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Temporal dynamics of bacterioplankton communities in response to excessive nitrate loading in oligotrophic coastal water

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ABSTRACT

Coastal ecosystems are receiving elevated loads of nitrogen (N) from anthropogenic sources. Understanding how excessive N loading affects bacterioplankton communities is critical to predict the biodiversity of marine ecosystems under conditions of eutrophic disturbance. In this study, oligotrophic coastal water microcosms were perturbed with nitrate in two loading modes: 1) one-off loading at the beginning of the incubation period; and 2) periodic loading every two days for 16 days. Turnover in the bacterioplankton community was investigated by 16S rDNA gene amplicon sequencing. The alpha diversity of the bacterioplankton community showed great temporal variability and similar responses to the different treatments. Bacterioplankton community composition was influenced remarkably by time and N loading mode. The effects of N loading on bacterioplankton community structure showed obvious temporal variation, probably because of the great temporal variation in environmental parameters. This study provides insights into the effects of N pollution in anthropogenically perturbed marine environments.

1. Introduction

Marine ecosystems, some of the most productive ecosystems in the world, are significant sources of materials on which man depends to satisfy his needs. In the past few decades, increasing anthropogenic activities have placed enormous pressure on marine ecosystems, and the loading of terrestrial pollutants has severely affected the physical and geochemical characteristics of nearshore aquatic environments. Excessive nutrient inputs have led to widespread eutrophication in many coastal areas (Howarth and Marino, 2006). Harmful algal blooms break out frequently, which seriously restrict the sustainable use of marine biological resources. Nutrient loading is regarded as a critical driver of coastal eutrophication (Lunau et al., 2013), and, as the most important nutrient element, nitrogen (N) is considered a primary limiting factors for seawater quality (Capone et al., 2008). Bacterioplankton play important roles in marine biogeochemical processes, including the N cycle (Falkowski et al., 2008), and rapidly respond to environmental changes (Paerl et al., 2003). Consequently, investigating on the dynamics of coastal bacterioplankton communities under different N loading modes (LM) can reveal the microecological effects of excessive N loading on the coastal biosphere.

Bacterioplankton communities have shown temporal variation in marine ecosystems (Wang et al., 2015b; Zhang et al., 2013) that was associated with environmental factors such as nutrients (Arrigo, 2005), heavy metals (Wang et al., 2015b), temperature (Lindh et al., 2013), and dissolved oxygen (DO) (Xiong et al., 2014a). Moreover, several studies have demonstrated that the richness, abundance, and production of bacteria are closely related to nitrate concentrations (Carlsson et al., 2012; Cavender-Bares et al., 2001). For example, species richness was shown to increase with an increase in nutrients (nitrate and phosphate) in lakes (Logue et al., 2011), whereas it decreased in the Petit Saut dam reservoir (Dumestre et al., 2002). High and unbalanced N loading can change the nutrient stoichiometry, resulting in substrates that resist decomposition by microorganisms (Xiong et al., 2014a). Additionally, substrates may drive bacterial population successions, and regulatory processes have an effect on the metabolic activities of whole communities (Schäfer et al., 2001). In recent years, the concentration of N has gradually increased, and N has diffused through coastal zones (Wang et al., 2015a; Xiong et al., 2014a; Zhang et al., 2007). It is crucial to understand how N loading influences bacterioplankton communities in oligotrophic coastal water, because this can provide insight into the consequences of increasing nutrient pollution in coastal areas. In this study, oligotrophic coastal water microcosms were used to investigate the effects of different N LM on bacterioplankton communities by 16S rDNA gene amplicon sequencing. We aimed to reveal the temporal dynamics

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of bacterioplankton communities' responses to N loading in oligotrophic coastal water.

2. Materials and methods

2.1. Microcosm set-up and N loading

Oligotrophic surface seawater was collected from the eastern coastal area of Xiangshan County (122°39′36.86″ E, 29°21′54.72″ N), East China Sea, on September 29, 2013, at a 0.5-m depth and transported to the laboratory within 4 h. The original physical and chemical parameters of the collected seawater were as follows: temperature in situ: 27.2 °C; salinity: 30.9; pH: 7.9; and the concentrations of nitrate, nitrite, ammonium (NH_4^+-N), phosphate, total phosphorous (TP), chemical oxygen demand (COD), and dissolved oxygen (DO) were not detectable, 0.002, 0.14, 0.002, 0.012, 0.51, and 8.12 mg L^{-1}, respectively. The acid-washed 40-L polyethylene tanks were filled with 35 L unfiltered seawater, randomly distributed and incubated in a temperature-controlled room at 27 ± 1 °C, which was close to the water temperature in situ. The seawater in the microcosms was automatically stirred by wave pumps, and the microcosms were stabilized for 3 days. Nitrate, the main form of bioavailable N in water of the northern coast of Zhejiang, East China Sea (Wang et al., 2015a), was loaded with a stock solution of sodium nitrate ($NaNO_3$) prepared in 0.2-μm filtered, autoclaved seawater to prepare 1.0 mg L^{-1} of N for administration in two LM: 1) one-off loading (OL) at the beginning of incubation and 2) periodic loading (PL) at a rate of 0.125 mg L^{-1} every two days for 16 days. Microcosms with no N loading were established as controls (CK). Each treatment included four biological replicates. The microcosms were exposed to a 14:10 light-dark cycle with a light intensity of 340 lx, similar to in situ conditions.

2.2. Sampling and seawater parameter measurement

Three liters of water was collected from each microcosm on days 2, 4, 8, and 16 (for PL treatment, before the addition of N). Salinity, pH, and DO were measured by a probe (YSI 550A, USA) at the time of each sampling. About 500 mL of sample was filtered through a 0.2-μm polycarbonate membrane (Millipore Type GTTP, USA) and stored at −80 °C before DNA extraction. Nitrite, nitrate, NH_4^+-N, phosphate, COD, suspended solids (SS), and TP were analyzed according to the standard methods (AQSIQ, 2007).

2.3. Bacterial 16S rRNA gene amplification and 16S rDNA gene amplicon sequencing

DNA was extracted using a Power Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. The extracts were quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA) and stored at −80 °C until amplification. The extracted DNA was used as a template for amplification. The V4 variable regions of the bacterial 16S rRNA gene were amplified using primers 515 F (5′-GTGCCAGCMGCCGCGGTAA-3′) with a unique 6-bp barcode sequence at the 5′ end and 806 R (5′-GGACTACHVGGGTWTCTAAT-3′). Each sample was amplified in triplicate to reduce error. The PCR reaction conditions included denaturing the DNA at 98 °C for 1 min, then 30 cycles of amplification at 98 °C for 10 s, annealing at 50 °C for 30 s, and extension at 72 °C for 30 s, followed by a final extension at 72 °C for 5 min. PCR products were mixed together and purified with a PCR fragment purification kit (Takara, Japan). Then, the PCR products were submitted to Novogene Co. Beijing generate a sequence library. Fragmented DNA was NEBNext End repaired, and NEBNext adaptors for Illumina were ligated, followed by PCR enrichment of the adaptor-ligated DNA (Chen et al., 2016). Then, cluster generation and 250-bp paired-end sequencing were performed on an Illumina Mi Seq platform.

2.4. Processing of 16S rDNA gene amplicon sequencing data

Raw Illumina FASTQ files were demultiplexed using QIIME v1.7.0 (Caporaso et al., 2010a), and the paired reads were joined using FLASh with default settings (Mago and Salzberg, 2011). Later, the joined pairs were quality controlled and analyzed in QIIME (Caporaso et al., 2010a). In short, the sequencing reads were truncated at any site of more than three successive bases that received a Phred quality score (Q) < 20, and any read containing ambiguous base calls was discarded, as were truncated reads that were <75% of the total read length (Bokulich et al., 2012). After the remaining sequences were checked for chimeras using UCHIME, non-chimera sequences were clustered into operational taxonomic units (OTUs, based on 97% sequence similarity) using UCLUST (Edgar, 2010). The most abundant sequence of each OTU was selected as the representative sequence, and then these sequences were taxonomically assigned based on similar sequences in Greengenes database 13.5 (Desantis et al., 2006) and aligned in PyNAST (Caporaso et al., 2010b). A phylogenic tree was constructed in FastTree (Price et al., 2010) from the filtered alignment. All singletons, Archaea, and chloroplast sequences were discarded. After the above procedures, 21,300 sequences per sample were obtained from 51 samples (with one sample in PL at day 8 discarded because of shallow sequencing) for further analysis. Alpha diversity indices (observed species, phylogenetic diversity, and Shannon diversity index) were calculated and the unweighted UniFrac distance matrix was generated based on OTUs and the phylogenetic tree using the *alpha_diversity.py* and *beta_diversity_through_plots.py* scripts, respectively, in the QIIME pipeline (Caporaso et al., 2010a; Faith, 1992; Lozupone and Knight, 2006). The sequence data were deposited in the Sequence Read Archive system in the DNA Data Bank of Japan (DDBJ, http://www.ddbj.nig.ac.jp/) and are available under the accession number DRA004922.

2.5. Statistical analyses

One-way analysis of variance (ANOVA) with Tukey's honest significant difference (HSD) post-hoc test was used to test the significance of differences between LM using SPSS 16.0. Two-way ANOVA was applied to evaluate the effects of LM, time, and their interaction on environmental parameters, alpha diversity indices, and the relative abundance of dominant bacterial families. Analysis of variance of the relative abundance of dominant bacterial families between the control and N loading treatments was based on a *t*-test. Non-metric multidimensional scaling (NMDS) was performed based on unweighted UniFrac distances between samples to visualize differences in the composition of bacterioplankton communities. Analysis of similarity (ANOSIM) was applied to investigate the effect of LM and sampling time on bacterioplankton community composition based on unweighted UniFrac distances using PRIMER V5 (Clarke and Gorley, 2001). Explanatory variables for the variation in bacterial communities were identified by forward selection with a distance-based multivariate linear model (DistLM). Spearman's rank correlations were used to analyze the relationship between the abundance of dominant bacterial families and nitrate concentration.

3. Results

3.1. Physicochemical characteristics of seawater

In general, the concentrations of nitrate in OL microcosms were constant, and the concentrations of nitrate in PL microcosms increased regularly over time, as we expected (Fig. S1-A). All of the physicochemical parameters changed significantly over time ($P < 0.001$, Table S1). The concentrations of nitrate and TP were significantly influenced by LM ($P < 0.05$), whereas the concentrations of nitrate, phosphate, and COD were significantly influenced by the interaction of sampling time and LM.

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