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Excess phosphate loading shifts bacterioplankton community composition in oligotrophic coastal water microcosms over time

Xinxin Chen [a], Kai Wang [a,b,*], Annan Guo [a], Zhiying Dong [a,c], Qunfen Zhao [a], Jie Qian [a], Demin Zhang [a,b]

[a] School of Marine Sciences, Ningbo University, Ningbo 315211, China
[b] Collaborative Innovation Center for Zhejiang Marine High-efficiency and Healthy Aquaculture, Ningbo 315211, China
[c] Faculty of Architectural, Civil Engineering and Environment, Ningbo University, Ningbo, 315211, China

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ABSTRACT

Phosphorus (P), primarily as phosphate, is considered as a key trophic factor of eutrophication in coastal environments. Information on the response of bacterial community to excess phosphate loading in oligotrophic coastal water could provide insights into the micro-ecological effects on the expanding trend of nutrient-enrichment in coastal water. In this study, temporal dynamic of bacterioplankton community composition was investigated using 16S Illumina MiSeq sequencing technique in oligotrophic seawater microcosms manipulated with no perturbation (control) and a certain level of phosphate by two loading modes: 1) one-off loading at the beginning of incubation; and 2) periodic loadings every two days over a 16-day duration. The results showed that overall bacterial alpha-diversity temporally changed and achieved the lowest value in phosphate loading (P-loading) microcosms at day 8; phosphate-addition increased the Shannon index and Pielou's evenness compared with those of the control at day 16. Bacterioplankton community turnover across all the treatments showed a similar temporal pattern. P-loading significantly affected bacterioplankton community composition with negative correlations of Methylophilaceae and Flavobacteriaceae and positive correlation of Saprospiraceae. Periodic loading treatments demonstrated a greater effect on community variation than one-off loading treatments did. Moreover, excess P-loading accelerated the temporal succession of the bacterioplankton community composition. These results provide insights into the pattern of bacterioplankton community phylogenetically responding to excess P-loading in the manipulated oligotrophic coastal system.

1. Introduction

Anthropogenic activities have accelerated the discharge of nutrients to coastal ecosystems, leading to widespread eutrophication (Claussen et al., 2009 and Meyer-Reil and Kster, 2000). Also, the rate of nutrients input has increased dramatically in some coastal areas in recent years (Glibert et al., 2005 and Sinkko et al., 2011), and excess nutrients are gradually spreading to offshore pelagic environments (Cotner and Biddanda, 2002). One of the great scientific challenges is how to mitigate the threats of eutrophication to the aquatic ecosystems. Detecting the influence of excess nutrients to biodiversity is a fundamental prerequisite to understand how eutrophication affects ecosystem health (Ager et al., 2010). Thus, it is important to reveal the response of biological components to excess nutrient stress (Meyer-Reil and Kster, 2000 and Ager et al., 2010). The crucial roles played by the bacterioplankton community in marine biogeochemical cycling and energy fluxing have been well established (Yeo et al., 2013). Both laboratory and field studies have shown that natural and/or anthropogenic inputs of nutrients generate modifications of bacterioplankton community composition in the marine ecosystems (Meyer-Reil and Kster, 2000; Ager et al., 2010 and Xiong et al., 2014). Therefore, assessing the dynamic changes in bacterial community composition in response to the nutrient-enrichment might be a promising way to evaluate the ecological effect of eutrophication in marine environment.

Historically, both nitrogen (N) and phosphorus (P) are of great importance in eutrophication (Glibert et al., 2005). Anthropogenic activities often cause imbalances in N and P loading, making it difficult to effectively control eutrophication by reducing only one nutrient (Paerl, 2009). The excess P input, primarily as phosphate, considered as the primary nutrient that controls marine productivity, has accelerated eutrophication in coastal areas (Sinkko et al., 2011 and Arrigo, 2004). Reducing phosphate supply from sources such as detergents would effectively slow coastal marine eutrophication (Ryther and Dunstan, 1971). In oligotrophic environments where inorganic nutrients are scarce, both heterotrophic bacterial productivity and biomass were co-limited by N and P, and addition of labile dissolved organic carbon provided no stimulation unless N and P were also added (Mills et al., 2008). Whereas, the opposite results were shown that neither bacterial

* Corresponding author at: School of Marine Sciences, Ningbo University, Ningbo, 315211, China.
E-mail address: wangkai@nbu.edu.cn (K. Wang).

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production nor utilization of dissolved organic carbon was enhanced with the addition of inorganic N or/and P (Carlson et al., 2002). These previous studies focused on impacts of inorganic nutrients on bacterial production and utilization of dissolved organic carbon in oligotrophic environments, but less attention was paid to bacterial composition and structure. Extensive reports focusing on coastal eutrophic areas observed an increase in taxa richness in nutrient-amended (nitrate and phosphate) water column (Zohary and Robarts, 1998; Hewson et al., 2003 and Logue et al., 2012), while other studies reported the opposite trend (Yeo et al., 2013 and Dumestre et al., 2001). Although the changes in species richness could be accompanied with the variation of bacterioplankton community composition (Lebaron et al., 2001), the impact of phosphate-enrichment on composition of bacterioplankton community in oligotrophic seawater is rarely revealed, which could be beneficial for understanding the possible microbial consequences due to the expanding trend of phosphate as a key limit factor of eutrophication in oligotrophic marine environments.

Temporal variability of bacterioplankton communities associated with nutrient conditions (Arrigo, 2004), water temperature (Hewson et al., 2003), phytoplankton biomass (Xiong et al., 2014), depth (Humayoun et al., 2003), and heavy metal (Wang et al., 2015) was commonly observed. Knowledge on how oligotrophic marine bacterioplankton communities phylogenetically respond to excess phosphate loading (P-loading) over time is still lacking. In this study, 16S Illumina MiSeq sequencing was used to profile the compositional variations of bacterioplankton community in the manipulated microcosms with different modes of phosphate-enrichment perturbations to reveal the temporal pattern of bacterioplankton community composition in response to excess phosphate loading in oligotrophic coastal system.

2. Methods

2.1. Microcosm set-up and seawater sampling

The oligotrophic surface seawater (at 0.5-m depth) was collected on September 29, 2013, at a coastal site (29°21′54.72″ N, 122°39′ 36.86″ E) located at eastern Xiangshan, Ningbo, East China Sea, and then transported to the lab within 6 h to construct the microcosms. The in situ salinity of seawater was 25.7. Each microcosm containing 35 L seawater in a 40-L polyethylene tank (acid-soaked and rinsed with deionized water prior to use) was incubated at a temperature-controlled (24.0 ± 1.5 °C) room over 16 days. Totally 12 microcosms were set up including four replicates of three treatments: i) control (no phosphate added); ii) one-off loading; and iii) periodic loading. 'One-off loading' microcosms were amended with a stock solution of NaH_2PO_4, which was prepared with 0.2-μm filtered pre-autoclaved original seawater, to achieve the target concentration (2.90 μM twice of the permissible concentration of the 4th grade (the worst) of seawater quality standard in China) (SEPA and SOA, 1998), while 'periodic loading' microcosms were designed to keep phosphate concentration increasing at a 0.36 μM rate every two days to achieve the actual concentration (2.90 μM) at the end of experimental duration (day 16). Seawater in the microcosms was continuously mixed by immersed pumps (wave makers) and incubated under a 14/10 h light/dark cycle with a light intensity of 350 Lux. At days 2, 4, 8, and 16, 3 L of water sample from each microcosm was collected, while at day 0 (original status), only water sample from the control microcosms was collected.

2.2. Seawater parameters analysis

Dissolved oxygen (DO) and pH of seawater were measured by a probe (YSI 550A, USA) in each microcosm at each sampling day. The concentrations of total phosphorus (TP), phosphate, nitrate, ammonium, and nitrite were analyzed following standard methods (AQSIQ, 2007). Nitrite, nitrate, and ammonium were determined with diazo-azo colorimetric method, zinc-cadmium reduction colorimetric method, and hypobromite oxidation colorimetric method, respectively, at the wavelength of 543 nm using UV-1800 type UV–vis spectrophotometer (Meipuda Instrument Corporation, Shanghai). Phosphate and TP were determined with ascorbic acid reductive phosphorus molybdenum blue method and potassium persulfate oxidation method, respectively, at the wavelength of 882 nm. External standard curves were generated using a series of standard solution with gradient concentrations to calculate the concentrations of target chemicals.

2.3. DNA extraction

Approximately 500 mL of seawater sample was filtered through 0.2-μm polycarbonate membranes (Millpore Type GTTP, USA) to collect microbial biomass. Total DNA on the membranes was extracted using a Power Soil® DNA isolation kit (MO BIO Laboratories, USA) following the manufacturer's recommended protocol. The quality and quantity of the DNA extracts were measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA). And then the DNA extracts were stored at −80 °C prior to amplification.

2.4. Bacterial 16S rRNA gene amplification and Illumina MiSeq sequencing

An aliquot of 10 ng purified DNA from each sample was used as the template for amplification. Bacterial 16S rRNA gene V4 regions were amplified, with the primers 515F (5′- GTGCCAGCMGCCGCGGTAA -3′) with a unique 6-bp barcode sequence at the 5′ end and 806R (5′-GGACTACHVGGGTWTCTAAT -3′) (Caporaso et al., 2011). Each sample was amplified in triplicate with a 30-μL reaction system containing 15 μL Phusion Master Mix (2×), 1.5 μL of each primer (6 μM final concentration), 10 μL genomic DNA (1 ng/μL), and 2 μL MO BIO PCR water. Reactions were held at 98 °C for 1 min to denature the DNA, with amplification proceeding for 35 cycles at 98 °C for 10 s, 50 °C for 30 s, and 72 °C for 30 s; with a final extension of 5 min at 72 °C. Following PCR amplification, the triplicate PCR products were pooled together and purified with a PCR fragment purification kit (Takara, Japan). Sequencing library was generated by Novogene Co. Beijing, China using NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations. Briefly, fragmented DNA was NEBNext-End prepared and then NEBNext adaptors for Illumina were ligated, followed by a PCR enrichment of adaptor ligated DNA. The PCR amplicons were purified, equivalently combined, and then size checked on an Agilent 2100 Bioanalyzer (Agilent, USA) priority to sequencing on an Illumina MiSeq platform.

2.5. Processing of Illumina sequencing data

Raw Illumina fastq files were demultiplexed with QIIME (Quantitative insights into microbial ecology, http://qiime.org) (Caporaso et al., 2010b) and the paired-end reads were joined with FLASH (fast length adjustment of short reads) (Magoc and Salzberg, 2011) using default setting. And then the pair-joined reads were quality filtered with QIIME. Briefly, the reads were truncated at any site of more than three consecutive bases receiving a Phred quality score (Q) < 20, and any read containing ambiguous base calls was discarded, as were truncated reads having <75% of their original length (Bokulich et al., 2013). The remaining sequences were chimera checked and removed using UCHIME method (Edgar et al., 2011). Bacteria phylotypes were identified using UCLUST (Edgar, 2010) and classified into the operational taxonomic units (OTUs) based on a sequence similarity >97%. The most abundant sequence of each OTU was selected as the representative sequence, and then was taxonomically assigned in Greengenes database (DeSantis et al., 2006) and aligned with PyNAST (Caporaso et al., 2010a). A phylogenic tree was generated from the filtered alignment