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The roles of cyanobacterial bloom in nitrogen removal

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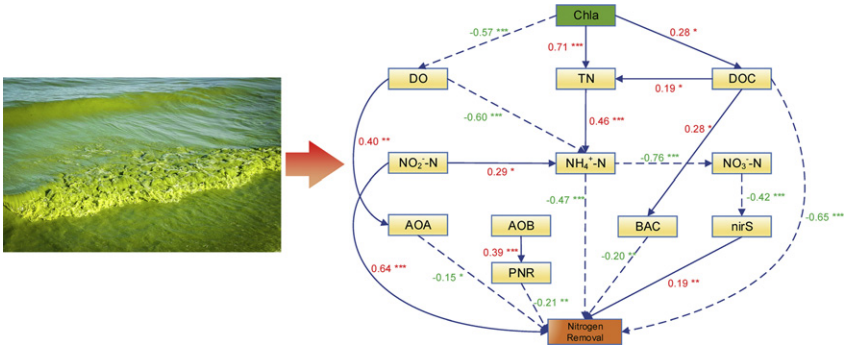
HIGHLIGHTS

- Cyanobacterial bloom released high level of N.
- Cyanobacterial bloom enhanced TN removal through nitrification-denitrification.
- Cyanobacterial bloom enhanced nitrification by providing NH_4^+-N and DO.

GRAPHICAL ABSTRACT



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ABSTRACT

Annually occurred cyanobacterial bloom aggravated eutrophication situation and changed the lacustrine ecosystem components. Recently, high concentration of bloom cyanobacteria had been found to accelerate total nitrogen (TN) removal. However, the contribution of cyanobacterial bloom to TN removal remained unclear. In this study, microcosms with different density of bloom cyanobacteria were constructed and quantitative PCR and structural equation modelling (SEM) were used to analyze the microbes, environmental variables and the causal relationship to TN removal. Total bacteria, ammonia-oxidizing archaea and nirS gene abundances were indirectly influenced by cyanobacteria biomass and all of them had a direct effect on TN removal. SEM confirmed that cyanobacteria made a direct contribution to ammonium nitrogen (NH_4^+-N) level in water and induced nitrification activity, which favored the process of denitrification by supplying substrate and aggravating the anoxic status. These results strongly suggested that an increased cyanobacteria biomass had strong impacts on mineralization, nitrification and denitrification by mediating TN, dissolved organic carbon and dissolved oxygen directly and subsequently influenced the nitrifiers and denitrifiers.

1. Introduction

Eutrophication, caused by overload of nutrient into lakes, has caused many notorious consequences, such as outbreak of cyanobacterial bloom, degradation of water quality, and disruption of ecosystem stability (Havens et al., 2003; Smith et al., 1999). Nitrogen (N) is a critical element controlling primary production in most lakes, therefore decreasing external and internal N loading is one of the most common measures to combat eutrophication (Conley et al., 2009; Paerl et al., 2011).

In many eutrophic lakes, cyanobacterial bloom usually occurred annually and influenced water chemo-physical properties as well as microbial communities (Anderson et al., 2002; Eiler and Bertilsson, 2004; Paerl and Otten, 2013), and a few studies had focused on the influence of cyanobacterial bloom on water nitrogen bank and microbial food web structure (McCarthy et al., 2007; Xin et al., 2010; Xu et al., 2010).

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On the one hand, the photosynthesis of algae and degradation of dead algal cells influenced lacustrine pH, dissolved oxygen, dissolved organic carbon concentration and redox potential (Chen et al., 2010; Chuai et al., 2011; de Figueiredo et al., 2006; Jensen et al., 1990). On the other hand, cyanobacteria absorb and accumulate a large amount of nitrogen in their cells (Ferber et al., 2004), and nitrogen is released into the water from decaying cyanobacterial bloom (Krivtsov et al., 2005). Recent studies (Han et al., 2015; Chen et al., 2012) had found that high nutrient level and algal density could accelerate nitrogen removal from Lake Taihu. These studies showed the pattern and pathway of nitrogen biogeochemical cycle could be changed by cyanobacterial bloom (Chen et al., 2010; Wu et al., 2007), and water bloom help to the capacity for self-purification of eutrophic lakes. Therefore, understanding the transformation and fate of nitrogen in lakes, especially process of nitrogen removal, under influence of algal bloom is important to understand the variation of whole-lake nutrient budgets and help to take steps to restore and protect water resources of lakes.

Nitrogen removal primarily involves a series of microbiological processes, including nitrification, denitrification and anaerobic ammonium oxidation (anammox) (Peng and Zhu, 2006; Verstraete and Philips, 1998). Ammonia oxidation, the first and rate-limiting step in nitrification and N removal, is driven by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (Francis et al., 2005; Könneke et al., 2005). Denitrification stepwisely reduces nitrate to nitrite, nitric oxide (NO), nitrous oxide (N_2O), and finally nitrogen (N_2), accounting for a major pathway for N removal. As is well-known, lake ecosystem is a network made up of multiple biota and abiota components and of the interactions between these components, in which the capacity of nitrification and denitrification is controlled by nitrifiers or denitrifiers, salinity, pH, concentration and stoichiometry of N, C, and O_2 (Bouskill et al., 2012; Caffrey et al., 2007; Knowles, 1982; Molina et al., 2010; Mosier et al., 2002; Weier et al., 1993; Wuchter et al., 2006), as well as the interactions between these microbes and environmental variables. The interactive impacts of microbial communities and altered environmental factors are of particular interest for they could modify the effects of cyanobacterial bloom on nitrogen removal. Although the importance of the effects of cyanobacterial bloom on nitrogen transformation and removal processes is widely accepted, the ranked contribution of environmental factors and microbes associated with nitrogen cycling, as well as how the interaction of environmental factors and microbes impacted on the removal of nitrogen still need further illustration.

In the present study, microcosms were constructed to simulate outbreak and decaying of cyanobacterial bloom, trying to study the dynamic of nitrogen transformation, as well as contribution of environmental variables and microbial functional genes associated with nitrogen removal during this process. Structural equation modelling analyses are used to analyze direct or indirect pathways of causality among multiple ecosystem components (Grace et al., 2010) or to test hypotheses about causal pathways of ecosystem functioning (Shipley, 2000). It has often been used for the testing of effects of ecosystem components or processes on community diversity and ecosystem functioning (Capmourteres and Anand, 2016; Hodapp et al., 2015; Jing et al., 2015; Thompson et al., 2015). Here SEM was also used trying to explore the direct and indirect effect of the causal associations between microbial functional genes and environmental variables involved in cyanobacterial bloom and nitrogen transformation.

2. Materials and methods

2.1. Microcosm and experimental design

The cyanobacterial bloom (dominated by *Microcystis*) were collected from Meiliang Bay in Lake Taihu in June 2011. Samples were collected in polyethylene bottles previously cleaned with distilled water and transported to lab immediately. The samples were filtered through a 100-μm nylon filter, and the filtered water and cyanobacteria biomass were collected separately. According to different density of cyanobacteria, three microcosms with different treatment levels were set up in 10-L glass water tanks (diameter 30 cm, height 40 cm) prefilled with 8 L filtered water: T1, adding high density of cyanobacteria to this treatment, and the initial density of cyanobacteria was about 10^8 cell L^{-1}; T2, adding low density of cyanobacteria to this treatment, and the initial density of cyanobacteria was about 10^6 cell L^{-1}; CK, the control treatment without supplement of cyanobacteria. Three replicate microcosms were performed at each treatment level. All the tanks were maintained at 25 °C, 12 h: 12 h light/dark cycle with light intensity of 2000 lx.

Cyanobacteria densities (cell L^{-1}) were determined with hemocytometer using Zeiss Axio Imager A2 microscope (×400; Zeiss, Germany) after the rapid disruption (20 s) of the aggregated structure of cyanobacteria by ultra-sonication (Reynolds and Jaworski, 1978).

2.2. Sampling and chemical properties analysis

Water samples were collected from 10 cm depth under the water surface on Day 0, 1, 2, 4, 7, 10, 15, 16, 17, 19, 22, 25, 30 and samples were divided into 3 subsamples for analysis of chemical properties, potential nitrification rate (PNR) and gene abundance.

Dissolved oxygen (DO) and pH value were recorded in situ using a digital multi parameter meter (Multi 3410, WTW, Germany). Nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), ammonium nitrogen (NH_4^+-N), phosphate (PO_4^{3-}-P), dissolved organic carbon (DOC) and chlorophyll-*a* (Chla) were measured according to standard methods (Jin and Tu, 1990).

2.3. Measurement of PNR

Potential nitrification rate (PNR) was measured using the chlorate inhibition method (Kurola et al., 2005). Briefly, 50 mL water sample was suction-filtered under diminished pressure (at a vacuum degree of −0.1 MPa) with a sterile 0.22-μm pore size filter, and then the filter was cut into pieces and put into a 50-mL centrifuge tube containing 20 mL phosphate buffer solution (1 mmol L^{-1}, pH 7.4), with a final concentration of 1 mmol L^{-1} $(NH_4)_2SO_4$ and 10 mmol L^{-1} $KClO_3$. The mixture was incubated in a dark incubator at 25 °C for 24 h, after which the samples were centrifuged and the supernatant were used for analysis of nitrite as described above.

2.4. DNA extractions and real-time quantitative PCR

Fifty mL water sample was filtered with a 0.22-μm pore size filter, and the total DNA was extracted from the filters using E.Z.N.A.® Water DNA Kit (OMEGA, USA). The quality of extracted DNA was evaluated by agarose gel electrophoresis and quantified by NanoDrop ND 2000 (Thermo Scientific, DE, USA).

qPCR was performed with Rotor Gene 6000 (Qiagen, Hilden, Germany). The primer sets (Bacterial 16S rRNA: 338F/518R; AOA: Arch-amoAF/Arch-amoAR; AOB: amoA-1F/amoA-2R; nirK: nirK-1F/nirK-5R; nirS: nirS-cd3af/nirS-r3cd) and thermal profiles used to amplify each target gene are listed in Table S1. For all assays, each reaction contained 10 μL 2× SYBR® Premix Ex Taq™ (Takara Bio, Otsu, Shiga, Japan), 0.5 μmol L^{-1} of each primer, 2 μL of template DNA and RNase-free water to final volume of 20 μL. Samples, standards, and non-template negative controls were run in triplicate. Melting curve analysis was carried out to ensure specific amplification. Standard curves were developed as described previously (He et al., 2007) using plasmids carrying AOA amoA, AOB amoA, nirK and nirS genes.

2.5. SEM analysis

SEM was selected to analyze the direct and indirect relationships. All the data processing and model testing were carried out according to