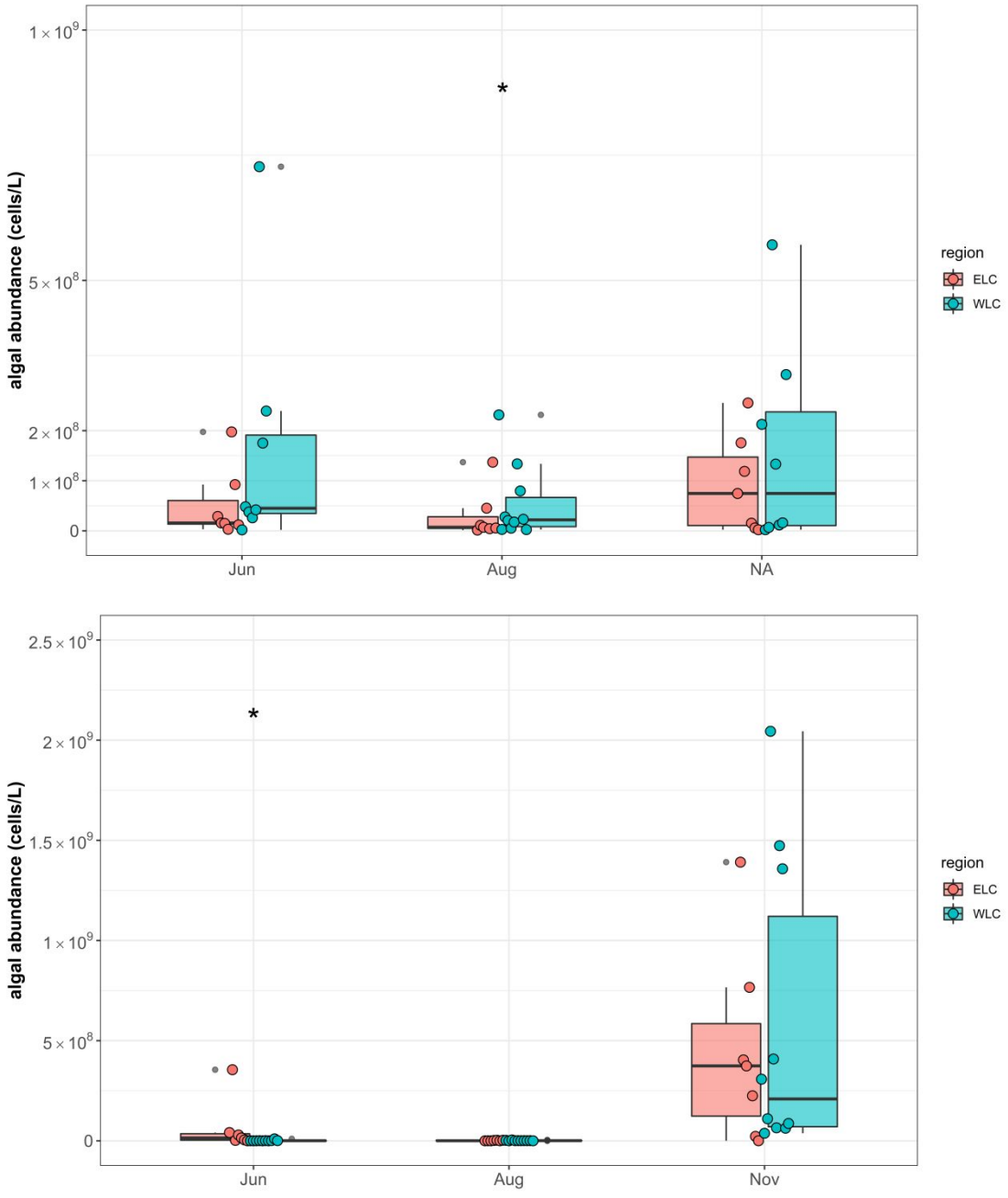

Figure S3. *Microcystis* abundance (top panel) and *Dolichospermum* abundance (bottom panel) in water columns of ELC and WLC from June, August and November. "*" above the column indicates there is a significant difference between ELC and WLC with $P<0.05$.

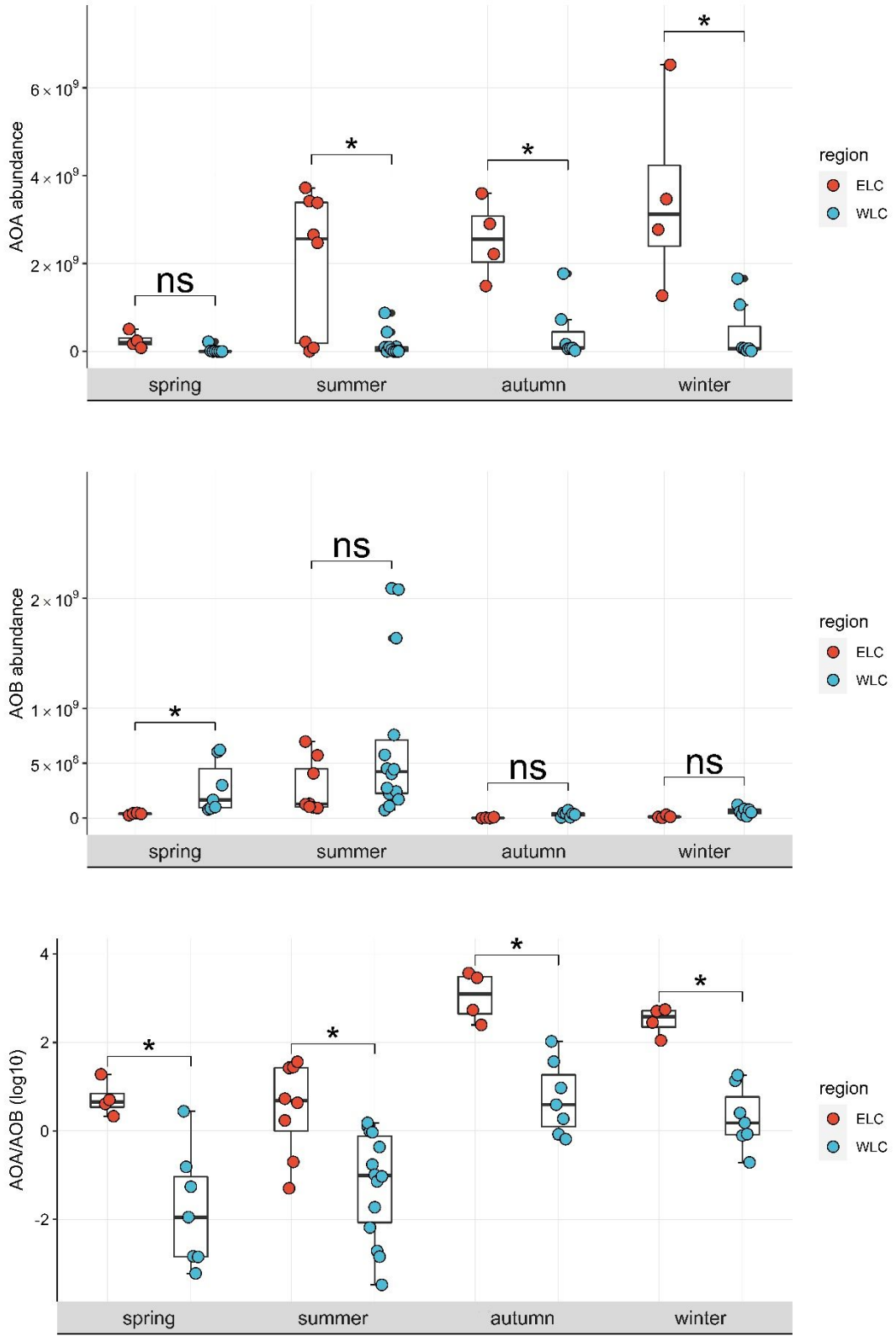

Figure S4. AOA (top panel), AOB (middle panel) abundance and ratios of AOA/AOB (log 10) (bottom panel) in sediments of Lake Chaohu from different

seasons. “ *” or “ns”above the column indicates there is a significant difference between ELC and WLC with $P<0.05$ or $P\geq0.05$, respectively.

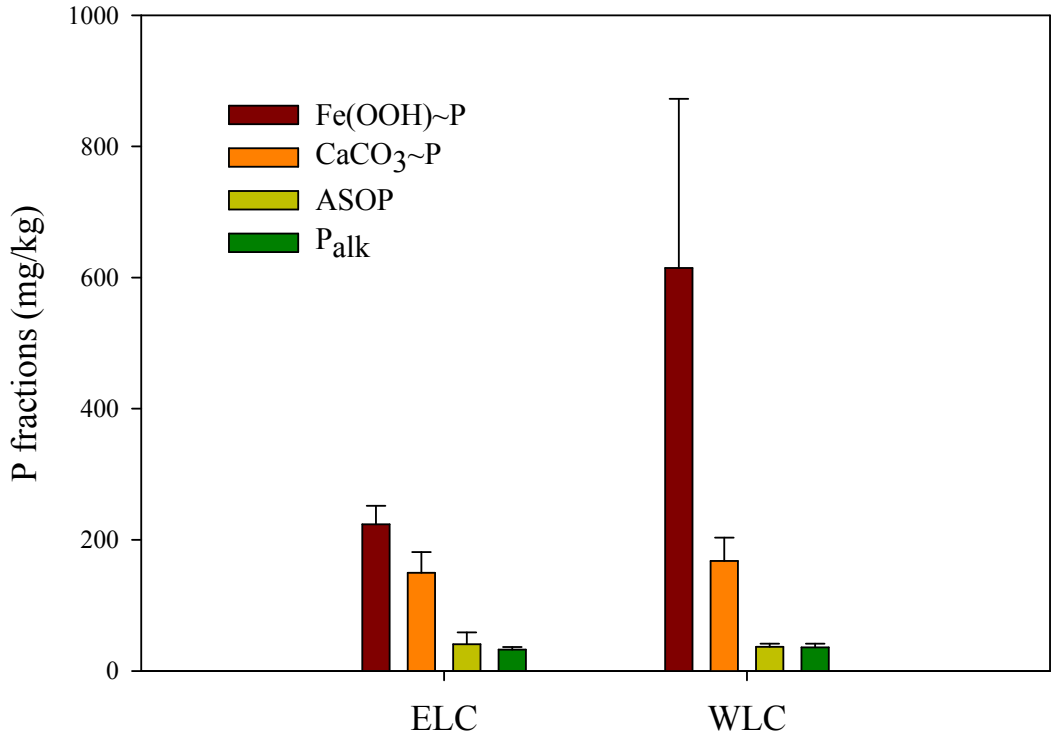

Figure S5. Content of different P fractions in sediment of east and west Chaohu. Fe(OOH)~P, $CaCO_3$~P, ASOP, and P_{alk} represent iron-bound P, calcium-bound P, acid-soluble organic P, and hot NaOH-extractable organic P, respectively. Bars indicate means with standard errors.

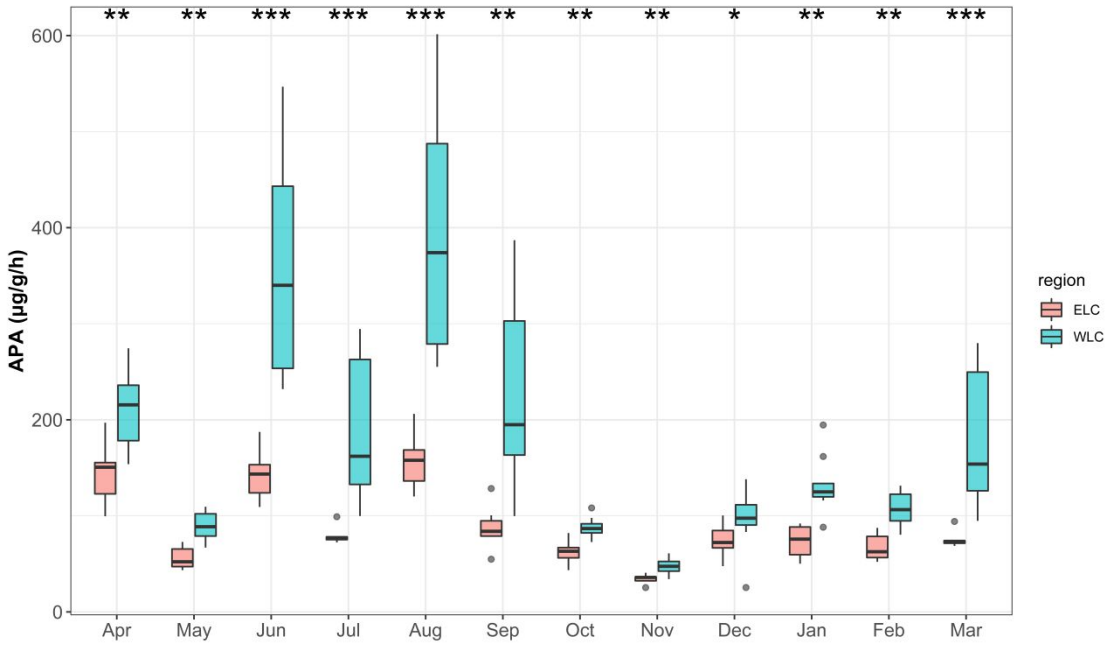

Figure S6. APA in sediment of ELC and WLC from different months. Significant levels are indicated by "*", "**" and "****" with $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

3. References for SI

[1] de Vet, W.; Loosdrecht, M.; Rietveld, L. C. Phosphorus limitation in nitrifying groundwater filters. Water Research. 2012, 46, 1061-1069.

[2] Shekhar, N. C. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. Fems Microbiology Letters. 1999, 170, 265-270.

[3] Yang, L.; Liu, Y. Q.; Cao, X. Y.; Zhou, Z. J.; Wang, S. Y.; Xiao, J.; Song, C. L.; Zhou, Y. Y. Community composition specificity and potential role of phosphorus solubilizing bacteria attached on the different bloom-forming cyanobacteria. Microbiological Research. 2017, 205, 59-65.