Acta Oceanol. Sin., 2018, Vol. 37, No. 12, P. 118–128
DOI: 10.1007/s13131-018-1272-7
http://www.hyxb.org.cn
E-mail: hyxbe@263.net

Variation of bacterial community associated with *Phaeodactylum tricornutum* in response to different inorganic nitrogen concentrations

SHI Feng[1], WEI Xiaoxue[1], FENG Jianfeng[1]*, SUN Yingxue[1], ZHU Lin[1]*

[1] Key Laboratory of Pollution Process and Environmental Criteria of Ministry of Education and Tianjin Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University, Tianjin 300350, China

Received 7 May 2018; accepted 27 August 2018

Abstract

Specific bacterial communities interact with phytoplankton in laboratory algal cultures. These communities influence phytoplankton physiology and metabolism by transforming and exchanging phytoplankton-derived organic matter. Functional bacterial groups may participate in various critical nutrients fluxes within these associations, including nitrogen (N) metabolism. However, it is unclear how bacterial communities and the associated algae respond to changes of phycosphere N conditions. This response may have far-reaching implications for global nutrient cycling, algal bloom formation, and ecosystem function. Here, we identified changes in the bacterial communities associated with *Phaeodactylum tricornutum* when co-cultured with different forms and concentrations of N based on the Illumina HiSeq sequencing of 16S rRNA amplicons. Phylogenetic analysis identified Proteobacteria and Bacteroidetes as the dominant phyla, accounting for 99.5% of all sequences. Importantly, bacterial abundance and community structure were more affected by algal abundance than by the form or concentration of inorganic N. The relative abundance of three gammaproteobacterial genera (*Marinobacter*, *Algiphilus* and *Methylophaga*) markedly increased in N-deficient cultures. Thus, some bacterial groups may play a role in the regulation of N metabolism when co-cultured with *P. tricornutum*.

Key words: *Phaeodactylum tricornutum*, nitrogen concentrations, nitrogen forms, bacterial diversity, community structure, Gammaproteobacteria

Citation: Shi Feng, Wei Xiaoxue, Feng Jianfeng, Sun Yingxue, Zhu Lin. 2018. Variation of bacterial community associated with *Phaeodactylum tricornutum* in response to different inorganic nitrogen concentrations. Acta Oceanologica Sinica, 37(12): 118–128, doi: 10.1007/s13131-018-1272-7

1 Introduction

Microbes are ubiquitous in ocean environments. Along with phytoplankton, microbes are critical components of various marine microbial food webs, and play key roles in several biogeochemical processes, including the cycling of carbon, nitrogen (N), and other nutrients (Buchan et al., 2014). Bacteria and phytoplankton are tightly linked in marine environments (Rooney-Varga et al., 2005), and the interactions between these taxa affect the structure and function of marine ecosystems (Ramanan et al., 2016). In aquatic systems, bacteria interact with phytoplankton primarily in the phycosphere, the region surrounding algal cells where many metabolic exchanges occur (Amin et al., 2012). The phycosphere is the aquatic analog of the soil rhizosphere and has a direct effect on nutrient flux to and from algal cells (Bell and Mitchell, 1972). In this niche, bacteria may live freely around algae, affecting algae through environmental metabolic and nutrient fluxes (Seymour et al., 2017). Alternatively, bacteria may attach to the algal surface or reside inside algal cells (as epiphytes and endophytes), forming tight functional associations (Lupette et al., 2016). Bacteria may positively or negatively influence phytoplankton population growth through a variety of mechanisms. For example, bacteria may stimulate phytoplankton growth by producing vitamins (Croft et al., 2005; Kuo and Lin, 2013), siderophores (Amin et al., 2009), phytohormones (de-Bashan et al., 2008), and inorganic N (Zehr and Ward, 2002). Alternatively, bacteria may inhibit algal growth or kill algae by secreting algicidal compounds (Mayali and Azam, 2004).

Some studies have identified specific bacterial species associated with algae during marine algal blooms and in unialgal cultures (Schäfer et al., 2002; Wemheuer et al., 2014; Yang et al., 2015; Bolch et al., 2017). Functional bacterial groups affect algal growth and metabolism in many different ways. However, it must be noted that the metabolic capacity of a single microbial species is limited, and that most microbes form complex and diverse communities, the effects of which far exceed those achieved by any single species (Chiu et al., 2014). The species comprising these communities often form tight relationships and metabolic dependencies (Little et al., 2008). These relationships endow the bacterial community with enhanced metabolic capacities, further influencing the stability and activity of neighboring communities (e.g., those of phytoplankton or plants; Liu et al., 2015). Due to these dependencies and synergistic relationships, the

Foundation item: The National Natural Science Foundation of China (NSFC) under contract No. 31470536; the National Key Research and Development Program of China under contract No. 2018YFC1406403.
*Corresponding author, E-mail: fengjf@nankai.edu.cn; zhulin@nankai.edu.cn

clear characterization of the metabolic capacity of a given microbial community in a given environment requires a deep understanding of the microbial diversity, community structure, and behavior.

Because bacterial-algal interactions play an important role in biogeochemical cycling and algal blooms, several recent studies have focused on identifying the specific physiological and metabolic mechanisms that regulate these interactions (Amin et al., 2012; Buchan et al., 2014). However, the bacterial communities associated with microalgae are still poorly known, especially those where the bacterial-algal interactions are involved in crucial metabolic processes, such as N metabolism. N is an important nutrient required for biological productivity, and its availability can limit primary production on a variety of spatial and temporal scales (Casciotti, 2016). The N cycle, which controls the availability of nitrogenous nutrients, is a key component of material circulation and energy flow in marine ecosystems (Capone et al., 2008). The N cycle includes multiple transformations of nitrogenous compounds catalyzed primarily by microbes, including N fixation, ammoniation, nitrification, denitrification, and anaerobic ammonia oxidation (Zehr and Ward, 2002).

Microbial communities are an inherently complex part of natural ecosystems, and these communities may be promising indicators of environmental change and aquatic ecosystem health, as they respond more rapidly to ecological fluctuations than larger animals and plants (Paerl et al., 2003). Moreover, different microbial communities have different responses to environmental change (Li et al., 2016). In particular, because many microbial communities are tightly associated with the utilization and transformations of diverse N nutrients, these communities are likely to be highly sensitive to changes in environmental N supply (Zehr and Kudela, 2010). Accordingly, changes in the abundance and activity of N-associated microbes resulting from fluctuations in available N will inevitably affect the structure of the entire bacterial community (Løvdal et al., 2008). This, in turn, may affect the abundance, diversity, community composition, and physiological characteristics of the associated phytoplankton communities (Liu et al., 2015). Indeed, changes in bacterial communities occur in response to variations in environmental N may have far-reaching implications in many areas, possibly affecting species competition and/or cooperation (Zehr and Ward, 2002; Donald et al., 2011), fluctuations in the N cycle (Fowler et al., 2013), and aquatic eutrophication (Liu et al., 2015).

However, few studies of N-related phycosphere bacteria in aquatic systems and unialgal cultures are available. Limited evidence has shown that some specific microbial groups (e.g., bacteria and cyanobacteria) supply N to coexistent algae (Foster et al., 2011; Amin et al., 2015), while other bacteria compete with the symbiont for N (Risgaard-Petersen et al., 2004; Martens-Habbena et al., 2009). These previous studies have mainly focused on the responses of specific bacterial taxa to N fluctuations or on the functional bacterial genes directly involved in N cycling. Thus, it is still unclear how the bacterial community responds to changes in N conditions in aquatic systems, even though similar studies have investigated terrestrial soil and rhizosphere microbial communities for decades (Ramirez et al., 2010; Sasaki et al., 2013; Ma et al., 2017).

Here, we used high throughput sequencing techniques to investigate the bacterial communities associated with a model diatom (*Phaeodactylum tricornutum*) cultured with different forms and concentrations of inorganic N. Diatoms are ubiquitous photosynthetic eukaryotes, which perform about 20% of global photosynthesis (De Martino et al., 2011). Diatoms and bacteria have co-occurred in marine ecosystems for more than 200 million years, fostering interactions between these groups over evolutionary time scales (Amin et al., 2012). For many decades, *P. tricornutum* has been used as a model of diatom physiology. The genome of *P. tricornutum* has recently been sequenced (Bowler et al., 2008) and more than 130 000 expressed sequence tags (ESTs), derived from cells grown in 16 different conditions, have been generated (Maheswari et al., 2010). The availability of these genetic resources allows gene function in diatoms, and diatom-bacteria interactions, to be better assessed, possibly leading to a better understanding of the ecological roles of these organism in aquatic environments. Such knowledge is vital for deciphering oceanic nutrient fluxes and biogeochemical cycles. In this study, therefore, we aimed to determine: (1) how the bacterial community responded to various N conditions in algal-bacterial co-cultures; (2) which bacterial taxa dominated each culture; and (3) whether any specific bacterial lineages showed dramatic responses to changes in environmental N. Our results indicated that bacterial growth and community structure changed significantly with shifts in algal abundance under different N conditions and suggested that some important bacterial groups may play a role in the regulation of N metabolism within this algal-bacterial co-culture.

2 Materials and methods

2.1 *Algal and bacterial cultures*

The algal strain used in this study was a xenic laboratory culture of the marine diatom *P. tricornutum* strain MASCC 17, originally isolated from an unspecified location in the Yellow Sea. This strain was purchased from the Marine Algae Stock Culture Collection Center (MASCCC, Qingdao, China), and was maintained in f/2+Si algal medium (Guillard and Ryther, 1962; Guillard, 1975) in an illuminated incubator at (22±1)°C, under a light intensity of 80 μmol m^{-2} s^{-1}, with a 12 h dark/12 h light cycle. All bacteria observed here were originally associated with the algal culture, no organisms were added, and no special processing was performed.

2.2 *Factorial experimental design*

Two N concentrations (N-sufficient: 500 μmol/L; and N-deficient: 40 μmol/L) were crossed with three N forms (ammonium, nitrate or both; Fig. 1). The three N-sufficient groups are herein referred to as HA (ammonium: 500 μmol/L NH_4^+), HN (nitrate: 500 μmol/L NO_3^-), and HAN (both: 250 μmol/L NH_4^+ plus 250 μmol/L NO_3^-), while the three N-deficient groups are referred to as LA (ammonium: 40 μmol/L NH_4^+), LN (nitrate: 40 μmol/L NO_3^-), and LAN (both: 20 μmol/L NH_4^+ plus 20 μmol/L NO_3^-). An additional group, with no added N (WN), was used as the negative control. The levels of N deemed sufficient and deficient used here were based on the preliminarily determined N requirements of *P. tricornutum* (Supplementary Methods and Fig. S1).

All experimental groups were cultured in triplicate in 800 mL volumes in 1 L flasks. The initial algal cell density in each culture was 4×10^4 cells/mL. To allow phycosphere bacteria and algae to adapt to our experimental conditions, algae were cultured under each experimental condition (without duplicates) for a 30-day acclimation period. During this period, generations were transferred every 10 days (3 generations in total). Following acclimation, cultures were immediately used as mother cultures for the experiment. All cultures were handled aseptically to prevent cross-contamination by external bacterial or the other cultures.

2.3 *Algal/bacterial enumeration and sample collection*

Algal cells were counted daily using a hemacytometer under a

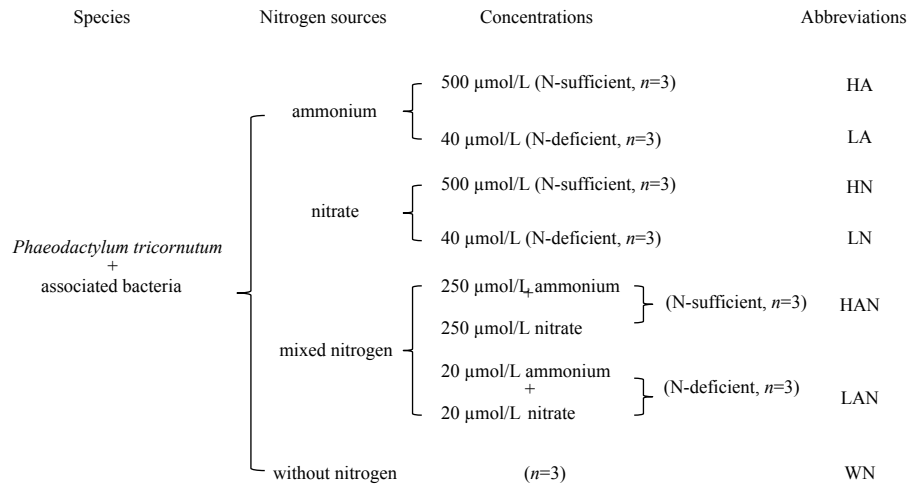

Fig. 1. Diagram of our 3×2 factorial experimental design. WN, the group without added nitrogen (N), was used as the negative control. Each group was cultured in triplicate.

light microscope (ECLIPSE Ci-S; Nikon, Japan), after being stained with Lugol's iodine solution. We counted at least 400 cells from each culture.

The total number of bacteria in each culture was determined using the DAPI (4′, 6-diamidino-2-phenylindole) direct count method (Wang et al., 2010). We fixed 0.2 mL aliquots of each of the triplicate samples by adding 5% (v/v) formalin. DAPI (Sigma-Aldrich, Germany) was added to the fixed samples to yield a final concentration of 5 μg/mL, and the samples were allowed to sit for 30 min. Samples were then filtered with black polycarbonate filters (pore size: 0.22 μm; diameter: 25 mm; Merkmillipore Inc., USA). At least 400 cells (10–20 randomly chosen fields) were counted per filter under an epifluorescence microscope (Eclipse 80i; Nikon, Japan). We calculated bacterial abundance (cells/mL) as $AS_1/(S_2V)$, where A was the average number of cells across 20 fields; S_1 was the effective filter area; S_2 was the area of field of vision; and V was the volume of the algal culture sample that was filtered.

Cells from all samples were harvested 168 h after inoculation by filtering an 100 mL aliquot of each culture through a sterile polycarbonate filter (pore size: 0.22 μm; Merkmillipore Inc., USA), to capture the major phycosphere bacteria. We chose 168 h after inoculation as this was the middle of the exponential phase of algal growth in the N-sufficient algal cultures, and was the onset of the stationary phase in the N-deficient algal cultures (Figs 2a and c). After filtration, filters were immediately frozen in liquid nitrogen and stored at –80°C.

2.4 *Bacterial DNA extraction*

Total bacterial genomic DNA was extracted from the filters using a FastDNA SPIN Kit for Soil (MP Biomedicals, USA), following the manufacturer's instructions. Crude products were visualized using 1% agarose gels stained with ethidium bromide, then purified using a TaKaRa MiniBEST Agarose Gel DNA Extraction Kit (Takara Bio Inc., Japan). All procedures were performed on ice. DNA concentrations were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), after which DNA samples were stored at –80°C.

2.5 *16S rRNA amplification and HiSeq sequencing*

The V4 region of the 16S rRNA gene was amplified using the primer pair 515F (5′-GTGCCAGCAGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2011), attaching a unique 12-bp barcode to the forward primer for each sample. All PCR reactions were performed using Phusion High-Fidelity PCR Master Mix Kits (New England Biolabs, USA), with the following cycling conditions: 94°C for 3 min; followed by 35 cycles of 94°C for 30 s, 52°C for 40 s, and 72°C for 1 min; and a final elongation at 72°C for 10 min. Amplified products were evaluated using electrophoresis on a 2% agarose gel, then purified with a QIAquick Gel Extraction Kit (Qiagen, Germany). After purification, sequencing libraries were generated using a TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, USA), following the manufacturer's instructions, and index codes were added. Library quality was assessed on a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, USA) and an Agilent Bioanalyzer 2 100 system (Agilent Technologies Inc., USA). Finally, libraries were sequenced on an Illumina HiSeq2500 platform (Novogene Co. Ltd., China).

2.6 *Data analysis*

Paired-end reads were merged with FLASH v1.2.7 (Magoč and Salzberg, 2011). QIIME v1.7.0 (Caporaso et al., 2010) was used to analyze the sequencing data and to demultiplex the raw sequences. Low quality and ambiguous reads were removed with QIIME, using default parameters. Chimeric reads were identified against the Gold database (Haas et al., 2011) and removed with the UCHIME algorithm (Edgar et al., 2011). UPARSE v7.0.1001 was used to determine operational taxonomic units (OTUs) at a similarity of 97% (Edgar, 2013). A single sequence was selected to represent each OTU. These representative sequences were taxonomically identified using Mothur (Wang et al., 2007), retrained with the SILVA database (Quast et al., 2013). After removing all OTUs represented by single sequences, as well as those identified as mitochondria or chloroplasts, we analyzed alpha diversity and beta diversity with QIIME. We used alpha diversity (the Shannon-Weaver and Chao1 indices) to assess the internal complexity of the microbial communities. We used beta diversity (based on weighted and unweighted Unifrac matrices) to identify differences in overall community composition. We performed principal coordinate analysis (PCoA) using the WGCNA, stat, and ggplot2 packages in R v3.4.0 (Borcard et al., 2011). We used the hierarchical clustering unweighted pair-group method with arithmetic means (UPGMA) in QIIME to interpret the distance matrix based on average linkages. We used permutational multivariate analyses of variance (PERMANOVAs) to determine the significance of variations in microbial communities based on

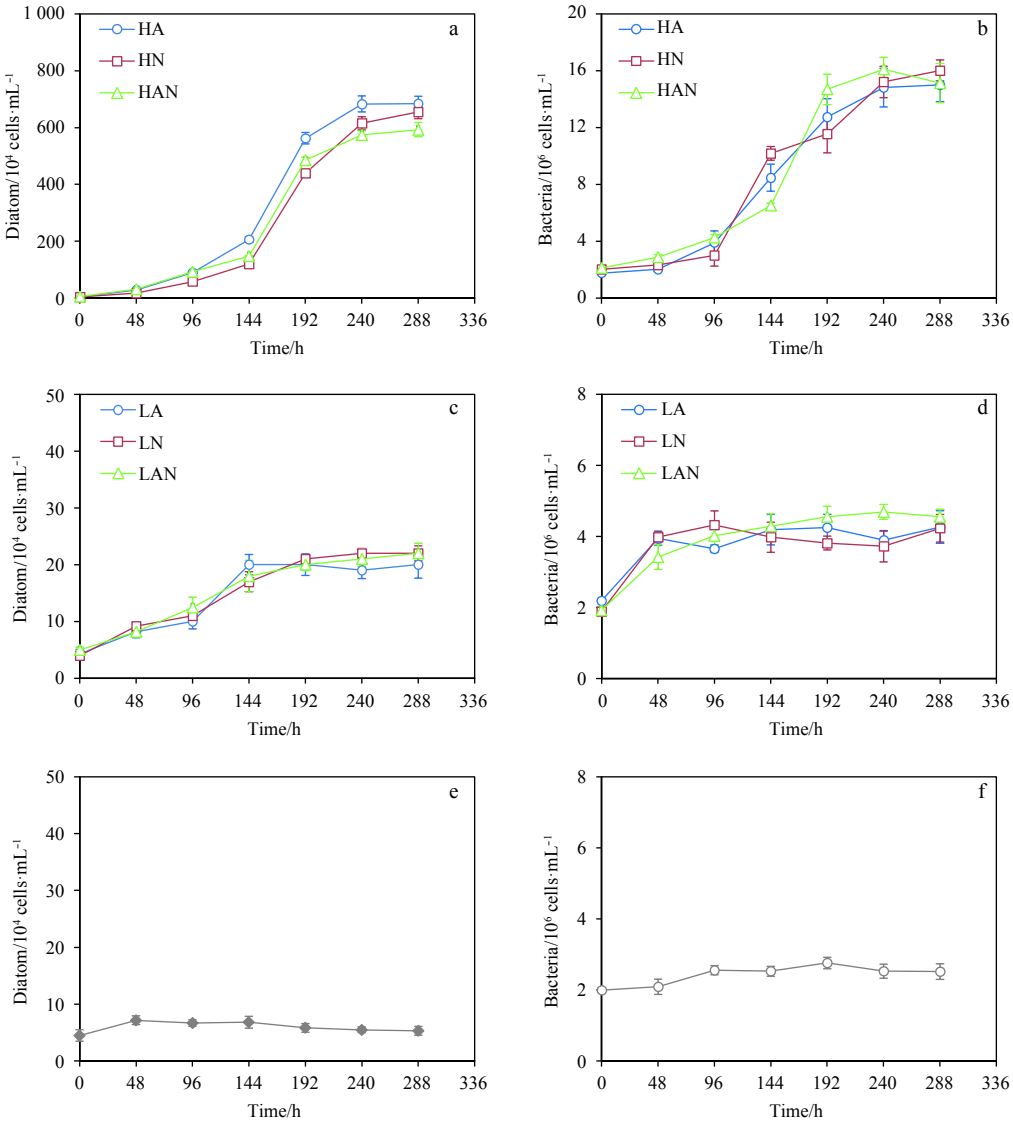

Fig. 2. Growth curves for *Phaeodactylum tricornutum* and total bacteria co-cultured under different nitrogen (N) conditions. a, c and e. Growth of *P. tricornutum* under N-sufficient (500 μmol/L N) conditions (a), N-deficient (40 μmol/L N) conditions (c), and without added N (e). b, d and f. Bacterial growth in *P. tricornutum* co-cultures under N-sufficient (500 μmol/L N) conditions (b), N-deficient (40 μmol/L N) conditions (d), and without added N (f). HA: N-sufficient culture with 500 μmol/L ammonium; HN: N-sufficient culture with 500 μmol/L nitrate; HAN: N-sufficient culture with 250 μmol/L ammonium plus 250 μmol/L nitrate; LA: N-deficient culture with 40 μmol/L ammonium; LN: N-deficient culture with 40 μmol/L nitrate; and LAN: N-deficient culture with 20 μmol/L ammonium plus 20 μmol/L nitrate. Error bars indicate the standard deviation (SD) of three independent cultures.

Bray-Curtis distances. The LDA Effect Size (LEfSe), based on the relative abundance of each microbial taxon, was calculated to identify the more abundant taxa in different cultured samples. Multiple comparisons and ANOVAs were performed with SPSS v20.0 software (IBM, USA). Other analyses were performed in R v3.4.0. All raw sequencing reads have been deposited in the sequence read archive of the National Center for Biotechnology (accession number: SRP119107).

3 Results

3.1 *Variations of algal/bacterial abundance in N-sufficient and N-deficient cultures*

Changes in bacterial abundance were strongly associated with the diatom growth phase in both N-sufficient and N-deficient cultures. In all N-sufficient cultures (HA, HN and HAN) *P. tricornutum* cell concentration increased from 48 h to 240 h after inoculation, at which point the stationary phase was reached (Fig. 2a). The highest algal cell densities were 6.83×10^6 cells/mL (Group HA), 6.55×10^6 cells/mL (Group HN), and 5.93×10^6 cells/mL (Group HAN). As diatom concentration increased, so did bacterial abundance. Bacterial abundance grew rapidly from 96 h to 240 h after inoculation (Fig. 2b). The highest bacterial cell densities were 1.48×10^7 cells/mL (Group HA), 1.52×10^7 cells/mL (Group HN), and 1.61×10^7 cells/mL (Group HAN).

In two of the N-deficient groups (LN and LAN), algal abundance increased slowly until 192 h post-inoculation; in the LA group, growth stopped at 144 h (Fig. 2c). The highest algal cell densities were 2.0×10^5 cells/mL (LA), 2.1×10^5 cells/mL (LN), and 2.0×10^5 cells/mL (LAN). Bacterial abundance increased from 0 h

to 48 h after inoculation, then stabilized (Fig. 2d). The highest bacterial cell densities were 3.95×10^6 cells/mL (Group LA), 3.97×10^6 cells/mL (Group LN), and 3.42×10^6 cells/mL (Group LAN).

There were significant differences in algal and bacterial growth between cultures exposed to different levels of N ($p<0.05$), but algal and bacterial growth varied little among cultures exposed to different forms of N ($p>0.05$). Total bacterial growth was positively correlated with algal growth in both the N-sufficient and the N-deficient co-cultures (Fig. 3). Algal and bacterial abundance in the control cultures (no N added) remained statistically unchanged ($p>0.05$) over the course of the experiment (Figs 2e and f).

3.2 *Diversity and composition of bacterial communities cultured with different N conditions*

We used Illumina sequencing to assess the phycosphere bacterial community composition based on the V4 region of the 16S rRNA gene. After the removal of chimeras, chloroplasts, and low quality reads, we obtained 1 177 575 high quality sequences, with an average length of 253 base pairs (bp), across all 21 samples (Table S1). These sequences were assigned to 614 OTUs. Calculated rarefaction curves indicated that sequencing was near saturation at a genetic distance of 3% across all samples (Fig. S2) (Edgar, 2013). According to the SILVA taxonomic database, the 614 OTUs fell into 22 phyla, 39 classes, 76 orders, 149 families, 201 genera, and 58 known species. Proteobacteria was the most abundant bacterial phylum across all samples (ca. 95.43% of all sequences), followed by Bacteroidetes (4.07%) and Planctomycetes (0.29%). Within the Proteobacteria, the dominant classes were Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria, representing 70.7%, 0.07%, 24.4%, 0.03%, and 0.03%, respectively, of all bacteria. Within the Bacteroidetes, the dominant classes were Cytophagia, Sphingobacteriia, Flavobacteriia, and Bacteroidia, representing 2.34%, 0.73%, 0.57% and 0.01%, respectively, of all bacteria (Fig. 4).

Using the accepted genetic distance of 3%, the Shannon-Weaver index value ranged from 2.29 to 4.72, and the estimated Chao1 ranged from approximately 71.25 to 404.15 (Table S2). Under N-sufficient conditions, the Chao1 index across all samples ranged from 71.75 to 222.26, with the HAN group having a significantly higher Chao1 than either the HA group or HN (Tukey test; $p<0.05$ for both). Under N-deficient conditions, the Chao1 index ranged from 71.25 to 404.15; the species richness of the mixed N source (MNS) groups (LAN) were significantly higher than that of single N source (SNS) groups (LA/LN) ($p<0.05$). Thus, bacterial species richness was significantly higher in the MNS groups than in the SNS groups under both N-sufficient and N-deficient conditions. There were no significant differences in species richness between the two SNS groups. The bacterial species richness was similar across groups grown with the same form of N at different concentrations, except for the MNS groups (HAN and LAN) (Fig. 5a). The species richness of the WN group was similar to that of the SNS groups. In contrast, the Shannon-Weaver index indicated that the LAN group had a significantly higher diversity than the other N-deficient groups (LA/LN) and the HAN group ($p<0.05$). No significant differences were observed among any other forms or concentrations of N groups (Fig. 5b). Additionally, the bacterial diversity of the WN group was very similar to that of the LAN group, and was significantly higher than that of the other groups because of its high evenness.

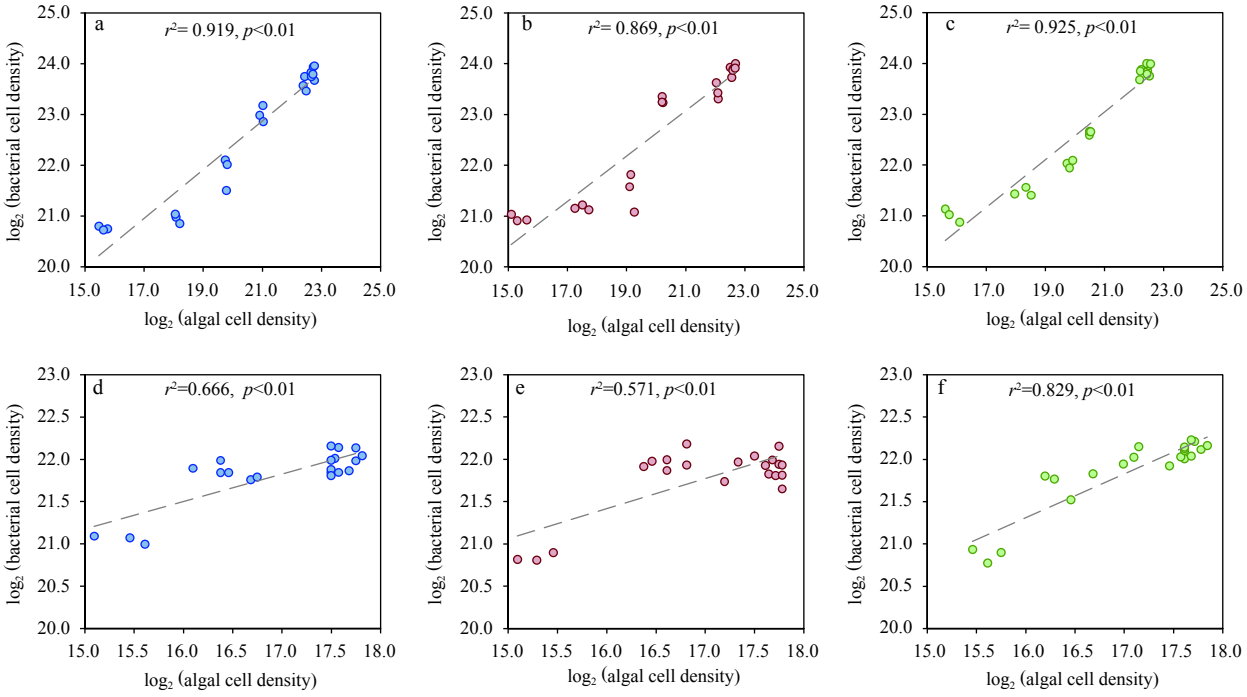

Fig. 3. Correlation of bacterial and algal cell concentrations during different phases of *Phaeodactylum tricornutum* growth in co-cultures with different levels/sources of nitrogen (N). a, b and c. Correlation of bacterial and algal abundance under N-sufficient conditions: 500 μmol/L ammonium (a), 500 μmol/L nitrate (b), and 250 μmol/L ammonium plus 250 μmol/L nitrate (c). d, e and f. Correlation of bacterial and algal abundance under N-deficient conditions: 40 μmol/L ammonium (d), 40 μmol/L nitrate (e), and 20 μmol/L ammonium plus 20 μmol/L nitrate (f). r^2 indicates the coefficient of determination adjusted for the degrees of freedom and p the level of statistical significance.

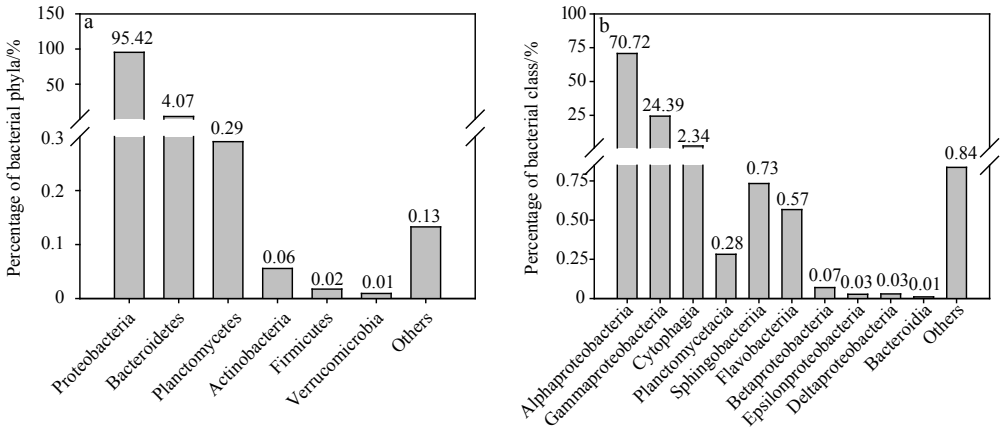

Fig. 4. The operational taxonomic units (OTUs) identified across all 21 samples that were assigned to major bacterial phyla (a) and classes (b) (97% similarity cutoff).

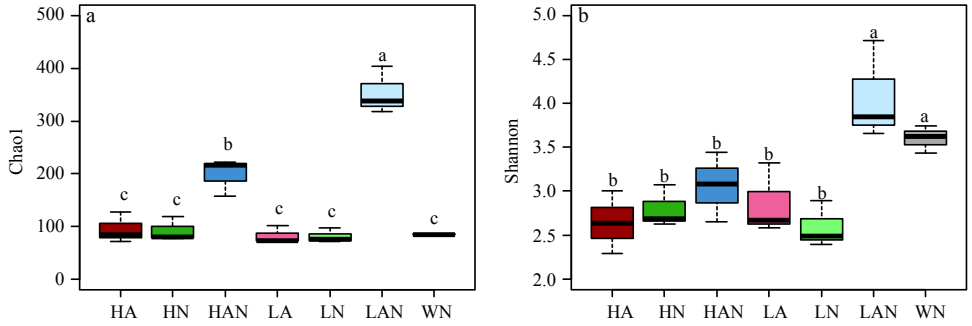

Fig. 5. Chao1 (a) and Shannon (b) indices across the seven groups containing different nitrogen (N) forms/concentrations. HA: N-sufficient culture with 500 μmol/L ammonium; HN: N-sufficient culture with 500 μmol/L nitrate; HAN: N-sufficient culture with 250 μmol/L ammonium plus 250 μmol/L nitrate; LA: N-deficient culture with 40 μmol/L ammonium; LN: N-deficient culture with 40 μmol/L nitrate; LAN: N-deficient culture with 20 μmol/L ammonium plus 20 μmol/L nitrate. From top to bottom, the horizontal lines of each box represent the upper-quartile, median, and lower-quartile, respectively. Whiskers extending from the top and bottom of each box represent maximum and minimum values. Different lowercase letters indicate significant differences (p<0.05).

β-diversity analyses suggested that bacterial communities among cultures grown with different forms of N at equivalent concentrations were more similar than those grown with the same forms of N at different concentrations (Fig. 6). Indeed, dissimilarity tests indicated that community differences between groups grown with same N form, but different N concentrations were significant (PERMANOVA; p<0.05). Moreover, the bacterial community of the WN group was similar to that of the N-deficient groups (PERMANOVA; p>0.05) but significantly different from that of the N-sufficient groups (PERMANOVA; p<0.05).

In the PCoA analysis, the bacterial communities of N-sufficient groups clustered together, indicating that these communities were similar; the N-deficient groups formed a separate cluster (Fig. 7a). However, samples grown with different N forms at identical concentrations were not separated, indicating that the bacterial communities of these samples were similar (Fig. 7a). The first principal component axis (PC1) explained 56.99% of the variation, while the second principal component axis (PC2) explained 13.47%. Our UPGMA results were consistent with the PCoA results (Fig. 7b).

3.3 *Typical taxa under different culture conditions*

We used LEfSe (Segata et al., 2011) to determine the significant taxa in each different treatment (N concentration/form). All taxa (average relative abundance>1%) with marked changes in relative abundance were Proteobacteria, and all significant changes in abundance were identified between the N-sufficient and N-deficient groups. Within the Proteobacteria, three gammaproteobacterial taxa were most differentially abundant in the N-deficient cultures: the genus *Marinobacter* (Fig. 8a), the genus *Algiphilus* (Fig. 8b) and the species *Methylophaga nitratireducenticrescens* (genus *Methylophaga*; Fig. 8c). One Alphaproteobacteria, the species *Sinorickettsia chlamys* (genus unidentified LWSR-14), was much more abundant in the N-sufficient groups (Fig. 8d).

4 Discussion

4.1 *Impact of different N conditions on bacterial abundance, diversity, and community structure*

Bacterial abundance is directly related to bacterial activity and aquatic ecosystem health (Liu et al., 2015). Investigations of bacterial abundance deepen our understanding of algal-bacterial interactions in the phycosphere. Here, we simultaneously monitored the growth of *P. tricornutum* and its associated bacteria under different N conditions. Bacterial growth closely coincided with that of algae in all cultures (N-sufficient, N-deficient, and without added N; Figs 2 and 3). Moreover, no obvious chang-

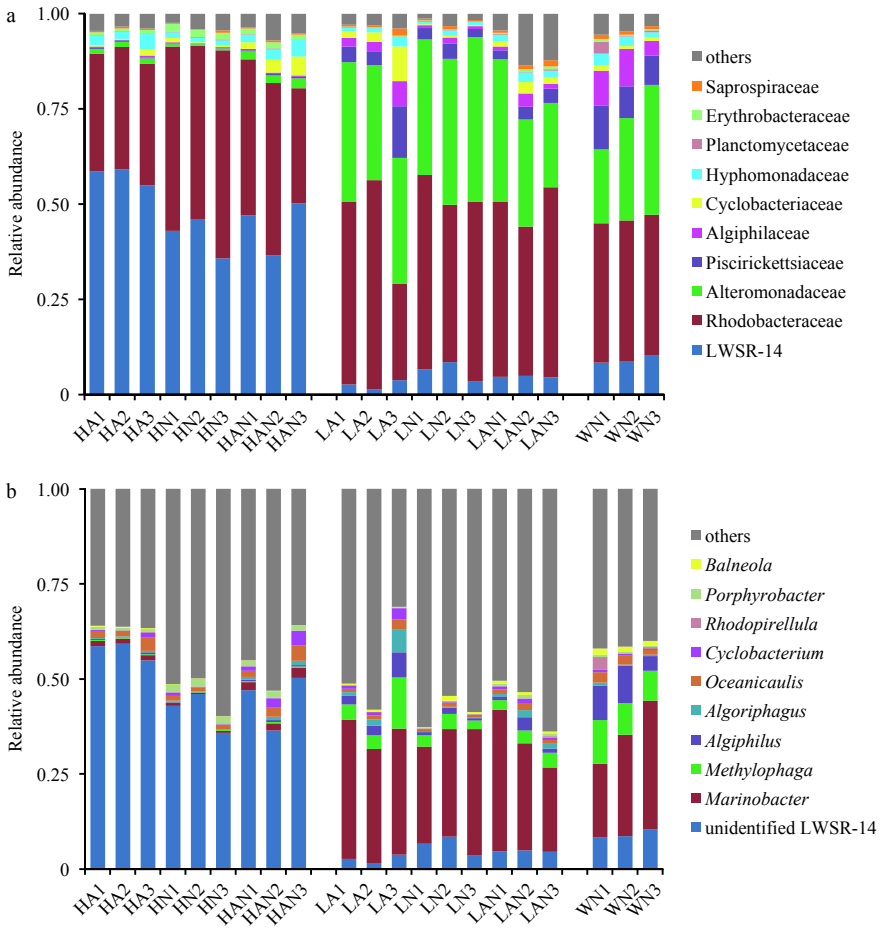

Fig. 6. Bacterial community structure in the different experimental groups at the family (a) and genus levels (b). Only the top 10 taxa are shown.

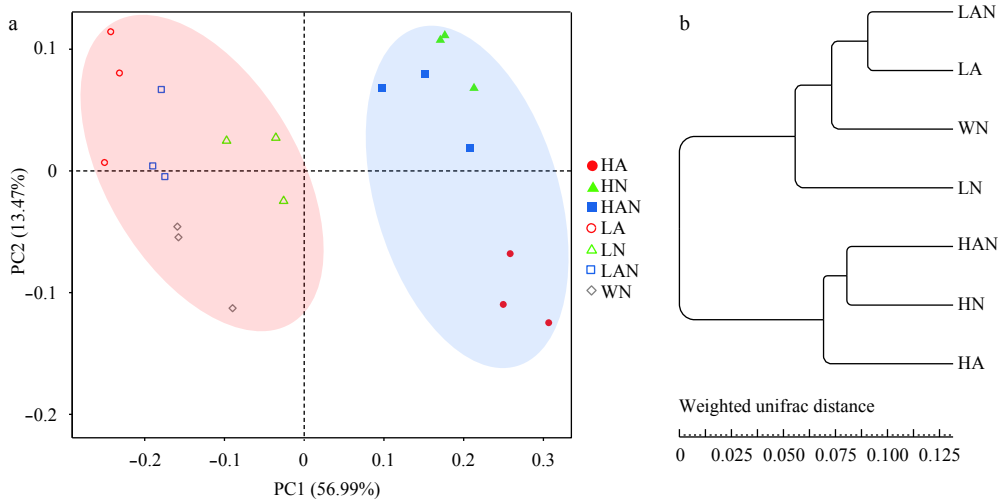

Fig. 7. Principal coordinate analysis of all 21 groups (Pink oval contains nitrogen (N)-sufficient bacterial communities and blue oval contains N-deficient bacterial communities) (a); and unweighted pair-group method with arithmetic means hierarchical clustering (UPGMA) of all seven groups, using the weighted UniFrac distance metric (each leaf represents three independent experiments) (b).

es were observed in the abundance of bacterial communities grown without *P. tricornutum* under different N conditions (Fig. S3). Based these results, it is likely that bacterial activity in the phycosphere is regulated by the extracellular products of *P. tricornutum*. Indeed, the f/2+Si media (used here), lacks a carbon source essential for bacterial growth (Guillard, 1975). Many other studies have suggested that bacterial growth is mainly dependent on the organic exudates of diatoms (Wang et al., 2010; Behringer et al., 2018).

Biodiversity and species composition influence ecosystem

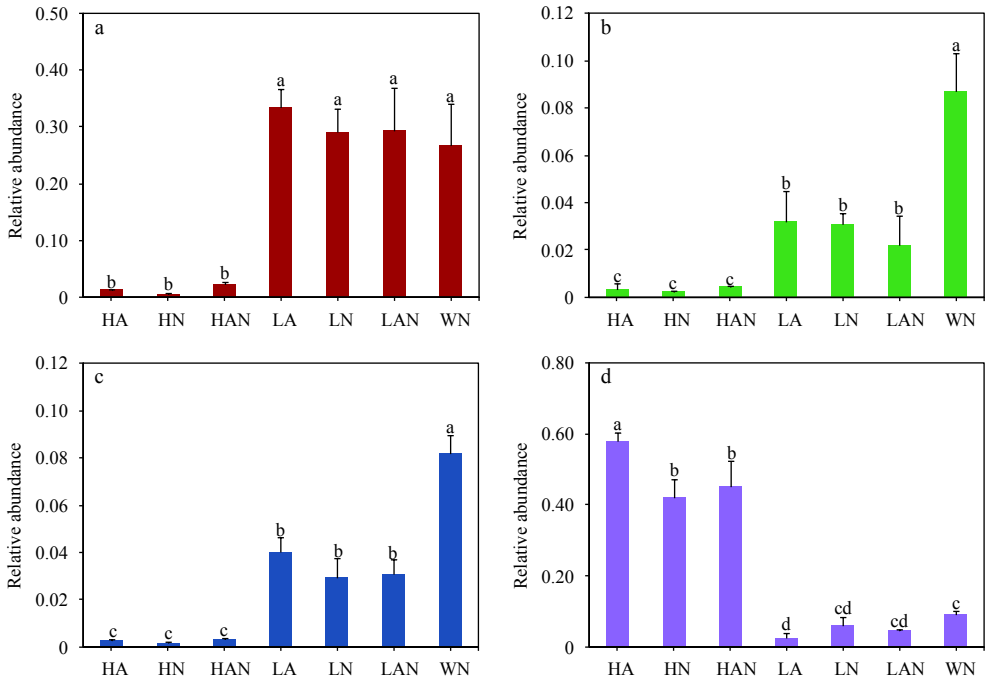

Fig. 8. Relative abundance of four typical bacterial taxa across the seven different nitrogen (N) treatment groups, filtered by the LDA Effect Size (LEfSe) method: *Marinobacter* (a), *Algiphilus* (b), *Methylophaga nitratireducenticrescens* (c), and *Sinorickettsia chlamys* (d). Error bars represent standard error of the mean of three independent experiments. Different lowercase letters indicate statistically significant differences (p<0.05).

structure and function, thereby raising concerns about the consequences of biodiversity changes caused by environmental perturbations (Chapin III et al., 2000; Balvanera et al., 2006). Here, bacterial species richness and diversity were significantly higher in the MNS groups than in the SNS groups (p<0.05), although no differences in the Shannon indices were observed between HAN and HA or HN. This suggested that the simultaneous presence of multiple N sources might influence algal-bacterial symbiosis state, which may benefit bacterial growth and diversification directly or indirectly. More bacterial species were present in the MNS phycosphere, although these were not the dominant flora.

Bacterial communities changed markedly with changes in N concentration (Fig. 6). It is possible that this was because bacterial community structure changed with *P. tricornutum* abundance. That is, phytoplankton release organic matter, providing bacteria with carbon, N, and other energy sources (Buchan et al., 2014), and bacterial community structure often depends on nutrient composition. Therefore, bacterial community composition may have changed with phytoplankton abundance in our cultures treated with different N concentrations. To test this, we performed a control experiment to determine the impact of different concentrations of inorganic N on phycosphere bacterial cultures without *P. tricornutum* (Supplementary Methods). We found that bacterial communities differed little at different N concentrations when grown without *P. tricornutum* (PERMANOVA; p>0.05; Fig. S4), indicating that the observed differences in bacterial communities between the algal-bacterial groups grown with different levels of N may have been caused by the algae.

We also found no differences in bacterial community structure when the algal-bacterial co-cultures were grown with different forms of N. This may have been because the dominant bacteria in our cultures were more affected by the algal exudates than by inorganic N. Although marine bacteria feed on a small set of low molecular mass compounds possibly excreted by algae (Bell, 1984), long-term selection should favor bacterial populations well-adapted to the spectrum of organic materials offered by algae. Additionally, no significant differences were found in algal abundance among the cultures grown with different N forms (Fig. 2). The similar algal densities in these groups probably provided bacteria with a similar range of algal exudates, resulting in similarly structured bacterial communities.

4.2 *Variations in typical bacterial taxa under different N conditions and their potential roles in the phycosphere*

Phylogenetic characterization of the bacteria obtained across all samples identified only a few dominant bacterial taxa. Proteobacteria and Bacteroidetes dominated all cultures, consistent with many other studies showing that Proteobacteria and Bacteroidetes are the main heterotrophic bacterial phyla associated with diatoms and dinoflagellates (Kaczmarska et al., 2005; Sapp et al., 2007; Green et al., 2010; Grossart et al., 2010). Diatom-associated bacteria are generally restricted to a small number of genera (e.g., *Rosebacter*, *Sulfitobacter*, *Marinobacter*, *Alteromonas*, and *Flavobacterium*) because of their specific phycosphere functions, including the decomposition of algal exudates (Buchan et al., 2000); the production and consumption of dimethyl sulfoniopropionate (DMSP) (González et al., 1999; Miller and Belas, 2004); the stimulation of phytoplankton growth via the production of vitamins (Haines and Guillard, 1974; Croft et al., 2005), iron chelators (siderophores) (Amin et al., 2009), and cytokinins (Maruyama et al., 1986); and the provision of nutrients such as N (as ammonium) to phytoplankton (Amin et al., 2015). Here, Rhodobacteraceae (class Alphaproteobacteria) was the most abundant bacterial family across all cultures, and its relative abundance did not change significantly among cultures grown with different forms/concentrations of N. A high incidence of

Rhodobacteraceae associated with diatom and dinoflagellate cultures has previously been reported in several studies (Schäfer et al., 2002; Green et al., 2004). Several common taxa in this family (especially the *Rosebacter* clade) have been shown to degrade aromatic compounds and DMSP, which may be excreted by algae as a source of both carbon and sulfur (Jasti et al., 2005). It is therefore possible that the large number of algal excretions and residues in our cultures were sufficient to support a stable Rhodobacteraceae community.

We used LEfSe to identify alterations in species composition and indicator species among different cultures. The most abundant phylogenetic cluster correlated with the different N concentrations was the gammaproteobacterial genus *Marinobacter* (Fig. 8a), which is the most diverse genus in the Alteromonadaceae (Gauthier et al., 1992). *Marinobacter* is common in oceans worldwide (Lupette et al., 2016), and species in this genus are associated with a range of algae, principally dinoflagellates (Alavi et al., 2001; Seibold et al., 2001; Green et al., 2010; Hatton et al., 2012), but also diatoms and coccolithophorids (Amin et al., 2009; Green et al., 2015). *Marinobacter* species dominate algal cultures because they perform functions important to algae, including providing vitamin B_{12} (Kuo and Lin, 2013), producing siderophores (Amin et al., 2009), and degrading hydrocarbons (Green et al., 2015). Here, *Marinobacter* was closely associated with the diatom *P. tricornutum*. Interestingly, the relative abundance of *Marinobacter* was markedly increased when N was limited. It has been suggested that *Marinobacter* species directly reduce nitrate to ammonium (dissimilatory nitrate reduction; De La Haba et al., 2011). We therefore postulate that *Marinobacter* might play a critical role in N metabolism in the phycosphere when N nutrients are limited. Additional studies are required to identify the metabolic functions of this specific genus in algal-bacterial associations.

The two other bacterial taxa identified were both Gammaproteobacteria: the genus *Algiphilus* (Fig. 8b) and the species *Methylophaga nitratireducenticrescens* (Fig. 8c). The *Algiphilus* genus was first isolated from a culture of the marine dinoflagellate *Lingulodinium polyedrum* (Gutierrez et al., 2012), while *Methylophaga nitratireducenticrescens* was first identified from the biofilm of a methanol-fed denitrification system (Villeneuve et al., 2013). Notably, both of these taxa reduce nitrate to nitrite. This is consistent with our speculation that specific bacteria groups might be involved in N metabolism when associated with *P. tricornutum*. Indeed, specific interactions between algae and their microbial counterparts related to N cycling have previously been reported. For example, Amin et al. (2015) demonstrated that, when co-cultured, the specific bacterial strain SA11 increased nitrate uptake and ammonium release, while *Pseudonitzschia multiseries* preferred to utilize bacterially-derived ammonium for growth, rather than exogenous nitrate. Foster et al. (2011) suggested that the N fixation rates of two cyanobacteria (*Richelia* and *Calothrix*) were 171–420 times higher when grown with diatoms as compared to when grown alone, and that the majority of the fixed N was transferred to their symbiont. Here, we used the PICRUSt software (Langille et al., 2013) to infer the potential functional role of the bacterial communities we identified. We detected some genes involved in N metabolism, and these were more abundant in N-deficient cultures (Fig. S5). These results further demonstrated that bacteria might participate in N metabolism in the phycosphere, and that these bacteria were more active when N was limited.

Another Alphaproteobacteria, *Sinorickettsia chlamys* (order: Rickettsiales; family: LWSR-14), was also identified. Interestingly, the abundance of this species varied inversely with the Gammaproteobacteria, as it was much more abundant in the N-sufficient cultures than in the N-deficient cultures (Fig. 8d). Unfortunately, information about this species is limited: its ecology and its relationship to algae are unclear.

Overall, although we detected some changes in specific bacterial floras and in bacterial community structures when cultured with different forms/concentrations of N, it remains unknown how and when these changes occurred. It also remains unclear if N metabolism regulated the interactions between bacteria and algae. Further studies, including time series co-cultures and metatranscriptomic analyses, are needed to more fully explore the influence of N utilization on bacterial-algal interactions, as well as to elucidate the mechanisms responsible for this association. It is also important to note that we studied the relationship between an alga and its phycosphere bacteria under laboratory conditions, and it is unlikely that the bacteria studied here accurately represent all of the bacterial taxa associated with a particular algal population over a range of ecological and environmental conditions (Jasti et al., 2005). Future field studies are required to better understand the functioning and community structure of phycosphere bacteria.

5 Conclusions

We show that the growth, distribution, and the formation of bacterial community structure in the phycosphere were greatly affected by *P. tricornutum*. N limitation inhibited algal growth, which in turn affected bacterial growth and lead to changes in bacterial community structure. The bacterial communities associated with all *P. tricornutum* co-cultures were dominated by two common marine groups, Proteobacteria and Bacteroidetes, which are associated with many dinoflagellates and diatoms. Three gammaproteobacterial genera (*Marinobacter*, *Algiphilus* and *Methylophaga*) play a potential role in N metabolism, and their relative abundances increased markedly in N-deficient cultures. Our results provide evidence that bacterial-algal interactions respond to shifts in environmental N. Further experiments are needed to explore the mechanisms underlying this association.

References

Alavi M, Miller T, Erlandson K, et al. 2001. Bacterial community associated with *Pfiesteria*-like dinoflagellate cultures. Environmental Microbiology, 3(6): 380–396

Amin S A, Green D H, Hart M C, et al. 2009. Photolysis of iron-siderophore chelates promotes bacterial-algal mutualism. Proceedings of the National Academy of Sciences of the United States of America, 106(40): 17071–17076

Amin S A, Hmelo L R, Van Tol H M, et al. 2015. Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. Nature, 522(7554): 98–101

Amin S A, Parker M S, Armbrust E V. 2012. Interactions between diatoms and bacteria. Microbiology and Molecular Biology Reviews, 76(3): 667–684

Balvanera P, Pfisterer A B, Buchmann N, et al. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters, 9(10): 1146–1156

Behringer G, Ochsenkühn M A, Fei Cong, et al. 2018. Bacterial communities of diatoms display strong conservation across strains and time. Frontiers in Microbiology, 9: 659

Bell W, Mitchell R. 1972. Chemotactic and growth responses of marine bacteria to algal extracellular products. The Biological Bulletin, 143(2): 265–277

Bell W H. 1984. Bacterial adaptation to low-nutrient conditions as studied with algal extracellular products. Microbial Ecology, 10(3): 217–230

Bolch C J S, Bejoy T A, Green D H. 2017. Bacterial associates modify

growth dynamics of the dinoflagellate *Gymnodinium catenatum*. Frontiers in Microbiology, 8: 670
Borcard D, Gillet F, Legendre P. 2011. Numerical Ecology with R. New York: Springer, 115–151
Bowler C, Allen A E, Badger J H, et al. 2008. The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. Nature, 456(7219): 239–244
Buchan A, Collier L S, Neidle E L, et al. 2000. Key aromatic-ring-cleaving enzyme, protocatechuate 3,4-Dioxygenase, in the ecologically important marine *Roseobacter* Lineage. Applied and Environmental Microbiology, 66(11): 4662–4672
Buchan A, LeCleir G R, Gulvik C A, et al. 2014. Master recyclers: features and functions of bacteria associated with phytoplankton blooms. Nature Reviews Microbiology, 12(10): 686–698
Capone D G, Bronk D A, Mulholland M R, et al. 2008. Nitrogen in the Marine Environment. 2nd ed. San Diego: Academic Press, 757
Caporaso J G, Kuczynski J, Stombaugh J, et al. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods, 7(5): 335–336
Caporaso J G, Lauber C L, Walters W A, et al. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences of the United States of America, 108(Suppl 1): 4516–4522
Casciotti K L. 2016. Nitrogen and oxygen isotopic studies of the marine nitrogen cycle. Annual Review of Marine Science, 8: 379–407
Chapin III F S I, Zavaleta E S, Eviner V T, et al. 2000. Consequences of changing biodiversity. Nature, 405(6783): 234–242
Chiu H C, Levy R, Borenstein E. 2014. Emergent biosynthetic capacity in simple microbial communities. PLoS Computational Biology, 10(7): e1003695
Croft M T, Lawrence A D, Raux-Deery E, et al. 2005. Algae acquire vitamin B12 through a symbiotic relationship with bacteria. Nature, 438(7064): 90–93
de-Bashan L E, Antoun H, Bashan Y. 2008. Involvement of indole-3-acetic acid produced by the growth-promoting bacterium *Azospirillum* spp. in promoting growth of *Chlorella vulgaris*. Journal of Phycology, 44(4): 938–947
De La Haba R R, Sánchez-Porro C, Marquez M C, et al. 2011. Taxonomy of halophiles. In: Horikoshi K, ed. Extremophiles Handbook. Tokyo: Springer, 255–265
De Martino A, Bartual A, Willis A, et al. 2011. Physiological and molecular evidence that environmental changes elicit morphological interconversion in the model diatom *Phaeodactylum tricornutum*. Protist, 162(3): 462–481
Donald D B, Bogard M J, Finlay K, et al. 2011. Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. Limnology and Oceanography, 56(6): 2161–2175
Edgar R C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods, 10(10): 996–998
Edgar R C, Haas B J, Clemente J C, et al. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics, 27(16): 2194–2200
Foster R A, Kuypers M M M, Vagner T, et al. 2011. Nitrogen fixation and transfer in open ocean diatom-cyanobacterial symbioses. ISME Journal, 5(9): 1484–1493
Fowler D, Coyle M, Skiba U, et al. 2013. The global nitrogen cycle in the twenty-first century. Philosophical Transactions of the Royal Society B: Biological Sciences, 368(1621): 20130164
Gauthier M J, Lafay B, Christen R, et al. 1992. *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. International Journal of Systematic and Evolutionary Microbiology, 42(4): 568–576
González J M, Kiene R P, Moran M A. 1999. Transformation of sulfur compounds by an abundant lineage of marine bacteria in the α-subclass of the class *Proteobacteria*. Applied and Environmental Microbiology, 65(9): 3810–3819
Green D H, Echavarri-Bravo V, Brennan D, et al. 2015. Bacterial diversity associated with the coccolithophorid algae *Emiliania huxleyi* and *Coccolithus pelagicus* f. *braarudii*. BioMed Research International, 2015: 194540
Green D H, Hart M C, Blackburn S I, et al. 2010. Bacterial diversity of *Gymnodinium catenatum* and its relationship to dinoflagellate toxicity. Aquatic Microbial Ecology, 61(1): 73–87
Green D H, Llewellyn L E, Negri A P, et al. 2004. Phylogenetic and functional diversity of the cultivable bacterial community associated with the paralytic shellfish poisoning dinoflagellate *Gymnodinium catenatum*. FEMS Microbiology Ecology, 47(3): 345–357
Grossart H P, Levold F, Allgaier M, et al. 2010. Marine diatom species harbour distinct bacterial communities. Environmental Microbiology, 7(6): 860–873
Guillard R R L. 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith W L, Chanley M H, ed. Culture of Marine Invertebrate Animals. Boston, MA: Springer, 29–60
Guillard R R L, Ryther J H. 1962. Studies of marine planktonic diatoms: I. Cyclotella nana Hustedt, and Detonula confervacea (cleve) Gran. Canadian Journal of Microbiology, 8(2): 229–239
Gutierrez T, Green D H, Whitman W B, et al. 2012. *Algiphilus aromaticivorans* gen. nov., sp. nov., an aromatic hydrocarbon-degrading bacterium isolated from a culture of the marine dinoflagellate *Lingulodinium polyedrum*, and proposal of *Algiphilaceae* fam. nov.. International Journal of Systematic and Evolutionary Microbiology, 62(11): 2743–2749
Haas B J, Gevers D, Earl A M, et al. 2011. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Research, 21(3): 494–504
Haines K C, Guillard R R L. 1974. Growth of vitamin B_{12}-requiring marine diatoms in mixed laboratory cultures with vitamin B_{12}-producing marine bacteria. Journal of Phycology, 10(3): 245–252
Hatton A D, Shenoy D M, Hart M C, et al. 2012. Metabolism of DMSP, DMS and DMSO by the cultivable bacterial community associated with the DMSP-producing dinoflagellate *Scrippsiella trochoidea*. Biogeochemistry, 110(1–3): 131–146
Jasti S, Sieracki M E, Poulton N J, et al. 2005. Phylogenetic diversity and specificity of bacteria closely associated with *Alexandrium* spp. and other phytoplankton. Applied and Environmental Microbiology, 71(7): 3483–3494
Kaczmarska I, Ehrman J M, Bates E S S, et al. 2005. Diversity and distribution of epibiotic bacteria on *Pseudo-nitzschia multiseries* (Bacillariophyceae) in culture, and comparison with those on diatoms in native seawater. Harmful Algae, 4(4): 725–741
Kuo R C, Lin Senjie. 2013. Ectobiotic and endobiotic bacteria associated with *Eutreptiella* sp. isolated from Long Island Sound. Protist, 164(1): 60–74
Langille M G I, Zaneveld J, Caporaso J G, et al. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology, 31(9): 814–821
Li Yang, Wang Zhen, Lin Xuezheng. 2016. Microbial community structure of Arctic seawater as revealed by pyrosequencing. Acta Oceanologica Sinica, 35(6): 78–84
Little A E F, Robinson C J, Peterson S B, et al. 2008. Rules of engagement: interspecies interactions that regulate microbial communities. Annual Review of Microbiology, 62: 375–401
Liu Lemian, Yang Jun, Lv Hong, et al. 2015. Phytoplankton communities exhibit a stronger response to environmental changes than bacterioplankton in three subtropical reservoirs. Environmental Science & Technology, 49(18): 10850–10858
Løvdal T, Eichner C, Grossart H P, et al. 2008. Competition for inorganic and organic forms of nitrogen and phosphorous between phytoplankton and bacteria during an *Emiliania huxleyi* spring bloom. Biogeosciences, 5(2): 371–383
Lupette J, Lami R, Krasovec M, et al. 2016. *Marinobacter* dominates the bacterial community of the *Ostreococcus tauri* phycosphere in culture. Frontiers in Microbiology, 7: 1414
Ma Yuexin, Tao Wei, Liu Changfa, et al. 2017. Response of microbial biomass and bacterial community composition to fertilization in a salt marsh in China. Acta Oceanologica Sinica, 36(6): 80–88

Magoč T, Salzberg S L. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics, 27(21): 2957–2963

Maheswari U, Jabbari K, Petit J L, et al. 2010. Digital expression profiling of novel diatom transcripts provides insight into their biological functions. Genome Biology, 11(8): R85

Martens-Habbena W, Berube P M, Urakawa H, et al. 2009. Ammonia oxidation kinetics determine niche separation of nitrifying archaea and bacteria. Nature, 461(7266): 976–979

Maruyama A, Maeda M, Simidu U. 1986. Occurrence of plant hormone (cytokinin)-producing bacteria in the sea. Journal of Applied Bacteriology, 61(6): 569–574

Mayali X, Azam F. 2004. Algicidal bacteria in the sea and their impact on algal blooms. Journal of Eukaryotic Microbiology, 51(2): 139–144

Miller T R, Belas R. 2004. Dimethylsulfoniopropionate metabolism by *Pfiesteria*-associated *Roseobacter* spp.. Applied and Environmental Microbiology, 70(6): 3383–3391

Paerl H W, Dyble J, Moisander P H, et al. 2003. Microbial indicators of aquatic ecosystem change: current applications to eutrophication studies. FEMS Microbiology Ecology, 46(3): 233–246

Quast C, Pruesse E, Yilmaz P, et al. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research, 41(D1): D590–D596

Ramanan R, Kim B H, Cho D H, et al. 2016. Algae-bacteria interactions: Evolution, ecology and emerging applications. Biotechnology Advances, 34(1): 14–29

Ramirez K S, Lauber C L, Knight R, et al. 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology, 91(12): 3463–3470

Risgaard-Petersen N, Nicolaisen M H, Revsbech N P, et al. 2004. Competition between ammonia-oxidizing bacteria and benthic microalgae. Applied and Environmental Microbiology, 70(9): 5528–5537

Rooney-Varga J N, Giewat M W, Savin M C, et al. 2005. Links between phytoplankton and bacterial community dynamics in a coastal marine environment. Microbial Ecology, 49(1): 163–175

Sapp M, Wichels A, Wiltshire K H, et al. 2007. Bacterial community dynamics during the winter-spring transition in the North Sea. FEMS Microbiology Ecology, 59(3): 622–637

Sasaki K, Ikeda S, Ohkubo T, et al. 2013. Effects of plant genotype and nitrogen level on bacterial communities in rice shoots and roots. Microbes and Environments, 28(3): 391–395

Schäfer H, Abbas B, Witte H, et al. 2002. Genetic diversity of 'satellite' bacteria present in cultures of marine diatoms. FEMS Microbiology Ecology, 42(1): 25–35

Segata N, Izard J, Waldron L, et al. 2011. Metagenomic biomarker discovery and explanation. Genome Biology, 12(6): R60

Seymour J R, Amin S A, Raina J B, et al. 2017. Zooming in on the phycosphere: the ecological interface for phytoplankton-bacteria relationships. Nature Microbiology, 2: 17065

Seibold A, Wichels A, Schütt C. 2001. Diversity of endocytic bacteria in the dinoflagellate *Noctiluca scintillans*. Aquatic Microbial Ecology, 25(3): 229–235

Villeneuve C, Martineau C, Mauffrey F, et al. 2013. *Methylophaga nitratireducenticrescens* sp. nov. and *Methylophaga frappieri* sp. nov., isolated from the biofilm of the methanol-fed denitrification system treating the seawater at the Montreal Biodome. International Journal of Systematic and Evolutionary Microbiology, 63(6): 2216–2222

Wang Qiong, Garrity G M, Tiedje J M, et al. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology, 73(16): 5261–5267

Wang Xin, Li Zhijiang, Su Jianqiang, et al. 2010. Lysis of a red-tide causing alga, *Alexandrium tamarense*, caused by bacteria from its phycosphere. Biological Control, 52(2): 123–130

Wemheuer B, Güllert S, Billerbeck S, et al. 2014. Impact of a phytoplankton bloom on the diversity of the active bacterial community in the southern North Sea as revealed by metatranscriptomic approaches. FEMS Microbiology Ecology, 87(2): 378–389

Yang Caiyun, Li Yi, Zhou B, et al. 2015. Illumina sequencing-based analysis of free-living bacterial community dynamics during an *Akashiwo sanguine* bloom in Xiamen sea, China. Scientific Reports, 5: 8476

Zehr J P, Kudela R M. 2010. Nitrogen cycle of the open ocean: from genes to ecosystems. Annual Review of Marine Science, 3: 197–225

Zehr J P, Ward B B. 2002. Nitrogen cycling in the ocean: new perspectives on processes and paradigms. Applied and Environmental Microbiology, 68(3): 1015–1024

Supplementary information:

Supplementary methods.

Table S1. Sequence statistics of the 16S rRNA datasets.

Table S2. Bacterial diversity (Shannon) and richness (Chao1) indices of all samples.

Fig. S1. Maximum algal biomass grown in different concentrations of nitrate (a) and ammonium (b).

Fig. S2. Rarefaction curves for all 21 samples at the 97% sequence identity cutoff.

Fig. S3. Total bacterial cells when grown without *Phaeodactylum tricornutum* under nitrogen (N)-sufficient (500 μmol/L) (a) and N-deficient (40 μmol/L) conditions (b).

Fig. S4. Bacterial communities grown without *Phaeodactylum tricornutum* at the family (a) and genus levels (b).

Fig. S5. Heatmap showing the predicted differences among the 21 investigated communities based on orthologous Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways associated with nitrogen (N).

The supplementary information is available online at www.hyxb.org.cn/aosen/ch/index.aspx. The supplementary information is published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.