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Insignificant effects of elevated CO_2 on bacterioplankton community in a eutrophic coastal mesocosm experiment

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Abstract

There is increasing concern about the effects of ocean acidification on marine biogeochemical and ecological processes and the organisms that drive them, including marine bacteria. Here, we examine the effects of elevated CO_2 on bacterioplankton community during a mesocosm experiment using an artificial phytoplankton community in subtropical, eutrophic coastal waters of Xiamen, Southern China. We found that the elevated CO_2 hardly altered the network structure of the bacterioplankton taxa present with high abundance but appeared to reassemble the community network of taxa present with low abundance by sequencing of the bacterial 16S rRNA gene V3-V4 region and ecological network analysis. This led to relatively high resilience of the whole bacterioplankton community to the elevated CO_2 level and associated chemical changes. We also observed that the Flavobacteriia group, which plays an important role in the microbial carbon pump, showed higher relative abundance under elevated CO_2 condition during the developing stage of the phytoplankton bloom in the mesocosms. Compared to the CO_2 enrichment, the phytoplankton bloom had more pronounced effects on baterioplankton community structure. Our results suggest that the bacterioplankton community in this subtropical, high nutrient coastal environment is relatively insensitive to changes in seawater carbonate chemistry.

Key words: elevated CO_2; mesocosm; bacterioplankton community; ecological network; Flavobacteriia

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1 Introduction

It is well established that ocean acidification is being caused by increased uptake of anthropogenically-derived carbon dioxide in the surface ocean. Consequently, it is predicted that under a "business-as-usual" CO_2 emission scenario, the present average surface pH value will drop 0.4 over the next century (Gattuso et al., 2015). Despite a growing interest in the importance of the roles of marine bacterioplankton in ocean ecosystems and biogeochemical cycles, our current understanding of their responses to ocean acidification is still limited. Over half of autotrophically-fixed oceanic CO_2 is processed by heterotrophic bacteria and archaea through the microbial loop and carbon pump (Azam, 1998; Jiao *et al.*, 2010). Furthermore, marine bacterioplankton play an essential role in marine ecosystems and global biogeochemical cycles central to the biological chemistry of Earth (Falkowski et al., 2008). The null hypothesis is that elevated CO_2 will not affect biogeochemistry processes (Liu *et al.*, 2010; Joint *et al.*, 2011), however more investigation is required. Ocean acidification mesocosm experiments provide good opportunities to explore the responses of marine organisms, including marine bacteria, to elevated CO_2. Mesocosm studies conducted in the Arctic Ocean, Norway, Sweden and the Mediterranean coastal sea using natural phytoplankton communities have found that elevated CO_2 has little direct effect on the bacterioplankton community (Zhang et al., 2013; Ray et al., 2012, Roy et al., 2013; Baltar et al., 2015). In contrast, phytoplankton blooms induced by high CO_2 can sometimes have significant indirect effects on heterotrophic microbes, thus altering bacterioplankton community structure (Allgaier et al., 2008).

Although most mesocosm studies have showed that elevated CO_2 had an insignificant impact on bacterioplankton community structure, microcosm experiments have demonstrated that small changes in pH can have direct effects on marine bacterial community composition (Krause et al., 2012). Ocean

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acidification experiments using natural biofilms showed bacterial community shifts, with decreasing relative abundance of Alphaproteobacteria and increasing Flavobacteriales (Witt et al., 2011). Coastal microbial biofilms grown at high CO_2 level also showed different community structures compared to those grown at ambient CO_2 level in a natural carbon dioxide vent ecosystem (Lidbury et al., 2012). Ocean acidification also affects the community structure of bacteria associated with corals. It has been reported that the relative abundance of bacteria associated with diseased and stressed corals increased under decreasing pH conditions (Meron et al., 2011). The effects of ocean acidification on isolated bacterial strains have also been investigated. Under lab conditions, growth of *Vibrio alginolyticus*, a species belonging to the class Gammaproteobacteria, was suppressed at low CO_2 levels (Labare et al., 2010). In contrast, stimulation of growth was observed for one Flavobacteriia species under high CO_2 levels (Teira et al., 2012).

Taken together, results from mesocosm, microcosm and cultured isolates experiments indicate a potentially complex interaction between different groups of marine bacteria in response to elevated CO_2. To begin to elucidate these complex interactions, network analysis methods would be beneficial. Ecological network approaches have been successfully applied to investigate the complexity of interactions among zooplankton and phytoplankton from different trophic levels in the Tara Oceans Expedition project (Lima-mendez *et al.*, 2015; Guidi *et al.*, 2015). Previous studies using ecological network analysis showed that elevated CO_2 significantly impacted soil bacterial/archaeal community networks, by decreasing the connections for dominant fungal species and reassembling unrelated fungal species in a grassland ecosystem (Tu et al., 2015). Elucidating the complex interactions between bacterioplankton and other marine organisms under anthropogenic perturbation will increase our understanding of their impact in a holistic way.

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It has been reported that eutrophication problems in coastal regions lead to complex cross-links between ocean acidification and eutrophication (Cai et al., 2011). The occurrence of ocean acidification combined with other environmental stressors such as eutrophication can potentially produce synergistic or antagonistic effects on bacterioplankton that differ from those caused by ocean acidification alone. Although there are some reports from mesocosm experiments describing the response of bacteria to elevated CO_2, there are limited studies on how the bacterial community responds to ocean acidification in eutrophic coastal seawater. In this study, the response of bacterial community to ocean acidification was investigated using a mesocosm experiment conducted in a eutrophic coastal area in Xiamen, China using V3-V4 region of 16S rRNA gene Illumina sequencing. The objective was to explore the effects of ocean acidification on the bacterioplankton community composition and ecological network structure in a eutrophic coastal mesocosm experiment.

2 Methods

2.1 Mesocosm setup and carbonate system manipulation

The mesocosm experiment was conducted in the FOANIC-XMU (Facility for the Study of Ocean Acidification Impacts of Xiamen University) mesocosm platform located in Wuyuan Bay, Xiamen, Fujian province, East China Sea (N24°31′48″, E118°10′47″) during the months of December 2014 and January 2015 (Fig. 1). Each transparent thermoplastic polyurethane (TPU) cylindrical mesocosm bag was 3 m deep and 1.5 m wide (~4000 L total volume). After setting up the mesocosm bags into steel frames, in situ seawater from Wuyuan Bay was filtered through a 0.01μm water purifying system and used to simultaneously fill eight bags within 24 hours. The initial in situ seawater pCO_2 in Wuyuan Bay was about 650 μatm. Wuyuan bay, located in the city centre, is strongly influenced by the human activities. The decomposition of land-sourced organic compounds is also active in Wuyuan Bay. All

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these reasons may lead to higher pCO_2 in Wuyuan bay than that in open ocean. In order to reach the target low pCO_2 associated with ambient air (400 ppm), Na_2CO_3 was added to each mesocosm to increase dissolved inorganic carbon (DIC) and total alkalinity (TA) by 100 μmol/L and 200 μmol/L respectively based on the carbonate system calculation (Lewis et al. 1998). To adjust seawater to the end of this century projected seawater under 1000 ppm CO_2 condition, about 5 L of CO_2 saturated filtered seawater was added to 4 mesocosms (#2, #4, #7, #9) respectively which were considered as the HC treatment, while the other 4 mesocosms (#1, #3, #6, #8) were considered as the LC treatment. Throughout the experiment, HC mesocosms and LC mesocosms were bubbled with air containing 1000 ppm and 400 ppm CO_2, respectively supplied by CO_2 Enrichlor (CE-100B, Wuhan Ruihua Instrument & Equipment Ltd, China) at a flow rate of 4.8 L per minute.

Two diatoms, *Phaeodactylum tricornutum* CCMA 106 (isolated from South China Sea in 2004) from the Centre for Collections of Marine Bacteria and Phytoplankton of the State Key Laboratory of Marine Environmental Science (Xiamen University, China), and *Thalassiosira weissflogii* CCMP 102 from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP, USA), as well as the coccolithophorid *Emiliania huxleyi* (CS-369) from the Commonwealth Scientific and Industrial Research Organization (CSIRO, Australia) were used as inoculum to construct a model phytoplankton community. The effects of ocean acidification on these phytoplankton species mentioned above have been intensively studied in the lab at physiological, biochemical and molecular levels. However, it is difficult to extrapolate the response of these species to ocean acidification in natural complex environments based on the lab single species experiments (Busch et al., 2015). In this study, the artificial phytoplankton were used to further investigate the effects of ocean acidification on these species in the community and ecosystem levels in the context of mescosm experiment (Jin et al., 2015;

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Liu et al., submitted). The initial concentration of *P. tricornutum* and *T. weissflogii* was 10 cells/mL respectively, and *E. huxleyi* was 20 cells/mL. Although 0.01μm filtered seawater were used in this mesocosm experiment, the bacterioplankton appeared on day 0. The bacterioplankton in this mesocosm experiment originated from both the lab culture and the natural seawater. The inoculated algal culture was also not axenic. The bacteria composition in the inoculated phytoplankton culture is shown in Fig. S1. The mesocosm and the CO_2 bubbling system were not sterile at the beginning of the experiment and not completely closed during the experiment. The natural bacterioplankton were undoubtedly introduced into the mesocosm system through bubbling and air-sea exchange. So the bacterioplankton community in this mesocosm experiment was derived from the bacteria added inoculated phytoplankton culture and the natural local prokaryotic assemblage.

2.2 Bacteria sampling, filtration and sample selection

A total of 500 ml to 2 L of water, depending on bacterial concentration, was collected from mesocosms. Six of the mesocosms (HC: #2, #4, #7 and LC: #1, #6, #8) were chosen for further study. Samples from days 4, 6, 8, 10, 13, 19, and 29 were collected in this study due to time, personnel and equipment constraints. Sequential size fractionated filtration (2 μm and 0.2 μm polycarbonate filters) by peristaltic pump was used to filter seawater collected from the mesocosm bags.

2.3 DNA extraction, 16S rDNA V3-V4 region amplification and Illumina MiSeq sequencing

Samples collected by 0.2 μm polycarbonate filter as described above were washed with PBS buffer then centrifuged at 9600g to obtain a cell pellet. A previously described DNA extraction protocol was utilized (Francis et al., 2005). Amplification, library construction and sequencing were performed offsite at ANNOROAD using the DNA samples isolated as described above. Primers were 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'), targeting

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the V3-V4 hyper variable regions of bacterial 16S rRNA gene. The PCR amplification condition was as follows: initial denaturation at 95°C for 3 min, 25 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s, then final extension at 72°C for 5 min. DNA library construction and sequencing followed the MiSeq Reagent Kit Preparation Guide (Illumina, USA).

2.4 Sequence assignment and sequence statistics analysis

Clean paired-end reads were merged using PEAR (Zhang et al., 2014). The remaining raw sequences were distinguished and sorted by unique sample tags. Unique operational taxonomic units (OTUs) were picked against Greengenes database (http://greengenes.lbl.gov/cgi-bin/JD_Tutorial/nph-16S.cgi) (McDonald et al., 2012) at 97% identity. OTUs with less than 2 reads were not considered. QIIME 1.8.0 was used for sequence analysis including OTUs extraction for bacterioplankton community structure analysis, OTUs overlapping analysis, phylogenetic analysis, species diversity and species richness analysis, Principal Components Analysis (PCA) (Caporaso et al., 2010). Bacterioplankton community composition differences were assessed by Unweighted UniFrac distance using QIIME 1.8.0 as well. Dissimilarity tests were based on the Bray-Curtis dissimilarity index using analysis of similarities (ANOSIM) (Clarke, 1993), non-parametric multivariate analysis of variance (ADONIS) (Anderson, 2001), and multi-response permutation procedures (MRPP) (Mielke, 1981). Observed species, Chao index, Shannon index and Simpson index were used for estimating the community diversity. Analysis of variance (ANOVA) followed by a T-test was performed to determine any significant differences between HC and LC treatments.

2.5 Ecological network construction and analysis

As previously described, ecological network analysis were performed to do ecological network construction and analysis based on relative abundance of OTUs in HC and LC treatments with three

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biological replicates (http://129.15.40.240/mena/). Firstly, a similarity matrix was created using Pearson correlation coefficient across time series by a random matrix theory (RMT)-based approach. Cut-off values were determined according to R^2 of power-law larger than 0.8 and equal between two manipulations to construct network structure. In order to ensure the constructed networks were not random biologically meaningless networks, 100 networks from the same matrix were constructed and randomized. This resulted in the experimental networks being different from random networks judging by significantly higher modularity, clustering coefficient and geodesic distance (Table 1). Then, module separation was produced using greedy modularity optimization, and *Z-P* values for all nodes were calculated. In addition, to compare networks, the network connection was randomly rewired and network topological properties were calculated. Finally, the bacteria network interaction was visualized by Cytoscape v.3.3.0. The *Z–P* plots were constructed based on within-module (*Z*) and among-module (*P*) values of each node derived from ecological network analysis. Ecological network analysis is a novel RMT-based framework for studying microbial interactions. A node in ecological network analysis shows an OTU and a link demonstrates a connection between two OTUs. The shortest path between nodes is indicated by geodesic distance. Since the network constructed by OTUs can be separated into several communities, or modules, the modularity value indicates how well a network can be divided into different communities. Clustering coefficients demonstrate how well a OTU is connected with other OTUs, while average clustering coefficients indicate the extent of connection in a network.

3 Results

3.1 Environmental parameters and experimental timeline

The initial inorganic nitrogen, PO_4^{3-}, and SiO_3^{2-} concentrations were 70–75 μmol/L, 2.5–2.6 μmol/L, and

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38–39 μmol/L, respectively. Because of the high nutrient levels, the effects of eutrophication cannot be ignored in this ocean acidification mesocosm experiment. Except SiO_3^{2-}, the other nutrient concentrations decreased with rapid growth of the phytoplankton and reached to low concentrations on day 15. The dissolved total inorganic nitrogen dropped from initial 74.9 ± 2.87 μmol/L to 57.2 ± 4.37 μmol/L in the HC condition and 72 ± 5.90 μmol/L to 53.6 ± 5.60 μmol/L in the LC condition by day 8, and then reached low concentrations on day 15 (average 3μmol/L in LC and average 6μmol/L in HC)

The pCO_2 in this study was calculated from DIC and pH by CO2SYS Program (Lewis et al. 1998). The initial pCO_2 of 373.0 ± 43.9 μatm in the LC treatment and 1296.0 ± 159.6 μatm in the HC treatment increased and reached the peak value of 922.5 ± 142.0 μatm in the LC treatment on day 8 and 1879.6 ± 145.4 μatm in the HC treatment on day 4. After reaching the peak, the pCO_2 values of both treatments decreased and were no longer statistically different from day 13 due to rapid CO_2 uptake by the phytoplankton, despite air containing 1000 ppm CO_2 being continuously bubbled into the HC treatments (Fig. 2). *P. tricornutum* and *T. weissflogii* were the dominant species throughout the whole phytoplankton bloom in both HC and LC conditions. Chlorophyll *a* (Chl*a*) concentration and diatom cell densities were used to identify changes in the diatom bloom following inoculation (Fig. 2, Nana Liu et al., submitted). Chl*a* concentration increased from 0.23 ± 0.12 μg/L to 5.33 ± 1.82 μg/L in the LC conditions, and from 0.19 ± 0.07 μg/L to 5.75 ± 1.17 μg/L in the HC conditions from day 4 to day 9. Thereafter, Chl*a* concentration increased significantly and peaked at 109.9 ± 38.04 μg/L in the LC treatment and 108.6 ± 46.07 μg/L in the HC treatment on day 15. Subsequently, Chl*a* concentrations in both treatments were maintained at high concentrations until day 25 and decreased progressively afterward. The bloom process identified by cell concentration of *P. tricornutum* and *T. weissflogii* was similar with that illustrated by Chl*a* concentration. The growth of these two diatom species entered into

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logarithmic phase from day 2. Cell density reached highest concentration on day 15 and day 19 for *T. weissflogii* and *P. tricornutum* respectively, and then dropped down slowly. The comprehensive analysis of phytoplankton cell density, Chla concentration, particle organic carbon (POC) and particle organic nitrogen (PON) during the experiment were described in Nana Liu et al., submitted.

3.2 Overview of sequencing analysis

Following sequencing, 828524 high quality sequences were kept after processing, and 39.3% of assembled reads were successfully aligned with the database. As a result, a total of 4992 OTUs were generated after clustering at a 97% similarity level, and 466 OTUs of them were unique. 49.1% of OTUs were classified to genera level with high taxonomic resolution. The phylogenetic tree was constructed based on the sequences derived from all of the samples (Fig. S2). The bacterioplankton from all of the samples in this study were identified as members of Bacteriodetes or Proteobacteria phylums. The most dominant OTUs were Alphaproteobacteria, Rhodobacterales, Rhodobacterceae and Sediminicola at class, order, family and genus level respectively (Fig. S3). The most abundant sequences at class, order, family and genus levels accounted for 43.4 %, 42.6 %, 41.7% and 32.8 % of all sequences respectively.

3.3 Bacterioplankton community structure throughout the phytoplankton bloom

Bacterioplankton community structure underwent dynamic changes during the diatom bloom in both the HC and LC treatments. The bacterioplankton community structure of all samples in different taxonomic levels is illustrated in Fig. 3. Significant variation in community structure was observed through the whole diatom bloom process, suggesting that the diatom bloom is a major driver for bacterioplankton community structure dynamics in both the HC and LC treatments. At the phylum level, the bacterioplankton were dominated by Proteobacteria, while the relative abundance of Bacteroidetes was very low when nutrients were replete and diatom biomass was not high. However, Bacteroidetes

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increased dramatically when diatom biomass increased dramatically, and began to drop down after reaching a peak on day 10 (Fig. 3 and Fig. 4). In contrast, Proteobacteria began to increase after reaching its lowest concentration on day 10.

The Alphaproteobacteria, Flavobacteriia, and Gammaproteobacteria classes with high abundance in all samples were selected for further analysis. The proportion of the Gammaproteobacteria class from the Proteobacteria phylum was very high at the beginning of the experiment (50.2 ± 13.8 % in the HC treatment and 44.1 ± 6.4 % in the LC treatment on day 6) and decreased throughout the duration of the experiment. On the other hand, the Alphaproteobacteria class, also from the Proteobacteria phylum, decreased from initial high proportions (46.9 ± 13.2 % in the HC treatment and 43.9 ± 11.6 % in the LC treatment) on day 6 to low proportions on day 10 (27.2 ± 2.8 %) in the HC treatment whereas remained almost unchanged (44.6 ± 7.5 %) in the LC treatment and increased to 63.2 ± 27.3 % in the HC treatment and 60.8 ± 32.7 % in the LC treatment on day 29 (Fig. 3 and Fig. 4). The relative abundance of the Flavobacteriia class from the Bacteroidetes increased from the beginning and reached a peak on day 10 (52.2 ± 4.2 % in the HC treatment and 24.8 ± 16.9 % in the LC treatment), then dropped down until day 19 (19.9 ± 2.2 % in the HC treatment and 18.0 ± 15.4% in the LC treatment) (Fig. 3 and Fig. 4). The proportional variation of the Flavobacteriales order and the Rhodobacterales order showed similar trends with the Flavobacteriia class and the Alphaproteobacteria class, respectively, as shown in Fig. 3 and Fig. 4.

3.4 The effects of elevated CO2 on bacterioplankton community structure

Bacterial community structures of the HC and LC treatments were compared at different sampling time-points. Briefly, CO_2 treatment was not a strong influence on bacterioplankton community structure, compared to the diatom bloom process (Fig. 3). A dissimilarity test based on ANOSIM, MRPP and

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ADONIS methods showed that no statistically significant differences were observed between HC and LC treatments at different time-points (Table 2). PCA analysis also agreed with the dissimilarity test (Fig. S6). The bacterioplankton community diversity in all samples was estimated by observed species, Chao index, Shannon index and Simpson index. Rarefaction curves showed no remarkable differences in in community diversity between HC and LC, regardless of the time point (Fig. S4). In general, the variation of the bacterioplankton community diversity in both HC and LC treatments followed the same trend, peaked on day 10 and declined for the remainder of the experiment (Fig. S5).

Although the general trend of bacterioplankton community structure variation was similar in both the HC and LC treatments as described above, some groups of bacterioplankton showed different responses to elevated CO_2 at some time points. Notably, Bacteroidetes, predominated by the Flavobacteriia had a higher average proportion in the HC treatment (52.2 % of Bacteroidetes and 52.2 % of Flavobacteriia) than that in the LC treatment (25.2% Bacteroidetes and 24.8% Flavobacteriia) at the early stage of the diatom bloom on day 10 (p=0.049 and 0.053 respectively). In contrast, Proteobacteria, especially the Alphaproteobacteria were observed to have lower proportion in the HC treatment (47.8 % of Proteobacteria and 27.2% of Alphaproteobacteria) than in the LC treatment (74.8 % of Proteobacteria and 44.6% of Alphaproteobacteria) on day 10 (p=0.049 and 0.019 respectively, Fig. 4). At a higher taxonomic level, Flavobacteriales, demonstrated higher proportions in the HC treatment (52.2 %) compared to the LC treatment (24.8 %) on day 10 (p=0.053), while for Rhodobacterales the inverse pattern was observed (p=0.020). Moreover, Flavobacteriaceae were observed to have a relatively higher ratio in the HC treatment (50.3 %) compared to the LC treatment (24.0 %) on day 10 (p=0.053), whereas Rhodobacteraceae demonstrated the opposite pattern (p=0.021, Fig. 4). It is notable that Alteromonadales, belonging to the Gammaproteobacteria, had a higher ratio in the HC treatment

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compared to the LC treatment on day 19 and day 29, although it was not statistically significant (p=0.24 and 0.34 on day 19 and 29 respectively).

3.5 The effects of elevated CO_2 on bacterioplankton community interactions

Both HC and LC networks were dominated by Alphaproteobacteria, Gammaproteobacteria and Flavobacteriia, suggesting their vital roles in maintaining stability of microbial ecosystems under both HC and LC conditions. The observation of more negative links compared to positive links indicates the dominant relationship among bacterioplankton is competitive rather than mutualistic under both the HC and LC treatments. Average connectivity and average clustering coefficient of the network under the HC treatment were higher than under LC treatment, while geodesic distance and modularity value was higher under the LC treatment. Bacterioplankton formed more modules under the LC treatment, but were densely connected in less modules under the HC treatment (Table 1, Fig. 5). However, as it shown in Fig. 5, the links among the abundant OTU 558885 (Rhodobacteraceae), 572670 (Rhodobacteraceae), 190052 (Flavobacteriaceae), 107130 (Flavobacteriaceae) and 4331023 (Rhodobacteraceae) were positive in both HC and LC.

Interestingly, some nodes that were sparsely distributed in independent modules in the LC network formed dense modules with high connectivity in the HC network (Fig. 5). As the OTUs connected within a module, they could be considered as a putative bacterioplankton ecological niche (Zhou et al., 2010). It is plausible that elevated CO_2 disrupted the connection between different bacterioplankton community niches, but enhanced alternative connections among species within certain ecological niches. Within module connectivity (*Zi*) and among-module connectivity (*Pi*) indexes were used to identify key module members (Olesen *et al.*, 2007, Fig. 6). In an ecological context, the peripherals may represent specialists, while module hubs and connectors may be more considered as intra-module and inter-module generalists

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respectively. Network hub are usually considered as super-generalists (Deng et al., 2012). It is interesting that the numbers of connectors that are considered as generalists were reduced whereas module hubs were increased under the HC treatment. However, two network hubs, the super-generalists that are more important than module hubs and connecters, were detected in the LC network but not in the HC network (Fig. 6).

4 Discussion

Although effects of elevated CO_2 on bacterioplankton communities have been reported (Allgaier et al., 2008; Tanaka *et al.*, 2008; Wang et al., 2016; Zhang et al., 2013; Ray et al., 2012; Roy et al., 2013; Baltar et al., 2015), how marine bacteria communities react to the occurrence of elevated CO_2 combined with other environmental perturbations is still uncertain. This mesocosm study comprehensively investigated the effects of elevated CO_2 on bacterioplankton community structure and networks using Illumina sequencing and ecological network analysis. The results indicate that the abundance and the community structure at different taxonomic levels were generally similar at different diatom bloom stages between the HC and LC treatment, in line with previous ocean acidification mesocosm bacterioplankton community studies (Tanaka *et al.*, 2008; Wang et al., 2016; Zhang et al., 2013; Ray et al., 2012; Roy et al., 2013; Baltar et al., 2015). The difference in bacterioplankton community diversity between the HC and LC treatments was also not remarkable as well. These results suggest the possibility that the whole bacterioplankton community has a certain degree of resilience to elevated CO_2, which is consistent with a previous stated hypothesis (Joint et al., 2011).

It has previously been proposed that the observed insignificant effects of ocean acidification on coastal bacterioplankton and their resilience to elevated CO_2 was due to their adaptation to strong natural variability in pH, with amplitudes of >0.3 units from diel fluctuation and seasonal dynamics found in

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coastal ecosystems (Hofmann et al., 2011). The comparative ecological network analysis in this study to some extent explains the resilience of the bacterioplankton community to elevated CO_2 levels. According to the present study, substantial amount of OTUs that were sparsely distributed in different and small modules in the LC network became connected with each other and formed less numbers of modules in the HC network, implying elevated CO_2 has the potential to reassemble the bacterioplankton community (Fig. 5). The positive relationship among these principle components were almost unaltered in the network analysis, suggesting that elevated CO_2 did not change the network of principal components of bacterioplankton and the positive relationship among them were vital for the whole bacterioplankton community's stability (Fig. 5).

It was also reported that sparsely distributed fungal species were reassembled into highly connected dense modules under long-term elevated CO_2 conditions (Tu et al., 2015). It is noteworthy that the OTUs involved in possible community reassembly were not very abundant, whereas the relationship between the abundant OTUs was virtually unaltered by elevated CO_2 in this study. Although elevated CO_2 promoted the reassembly of the bacterioplankton community, the network constructed by abundant OTUs which are usually considered as the foundation of the whole bacterioplankton community was still stable in response to elevated CO_2. This to some extent led to the stability of the bacterioplankton community under the ocean acidification stimuli in the context of eutrophic conditions in the current study. Additionally, this data indicates that more negative than positive relationships between OTUs were observed in both HC and LC treatments, which is consistent with a previous ocean acidification mesocosm study conducted in the Arctic Ocean (Wang et al., 2016). It was proposed that a community with more competitors would be more stable and yield less variation under environmental fluctuations (Gonzalez and Loreau, 2009). So, it could be speculated that the dominant competition relationship

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between bacterioplankton species in this mesocosm experiment helped the whole bacterioplankton community to adapt to pH perturbations, with less variation in total biomass and diversity.

Although the effects of elevated CO_2 on bacterioplankton community structure were not significant, the proportion of some groups of bacterioplankton varied between the HC and LC treatments in the early stages of the diatom bloom. Elevated CO_2 significantly increased the proportion of Flavobacteriia dominated by Flavobacteriales, in the HC treatment on day 10 when the diatoms cells began to grow rapidly. In contrast, the HC treatment had negative effects on the growth of Alphaproteobacteria compared to the LC treatment. The results reported here are in line with previous reports about the response of Flavobacteriia to ocean acidification in biofilm and single species experiments (Witt et al., 2011; Teira *et al.*, 2012). Flavobacteriia are considered as the "first responders" to phytoplankton blooms because they specialize in attacking algal cells and further degrading biopolymers and organic matter derived from algal detrital particles (Kirchman, 2002; Teeling *et al.*, 2012). Flavobacteriia are especially good at converting high molecular weight (HMW) dissolved organic matter (DOM) to low molecular weight (LMW) DOM using the highly efficient, extracellular, multi-protein complex TonB-dependent transporter (TBDT) system based on previous in situ proteomics and metatranscriptomics data (Teeling et al., 2012). Higher abundance of Flavobacteriia under elevated CO_2 means more HMW DOM could be degraded and so enter into the carbon cycle (Buchan et al., 2014). Based on the results reported here, it can be speculated that increased amounts of Flavobacteriia under the elevated CO_2 treatment combined with eutrophication could promote the TBDT system to break down HMW DOM and lead to improved efficiency of the Microbial Carbon Pump (MCP), and possibly further influence the carbon storage in the ocean (Jiao et al., 2010).

Additionally, it has been postulated that the Flavobacteriia originated light-driven proton pump

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proteorhodopsin could be involved in dealing with ocean acidification and pH perturbation (Fuhrman et al., 2008). Recent metatranscriptomic data further emphasize the role of proteorhodopsin in pH homeostasis in bacterioplankton under elevated CO_2 (Bunse *et al.*, 2016; Gómez-Consarnau *et al.*, 2007). In this study, we speculate that the stimulated growth of Flavobacteriia could have been due to the enhanced activation of proteorhodopsin under the HC treatment at the early stage of diatom bloom. The mechanisms of proteorhodopsin in pH homeostasis in bacterioplankton under elevated CO_2 need further investigation in the future.

Interestingly, Flavobacteriia in our study showed higher abundance in the HC treatment in the early phytoplankton bloom stage. However, a negative relationship between CO_2 level and relative abundance of Bacteroidetes based on terminal restriction fragment length polymorphism (T-RFLP) method was observed in a mesocosm experiment conducted in the Arctic region with low nutrient levels (Roy *et al.*, 2013). Moreover, the effects of elevated CO_2 on bacterioplankton community interaction webs in this study were not observed in a previous mesocosm experiment conducted in the Arctic Ocean (Wang *et al.*, 2016; Roy *et al.*, 2013).

Conclusion

Briefly, elevated CO_2 was not a strong influence on bacterioplankton community structure, compared to the diatom bloom process based on 16S V3-V4 region Illumima sequencing. We also found that the elevated CO_2 appeared to reassemble the community network of taxa present with low abundance but hardly altered the network structure of the bacterioplankton taxa present with high abundance based on ecological network analysis. The results showed that the effects of elevated CO_2 in the context of eutrophication in the current study were different compared to elevated CO_2 on bacterioplankton community networks in mesocosm study carried out in the oligotrophic Arctic Ocean. The data here and

previous reported seemingly contradictory results highlight the importance of including the combined effects of ocean acidification and other anthropogenic perturbations to interpret and predict the impact of global change on marine life.

In this study, a simplified model phytoplankton community was used, so two diatom species *P. tricornutum* and *T. weissflogii* dominated in both LC and HC treatments. It is possible that the similarity of the two bacterial communities in the two treatments was due to the similar composition and quality of DOM produced by these two diatoms. With a more diverse natural phytoplankton community experimental system, perhaps different phytoplankton taxa would have dominated in the HC and LC treatments, leading to different bacterial communities.

Author contributions

Conceived and designed the experiments: K.G., X.L., M.D.. Performed the experiments: R.P.H., X.L., Y.P.W.,Y.L.. Analysed data: R.P.H. and X.L.. Wrote the paper: X.L.. Revised the paper: D.H. and K.G. All authors reviewed the manuscript.

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Competing interests:

The authors declare no competing financial interests.

Reference

Allgaier, M., Riebesell, U., Vogt, M., Thyrhaug, R. and Grossart, H.-P.: Coupling of heterotrophic bacteria to phytoplankton bloom development at different pCO2 levels: a mesocosm study, Biogeosciences, 5(4), 1007–1022, doi:10.5194/bgd-5-317-2008, 2008.

Anderson, M. J.: A new method for non-parametric multivariate analysis of variance, Austral Ecol., 26(2001), 32–46, doi:10.1046/j.1442-9993.2001.01070.x, 2001.

Azam, F.: Microbial control of oceanic carbon flux: the plot thickens., Science (80-.)., 280(5364), 694–696, 1998.

Baltar, F., Palovaara, J., Vila-Costa, M., Salazar, G., Calvo, E., Pelejero, C., Marrasé, C., Gasol, J. M. and Pinhassil, J.: Response of rare, common and abundant bacterioplankton to anthropogenic perturbations in a Mediterranean coastal site, FEMS Microbiol. Ecol., 91(6), 1–12, doi:10.1093/femsec/fiv058, 2015.

Buchan, A., LeCleir, G. R., Gulvik, C. A. and González, J. M.: Master recyclers: features and functions of bacteria associated with phytoplankton blooms, Nat. Rev. Microbiol., 12(10), 686–698, doi:10.1038/nrmicro3326, 2014.

Bunse, C., Lundin, D., Karlsson, C. M. G., Vila-Costa, M., Palovaara, J., Akram, N., Svensson, L.,

Holmfeldt, K., González, J. M., Calvo, E., Pelejero, C., Marrasé, C., Dopson, M., Gasol, J. M. and Pinhassi, J.: Response of marine bacterioplankton pH homeostasis gene expression to elevated CO2, Nat. Clim. Chang., (January), doi:10.1038/nclimate2914, 2016.

Busch, D. S., O'Donnell, M. J., Hauri, C., Mach, K. J., Poach, M., Doney, S. C. and Signorini, S. R.: Understanding, characterizing, and communicating responses to ocean acidification: Challenges and uncertainties, Ocenaography, 28(2), 30–39, doi:http://dx.doi.org/10.5670/oceanog.2015.29, 2015.

Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M. C., Lehrter, J. C., Lohrenz, S. E., Chou, W.-C., Zhai, W., Hollibaugh, J. T., Wang, Y., Zhao, P., Guo, X., Gundersen, K., Dai, M. and Gong, G.-C.: Acidification of subsurface coastal waters enhanced by eutrophication, Nat. Geosci., 4(11), 766–770, doi:10.1038/ngeo1297, 2011.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. a, Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. a, Mcdonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. a, Widmann, J., Yatsunenko, T., Zaneveld, J. and Knight, R.: correspondence QIIME allows analysis of high- throughput community sequencing data Intensity normalization improves color calling in SOLiD sequencing, Nat. Publ. Gr., 7(5), 335–336, doi:10.1038/nmeth0510-335, 2010.

Clarke, K. R.: Non-parametric multivariate analyses of changes in community, Aust. J. Ecol., (18), 117–143, 1993.

Deng, Y., Jiang, Y.-H., Yang, Y., He, Z., Luo, F. and Zhou, J.: Molecular ecological network analyses, BMC Bioinformatics, 13, 113, doi:10.1186/1471-2105-13-113, 2012.

Biogeosciences Discuss., doi:10.5194/bg-2017-10, 2017
Manuscript under review for journal Biogeosciences
Published: 30 January 2017

Falkowski, P. G., Fenchel, T. and Delong, E. F.: The Microbial Engines That Drive Earth's Biogeochemical Cycles, Science (80-.)., 320(5879), 1034–1039, doi:10.1126/science.1153213, 2008.

Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E. and Oakley, B. B.: Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean, , 102(41), 14683–14688, doi:10.1073/pnas.0506625102, 2005.

Fuhrman, J. a, Schwalbach, M. S. and Stingl, U.: Proteorhodopsins: an array of physiological roles?, Nat. Rev. Microbiol., 6(6), 488–494, doi:10.1038/nrmicro1893, 2008.

Gattuso, J.-P., Magnan, A., Bille, R., Cheung, W. W. L., Howes, E. L., Joos, F., Allemand, D., Bopp, L., Cooley, S. R., Eakin, C. M., Hoegh-Guldberg, O., Kelly, R. P., Portner, H.-O., Rogers, a. D., Baxter, J. M., Laffoley, D., Osborn, D., Rankovic, A., Rochette, J., Sumaila, U. R., Treyer, S. and Turley, C.: Contrasting futures for ocean and society from different anthropogenic CO2 emissions scenarios, Science (80-.)., 349(6243), aac4722-1-aac4722-10, doi:10.1126/science.aac4722, 2015.

Gómez-Consarnau, L., González, J. M., Coll-Lladó, M., Gourdon, P., Pascher, T., Neutze, R., Pedrós-Alió, C. and Pinhassi, J.: Light stimulates growth of proteorhodopsin-containing marine Flavobacteria, Nature, 445(7124), 210–213, doi:10.1038/nature05381, 2007.

Gonzalez, A. and Loreau, M.: The Causes and Consequences of Compensatory Dynamics in Ecological Communities, Annu. Rev. Ecol. Evol. Syst., 40(1), 393–414, doi:10.1146/annurev.ecolsys.39.110707.173349, 2009.

Guidi, L., Chaffron, S., Bittner, L., Eveillard, D., Larhlimi, A., Roux, S., Darzi, Y., Audic, S., Berline, L., Brum, J., Coelho, L. P., Espinoza, J. C. I., Malviya, S., Sunagawa, S., Dimier, C., Kandels-Lewis, S., Picheral, M., Poulain, J., Searson, S., Coordinators, T. O., Stemmann, L., Not,

F., Hingamp, P., Speich, S., Follows, M., Karp-Boss, L., Boss, E., Ogata, H., Pesant, S., Weissenbach, J., Wincker, P., Acinas, S. G., Bork, P., de Vargas, C., Iudicone, D., Sullivan, M. B., Raes, J., Karsenti, E., Bowler, C. and Gorsky, G.: Plankton networks driving carbon export in the oligotrophic ocean, Nature, 532(7600), in review, doi:10.1038/nature16942, 2015.

Hofmann, G. E., Smith, J. E., Johnson, K. S., Send, U., Levin, L. A., Micheli, F., Paytan, A., Price, N. N., Peterson, B., Takeshita, Y., Matson, P. G., Crook, E. D., Kroeker, K. J., Gambi, M. C., Rivest, E. B., Frieder, C. A., Yu, P. C. and Martz, T. R.: High-Frequency Dynamics of Ocean pH: A Multi-Ecosystem Comparison, PLoS One, 6(12), e28983, doi:10.1371/journal.pone.0028983, 2011.

Jiao, N., Herndl, G. J., Hansell, D. A., Benner, R., Kattner, G., Wilhelm, S. W., Kirchman, D. L., Weinbauer, M. G., Luo, T., Chen, F. and Azam, F.: Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean, Nat. Rev. Microbiol., 8(8), 593–599, doi:10.1038/nrmicro2386, 2010.

Jin, P., Wang, T., Liu, N., Dupont, S., Beardall, J., Boyd, P. W., Riebesell, U. and Gao, K.: Ocean acidification increases the accumulation of toxic phenolic compounds across trophic levels., Nat. Commun., 6(October), 8714, doi:10.1038/ncomms9714, 2015.

Joint, I., Doney, S. C. and Karl, D. M.: Will ocean acidification affect marine microbes?, ISME J., 5(1), 1–7, doi:10.1038/ismej.2010.79, 2011.

Kirchman, D. L.: The ecology of Cytophaga-Flavobacteria in aquatic environments, FEMS Microbiol. Ecol., 39(2), 91–100, doi:10.1016/S0168-6496(01)00206-9, 2002.

Krause, E., Wichels, A., Giménez, L., Lunau, M., Schilhabel, M. B. and Gerdts, G.: Small Changes in pH Have Direct Effects on Marine Bacterial Community Composition: A Microcosm Approach,

PLoS One, 7(10), e47035, doi:10.1371/journal.pone.0047035, 2012.

Labare, M. P., Bays, J. T., Butkus, M. a., Snyder-Leiby, T., Smith, A., Goldstein, A., Schwartz, J. D., Wilson, K. C., Ginter, M. R., Bare, E. a., Watts, R. E., Michealson, E., Miller, N. and LaBranche, R.: The effects of elevated carbon dioxide levels on a Vibrio sp. isolated from the deep-sea, Environ. Sci. Pollut. Res., 17(4), 1009–1015, doi:10.1007/s11356-010-0297-z, 2010.

Lewis, E., and D. W. R. Wallace: Program developed for CO2 system calculations, Rep. ORNL/CDIAC-105, Carbon Dioxide Inf. Anal. Cent., Oak Ridge Natl. Lab., Oak Ridge, Tenn., doi:10.2172/639712, 1998.

Lidbury, I., Johnson, V., Hall-spencer, J. M., Munn, C. B. and Cunliffe, M.: Community-level response of coastal microbial biofilms to ocean acidification in a natural carbon dioxide vent ecosystem, Mar. Pollut. Bull., 64(5), 1063–1066, doi:10.1016/j.marpolbul.2012.02.011, 2012.

Lima-Mendez, G., Faust, K., Henry, N., Decelle, J., Colin, S., Carcillo, F., Chaffron, S., Ignacio-Espinosa, J. C., Roux, S., Vincent, F., Bittner, L., Darzi, Y., Wang, J., Audic, S., Berline, L., Bontempi, G., Cabello, A. M., Coppola, L., Cornejo-Castillo, F. M., D'Ovidio, F., De Meester, L., Ferrera, I., Garet-Delmas, M.-J., Guidi, L., Lara, E., Pesant, S., Royo-Llonch, M., Salazar, G., Sanchez, P., Sebastian, M., Souffreau, C., Dimier, C., Picheral, M., Searson, S., Kandels-Lewis, S., Gorsky, G., Not, F., Ogata, H., Speich, S., Stemmann, L., Weissenbach, J., Wincker, P., Acinas, S. G., Sunagawa, S., Bork, P., Sullivan, M. B., Karsenti, E., Bowler, C., de Vargas, C. and Raes, J.: Determinants of community structure in the global plankton interactome, Science (80-.)., 348(6237), 1262073–1262073, doi:10.1126/science.1262073, 2015.

Liu, J., Weinbauer, M., Maier, C., Dai, M. and Gattuso, J.: Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning, Aquat. Microb.

Ecol., 61(3), 291–305, doi:10.3354/ame01446, 2010.

Liu, N., Tong S., Yi, X., Li, Y., Li, Z., Miao, H., Wang, T., Li F., Yan, D., Huang, R., Wu, Y., Hutchins, D., Beardall, J., Dai M., Gao, K.: Carbon assimilation and losses during an ocean acidification mesocosm experiment, with special reference to algal blooms, submmitted.

McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., Andersen, G. L., Knight, R. and Hugenholtz, P.: An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea, ISME J., 6(3), 610–618, doi:10.1038/ismej.2011.139, 2012.

Meron, D., Atias, E., Iasur Kruh, L., Elifantz, H., Minz, D., Fine, M. and Banin, E.: The impact of reduced pH on the microbial community of the coral Acropora eurystoma, ISME J., 5(1), 51–60, doi:10.1038/ismej.2010.102, 2011.

Mielke, P. W., Berry, K. J., Brockwell, P. J. & Williams, J. S.: A class of nonparametric tests based on multiresponse permutation procedures, Biometrika, (68), 720–724, 1981.

Ray, J. L., Töpper, B., An, S., Silyakova, A., Spindelböck, J., Thyrhaug, R., Dubow, M. S., Thingstad, T. F. and Sandaa, R. A.: Effect of increased pCO2 on bacterial assemblage shifts in response to glucose addition in Fram Strait seawater mesocosms, FEMS Microbiol. Ecol., 82(3), 713–723, doi:10.1111/j.1574-6941.2012.01443.x, 2012.

Roy, A.-S., Gibbons, S. M., Schunck, H., Owens, S., Caporaso, J. G., Sperling, M., Nissimov, J. I., Romac, S., Bittner, L., Mühling, M., Riebesell, U., LaRoche, J. and Gilbert, J. a.: Ocean acidification shows negligible impacts on high-latitude bacterial community structure in coastal pelagic mesocosms, Biogeosciences, 10(1), 555–566, doi:10.5194/bg-10-555-2013, 2013.

Tanaka, T., Thingstad, T. F., Løvdal, T., Grossart, H.-P., Larsen, A., Schulz, K. G. and Riebesell, U.:

Availability of phosphate for phytoplankton and bacteria and of labile organic carbon for bacteria at different pCO2 levels in a mesocosm study, Biogeosciences, (5), 669–678, doi:10.5194/bgd-4-3937-2007, 2007.

Teeling, H., Fuchs, B. M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C. M., Kassabgy, M., Huang, S., Mann, A. J., Waldmann, J., Weber, M., Klindworth, A., Otto, A., Lange, J., Bernhardt, J., Reinsch, C., Hecker, M., Peplies, J., Bockelmann, F. D., Callies, U., Gerdts, G., Wichels, A., Wiltshire, K. H., Glockner, F. O., Schweder, T. and Amann, R.: Substrate-Controlled Succession of Marine Bacterioplankton Populations Induced by a Phytoplankton Bloom, Science (80-.)., 336(6081), 608–611, doi:10.1126/science.1218344, 2012.

Teira, E., Fernández, A., Álvarez-Salgado, X. A., García-Martín, E. E., Serret, P. and Sobrino, C.: Response of two marine bacterial isolates to high CO 2 concentration, Mar. Ecol. Prog. Ser., 453, 27–36, doi:10.3354/meps09644, 2012.

Tu, Q., Yuan, M., He, Z., Deng, Y., Xue, K., Wu, L., Hobbie, S. E., Reich, P. B. and Zhou, J.: Fungal Communities Respond to Long-Term CO_2 Elevation by Community Reassembly, Appl. Environ. Microbiol., 81(7), 2445–2454, doi:10.1128/AEM.04040-14, 2015.

Wang, Y., Zhang, R., Zheng, Q., Deng, Y., Van Nostrand, J. D., Zhou, J. and Jiao, N.: Bacterioplantkon community resilience to ocean acidification: evidence from microbial network analysis, ICES J. Mar. Sci., 73(3), 865–875, doi:10.1093/icesjms/fst176, 2016.

Witt, V., Wild, C., Anthony, K. R. N., Diaz-Pulido, G. and Uthicke, S.: Effects of ocean acidification on microbial community composition of, and oxygen fluxes through, biofilms from the Great Barrier Reef, Environ. Microbiol., 13(11), 2976–2989, doi:10.1111/j.1462-2920.2011.02571.x, 2011.

Biogeosciences Discuss., doi:10.5194/bg-2017-10, 2017
Manuscript under review for journal Biogeosciences
Published: 30 January 2017

Zhang, J., Kobert, K., Flouri, T. and Stamatakis, A.: PEAR: a fast and accurate Illumina Paired-End reAd mergeR, Bioinformatics, 30(5), 614–620, doi:10.1093/bioinformatics/btt593, 2014.

Zhang, R., Xia, X., Lau, S. C. K., Motegi, C., Weinbauer, M. G. and Jiao, N.: Response of bacterioplankton community structure to an artificial gradient of pCO2 in the Arctic Ocean, Biogeosciences, 10(6), 3679–3689, doi:10.5194/bg-10-3679-2013, 2013.

Zhou, J., Deng, Y., Luo, F., He, Z., Tu, Q. and Zhi, X.: Functional molecular ecological networks, MBio, 1(4), e00169-10, doi:10.1128/mBio.00169-10.Editor, 2010.

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Figure legends

Figure 1 Location of the FOANIC-XMU (Facility for the Study of Ocean Acidification Impacts of Xiamen University) mesocosm platform (Wuyuan Bay, Xiamen, Fujian province, East China Sea (N24°31′48″, E118°10′47″)).

Figure 2 Temporal variations of pCO_2 and Chl*a* during the whole experiment. The pCO2 was calculated from DIC and pH by CO2SYS Program (Lewis et al. 1998).

Figure 3 Bacterioplankton community structure overview at different taxonomic levels during day 4, 6, 8, 10, 13, 19 and 29 under LC and HC. X-axis represents sample name (for example, D4. 1 refers to bacterioplankton in mesocosm bag 1 collected on day 4) and the Y-axis represents relative abundance of different groups of bacterioplankton.

Figure 4 The relative abundance over time of primary taxa of the bacterioplankton community; HC (2, 4, 7 mesocosm bags)in red and LC (1, 6, 8 mesocosm bags) in black. Data are the means ± SD, Proteobacteria (a) and Bacteroidetes (b) are phylum level; Flavobacteriia (c) and Alphabacteria (d) are class level; Flavobacteriales (e) and Rhodobacteriales (f) are order level; Flavobacteriaceae (g) and Rhodobacteraceae (h) are family level. The asterisk represents a difference at $p < 0.05$.

Figure 5 Bacterioplankton network interactions under LC (a) and HC (b) conditions. Each node represents an OUT. Node colors demonstrate different taxon. Each line connects two OTUs. A blue line

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indicate a negative interaction between nodes suggesting a predation or competition while a red line indicates a positive interaction suggesting mutualism or cooperation. OTUs with importance are marked with OTU identification numbers.

Figure 6 Sub-modules in ecological network analysis under LC (a) and HC (b) conditions. Each dot represents an OTU. The *Z–P* plot shows OTU distribution based on their module-based topological role according to within-module (*Z*) and among-module (*P*) connectivity. The nodes were defined as module hubs with $Zi > 2.5$ and $Pi < 0.625$, which were more closely connected within the module, while the connectors were nodes with $Zi < 2.5$ and $Pi > 0.625$ were more closely connected to nodes in other modules. Network hubs are super-generalist with a $Zi > 2.5$ and $Pi > 0.625$. The other nodes were considered peripheral.

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Figure 1

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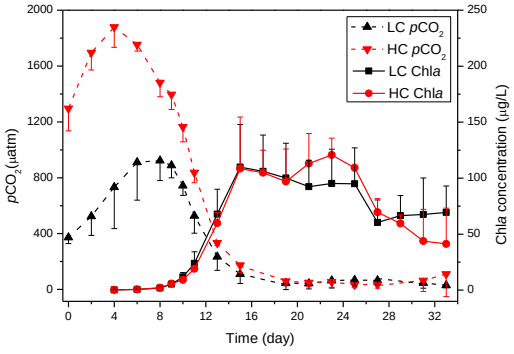

Figure 2

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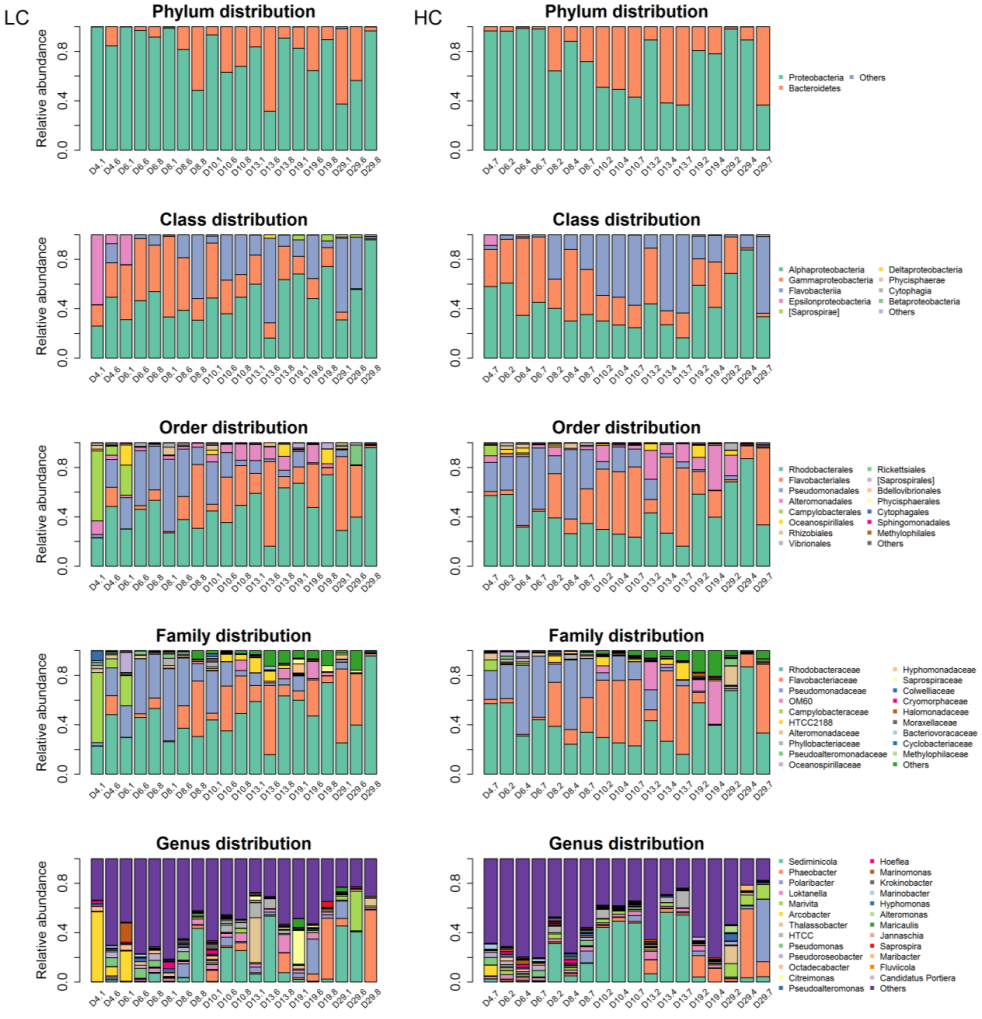

Figure 3

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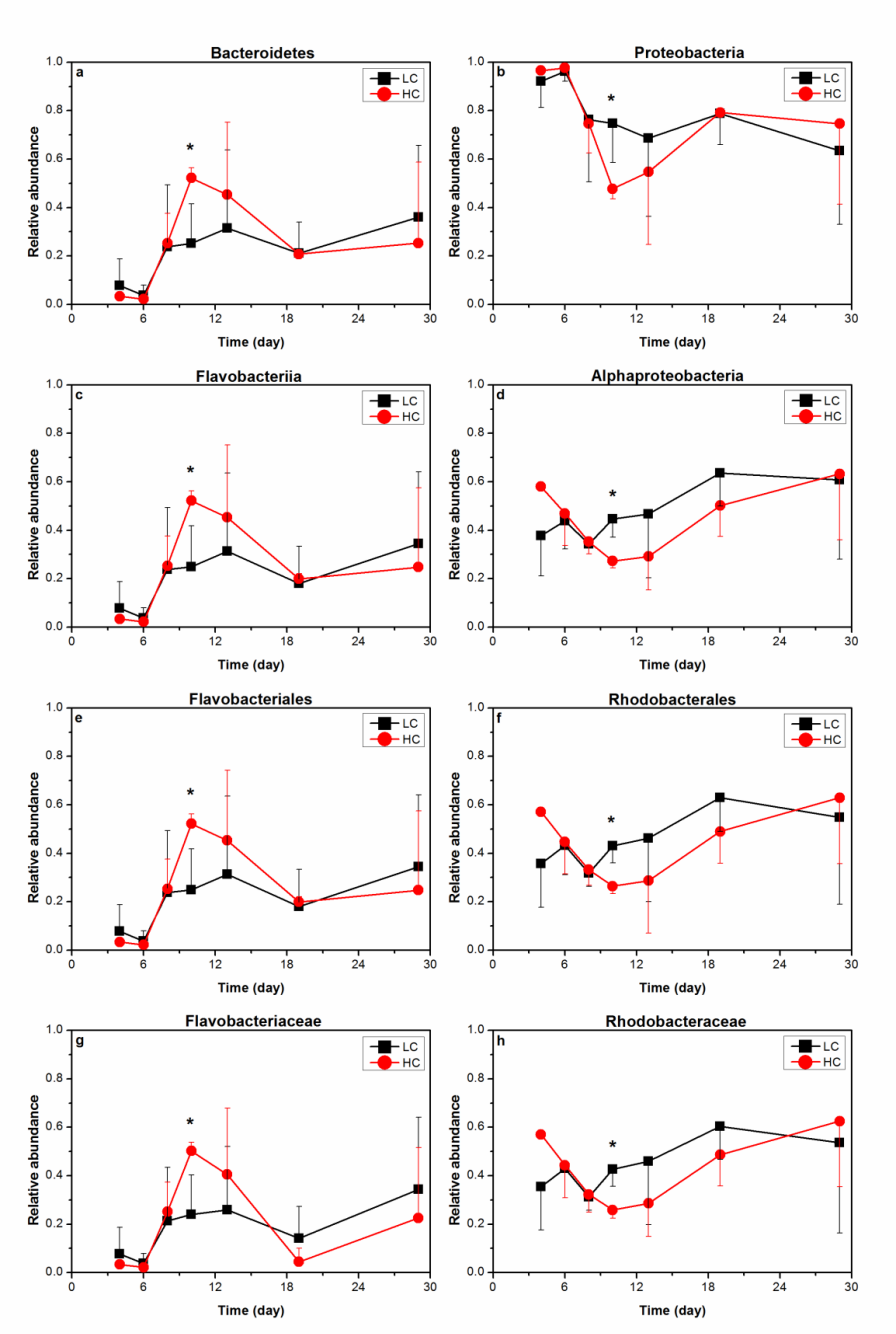

Figure 4

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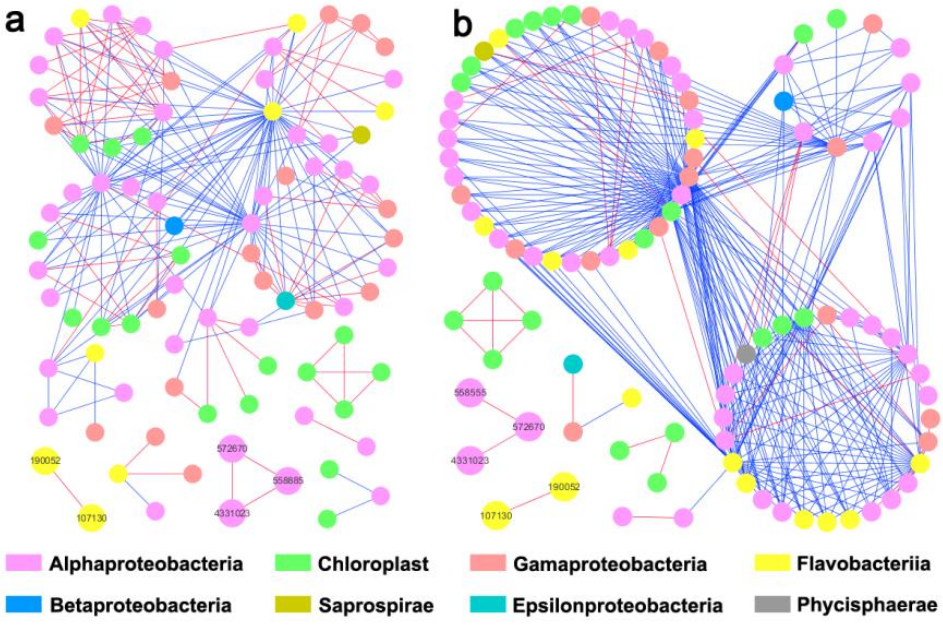

Figure 5

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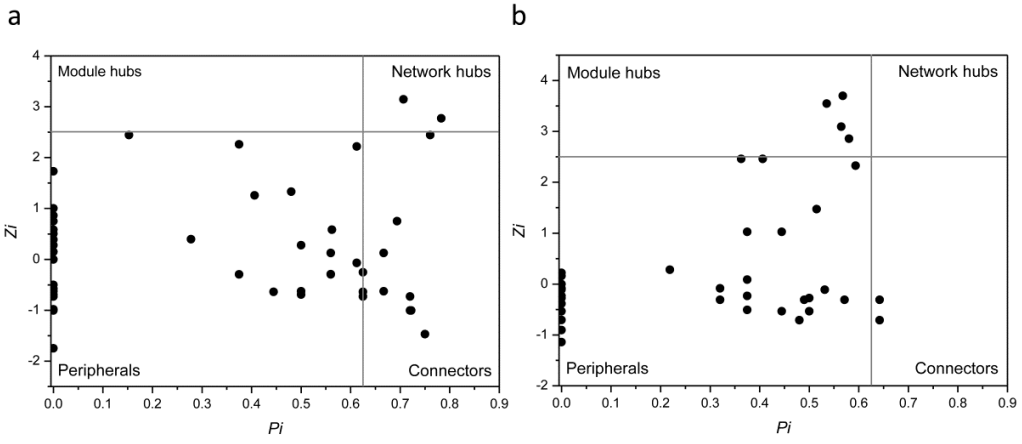

Figure 6

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Table 1 Topological properties of the bacterioplankton communities as represented by molecular networks under HC and LC treatments; also their rewired random networks.

	Experimental network							**Random network**		
	Total nodes	**Total links**	**R2 of power-law**	**Average clustering coefficient (avgCC)**	**Average connectivity**	**Harmonic geodesic distance (HD)**	**Modularity**	**Average clustering coefficient (avgCC)**	**Harmonic geodesic distance (HD)**	**Modularity**
LC	85	209	0.817	0.402	0.625	3.397	0.414	0.424 +/- 0.023	2.187 +/- 0.049	0.249 +/- 0.010
HC	96	310	0.817	0.448	0.714	2.956	0.303	0.292 +/- 0.023	2.306 +/- 0.059	0.323 +/- 0.008

Table 2 Dissimilarity tests of bacterial communities under HC and LC treatment at various time points.

	Anosim		**MRPP**		**Adonis**	
Time	R	P-value	δ	P-value	R^2	P
day6	-0.111	0.602	0.3952	1	0.15447	1
day8	0.111	0.284	0.438	0.6	0.2	0.5
day10	0.037	0.613	0.4929	0.7	0.17829	0.7
day13	0.111	0.309	0.412	0.5	0.19714	0.5
day19	0	0.693	0.4336	0.3	0.28263	0.3
day29	-0.259	1	0.4513	0.9	0.15517	0.9