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# Community structure of aerobic anoxygenic phototrophic bacteria in algae- and macrophyte-dominated areas in Taihu Lake, China*

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**Abstract** Aerobic anoxygenic phototrophic bacteria (AAPB) represent a major group of bacterioplankton assemblages in many water systems and some are assumed to be closely associated with phytoplankton. However, studies on relationships between AAPB and cyanobacterial blooms are in scarcity. The dynamics of the abundance and diversity of AAPB was compared based on *pufM* gene in Meiliang Bay (featured by cyanobacterial blooms) and East Bay (featured by macrophyte) of Taihu Lake, a shallow subtropical lake in the East China plain. AAPB abundance was not significantly different between the two sites, and they were positively correlated with dissolved organic carbon (DOC) concentration. The ratios of AAPB to total bacteria varied from 3.4% to 11.5% and peaked in winter in both sites. No significant differences of AAPB community compositions were detected between the two sites, but there was a separation between warm seasons (June, August, and October) and cold seasons (December, February, and April). Rhizobiales and *Limnohabitans*-like *pufM* sequences were significantly contributors for the difference between two seasons, and specially enriched in cold seasons. Chlorophyll *a* (Chl *a*) and DOC were the most significant variables influencing the AAPB community structure. Furthermore, *Porphyrobacter* and Rhodospirillales-like *pufM* sequences were positively correlated with Chl *a*, indicating potential influence of cyanobacterial blooms on these AAPB taxa. These results suggested that diverse AAPB ecotypes coexisted in Taihu Lake, and their ecological role in carbon cycling in the lake may not be ignored.

**Keyword**: aerobic anoxygenic phototrophic bacteria; cyanobacterial blooms; dissolved organic carbon; Alphaproteobacteria; chlorophyll *a*

## 1 INTRODUCTION

Aerobic anoxygenic phototrophic bacteria (AAPB) are heterotrophs that can obtain additional energy from light, and exhibit phototrophy and heterotrophy without producing oxygen (Yurkov and Beatty, 1998; Kolber et al., 2001). They are widely distributed in oceans, estuaries, lakes, and rivers (Koblížek et al., 2006; Mašín et al., 2008), and may be remarkable contributors to the productivity and carbon cycling of aquatic ecosystems (Jiao et al., 2010). The growth rate and efficiency in organic carbon utilization of these bacteria are higher than those of other strict

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heterotrophs, and can survive diverse environmental conditions and outgrow competitors (Koblížek et al., 2007; Cepáková et al., 2016).

In freshwater systems, AAPB may constitute various fractions ranging from 1% to 80% of the total bacterioplanktonic community in different trophic lakes (Mašín et al., 2008, 2012; Čuperová et al., 2013; Fauteux et al., 2015). AAPB are phylogenetically diverse and mainly composed of Alpha-, Beta-, and Gammaproteobacteria. The taxonomic compositional structure of AAPB varies in different aquatic systems. Alpha- and Gammaproteobacteria-like AAPB are generally dominant in saline water systems (Allgaier et al., 2003; Jiao et al., 2007; Jiang et al., 2009; Lehours et al., 2010; Jeanthon et al., 2011; Boeuf et al., 2013), while Betaproteobacteria-like AAPB are dominant in freshwater systems (Waidner and Kirchman, 2005, 2008; Ferrera et al., 2017b). Betaproteobacteria-like AAPB are dominant in Lake Stechlin, an oligotrophic freshwater lake, whereas Alphaproteobacteria-like AAPB dominate the humic matter-rich southwest basin of Lake Grosse Fuchskule, another freshwater body (Salka et al., 2011). Sphingomonadales-like AAPB affiliated with Alphaproteobacteria are dominant in an alpine oligotrophic lake (Čuperová et al., 2013). The variability in the AAPB community structure is likely attributed to environmental factors, such as trophic status, light attenuation, salinity gradients, inorganic nitrogen levels, total phosphorus contents, dissolved organic carbon (DOC), and temperature of aquatic environment where they inhabit (Jiang et al., 2010; Lehours et al., 2010; Caliz and Casamayor, 2014).

Additional to the above-mentioned abiotic factors, close associations between AAPB and phytoplankton has been demonstrated in cyanobacterial mats, microalgae, and dinoflagellate cultures in some pioneer works (Allgaier et al., 2003; Green et al., 2004; Goecke et al., 2013). Specific response of AAPB to different algal species has also been observed (Chen et al., 2011). Due to the increased relative abundance of light-dependent AAPB, bacterial community composition in phycosphere was linked closely to light intensity (Piwosz et al., 2020). Distribution of AAPB is affected by the availability of organic carbon derived from phytoplankton found in global oceans (Kolber et al., 2001; Jiao et al., 2007; Zhang and Jiao, 2007). Rhodobacteracea-like AAPB affiliated with Alphaproteobacteria become dominant when chlorophyll *a* (Chl *a*) and nutrient concentrations in an oligotrophic area in the Northwestern Mediterranean increase (Ferrera et al., 2014). These studies indicated close relationships between AAPB and phytoplankton. Moreover, in our study on bacteria in phycosphere of cyanobacteria, we also observed that AAPB are associated not only with cyanobacterial cultures but also with cyanobacterial colonies (Shi et al., 2010). So we proposed that there may be some association between AAPB and cyanobacterial blooms which occur in freshwaters and spread worldwide quickly under climate warming (Paerl and Paul, 2012).

Taihu Lake is well known for annual cyanobacterial blooms, which have greatly changed bacterial communities. However, whether the compositions of AAPB are influenced by cyanobacterial blooms is still unknown. Given special feature of AAPB and their importance in carbon and nutrient cycling, it is urgent to reveal their distribution pattern in Taihu Lake. In this study, we compared AAPB populations in Meiliang Bay characterized by dense cyanobacterial blooms and East Bay characterized by macrophyte in Taihu Lake in China. In Meiliang Bay, with annual average Chl-*a* concentrations throughout the water column increased from 23 μg/L in 1992 to peaks around 50 μg/L in 1998, cyanobacterial bloom biomass steadily remained at high levels up to 2010 (Xu et al., 2017). Coverage of cyanobacterial blooms remained moderate and stable until 2015, and then reached another peak around 2017 (Jia et al., 2019). In East Bay, remote sensing mapping revealed a decreased aquatic vegetation presence frequency during 2003–2014 (Zhang et al., 2016). Macrophyte cover declined linearly from 36% in 2002 to 17% in 2015, which was largely due to the loss of submerged macrophytes, which decreased significantly from 88.5% in 2000 to 45.8% in 2013 (Zhang et al., 2019). Here, we performed distance-based redundancy analysis (dbRDA) and Pearson correlation analysis to determine the key environmental factors driving AAPB distribution.

## 2 MATERIAL AND METHOD

### 2.1 Study area

Taihu Lake is located between 30°56′N–31°33′N and 119°53′E–120°36′E in Jiangsu Province, East China, and is the third-largest freshwater lake in China. With a surface area of 2 338 km$^2$, a mean depth of 1.9 m, and a water residence time of approximately 284 days (Qin et al., 2007), the lake is used as the drinking water source of several cities, including Shanghai, Suzhou, Wuxi, and Huzhou. In addition, it is an important water source for irrigation for farming,

industry, and recreational activities (Qin et al., 2007). However, with rapid economic development, the lake water has been seriously polluted. The lake as a whole is eutrophic and affected by cyanobacterial blooms dominated by *Microcystis* spp. (Chen et al., 2003; Ma et al., 2016). Most of the lakes, especially Meiliang Bay, suffered from cyanobacterial blooms. The eastern region (the East Bay) is mesotrophic and mainly covered by submerged macrophytes. In our study, water samples were collected in the Meiliang Bay (Site N, 31°25′53.8″N, 120°12′42.5″E) and the East Bay (Site E, 31°3′5″N, 120°27′59.8″E) of Taihu Lake (Fig.1).

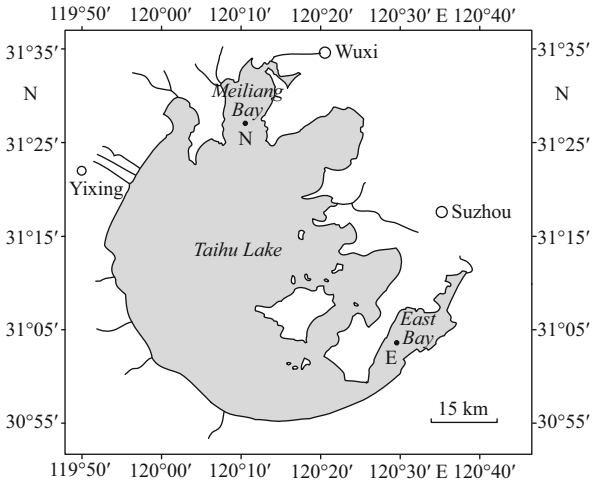


**Fig.1 Map of Taihu Lake and the sampling sites (N and E)**

## 2.2 Sample collection and handling

Water samples (0–0.5-m depth) were collected using water sampler bimonthly during August 2011 to June 2012. Water temperature, pH, and dissolved oxygen (DO) were determined in situ using an YSI 6600 multiparameter underwater sensor (Yellow Springs Instrument, Model, USA). Nutrient concentration including dissolved inorganic nitrogen, phosphorus, total nitrogen (TN), and phosphorus (TP) were measured using a Skalar auto analyzer (Skalar, San Plus System). For determination of Chl *a* and phycocyanin (PC), 100 mL of water samples were filtered using GF/F filters (Whatman, 47 mm), and the filters were extracting with 90% acetone for Chl *a* and 0.1-mol/L phosphate buffer (pH 6.8) for PC respectively, and absorbance of the extracts was measured accordingly using a spectrofluorophotometer (ModelRF-5301PC; Shimadzu) (Asai et al., 2001). The dissolved organic carbon (DOC) concentration in filtrate was analyzed with a total organic carbon analyzer (TOC-5000, Shimadzu, Tokyo, Japan). For determination of AAPB, 100 mL of water samples was filtered through polycarbonate filters (Millipore, 0.2-μm pore size, 47-mm diameter) using moderate vacuum, and the filters were stored at -70 °C until DNA extraction.

## 2.3 DNA extraction

Community DNA was extracted from the 0.2-μm filters using xanthogenate-sodium dodecyl sulfate (SDS) DNA extraction protocols according to Tillett and Neilan (2000). Briefly, filters were cut into small pieces, put into a 2-mL sterile Eppendorf tube with 50-μL TER (10-mmol/L Tris-HCl, pH 7.4; 1-mmol/L EDTA, pH 8; 100-μg m/L RNase A), 750-μL freshly made XS buffer (1% potassium ethylxanthogenate; 100-mmol/L Tris-HCl, pH 7.4; 20-mmol/L EDTA, pH 8; 1% sodium dodecyl sulfate; 800-mmol/L ammonium acetate) were added, then the tubes were incubated at 70 °C for 120 min, after that the tubes were vortexed for 10 s before placed on ice for 30 min. The supernant DNA was precipitated with isopropanol, washed with 70% ethanol, and dissolved in 50-μL sterile distilled water.

## 2.4 Quantitative real-time PCR (qPCR)

The abundances of AAPB, total bacteria were determined by qPCR based on their specific primers using Master cycler ep Realplex (Eppendorf, Germany). For AAPB, PCR amplifications of the partial sequences of the *pufM* gene were conducted using the forward primer *pufM*557F (5′-TACGGSAACCTGTWCTAC-3′) and reverse primer *pufM*750R (5′-CCATSGTCCAGCGCCAG-AA-3′) (Achenbach et al., 2001; Béjà et al., 2002; Du et al., 2006; Hu et al., 2006; Jiao et al., 2007). The PCR products were 193-bp fragments. The reaction mixtures (25 μL) contained 12.5-μL SYBR Premix Ex Taq (TaKaRa, Japan) and 2.5 pmol of each primer. The amplification conditions consisted of 94 °C for 4 min, followed by 30 cycles at 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 1 min, and an extension at 72 °C for 5 min (Du et al., 2006). For total bacteria, qPCR amplifications was conducted by using Bac331F (5′-TCCTACGGGAGGCAGCAGT-3′)/Bac797R (5′-GGACTACCAGGGTCTAATCCTGTT-3′) (Nadkarni et al., 2002; Jiang et al., 2009). The PCR products were gel-purified, and cloned into *Escherichia coli* DH5α cells following the manufacturer's instructions. Inserts in the clones were confirmed through PCR using the above specific primers and subsequent electrophoresis. Dilution series ($10^3$ to $10^8$ copies/μL) of the purified plasmids containing the target genes

were used as standard DNA templates to make the standard curves. QPCR for AAPB and total bacteria was conducted in triplicate as described above except the amplification cycles were changed from 30 to 45. The standard curve was determined by plotting the threshold cycle number ($C_t$) values versus the standard DNA concentrations, thus DNA concentrations in samples can be calculated based on their $C_t$ values.

### 2.5 PCR and clone library construction

The partial sequences of *pufM* gene of AAPB were amplified using the primer set *pufM*557F and *pufM*750R, as described above. PCR reaction mixtures (25 μL) contained 0.2 mmol/L of each primer, 20-ng template DNA, 1.5-mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, and 5-U Taq DNA polymerase (TaKaRa, Japan). A total of 12 *pufM* gene clone libraries were constructed from the 12 samples. For each sample, the PCR products of *pufM* gene amplified in triplicate were pooled, ligated to a pGEM-T easy vector (Promega Corp., USA), and cloned into *E. coli* DH5α cells. Fifty positive clones were randomly selected from each clone library, examined through PCR by using the vector primers T7 and SP6, and sequenced with an ABI DNA sequencer. All of the clone sequences retrieved from this study were subjected to a chimera test with the CHIMERA_CHECK program available in the Ribosomal Database Project (RDP) website (Cole et al., 2003). A total of 482 nonchimeric *pufM* sequences were grouped into operational taxonomic units (OTUs) with 6% divergence of nucleic acid sequence (Zeng et al., 2007) by using Mothur (Schloss et al., 2009). Rarefaction analysis computed in Mothur was conducted to estimate the total diversity in each clone library. The coverage of each clone library was determined with the formula $[1-(OTU_N/sequence_N)]\times100$, where $OTU_N$ is the number of OTUs, and $sequence_N$ is the total number of sequences (Jiang et al., 2010).

### 2.6 Phylogenetic analysis

Almost the complete *pufM* sequences longer than 900 bp of the cultured species and the environmental clones were retrieved from GenBank database (http://www.ncbi.nlm.nih.gov/Genbank/). Sequences were automatically aligned with Clustal W in Mega 4.0. A *pufM* database containing 190 aligned sequences was then imported into the ARB database (http://www.arb-home.de/). A backbone tree was calculated from these sequences with a maximum-parsimony algorithm and saved as a positional tree server in the ARB software package. The partial *pufM* sequences obtained in this study were then inserted into the pre-established core tree by using the ARB parsimony tool and maintaining the overall tree topology without changes. The trees were then exported as Newick files and edited online via the Interactive Tree of Life (iTOL) (http://itol.embl.de) (Letunic and Bork, 2007). The phylogenetic affiliations of *pufM* sequences were based on the phylogenetic tree and compared with the sequences in the National Center for Biotechnology Information (NCBI) database via the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences obtained in this study were deposited in the European Molecular Biology Laboratory (EMBL) database under accession numbers HF947097 to HF947271.

### 2.7 Statistical analysis

Nonmetric multidimensional scaling (NMDS) analysis based on Bray-Curtis algorithm distance matrix was performed for all samples on the OTU level. Distance-based redundancy analysis (dbRDA) was used to examine the influence of detected environmental factors including temperature, pH, DO, TN, $NH_4^+$, $NO_x^-$ ($NO_2^-$+$NO_3^-$), TP, $PO_4^{3-}$, DOC, and Chl *a* on the dynamics of AAPB composition. Significance of variables was assessed with Monte-Carlo permutation tests (999 unrestricted permutations). All these analyses were performed with the "vegan" package (Oksanen et al., 2013) of R software (R Development Core Team 2012). Taxa that were significantly different between the two sites and two major different seasons were detected using the Bioconductor-edgeR package (version 3.2.4) (Robinson et al., 2010). In addition, Pearson correlations were run to investigate the relationship between environmental factors and dominant *pufM* genotypes using SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA).

## 3 RESULT

### 3.1 Environmental parameter

Seasonal variation of environmental variables and nutrient concentrations of surface water are shown in Table 1. Water temperature varied from 4.4 °C to 30.9 °C throughout the year. The oxygen concentration varied from 5.7 to 13.6 mg/L, and was significantly negatively correlated with temperature ($R$=-0.88, $P$<0.01, $n$=12). In Site N, cyanobacterial blooms dominated by *Microcystis* occurred from June

**Table 1 Basic physico-chemical characteristics of water samples from Taihu Lake**

| Sample | DOC (mg/L) | $PO_4^{3-}$(μg/L) | $NH_4^+$ (mg/L) | $NO_3^-$ (mg/L) | $NO_2^-$ (mg/L) | TN (mg/L) | TP (mg/L) | Chl *a* (μg/L) | PC (μg/L) | Temp (°C) | pH | DO (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aug-N | 3.87 | 28.34 | 0.019 | 0.16 | 0.044 | 1.83 | 0.14 | 23.21 | 37.37 | 27.8 | 9.6 | 7.3 |
| Oct-N | 6.28 | 16.73 | 0.035 | 0.09 | 0.003 | 0.54 | 0.04 | 31.76 | 65.99 | 17.8 | 7.3 | 9.1 |
| Dec-N | 4.49 | 8.59 | 0.034 | 0.41 | 0.005 | 2.01 | 0.10 | 31.06 | 57.77 | 4.5 | 8.0 | 12.9 |
| Feb-N | 4.40 | 3.09 | 0.023 | 1.54 | 0.014 | 2.43 | 0.04 | 6.81 | 3.62 | 4.4 | 8.2 | 13.6 |
| Apr-N | 4.65 | 5.70 | 0.069 | 0.37 | 0.030 | 1.07 | 0.01 | 1.96 | 4.37 | 18.8 | 7.9 | 9.2 |
| Jun-N | 4.45 | 0.09 | 0.013 | 0.80 | 0.008 | 1.52 | 0.04 | 65.15 | 157.80 | 25.1 | 8.7 | 8.8 |
| Aug-E | 2.99 | 4.33 | 0.015 | 0.17 | 0.002 | 0.24 | 0.01 | 4.93 | 2.14 | 30.9 | 8.1 | 5.7 |
| Oct-E | 3.61 | 29.73 | 0.036 | 0.16 | 0.003 | 0.25 | 0.02 | 3.39 | 2.99 | 20.0 | 8.1 | 7.2 |
| Dec-E | 5.30 | 1.58 | 0.032 | 0.26 | 0.006 | 0.90 | 0.08 | 5.01 | 2.03 | 5.7 | 8.3 | 9.3 |
| Feb-E | 10.72 | 2.11 | 0.083 | 0.17 | 0.003 | 0.72 | 0.02 | 3.30 | 0.85 | 6.4 | 7.6 | 10.9 |
| Apr-E | 10.11 | 1.66 | 0.004 | 1.51 | 0.052 | 0.84 | 0.01 | 5.59 | 1.99 | 19.6 | 8.5 | 7.9 |
| Jun-E | 6.13 | 3.23 | 0.060 | 1.42 | 0.017 | 0.62 | 0.01 | 5.30 | 2.49 | 26.1 | 8.0 | 6.3 |

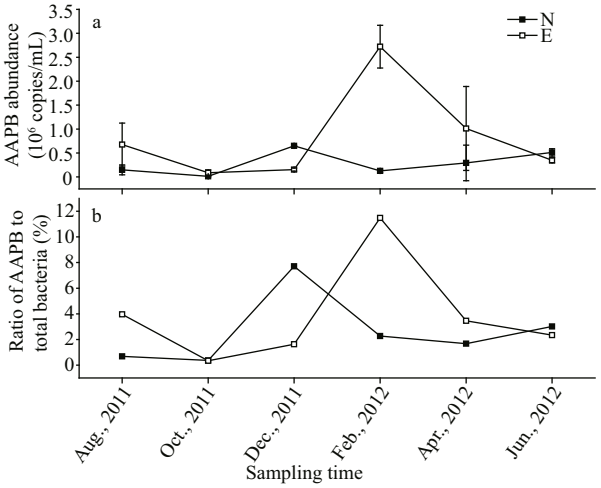


**Fig.2 Abundance of AAPB based on *pufM* gene (a) and the ratio of AAPB to total bacteria (b) in the two sampling sites in Taihu Lake**

Meiliang Bay (N) and East Taihu (E).

to October. The Chl-*a* and PC concentration varied from 1.96 to 65.15 μg/L and 3.62 to 157.80 μg/L respectively, with a maximum value in June. In Site E, submersed macrophytes were dominant with relatively low cyanobacterial biomass. The concentrations of Chl *a* and PC varied from 3.30 to 5.59 μg/L and 0.85 to 2.99 μg/L respectively. There were significantly correlated relationships between PC and Chl *a* (Pearson correlation, $R$=0.993, $P$<0.001). TN in Site N was significantly higher than that in Site E ($P$<0.01, ANOVA). Temperature was significantly higher while DO was significantly lower in warm seasons (June, August, and October) than that in cold seasons (December, February, and April) ($P$<0.05, ANOVA). There were no significant differences of other detected environmental variables between the two sites, or between warm and cold seasons.

### 3.2 Abundance of AAPB

QPCR based on the *pufM* gene showed that AAPB in Site N varied from $0.01\times10^6$ (±10 260) to $0.65\times10^6$ (±10 360) copies/mL, whereas that in Site E varied from $0.08\times10^6$ (±58 900) to $2.72\times10^6$ (±446 000) copies/mL (Fig.2a). Amplification efficiencies of 1.11–1.32 were obtained with $R^2$ values of 0.985 to 0.990. The ratio of AAPB to total bacteria in Sites N and E ranged from 0.68% to 7.70%, and from 0.34% to 11.47%, respectively, with peak values in December or February when temperature was around 4 °C in Site N and E, respectively (Fig.2b). Pearson correlation analysis revealed that abundance of AAPB was positively correlated with DOC concentrations ($R$=0.741, $P$<0.01, $n$=12).

### 3.3 Phylogenetic analyse of *pufM* gene

A total of 482 clones (243 clones from Site N and 239 clones from Site E) were obtained from the 12 *pufM* gene clone libraries, and a total of 136 distinct OTUs were identified after the sequences were grouped at 94% nucleic acid sequence similarity. Among the 136 OTUs, 57 and 43 were unique in Sites N and E, respectively, and 36 were shared by the two sites (Fig.3a). The genetic diversity of *pufM* in Sites N and E varied from 2.26 to 3.19 and from 1.73 to 2.88, respectively. The coverages of *pufM* in Sites N and E varied from 33% to 57% and from 41% to 70%, respectively (Table 2). The rarefaction curves appeared to be far from the saturation level (Fig.3b).

Most OTUs exhibited 80% to 97% similarity

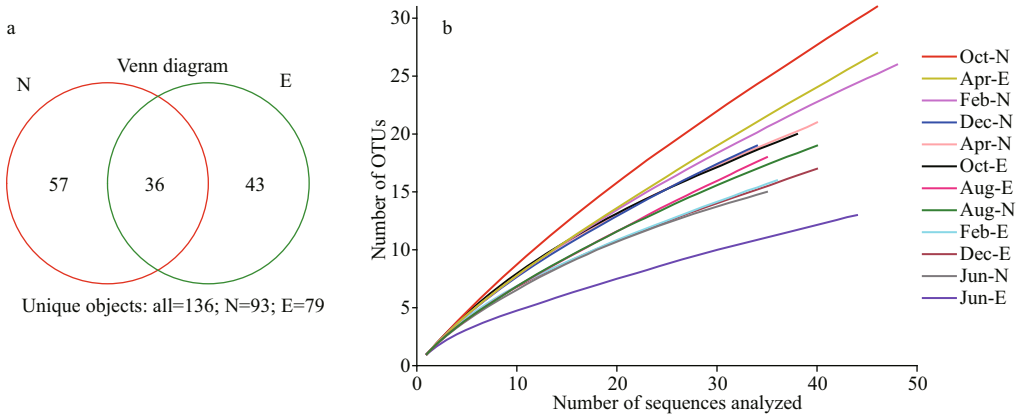


**Fig.3 Diversity of AAPB genotypes**

a. the Venn diagram showing the number and abundance of the OTUs based on *pufM* gene found in the two sites; b. rarefaction curves of the observed species (94% OTUs) from AAPB based on *pufM* gene in the 12 clone libraries.

with *pufM* of the cultured anoxygenic phototrophs. Phylogenetic analysis demonstrated that most *pufM* gene sequences were affiliated with the Alphaproteobacteria clade (Fig.4). Alphaproteobacteria-like *pufM* sequences were dominated by three orders/genera, namely, Rhizobiales-, *Rhodobacter*-, and *Porphyrobacter*-like organisms (Fig.5). *Limnohabitans*-like *pufM* sequences affiliated with Betaproteobacteria were also a dominant group from December to April in both sites (Fig.5).

No significantly different genotypes were observed between the two sites. Most *pufM* sequences were present in both sites, but *Sandarakinorhabdus* and *Methylobacterium*-like *pufM* sequences were only detected in Site N, and *Blastomonas*-like *pufM* sequences were only detected in Site E (Fig.5). NMDS analysis revealed that no significiant difference occurred between the two sampling sites, but a separation between warm seasons (June, August, and October) and cold seasons (December, February, and April) existed (Fig.6). EdgeR analysis revealed that 1 OTU affiliated with Rhizobiales and 5 OTUs affiliated with *Limnohabitans*-like *pufM* sequences significantly contributed to the difference between the two seasons, and they were specially enriched in cold seasons ($P$<0.05, false discovery rate (FDR)-corrected) (Table 3).

### 3.4 Distribution of *pufM* diveristy in relation to environmental factors

DbRDA results illustrated that Chl *a* and DOC were the most significant variables (Monte Carlo test, $P$<0.05) in the community composition (Fig.7). Besides, temperature and $PO_4^{3-}$ were also major contributors to the variance. The first two axes accounted for 52.7% (Axis 1, eigenvalue=0.13) and 22.4% (Axis 2, eigenvalue=0.06) of the variations of AAPB community composition. *Porphyrobacter*, Rhodospirillales, *Sandarakinorhabdus*-like *pufM* sequences were closely associated with Chl *a*, whereas Gammaproteobacteria, *Sphingomonas*, *Novosphingobium*-like *pufM* sequences were closely related with temperature and DOC (Fig.7). Pearson correlation analyses revealed that *Limnohabitans*-like *pufM* sequences were negatively correlated with temperature ($R$=-0.69, $P$<0.05) and positively correlated with DOC ($R$=0.61, $P$<0.05). *Rhodobacter*-like *pufM* sequences were positively correlated with temperature ($R$=0.58, $P$<0.05). Rhizobiales-like *pufM* sequences were negatively correlated with Chl *a* (Pearson correlation, $R$=-0.6, $P$<0.05), whereas *Porphyrobacter* and Rhodospirillales-like *pufM* sequences were positively correlated with Chl *a* (Pearson correlation, $R$=0.85 and $R$=0.72, respectively, $P$<0.01). *Sandarakinorhabdus*-like *pufM* sequences were also positively correlated with Chl *a* (Pearson correlation, $R$=0.61, $P$<0.05).

## 4 DISCUSSION

The higher concentrations of both Chl *a* and PC in Site N than that in Site E of Taihu Lake confirmed that cyanobacterial blooms occurs in Site N, and there are relatively low cyanobacterial biomass in Site E. PC is an accurate probe for monitoring cyanobacteria for significant correlation between PC and cyanobacterial biomass (Brient et al., 2008). In particular, PC was not displayed in the dbRDA results due to significantly correlation between PC and Chl *a* (Pearson correlation, $R$=0.993, $P$<0.001). The well correlated

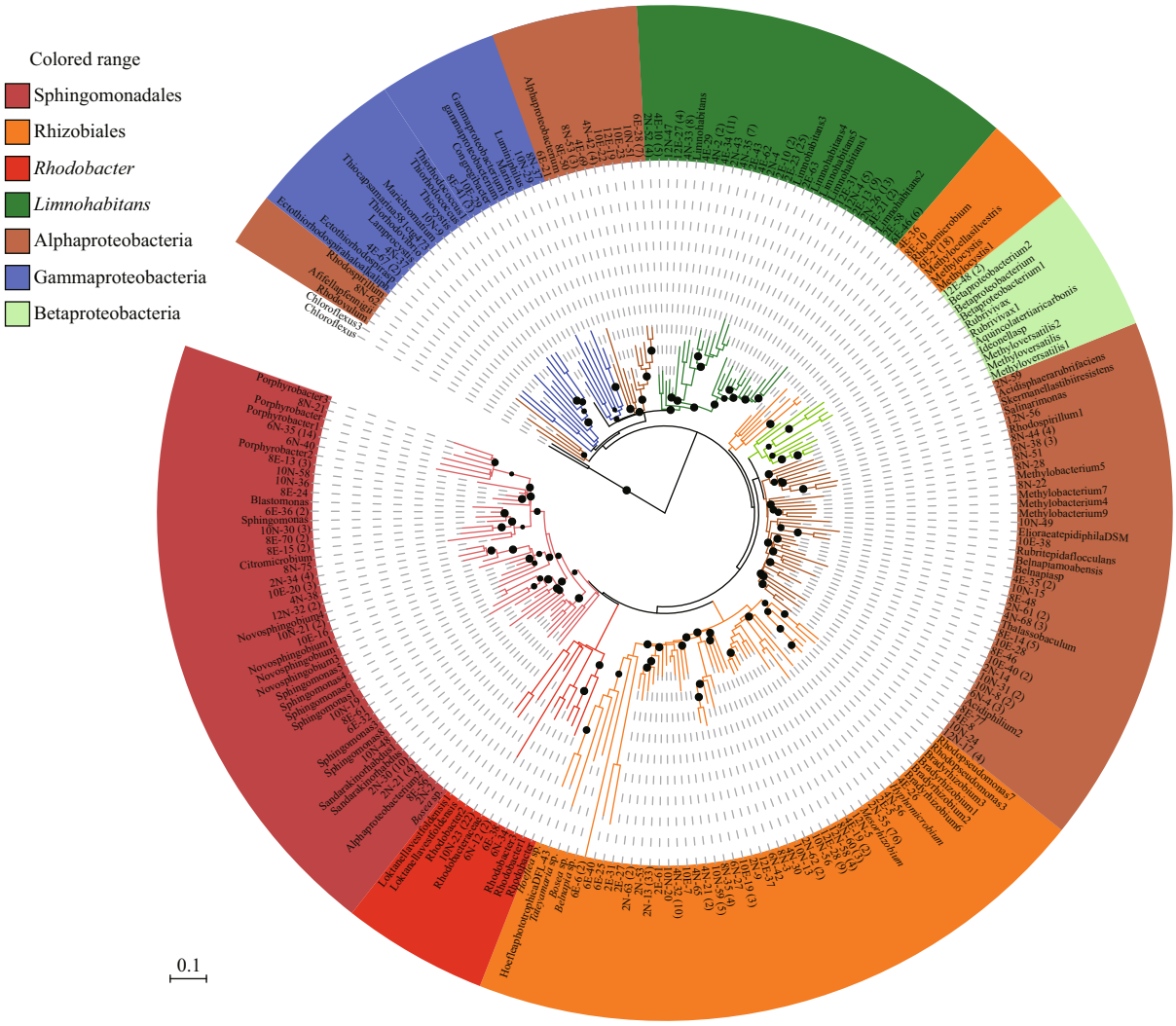


**Fig.4 Phylogenetic tree inferred by the neighbor-joining analysis of partial *pufM* sequences based on a backbone tree by using the ARB software package (http://www.arb-home.de)**

The tree was exported and edited online via iTOL to define color ranges and export image files. The corresponding clone numbers of each operational taxonomic unit (OTU) were shown in parenthesis. Bootstrap values (1 000 replicates) that exceeded 50% were indicated above the branches (dark dots). *Chloroflexus aurantiacus* were set as outgroups. The scale bar corresponded to a 10% nucleotide substitution percentage. 8N, 10N, 12N, 2N, 4N, 6N, 8E, 10E, 12E, 2E, 4E, and 6E were the representatives of the samples collected in August 2011, October 2011, December 2011, February 2012, April 2012, and June 2012 in Sites N and E, respectively.

relationship between PC and Chl *a* also convinced dominance of cyanobacteria in total phytoplankton. Our results demonstrated the high abundance of *pufM* gene and their positive correlations with DOC in the investigated regions of Taihu Lake. The abundance of AAPB in Taihu Lake was comparable with that in the Waihai and Chaohai areas of Dianchi Lake, a eutrophic lake (Tian et al., 2018) and mesotrophic lake Vlkov (Kolářová et al., 2019), but was considerably higher than that in oligotrophic saline lakes in the Tibetan plateau (Jiang et al., 2009). The ratio of AAPB to the total bacteria in Taihu Lake was similar to that in glacial lakes in Northern Europe (Mašín et al., 2012) and lakes in Northern Québec (Fauteux et al., 2015) (Table 4). Moreover, the result that ratio of AAPB to the total bacteria was higher in winter than other seasons was consistent to previous study (Zhang and Jiao, 2007), further indicating less dependent of some AAPB on temperature. The result that AAPB abundance was significantly positively related to DOC in Taihu Lake was similar to the observation that has been noted in lakes in three distinct regions in Northern Québec (Fauteux et al., 2015), in the alpine lake Gossenköllesee located in Tyrolean Alps, Austria (Čuperová et al., 2013), and in freshwater lake Vlkov (Kolářová et al., 2019). AAPB may rely on labile DOC excreted by phytoplankton and contribute to refractory DOC production (Jiao et al., 2007, 2010).

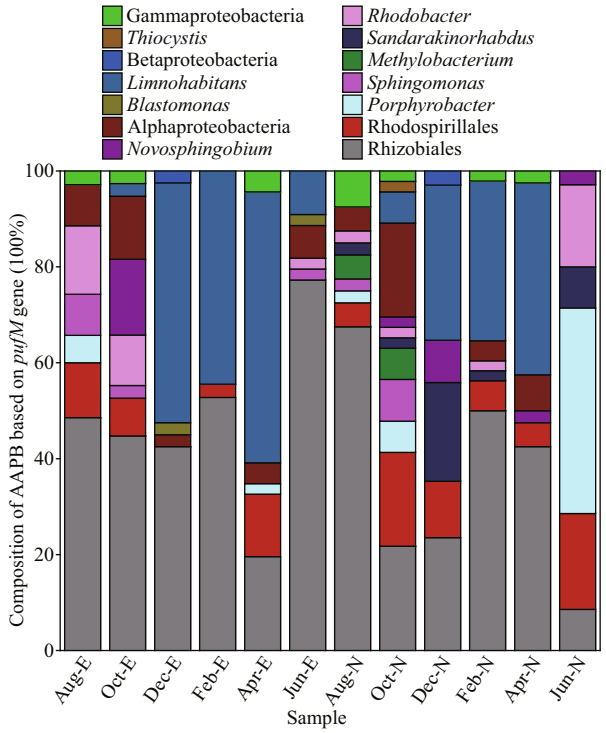


**Fig.5 Community compositions of AAPB based on *pufM* sequences in the two sites in Taihu Lake**

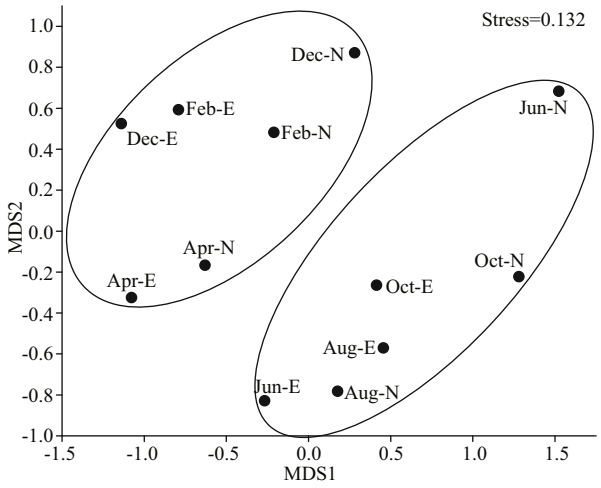


**Fig.6 Nonmetric multidimensional scaling plot based on Bray-Curtis dissimilarity**

**Table 2 Properties of the distribution of phylotypes in both Sites N and E**

| Sample | Number of distinct OTUs | Total clone numbers | *H'* (Shannon diversity index) | Coverage (%) |
|---|---|---|---|---|
| Aug-N | 19 | 40 | 2.44 | 53 |
| Oct-N | 31 | 46 | 3.19 | 33 |
| Dec-N | 19 | 34 | 2.65 | 44 |
| Feb-N | 26 | 48 | 2.86 | 46 |
| Apr-N | 21 | 40 | 2.76 | 48 |
| Jun-N | 15 | 35 | 2.26 | 57 |
| Aug-E | 18 | 35 | 2.39 | 49 |
| Oct-E | 20 | 38 | 2.75 | 47 |
| Dec-E | 17 | 40 | 2.40 | 58 |
| Feb-E | 16 | 36 | 2.38 | 56 |
| Apr-E | 27 | 46 | 2.88 | 41 |
| Jun-E | 13 | 44 | 1.73 | 70 |

In addition, AAPB utilize light energy under natural conditions to maintain much higher growth rates than many other bacterioplankton groups (Ferrera et al., 2017a), and may be even higher under elevated water temperatures in the absence of grazers (Sato-Takabe et al., 2019). Therefore, we suggested that AAPB may contribute significantly to the alteration and regeneration of carbon forms in these ecosystems.

The phylogenetic analyses of the *pufM* sequences revealed that AAPB in both sites of Taihu Lake were dominated by Rhizobiales-like sequences in Alphaproteobacteria, followed by *Limnohabitans*-like sequences in Betaproteobacteria. This result was different from that in most German freshwater lakes including the alkaline to pH neutral lakes Stolp, Stechlin, and Fuchskuhle NE basin where *Rhodoferax*-related *pufM* sequences in Betaproteobacteria are predominant (Salka et al., 2011). Alphaproteobacteria-like *pufM* sequences were observed to be dominant in Taihu Lake in our preliminary study (Shi et al., 2010) and other freshwater lakes, such as southwestern basin of Lake Grosse Fuchskule in Germany (Salka et al., 2011) and Lugu Lake, Erhai Lake, and Chenghai Lake in China (Tian et al., 2018). These results further indicated lake specific diversity of AAPB community. Some Alphaproteobacteria AAPB may possess the genomic potential for anoxygenic phototrophy and carbon fixation via the Calvin-Benson-Bassham cycle and for sulfite and thiosulfate oxidation (Graham et al., 2018; Imhoff et al., 2018). Thus, the effect of the predominance of Alphaproteobacteria-like *pufM* sequences on the primary production in these lakes should be considered, especially in the calculation of the carbon budget in these lakes.

*Sandarakinorhabdus* and *Methylobacterium*-like *pufM* sequences were detected in Site N only. *Porphyrobacter*-like *pufM* sequences was dominant in Site N in June when Chl-*a* concentrations exhibited a peak value of 65 μg/L, which indicated serious cyanobacterial blooms. Bacteria affiliated to *Sandarakinorhabdus*, *Methylobacterium*, and *Porphyrobacter* are dominant bacteria attached to cyanobacterial phycosphere (Berg et al., 2009; Shi et al., 2009a, 2009b). Therefore, these AAPB may

**Table 3 EdgeR analysis revealed *pufM* sequences that were significantly different between warm and cold seasons ($P$<0.05, FDR-corrected)**

| Group | LogFC | LogCPM | F | *P* value | FDR | Affiliation |
|---|---|---|---|---|---|---|
| Otu002 | -6.133 97 | 17.266 56 | 24.066 75 | 0.000 001 | 0.000 062 | Rhizobiales |
| Otu003 | -4.954 46 | 17.044 54 | 15.929 96 | 0.000 076 | 0.001 857 | *Limnohabitans* |
| Otu008 | -4.935 00 | 16.542 77 | 14.192 14 | 0.000 185 | 0.003 024 | *Limnohabitans* |
| Otu007 | -4.680 13 | 16.427 74 | 12.089 01 | 0.000 552 | 0.006 766 | *Limnohabitans* |
| Otu011 | -4.296 42 | 16.267 92 | 9.213 99 | 0.002 529 | 0.024 789 | *Limnohabitans* |
| Otu013 | -4.219 64 | 16.239 96 | 8.707 62 | 0.003 321 | 0.027 118 | *Limnohabitans* |

LogFC: log2-fold-change; LogCPM: log2-counts-per-million; FDR: the false discovery rate, multiple testing correction is performed using the Benjamini-Hochberg method.

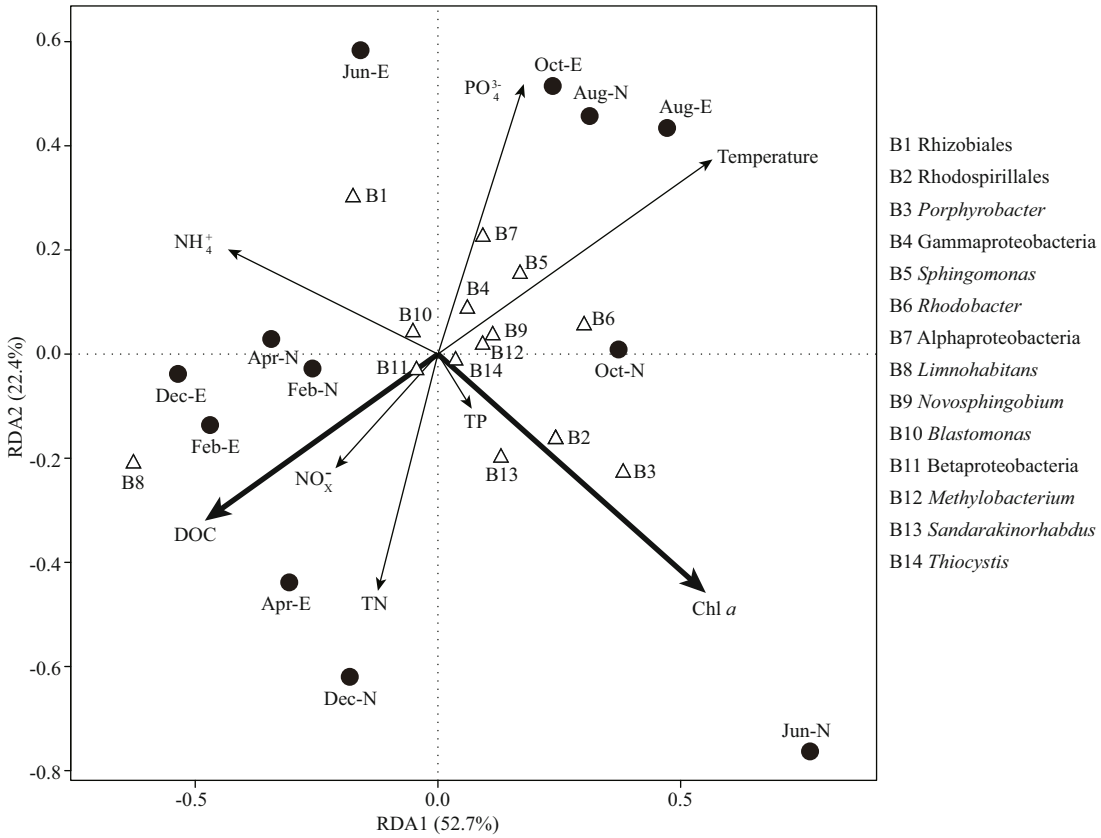


**Fig.7 Distance-based redundancy analysis ordination plot showing the relationship of the samples, AAPB, and water environmental parameters**

originate from cyanobacterial phycosphere, which is a special microenvironment that feeds some unique bacterial flora (Shi et al., 2009b). These results indicated influence of cyanobacterial blooms on certain AAPB communities. Furthermore, *Sandarakinorhabdus* and *Porphyrobacter* in cyanobacterial phycosphere participated in forming the phosphorous cycling co-pathway as their functional links to cyanobacteria (Zhu et al., 2021). Dominance of *Methylobacterium* that could utilize single-carbon compounds was observed in small cyanobacterial aggregates (Cai et al., 2014). Moreover, association between AAPB and cyanobacteria have also been observed in Tama River, Japan (Sato-Takabe et al., 2020), and global ocean (Jiao et al., 2007). AAPB have high efficiency in organic carbon utilization (Koblížek et al., 2007; Cepáková et al., 2016); thus, the presence of these functional bacteria in cyanobacterial phycosphere may accelerate nutrient cycling and facilitate cyanobacterial growth. Future studies on the ecological roles of these AAPB attached to cyanobacteria should be conducted.

Our results demonstrate the high diversity of *pufM* in Taihu Lake. Rarefaction curves indicated that diversity was not fully captured in most communities,

**Table 4 Comparison of AAPB abundance and ratios of AAPB abundance to total bacteria in Taihu Lake and other lakes**

| Area | AAPB abundance | Ratio of AAPB abundance to total bacteria | Reference |
|---|---|---|---|
| Meiliang Bay of Taihu Lake | $(0.01–0.65)\times10^6$ copies/mL | 0.68%–7.70% | This study |
| East Lake of Taihu Lake | $(0.08–2.72)\times10^6$ copies/mL | 0.34%–11.47% | This study |
| Caohai of Dianchi Lake | $1.59\times10^7$ copies/mL | 6.3%–6.8% | Tian et al., 2018 |
| Waihai of Dianchi Lake | $3.16\times10^6$ copies/mL | 5.4%–5.8% | Tian et al., 2018 |
| Qinghai Lake | $4.60\times10^4$ copies/mL | 5.23% | Jiang et al., 2009 |
| Glacial lakes of northern Europe | $(1–12)\times10^5$ cells/mL | 2%–12% | Mašín et al., 2012 |
| Lakes of Québec | $(1.51–5.49)\times10^5$ cells/mL | 1%–37% | Fauteux et al., 2015 |
| Alpine lake Gossenköllesee | $(0.01–1.30)\times10^5$ cells/mL | 1%–29% | Čuperová et al., 2013 |
| Freshwater lake Vlkov | $(0.35–1.78)\times10^6$ cells/mL | 0.3%–20.1% | Kolářová et al., 2019 |

AAPB abundance is displayed as *pufM* gene copy numbers based on qPCR (copies/mL) or as cell numbers based on infra-red epifluorescence microscopy (cells/mL).

and our clone libraries were incomplete. Future studies involving high-throughput sequencing based on *pufM* should also be conducted. No obvious separation was observed between the samples in the two sites. This may be due to sample scale limitation; more samples are needed in future for accurately analysis of AAPB distribution pattern in these two sites. However, NMDS analysis revealed a separation between warm seasons (June, August, and October) and cold seasons (December, February, and April). Rhizobiales and *Limnohabitans*-like *pufM* sequences were found to contribute to the difference between the two seasons, especially enriched in cold seasons. Rhizobiales associated with plant shoot and root systems having photosynthetic systems may provide additional energy for nitrogen fixation, and promote plant growth and stem nodulation (Fleischman and Kramer, 1998; Giraud and Fleischman, 2004). *Limnohabitans* was considered a ubiquitous photoheterotrophic bacterium in various freshwater habitats (Kasalický et al., 2018). *Limnohabitans*-like *pufM* sequences are predominant in ultraoligotrophic high-altitude lakes (Central Pyrenees), where Chl *a* is lower than 5 μg/L when temperature is lower than 15 °C (Caliz and Casamayor, 2014). *Limnohabitans* is also assumed a photoautotroph and ammonia oxidizer (Zeng et al., 2012). Therefore, these genotypes specifically enriched in cold seasons indicated separated ecological niches might exist between them and cyanobacteria.

DbRDA results showed that Chl *a* and DOC were the most significant variables associated with the community structure of AAPB indicating influence of cyanobacterial blooms on AAPB composition. Furthermore, certain AAPB genotypes were significantly correlated with Chl *a*, DOC, or temperature. These results suggested that diverse AAPB ecotypes were present in Taihu Lake and they may be influenced by different environmental factors. Some AAPB may share a similar ecological niche with phytoplankton or be specifically stimulated by cyanobacterium-derived DOC because they are mixotrophic (Yurkov and Beatty, 1998; Graham et al., 2018). Specific clades of *pufM* were significantly associated with high abundances of *Synechococcus* and chlorophyll in coastal regions of the Pacific Ocean (Ritchie and Johnson, 2012). Taxonomic specific response of AAPB to light and predation were also observed (Ruiz-González et al., 2020). The close correlation between some AAPB and Chl *a* may indicate that they coexist in a tightly linked nutrient cycle (Kolber et al., 2001). Given the rapid growth and photoheterotrophic characteristics of AAPB, they can form large sinks for dissolved organic matter (Li et al., 2017) and influence carbon and nitrogen cycle, especially the microbial loop in aquatic systems (Koblížek, 2015). Furthermore, mixed AAPB may have higher DOC and nitrogen removal efficiencies (Zhang et al., 2020). Therefore, the ecological role of the diverse AAPB in biogeochemical cycles in these large shallow eutrophic lakes should be considered in future.

## 5 CONCLUSION

In summary, this study revealed the presence of abundant and diverse *pufM* sequences in Meiliang Bay and East Bay of Taihu Lake. The abundance of *pufM* sequences was influenced by DOC, and the ratio of *pufM* sequences to total bacteria peaked in winter in both sites. Alphaproteobacteria-like *pufM* sequences were predominant in Taihu Lake. A clear separation between the samples collected in the two

sites was not observed, but a separation between warm seasons (June, August, and October) and cold seasons (December, February, and April) was revealed. The community structure of AAPB in the investigated samples was significantly influenced by Chl *a* and DOC, and certain AAPB genotypes were significantly correlated with temperature, Chl *a* or DOC, respectively. These results indicated that *pufM* sequences were abundant and diverse in Taihu Lake, and some ecotypes were influenced by cyanobacterial blooms. Thus, their ecological role in carbon and nutrient cycling, especially in these lakes, should be considered.

## 6 DATA AVAILABILITY STATEMENT

The data supporting the conclusions are presented in the main article.

## 7 ACKNOWLEDGMENT

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