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Ocean acidification alters microeukaryotic and bacterial food web interactions in a eutrophic subtropical mesocosm

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ABSTRACT

Ocean acidification (OA) is known to influence biological and ecological processes, mainly focusing on its impacts on single species, but little has been documented on how OA may alter plankton community interactions. Here, we conducted a mesocosm experiment with ambient (~410 ppmv) and high (1000 ppmv) CO_2 concentrations in a subtropical eutrophic region of the East China Sea and examined the community dynamics of microeukaryotes, bacterioplankton and microeukaryote-attached bacteria in the enclosed coastal seawater. The OA treatment with elevated CO_2 affected taxa as the phytoplankton bloom stages progressed, with a 72.89% decrease in relative abundance of the protist Cercozoa on day 10 and a 322% increase in relative abundance of Stramenopile dominated by diatoms, accompanied by a 29.54% decrease in relative abundance of attached Alphaproteobacteria on day 28. Our study revealed that protozoans with different prey preferences had differing sensitivity to high CO_2, and attached bacteria were more significantly affected by high CO_2 compared to bacterioplankton. Our findings indicate that high CO_2 changed the co-occurrence network complexity and stability of microeukaryotes more than those of bacteria. Furthermore, high CO_2 was found to alter the proportions of potential interactions between phytoplankton and their predators, as well as microeukaryotes and their attached bacteria in the networks. The changes in the relative abundances and interactions of microeukaryotes between their predators in response to high CO_2 revealed in our study suggest that high CO_2 may have profound impacts on marine food webs.

1. Introduction

Atmospheric CO_2 concentrations are higher today than at any point in at least the past 800,000 years (Lüthi et al., 2008) and the ocean has absorbed about one-third of the anthropogenic CO_2 emitted since the Industrial Revolution (Gruber et al., 2019). Uptake of anthropogenic CO_2 has caused a decrease in surface seawater pH and has altered marine carbonate chemistry, in a process known as ocean acidification. Under the IPCC 'business as usual' scenario average surface seawater pH is projected to drop by a further 0.3 units during this century, with an even greater fall in pH in eutrophic waters (Pörtner et al., 2021). The effects of high CO_2, explored using such methods as cellular metabolism and organismal physiology, differ widely within and between species (Doney et al., 2020; Gao et al., 2019; Mostofa et al., 2016). Marine microeukaryotes and bacteria have important global roles in food webs and nutrient cycling. Phytoplankton convert CO_2 into organic compounds, contributing ~50% of the total global primary production and providing the base of the ocean food web (Field et al., 1998). Between 5% and 95% of the organic carbon fixed by phytoplankton is released into seawater as dissolved organic matter, which can then be used by bacteria (Thornton, 2014) which are in turn consumed by zooplankton and flagellates, forming an important part of the 'microbial loop' (Fenchel, 2008).

Mesocosm experiments allow studies of marine communities and so

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can be used to assess both the direct and indirect influences of high CO_2 on ecosystem function (Baltar et al., 2015; Doney et al., 2020; H P Grossart et al., 2006; Riebesell et al., 2017; Spisla et al., 2021). The effects of high CO_2 on phytoplankton species can be positive, negative or neutral (Gao et al., 2019; Hutchins and Sañudo-Wilhelmy, 2022; O'Brien et al., 2016). Pronounced beneficial effects of high CO_2 levels have been observed on some phytoplankton with small sizes but not others (Hyun et al., 2020; Schulz et al., 2017). Within bacterial communities, high CO_2 has been found to influence the relative abundance of certain taxa (Bach et al., 2019; Baltar et al., 2015; Maugendre et al., 2017a; Meakin and Wyman, 2011; Riebesell et al., 2017; A. S. Roy et al., 2013; Spisla et al., 2021; Zhang et al., 2013).

Marine species exchange materials, energy and information-carrying chemicals in a range of interactions including competition, mutualism, predation and commensalism (Lima-Mendez et al., 2015). The interactions between species and their environment are altered by ocean acidification (Nagelkerken et al., 2016; Sswat et al., 2018), but comprehensive studies of the potential effects of ocean acidification on these interactions are lacking. Molecular ecological network analysis can elucidate network interaction in microbial communities and their responses to environmental changes (Djurhuus et al., 2020; Fuhrman et al., 2015; Lentendu and Dunthorn, 2021). In soil studies, warming (Yuan et al., 2021) and elevated CO_2 (Tu et al., 2015; Zhou et al., 2011) altered biological communities, interaction network structures and ecosystem function. Prior to our study, very little was known about the potential effects of high CO_2 on ecological networks in the plankton although an Arctic mesocosm experiment indicated that bacterioplankton interaction networks were not significantly affected by high CO_2 (Wang et al., 2016). However, in subtropical eutrophic coastal waters high CO_2 was found to cause extensive changes in bacterioplankton community networks (Lin et al., 2018). Until now, network analysis of marine eukaryote-prokaryote community interactions has been lacking in the context of global human impacts on marine ecosystems.

Most high CO_2 mesocosm experiments have used a starting oligotrophic seawater with or without the experimental addition of nutrients (Gazeau et al., 2017; Kim et al., 2006; Maugendre et al., 2017a; Spisla et al., 2021; Zhang et al., 2013). Here we examined a coastal area that is strongly influenced by eutrophication, as it is known that high nutrient availability can interact with the effects of ocean acidification (Cai et al., 2011; Wallace et al., 2014). Coastal waters are projected to be 0.13 pH units more acidic than the open ocean by the end of this century, due to eutrophication (Cai et al., 2011). In mesocosm experiments conducted in oligotrophic seawater with nutrient addition, nitrate, phosphate and Si or Fe were added before or during the experiment to stimulate phytoplankton growth (Alvarez-Fernandez et al., 2018). In those experiments the concentrations of nutrients, elements and dissolved organic matter were different to those found in eutrophic coastal waters. The Facility for the Study of Ocean Acidification Impacts of Xiamen University (FOANICXMU) mesocosm platform, located in a subtropical eutrophic coastal region (Chen et al., 2021) of the East China Sea, provided an opportunity to study the effects of ocean acidification in the context of eutrophication. This is pertinent to the real-world conditions presently found around coastlines worldwide. Therefore, the simulated ocean acidification mesocosm experiments conducted in eutrophic seawater in our study should better reflect the potential effects of ocean acidification in real-world eutrophic conditions. During the five-week experiment that simulated ocean acidification by manipulating seawater pCO_2, the 18S ribosomal DNA (V9 region) was used for determining microeukaryotic communities, and the 16S ribosomal DNA (V4–V5 region) was used for determining the communities of bacterioplankton and microeukaryote-attached bacteria under ambient CO_2 and high CO_2. We then used these data to investigate changes in community composition and interaction network structures due to high CO_2. This study is the first to use co-occurrence network analysis to fully explore how interactions between various microeukaryotes and bacteria in the marine environment are impacted by elevated CO_2 based on the mesocosm experiment. Our study highlights the potential changes in interactions between marine microbes in the context of global change and their implications for the marine ecosystem.

2. Materials and methods

2.1. Mesocosm setup

Our mesocosm experiment ran from April 9th to May 15th' 2018, using the FOANICXMU mesocosm platform in Wuyuan Bay, Xiamen, Fujian Province, East China Sea (24°31′48″ N, 118°10′47″ E). Each transparent thermoplastic polyurethane cylindrical mesocosm bag was 3 m deep and 1.5 m wide (4000 L total volume). Seawater from Wuyuan Bay was filtered through a 0.01 μm water purifying system (MU801-4T, Midea, China) and used to simultaneously fill 9 bags within 36 h. Bags 2, 4, 6, and 8 were controls, aerated with ambient air pCO_2. Bags 1, 3, 5, 7, and 9 were acidified with approximately 11 L of CO_2 saturated seawater to attain 1000 μatm CO_2. Subsequently, Ambient and high CO_2 mesocosm bags were aerated (5 L min^{-1}) with ambient air of 410 ppmv pCO_2 and premixed air-CO_2 of 1000 ppmv pCO_2, respectively, till the end of the experiment. Subsequently, 720 L of in situ seawater filtered by 180 μm mesh was added simultaneously into 9 mesocosm bags after about 2–3 h of aeration for carbonate system homogenization. Therefore, each bag was inoculated with 80 L of in situ seawater containing a natural community of microeukaryotes and microbes. Every 1–3 days, the mesocosm samples were collected from 0.5 m depth in each bag at 10:00 a.m. for physical, chemical and biological analysis, as described in Huang et al. (2021) and supplementary information. The initial concentrations of NO_3^-, NO_2^-, NH_4^+, PO_4^{3-} and $Si(OH)_4$ in all mesocosm bags were 6.73–10.65, 0.49–0.79, 6.07–8.71, 0.18–0.47, and 5.42–9.50 μmol kg^{-1} respectively (Supplementary Fig. 1), and matched the eutrophic conditions of the surrounding coastal seawater. The large variation in nutrient concentration among these mesocosm bags was caused by difficulties in seawater filtration in eutrophic coastal regions with high concentrations of organisms and particles.

2.2. Sample filtration, DNA extraction, amplification and sequencing

A total of 500 mL to 2 L of seawater, depending on biomass concentration, was sampled from each mesocosm bag. Samples from days 3, 6, 8, 10, 16, 20, and 28, which could represent different stages of phytoplankton blooms according to temporal variations in Chl *a*, were collected and used for DNA extraction and sequencing in this study. Sequential size fractionated filtration (2-μm and 0.2-μm polycarbonate filters) by a peristaltic pump was used to filter seawater collected from the mesocosm bags. DNA from samples was extracted as follows. 1 mL of 70 °C preheated lysis buffer (100 mM Tris, 40 mM EDTA, 100 mM NaCl, 1% SDS) was added to the centrifuge tube with the filter. After 3 times of 30s vortex and 5 min water bath at 70 °C, NaCl 0.7 M and 1% CTAB (finial concentrations) were added. The filter was then water-bathed at 70 °C for 10 min. Phenol-chloroform extraction and ethanol precipitation was then used for DNA extraction. The DNA from samples collected by 0.2-μm and 2-μm filters was used for 16S V4–V5 region PCR amplification representing the community of bacterioplankton and microeukaryote-attached bacteria, respectively. DNA from samples collected by 2-μm filters were also used for 18S V9 region PCR amplification for the microeukaryotic community. 515AF (GTGYCAGCMGCCGCGGTAA), and 926R (CCGYCAATTYMTTTRAGTTT) targeting the 16S rDNA V4–V5 region of prokaryotes were used. 1389F (TTGTACACACCGCCC) and 1510R (CCTTCYGCAGGTTCACCTAC) targeting the 18S rDNA V9 region of eukaryotes were used. The amplification conditions were as follows: initial denaturation at 95 °C for 3 min, 29 cycles for 16S V4–V5 region and 30 cycles for 18S V9 region of denaturation at 95 °C for 30s, annealing at 53 °C for 30s extension at 72 °C for 45s, and then final extension at 72 °C for 10 min. PCR products

were purified using an AxyPrepDNA gel extraction kit (Axygen, Union City, CA, United States) from 2% agarose gel after electrophoresis. DNA library was constructed following the MiSeq Reagent Kit preparation guide (Illumina, San Diego, CA, United States). The sequencing was conducted using an Illumina MiSeq PE300 platform (Shanghai Majorbio Bio-pharm Technology Co. Ltd., Shanghai, China) after the purification and quantification of PCR products.

2.3. Sequence assignment and data analysis

Raw fastq sequences were quality filtered by fastp (v0.19.6) and merged by FLASH (v1.2.7) before analysis. The filtered reads were imported to QIIME 2, and DADA2 was used to de-noise the sequences, resulting in high-resolution amplicon sequence variants (ASVs). For taxonomic classification, we used the SILVA database138 with 97% identity for 16S V4–V5 region gene sequencing and the TARA 18S V9 database with 97% identity for 18S V9 region gene sequencing data (http://taraoceans.sb-roscoff.fr/EukDiv/index.html). To minimize the effects of sequencing depth on subsequent analyses, the number of sequences from each sample was rarefied to the smallest number of sequences in all samples. Alpha diversity was estimated by Shannon index and Simpson index using Mothur 1.30. Beta diversity was calculated by non-metric multidimensional scaling (NMDS) and analysis of similarities (ANOSIM) using Bray distance matrices. We also used canonical correlation analysis (CCA) to identify and measure the associations between environmental variables and the bacterial and microeukaryotic communities. The species with significant differences in relative abundance between ambient and high CO_2 treatments were identified using Wilcoxon sum-rank test. To eliminate the effects of phytoplankton bloom stages on the community structure, LEfSe analysis were carried out regardless of different stages. The linear discriminant analysis (LDA) effect size (LEfSe) is used to estimate the impact strength of the abundance with differences between ambient and high CO_2 treatments, with the setting of LDA score >2.0, based on the results of Wilcoxon sum-rank test at the level of $P < 0.05$ (Segata et al., 2011).

2.4. Network construction

Co-occurrence networks of microeukaryotes, microeukaryote-attached bacteria and bacterioplankton were constructed under high CO_2 and ambient CO_2 treatments respectively and throughout the experiment, in the R environment. The package 'Hmisc' was used to calculate the Spearman rank coefficient (r) between rarefied ASVs. The package "igraph" was used to construct network, based on the correlations that were robust ($|r| > 0.6$) and significant ($p_{adj} < 0.01$). The supplemental material contains a description of the network's parameters. To determine how high CO_2 affected network complexity, network topological characteristics were analyzed using the package 'igraph', and the differences in 500 bootstrapped node attributes between different CO_2 treatments were performed by the Kolmogorov-Smirnov test. To evaluate how high CO_2 influenced network stability, robustness and vulnerability which can reflect the sensitivity of networks toward node removal, were compared under different CO_2 concentrations (Yuan et al., 2021). Robustness was expressed by the proportion of nodes remained by randomly removing 50% nodes for 1000 times, and then compared between ambient and high CO_2 using Wilcoxon test. Vulnerability was represented by the maximum decrease in network efficiency when a single node is deleted from the network, for running times which were equal with number of nodes. The proportions of interactions of phytoplankton and their predators, were extracted from microeukaryote networks and cross domain networks respectively, and then compared under high CO_2 and ambient CO_2. To obtain the interactions of microeukaryotes and their attached bacteria, we also constructed cross domain networks using 18S V9 and 16S V4-5 sequencing results of samples with diameter larger than 2 μm.

3. Results

3.1. Experimental timeline and dynamic of biotic and abiotic parameters

The differences in carbonate system parameters pH_{NBS} and pCO_2 between high CO_2 and ambient CO_2 treatments maintained relatively stable with the values of 0.22 ± 0.05 units and 455 ± 100 μatm respectively (Fig. 1 a, b). The Chl *a* concentration peaked on day 12 with 6.35 μg/L under high CO_2 and 7.91 μg/L under ambient CO_2 and then decreased gradually (Fig. 1 c). The bacterioplankton cell abundance peaked on day two and then decreased, reacheding another peak on day eight under both high CO_2 and ambient CO_2 treatments (Fig. 1 d). The rapid growth of the bacterioplankton in the initial days may be due to the initial filtered seawater with a low concentration of bacteria predators.

3.2. The biodiversity of microeukaryotes, bacterioplankton and attached bacteria

By removing bacterial sequence from 18S V9 region raw data and rarefaction, we obtained 629 amplicon sequence variants (ASVs), 250 genera, 169 families, 109 orders, 67 classes and 24 phyla for microeukaryotes community (Supplementary Table 1). The most abundant microeukaryotic phyla were Stramenopiles (42.0%), Dinophyta (16.9%), Cercozoa (15.7%), Metazoa (5.9%), Ciliophora (5.9%) and Choanoflagellida (3.7%) (Supplementary Fig. 1). By removing chloroplast sequence elimination from 16S V4–V5 region raw data and rarefaction, we obtained 2891 ASVs, 532 genera, 224 families, 135 orders,

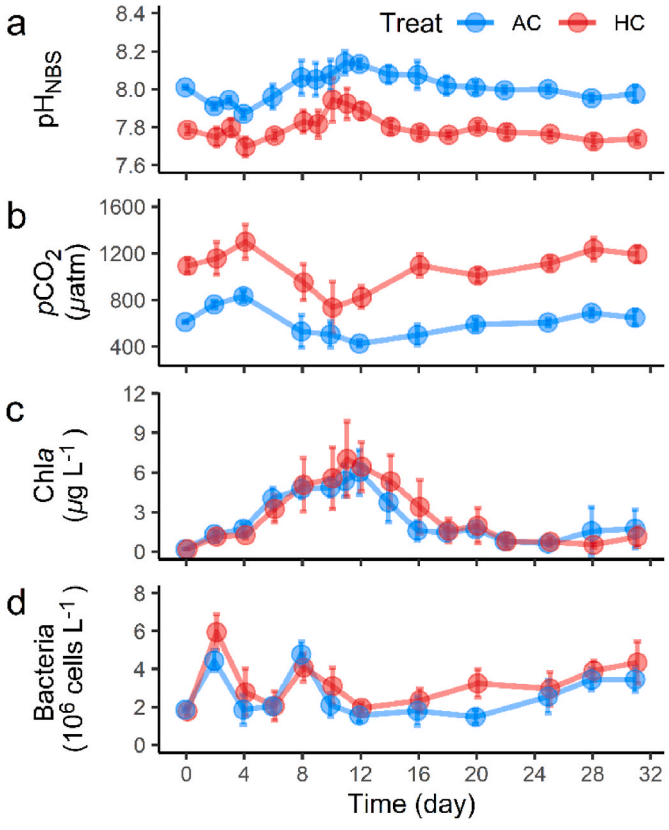

Fig. 1. Temporal variations of pH_{NBS} (a), pCO_2 (b), Chl *a* concentration (c), and the absolute abundance of bacterioplankton (d) under high CO_2 (HC, 1000 ppmv) and ambient CO_2 (AC, 410 ppmv) mesocosm treatments in Wuyuan Bay, East China Sea from April 9th to May 15th, 2018. The pCO_2 was estimated from the measured pH and DIC concentration using CO2SYS program. Data are means ± SD of replicates for HC treatments and for AC treatments (Huang et al., 2021).

52 classes and 24 phyla for bacterial community (Supplementary Table 1). Proteobacteria and Bacteroidota were the most abundant phyla, with the proportion of 48.8% and 46.8% in bacterioplankton community and 63.0% and 28.6% in microeukaryote-attached bacteria community, respectively (Supplementary Fig. 2).

3.3. The time series dynamics of community composition

Evident in the NMDS results, significant differences in beta diversity were observed among different sampling time points, representing different stages of phytoplankton bloom (ANOSIM: R = 0.53 and P = 0.001 for microeukaryotes, R = 0.7246 and P = 0.001 for bacterioplankton, and R = 0.5253 and P = 0.001 for attached bacteria, Fig. 2). The results of CCA were consistent with those of NMDS, in which community compositions were significantly correlated with different sampling time points (R^2 = 0.375 and P = 0.001, R^2 = 0.892 and P = 0.001, and R^2 = 0.333 and P = 0.001 for microeukaryotes, bacterioplankton and attached bacteria, respectively, Supplementary Fig. 3). Both NMDS and CCA results indicate that the community composition of microeukaryotes, bacterioplankton and attached bacteria varied along with the development of phytoplankton bloom.

The relative abundance of Stramenopiles, mainly composed by Bacillariophyta, decreased from 66.4 ± 31.1% on day three to the lowest levels 10.3 ± 9.5% on day 16, then reached a peak of 59.2 ± 31.3% on day 20 and decreased to 17.7 ± 18.3% on day 28 (Fig. 3 a). The relative abundance of Dinophyta maintained stable and low with average of 11.5% during day 3–20, and increased to 48.7 ± 27.8% since then (Fig. 3 b). The relative abundance of Cercozoa, dominated by Filosa-Sarcomonadea, increased from the average value of 6.5% on day three, peaked on day 10 under ambient CO_2 (62.7 ± 20.2%) and on day 16 under high CO_2 (34.1 ± 41.0%), and decreased to a low level till the end of the experiment (Fig. 3 c). The relative abundances of Metazoa maintained nearly 0 through the whole processes under ambient CO_2, and appeared a peak with the value of 0.6 ± 1.0% during day 8–20 under high CO_2 (Fig. 3 d).

In both bacterioplankton community and attached bacteria community, the relative abundance of Proteobacteria showed a general increase throughout the experiment, while the relative abundance of Bacteroidota had an opposite trend (Fig. 4). In bacterioplankton community, the relative abundance of Proteobacteria, with 56.3 ± 12.2% on day three, appeared a small peak of 70.0 ± 12.7% on day eight, and decreased to 36.5 ± 11.2% since then (Fig. 4 a). Correspondingly, the relative abundance of planktonic Bacteroidota, reached a vale with an average of 24.8% on day eight, and then increased to 56.6 ± 12.3% (Fig. 4 b). The time series changes in the relative abundance were more pronounced in attached bacteria community, with a decrease from 93.8 ± 5.6% to 40.5 ± 8.8% for Proteobacteria, and an increase from 5.9 ± 5.6% to 35.7 ± 8.3% for Bacteroidota (Fig. 4 c-d).

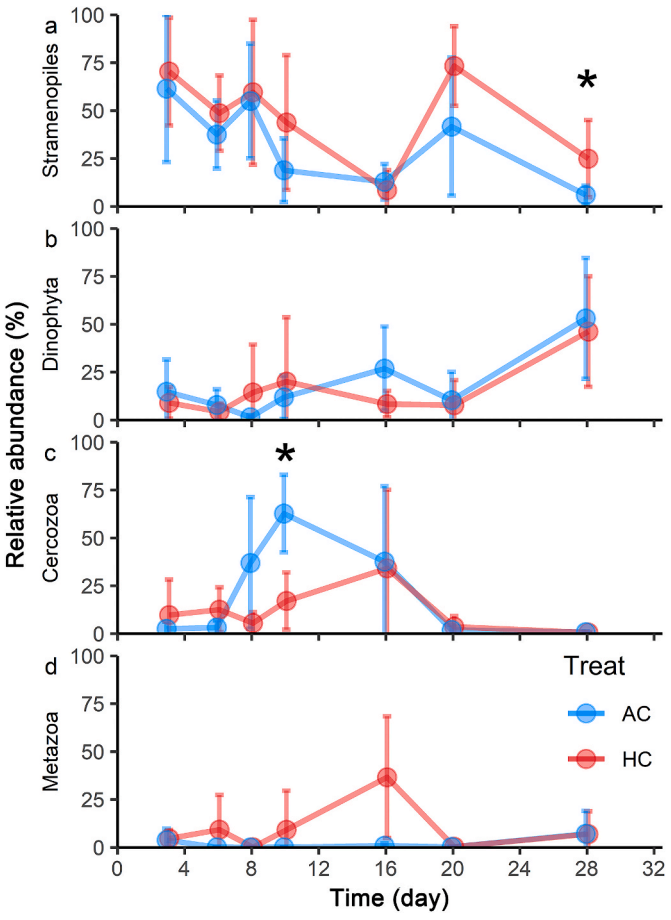

Fig. 3. Relative abundance of the most abundant microeukaryotic phyla under high CO_2 (HC) and ambient CO_2 (AC) throughout the experiment: Stramenopiles (a), Dinophyta (b), Cercozoa (c) and Metazoa (d). Data are means ± SD of replicates for HC treatments and for AC treatments. *Indicates significant difference between HC and AC at that time point (P < 0.05).

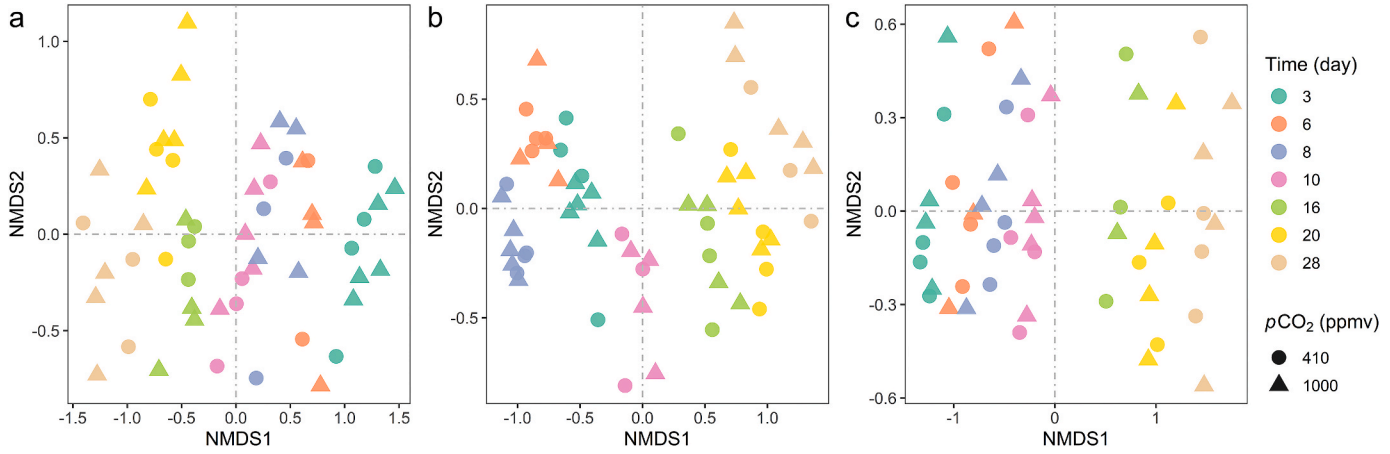

Fig. 2. The beta diversity of different samples from different time points (Day 3, 6, 8, 10, 16, 20 and 28) is represented using non-metric multidimensional scaling plots (NMDS) of microeukaryotes (a), bacterioplankton (b) and microeukaryote-attached bacteria (c) under high CO_2 (1000 ppmv) and ambient CO_2 (410 ppmv) treatments.

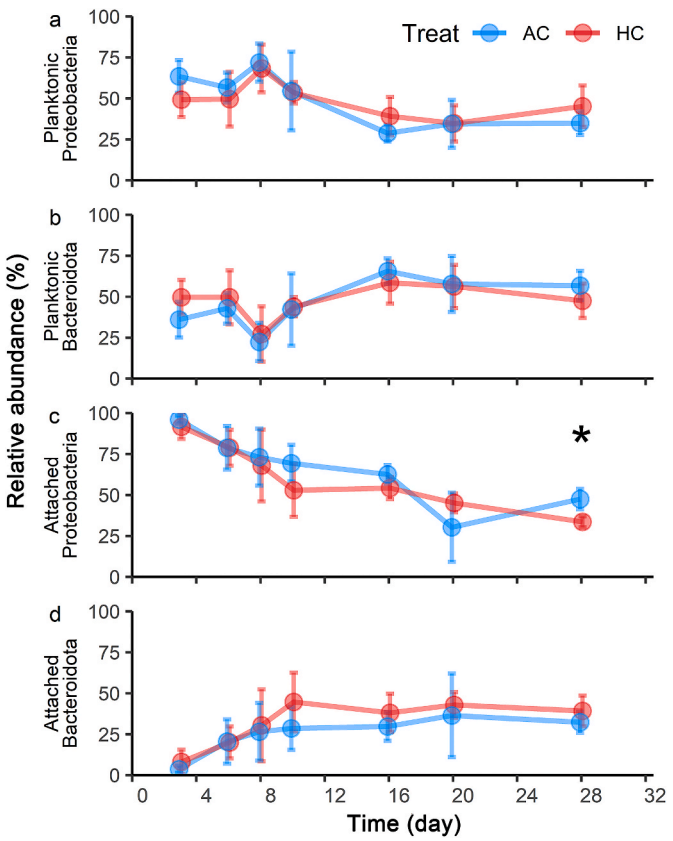

Fig. 4. Temporal changes of relative abundance of planktonic Proteobacteria (a), planktonic Bacteriodota (b), attached Proteobacteria (c), attached Bacteriodota under high CO_2 (HC, 1000 ppmv) and ambient CO_2 (AC, 410 ppmv) treatments during the eutrophic mesocosm experiment. Data are means ± SD of replicates for HC treatments and AC treatments. *Indicates significant differences between HC and AC at certain time points ($P < 0.05$).

3.4. The effects of different CO_2 concentrations on microeukaryote

We found that high CO_2 had significant effects on some microeukaryotic taxa at some phytolankton bloom stages based on the Wilcoxon sum-rank analysis although non significant correlation between CO_2 treatments and the whole community composition. High CO_2 significantly influenced Cercozoa at the developing stage of phytoplankton bloom, and Stramenopiles at the decline stage. Compared to AC, the relative abundance of Cercozoa under HC was 73% lower on day 10 ($P = 0.016$), and peaked more tardily during the experiment (Fig. 3 c). The differences in the relative abundance of Cercozoa were mainly attributed to the dominant species, *Cercomonas volcana* (belonging to Filosa-sarcomonadea) accounting for 70.8–100% (Supplementary Fig. 4 a-b). At the decline stage of phytoplankton bloom represented by day 28, the relative abundance of Stramenopiles was 3.2 times higher under HC compared to AC ($P = 0.036$, Fig. 3 a), in which the second dominant species diatom *Cylindrotheca closterium* showed higher relative abundance under HC ($P = 0.036$, Supplementary Fig. 4 c-d).

High CO_2 had significant effects on some microeukaryotic taxa, as evidenced by the LEfSe analysis regardless of phytoplankton bloom stages. In microeukaryotes community, Genus *Cirripedia* and Class Annelida which were the dominant taxa of Metazoa, as well as Family Tintinnidae belonging to Cilliphora, significantly benefited under HC (Fig. 5). While Order Choanoflagellatea_X considered to be the closest living relative of animals and Genus *Minorisa* belonging to Cercozoa were more common in AC (Fig. 5). In addition, several primary producers were enriched under HC, for example Genus *Oltmannsiellopsis* belonging to Class Ulvophyceae, Genus *Chrysolepidomonas* which are single-celled flagellate 'golden algae' and Genus *Cymbella* which is a genus of pennate diatom (Fig. 5).

3.5. The effects of different CO_2 concentrations on the attached bacteria and bacterioplankton

Using Wilcoxon sum-rank analysis, we discovered that HC had a substantial effect on some attached bacterial taxa at the decline stage of phytoplankton bloom. On day 28, the relative abundance of attached Proteobacteria dominated by Alphaproteobacteria was 29% lower under HC compared to AC ($P = 0.029$, Fig. 4 c, Supplementary Fig. 5 a-b). However, attached Desulfobacterota dominated by Desulfuromonadia, and *Phaeodaylibacter xiamenensis* had a higher relative abundance under HC (2.3 ± 1.4%) compared to AC (0.2 ± 0.1%) ($P = 0.029$, Supplementary Fig. 5 c-e) on day 28.

In bacterioplanktonic community, HC significantly influenced 20 species, 15 genera, 6 families and 3 orders (Supplementary Table 2). Attached bacteria community were more significantly influenced by CO_2, indicated by the number of biomarkers were 1.2 times larger than that in bacterioplankton community (Supplementary Table 2). For attached bacteria, high CO_2 had negative effects on Alphaproteobacteria and Bacteroidales, and BD7-11 belonging to Planctomycetota, but positive effects on Bdellovibrionota, Actinobacteriota and PB19 belonging to Desulfobacterota (Supplementary Table 2).

3.6. The effects of different CO_2 on co-occurrence networks

Each network exhibited 'small world' characteristics, indicated by the average path length between two nodes ranging between 3.4 and 6.6 (Table 1). The dominant compositions of networks were similar under different CO_2 concentrations (Supplementary Fig. 5). Positive interactions were dominant in all co-occurrence networks, especially in microeukaryote networks with more than 99% positive correlations (Table 1). Compared to bacterioplankton networks, attached bacteria networks possessed similar node numbers and more edge numbers, indicating more interactions existed in attached bacteria networks (Table 1).

Both topological properties and the Kolmogorov–Smirnov test illustrated that HC influenced network complexity, with the largest degrees for microeukaryotes networks, followed by bacterioplankton networks (Tables 1 and 2). HC expanded the scale of microeukaryotes network, as evidenced by the larger numbers of nodes and edge (Table 1). The average clustering coefficient and network density were lower in HC_2 than in AC, indicating that HC decreased the compaction of microeukaryotes network (Table 1). However, HC increased module compaction in microeukaryotes network, evidenced by lower network diameter, modularity and average path length (Table 1). For the bacterioplankton network, increases in the number of nodes and edges, average degree, modularity, average clustering coefficient and average path length were detected under HC (Table 1), demonstrating that HC may increase the complexity of bacterioplankton networks.

Under HC, robustness was significantly higher ($P < 0.0001$, Fig. 6) and vulnerability was lower for microeukaryote networks (Supplementary Table 3) indicating that high CO_2 enhanced the stability of microeukaryote networks. HC significantly induced higher robustness for bacterioplankton networks but lower robustness for attached bacteria networks ($P < 0.0001$ and $P < 0.05$ respectively, Fig. 6). However, higher vulnerability for bacterioplankton network but lower vulnerability for attached bacteria network were detected under HC (Supplementary Table 3).

In addition, we analyzed the effects of different CO_2 concentrations on the interactions between phytoplankton and their predators by extracting and analyzing the changes of co-occurrence between phytoplankton and their predators from microeukaryote networks. We found that HC increased the proportion of Metazoa and Bacillariophyta/Dinophyta links by 58% and Cercozoa and Bacillariophyta/Dinophyta

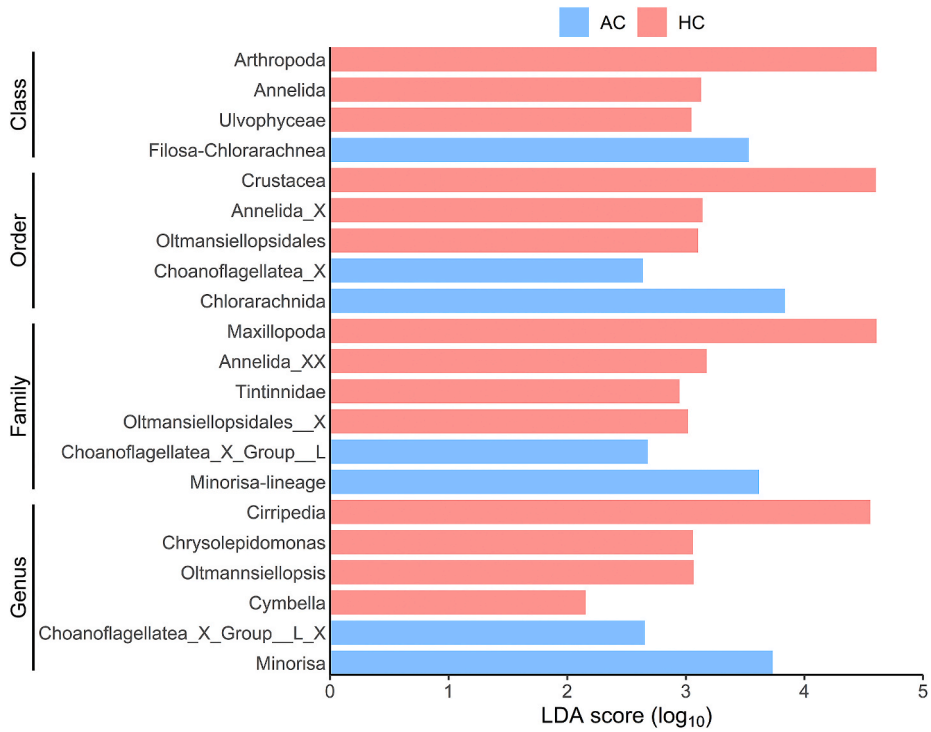

Fig. 5. Biomarker taxa under high CO_2 (HC, 1000 ppmv, red) and ambient CO_2 (AC, 410 ppmv, blue) identified by LEfSe analysis (LDA >2, $P < 0.05$) from different taxonomic levels in microeukaryotes.

Table 1
Topological properties of networks of microeukaryotes (18S_2 μm), attached bacteria (16S_2 μm) and bacterioplankton (16S_0.2 μm) under high CO_2 (HC) and ambient CO_2 (AC) conditions.

Network	Nodes	Edges	negative	positive	Modularity	Average clustering coefficient	Average path length	Network diameter	Average degree	Network density
18S_2 μm_HC	485	3604	5	3599	0.696	0.678	4.972	12	14.862	0.031
18S_2 μm_AC	361	2596	4	2592	0.775	0.826	6.607	18	14.382	0.040
16S_2 μm_HC	1563	54541	1347	53194	0.570	0.653	3.540	8	69.790	0.045
16S_2 μm_AC	1493	58051	1239	56812	0.576	0.676	3.372	8	77.764	0.052
16S_0.2 μm_HC	1500	32843	595	32248	0.661	0.727	3.813	9	43.791	0.029
16S_0.2 μm_AC	1211	24011	325	23686	0.652	0.693	3.790	9	39.655	0.049

Table 2
Results of the Kolmogorov-Smirnov test comparing bootstrapped node attributes in networks of microeukaryotes (18S_2 μm), attached bacteria (16S_2 μm) and bacterioplankton (16S_0.2 μm) under high CO_2 (HC) and ambient CO_2 (AC) conditions. Node attributes were bootstrapped with 500 iterations.

	Degree		Betweenness		Closeness		Transitivity	
	D.	p.value	D.	p.value	D.	p.value	D.	p.value
18S_2 μm_HC vs 18S_2 μm_AC	0.138	1.46e-4	0.222	3.97e-11	1	0	0.244	2.36e-13
16S_2 μm_HC vs 16S_2 μm_AC	0.112	3.78e-3	0.178	2.64e-7	1	0	0.072	1.50e-1
16S_0.2 μm_HC vs 16S_0.2 μm_AC	0.164	2.89e-6	0.216	1.48e-10	0.178	2.64e-7	0.156	1.04e-5

links by 4%. High CO_2, on the other hand, reduced the proportion of Ciliophora and Bacillariophyta/Dinophyta links by 5% and Choanoflagellida and Bacillariophyta/Dinophyta links by 47% (Fig. 7 a). The interactions between microeukaryotes and their attached bacteria were extracted from the cross networks including microeukaryotes and attached bacteria. We found that the proportions of Metazoa and bacteria links increased by 10%, while the proportions of Choanoflagellida and bacteria links increased by 12%, under HC. In contrast, HC decreased the proportions of Ciliophora and bacteria links by 17%, as well as the proportions of Cercozoa and bacteria links by 22% (Fig. 7 b).

4. Discussion

4.1. The effects of elevated CO_2 on the microeukaryotic community

We found that high CO_2 had significant effects on Cercozoa and diatoms at the developing stage and the decline stage respectively. Cercozoa, a biflagellate heterotroph found globally, increased on day 10 and advanced the peak by ambient CO_2. Cercozoa were less resistant to acidic environments in our study, which is consistent with that fact that most of Cercozoa prefer basic or neutral soil environments (Dupont et al., 2016; Xu et al., 2022). Genus Cirripedia belonging to Class

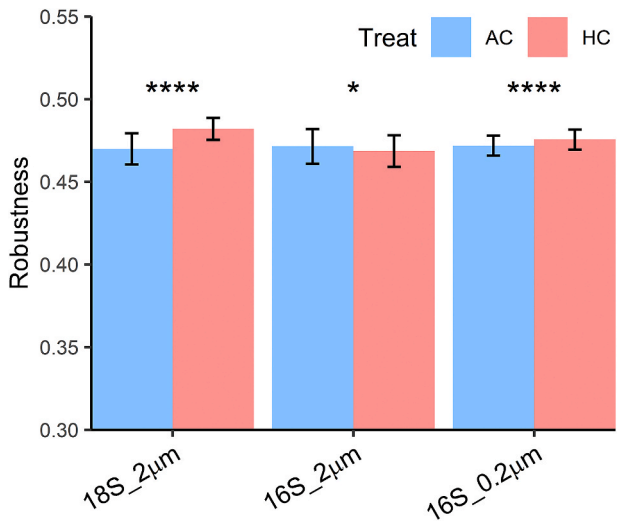

Fig. 6. Robustness of microeukaryotes (18S_2 μm), attached bacteria (16S_2 μm) and bacterioplankton (16S_0.2 μm) networks under high CO_2 (HC) and ambient CO_2 (AC) conditions, indicated by the proportion of remaining species after 50% nodes randomly removal. * and **** indicate $P < 0.05$ and $P < 0.0001$ respectively.

Arthopoda and Class Annelida were more abundant under HC based on LEfSe analysis. Crustaceans are generally less vulnerable to elevated pCO_2/low pH although they are calcifying organisms, because they have evolved a relatively good control of their extracellular pH through active ion transport (Whiteley, 2011). Annelida was considered the most resilient taxa to elevated pCO_2/low pH (Kroeker et al., 2011; Widdicombe and Needham, 2007), which may be a by-product of an increase in 'ecological space' (Hale et al., 2011). In addition, the in-situ organisms experience fluctuating conditions with 0.11–0.18 units of daily △pH, due to the high flux of day-time photosynthetic CO_2 removal and night-time respiratory CO_2 release. The preference of Arthopoda and Annelida to elevated CO_2 in this study may be influenced by both local conditions and organism physiology.

At the decline stages of phytoplankton bloom, elevated CO_2 increased the relative abundance of Stramnopiles mainly composed of Bacilliriophyta, which was in agreement with microscope observations (Huang et al., 2021). The effects of high CO_2 on diatoms are likely to vary regionally (Bach et al., 2019). In our study, nutrient conditions, prevailing diatom species with higher CO_2 affinity for carboxylation (Raven et al., 2011), lack of CCMs plasticity (Van de Waal et al., 2019) and lower mortality rates (Wang et al., 2022) compared to dinoflagellates may result in higher relative abundance of diatoms under elevated CO_2.

4.2. *The effects of elevated CO_2 on the prokaryotic community*

Our previous study revealed a higher cell abundance of bacterioplankton under high CO_2 throughout the experiment based on GAMM analysis (Huang et al., 2021). However, the overall community structures of both bacterioplankton and attached bacteria were not strongly affected by high CO_2. However, we found that more attached bacteria taxa than bacterioplankton taxa were recognized as biomarker taxa under two CO_2 treatments. These findings are consistent with previous studies that the effects of elevated CO_2 on attached bacteria were much more pronounced than on bacterioplankton in terms of cell-specific production rate (Grossart et al., 2006). Compared to bacterioplankton, attached bacteria have bigger genome sizes, and contain more transporters that may be linked to the successive decomposition of phytoplankton blooms (Smith et al., 2013; Teeling et al., 2012). This may

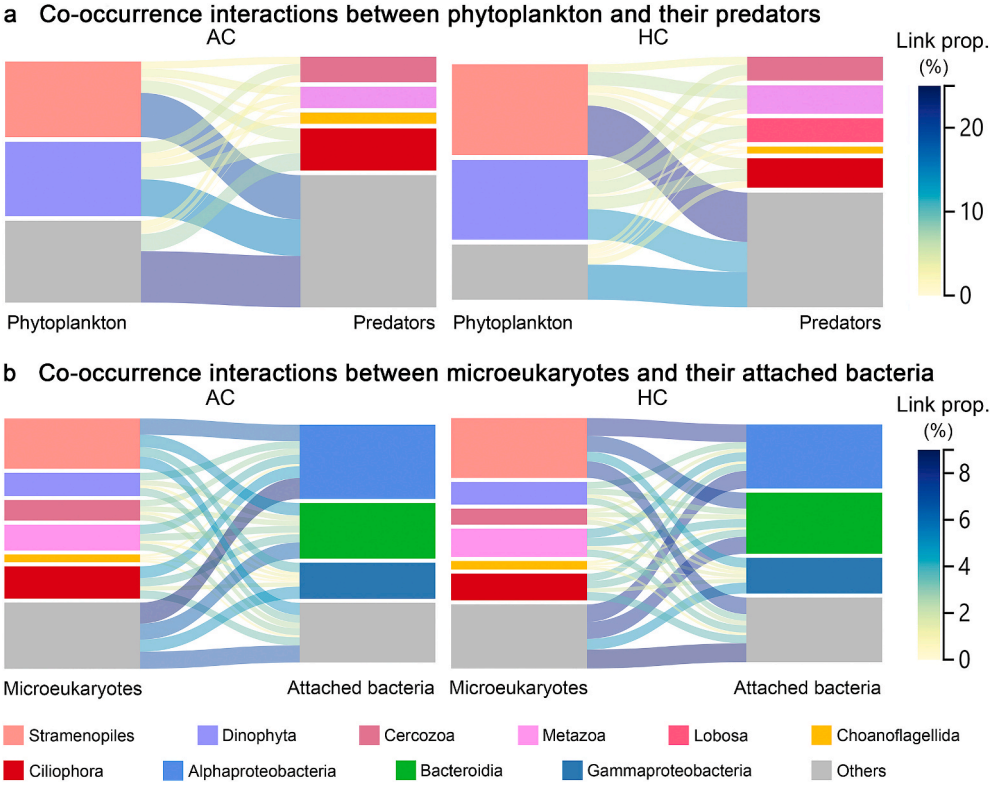

Fig. 7. Co-occurrence interactions between phytoplankton and their predators, and microeukaryotes and their attached bacteria under high CO_2 (HC) and ambient CO_2 (AC) conditions. The colors of columns correspond to different phyla for microeukaryotes and classes for attached bacteria, the colors of ribbons correspond to the proportions of interactions. In the panels of Co-occurrence nteractions between phytoplankton and their predators, Stramenopiles represents the dominant Class Bacillariophyta.

allow for quick and flexible acclimation of attached bacteria to changes in various environmental conditions, such as high CO_2. In attached bacterial community, Proteobacteria, especially Alphaproteobacteria, had significantly lower relative abundance under high CO_2 on day 28 at late stage of phytoplankton bloom. Meanwhile, the relative abundance of attached Desulfobacterota dominated by Desulfuromonadia had a higher relative abundance under high CO_2. The implication of the changes in the relative abundance of Alphaproteobacteria and Desulfuromonadia under elevated CO_2 on biogeochemical cycles is worth further exploration.

In this study, we did not filter out zooplankton from the samples collected on 2 μm filters. Therefore, 'attached bacteria' in our study could include bacteria not only attached to microeukaryotes surfaces but also internal zooplankton-associated bacteria. Previous studies have shown that both environmental fluctuation and zooplankton host genotype can induce variations in gut microbiota structure (Grossart et al., 2009; Li et al., 2021; MacKe et al., 2017). If zooplankton species with a specific microbiome become more abundant in high CO_2 environments, this may have an indirect effect on the zooplankton-associated bacteria community we discovered, even though the zooplankton-associated bacteria are not sensitive to changes in seawater pH (De Corte et al., 2018).

4.3. The effects of elevated CO_2 on the interaction networks

Both direct and indirect effects of elevated CO_2 on marine ecosystems at the ecological levels should be evaluated (Cripps et al., 2014, 2016; Ellis et al., 2017; Hammill et al., 2018). Co-occurrence ecological networks can assess the potential indirect effects under different treatments. In our study, elevated CO_2 changed network complexity and stability, to a greater extent than bacteria (Fig. 6). These results are in line with previous studies at marine CO_2 seeps (Kerfahi et al., 2022) and differs from work on macro organisms that form more stable but less complex networks with low biodiversity under elevated CO_2 (Harvey et al., 2019). These results may be due to the higher resilience of bacteria and the cascading effects of food webs in microeukaryotes (Bach et al., 2017; Boxhammer et al., 2018; Teixidó et al., 2018).

Changes in the proportions of co-occurrences in networks imply that high CO_2 may alter the potential interactions between phytoplankton, zooplankton and bacteria. A higher proportion of Metazoa and attached bacteria co-occurrence under high CO_2 implies that the trophic webs formed by Metazoa and bacteria, as microbivores and decomposers (Jeong and Kim, 2021), may be enhanced under elevated CO_2. The proportion of Metazoa and attached phytoplankton was also higher under high CO_2, implying that high CO_2 may enhance their predator-prey relationship (Fig. 5). In contrast with Metazoa, high CO_2 may be unfavorable to Ciliphora, a phenomenon was also observed in acidic soils (Xu et al., 2022). These may result from the decreased proportions of Ciliphora's co-occurrence with both phytoplankton and bacteria, which could constitute Ciliphora's food resources (Fig. 5). Cercozoa also feeds on phytoplankton as well as bacteria (Pescador et al., 2022). The decreased proportion of Cercozoa and bacteria co-occurrence but increased proportion of Cercozoa and phytoplankton occurrence under high CO_2. The results demonstrated that the decrease in the relative abundance of Cercozoa was mainly attributed to the changes in proportion of Cercozoa and bacteria rather than Cercozoa and phytoplankton. Choanoflagellida is a microeukaryote that feeds on bacteria and detritus (King, 2005). The increase in the proportion of Choanoflagellida and attached bacteria co-occurrence, indicates that elevated CO_2 may enhance the potential interactions between Choanoflagellida and attached bacteria, but could not explain the decrease in the relative abundance of Choanoflagellatea.

In our experimental system, zooplankton larger than 180 μm were removed from the original community, resulting in a reduction of top-down control in the system, such as the prey of large zooplankton on phytoplankton was not well presented in the networks (MacKe et al., 2017). In summary, high CO_2 may have an influence on potential interactions between bacteria, phytoplankton and zooplankton in eutrophic coastal conditions based on the network analysis approach (Fig. 8). This approach could be usefully applied to assess the ecological effects and advantages of reducing multiple stressors (e.g. eutrophication, warming, bottom-towed fishing gear, ocean acidification and deoxygenation) in coastal regions worldwide. It should be noted that interactions revealed by co-occurrence network analysis should be combined with empirical experiments to comprehensively understand the effects of stressors on the interactions between marine organisms (Vincent and Bowler, 2020).

Altered food web structures and interactions between different species found in our mesocosm experiment may influence the effects of high CO_2 on the entire community (Doney et al., 2020; Hammill et al., 2018; Williams et al., 2012), which may have profound implications for the marine ecosystem. However, our mesocosm experiment has its limitations. Our mesocosm experiment excluded large organisms and thus we do not know how increases in micro-zooplankton populations would then affect large zooplankton or fish, but increased secondary productivity is likely to be fed on through the eutrophic food webs of the East China Sea as CO_2 levels continue to rise. Furthermore, the short duration of the experiment in this study did not allow for any potential adaptation of planktonic communities that might take place over extended periods of time.

To conclude, we found that in eutrophic subtropical coastal waters, which are common worldwide, ocean acidification has potential effects on food web interactions and the network structure of microeukaryotic and bacterial communities, based on a mesocosm experiment. Our study's findings regarding the responses of microeukaryotes to elevated CO_2 in terms of their relative abundances and interactions with their predators imply that elevated CO_2 levels could have significant effects on marine food webs and marine ecosystem.

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CRediT authorship contribution statement

Ruiping Huang: Data curation, Formal analysis, Methodology, Writing – original draft. **Ping Zhang:** Methodology. **Xu Zhang:** Methodology. **Shouchang Chen:** Methodology. **Jiazhen Sun:** Methodology. **Xiaowen Jiang:** Methodology. **Di Zhang:** Methodology. **He Li:** Methodology. **Xiangqi Yi:** Methodology. **Liming Qu:** Methodology. **Tifeng Wang:** Methodology. **Kunshan Gao:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision. **Jason M. Hall-Spencer:** Conceptualization, Writing – review & editing. **Jonathan Adams:** Writing – review & editing. **Guang Gao:** Conceptualization, Funding acquisition, Investigation. **Xin Lin:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Software, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Xin Lin, Kunshan Gao, Jason M Hall-Spencer reports was provided by Xiamen University.

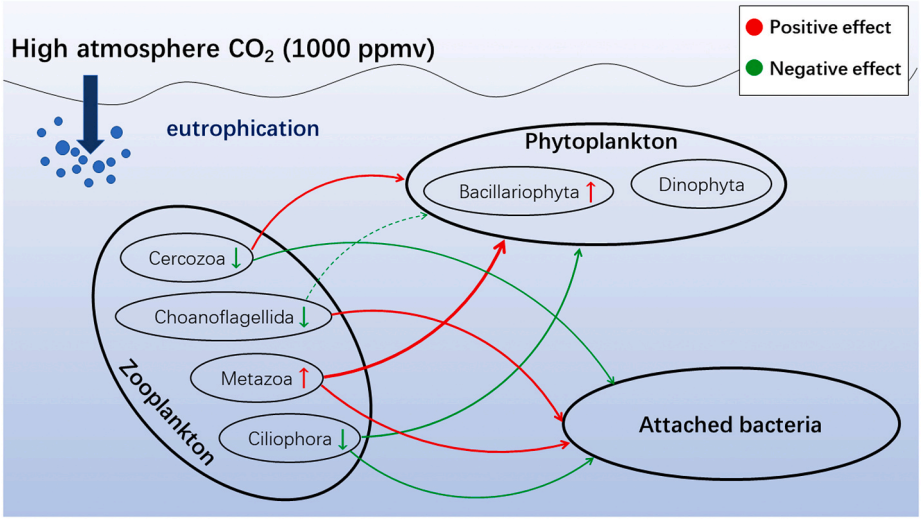

Fig. 8. Schematic illustration of the microeukaryotic community and bacterial community response to the elevated pCO_2 in our study. The red arrow indicates positive (increased) effects and the green arrow indicates negative (reduced) effects.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.119084.

References

Alvarez-Fernandez, S., Bach, L.T., Taucher, J., Riebesell, U., Sommer, U., Aberle, N., Brussaard, C.P.D., Boersma, M., 2018. Plankton responses to ocean acidification: the role of nutrient limitation. Prog. Oceanogr. 165, 11–18. https://doi.org/10.1016/j.pocean.2018.04.006.

Bach, L.T., Alvarez-Fernandez, S., Hornick, T., Stuhr, A., Riebesell, U., 2017. Simulated ocean acidification reveals winners and losers in coastal phytoplankton. PLoS One 12, 1–22. https://doi.org/10.1371/journal.pone.0188198.

Bach, L.T., Hernández-Hernández, N., Taucher, J., Spisla, C., Sforna, C., Riebesell, U., Arístegui, J., 2019. Effects of elevated CO_2 on a natural diatom community in the subtropical NE Atlantic. Front. Mar. Sci. 6, 1–16. https://doi.org/10.3389/fmars.2019.00075.

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

Boxhammer, T., Taucher, J., Bach, L.T., Achterberg, E.P., Algueró-Muñiz, M., Bellworthy, J., Czerny, J., Esposito, M., Haunost, M., Hellemann, D., Ludwig, A., Yong, J.C., Zark, M., Riebesell, U., Anderson, L.G., 2018. Enhanced transfer of organic matter to higher trophic levels caused by ocean acidification and its implications for export production: a mass balance approach. PLoS One 13, 1–25. https://doi.org/10.1371/journal.pone.0197502.

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., Gong, G.-C., 2011. Acidification of subsurface coastal waters enhanced by eutrophication. Nat. Geosci. 4, 766–770. https://doi.org/10.1038/ngeo1297.

Chen, B., Wang, K., Dong, X., Lin, H., 2021. Long-term changes in red tide outbreaks in Xiamen Bay in China from 1986 to 2017. Estuar. Coast Shelf Sci. 249, 107095 https://doi.org/10.1016/j.ecss.2020.107095.

Cripps, G., Lindeque, P., Flynn, K.J., 2014. Have we been underestimating the effects of ocean acidification in zooplankton? Global Change Biol. 20, 3377–3385. https://doi.org/10.1111/gcb.12582.

Cripps, G., Flynn, K.J., Lindeque, P.K., 2016. Ocean acidification affects the phyto-zoo plankton trophic transfer efficiency. PLoS One 11, 1–15. https://doi.org/10.1371/journal.pone.0151739.

De Corte, D., Srivastava, A., Koski, M., Garcia, J.A.L., Takaki, Y., Yokokawa, T., Nunoura, T., Elisabeth, N.H., Sintes, E., Herndl, G.J., 2018. Metagenomic insights into zooplankton-associated bacterial communities. Environ. Microbiol. 20, 492–505. https://doi.org/10.1111/1462-2920.13944.

Djurhuus, A., Closek, C.J., Kelly, R.P., Pitz, K.J., Michisaki, R.P., Starks, H.A., Walz, K.R., Andruszkiewicz, E.A., Olesin, E., Hubbard, K., Montes, E., Otis, D., Muller-Karger, F. E., Chavez, F.P., Boehm, A.B., Breitbart, M., 2020. Environmental DNA reveals seasonal shifts and potential interactions in a marine community. Nat. Commun. 11, 1–9. https://doi.org/10.1038/s41467-019-14105-1.

Doney, S.C., Busch, D.S., Cooley, S.R., Kroeker, K.J., 2020. The impacts of ocean acidification on marine ecosystems and reliant human communities. Annu. Rev. Environ. Resour. 45, 83–112. https://doi.org/10.1146/annurev-environ-012320-083019.

Dupont, A.O., et al., 2016. Differences in soil micro-eukaryotic communities over soil pH gradients are strongly driven by parasites and saprotrophs. Environ. Microbiol. 18, 2010–2024. https://doi.org/10.1111/1462-2920.13220.

Ellis, R.P., Davison, W., Queirós, A.M., Kroeker, K.J., Calosi, P., Dupont, S., Spicer, J.I., Wilson, R.W., Widdicombe, S., Urbina, M.A., 2017. Does sex really matter? Explaining intraspecies variation in ocean acidification responses. Biol. Lett. 13 https://doi.org/10.1098/rsbl.2016.0761.

Fenchel, T., 2008. The microbial loop - 25 years later. J. Exp. Mar. Biol. Ecol. 366, 99–103. https://doi.org/10.1016/j.jembe.2008.07.013.

Field, C.B., Behrenfeld, M.J., Randerson, J.T., Falkowski, P., 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281, 237–240. https://doi.org/10.1126/science.281.5374.237.

Fuhrman, J.A., Cram, J.A., Needham, D.M., 2015. Marine microbial community dynamics and their ecological interpretation. Nat. Rev. Microbiol. 13, 133–146. https://doi.org/10.1038/nrmicro3417.

Gao, K., Beardall, J., Häder, D.P., Hall-Spencer, J.M., Gao, G., Hutchins, D.A., 2019. Effects of ocean acidification on marine photosynthetic organisms under the concurrent influences of warming, UV radiation, and deoxygenation. Front. Mar. Sci. 6, 1–18. https://doi.org/10.3389/fmars.2019.00322.

Gazeau, F., Sallon, A., Maugendre, L., Louis, J., Dellisanti, W., Gaubert, M., Lejeune, P., Gobert, S., Borges, A.V., Harlay, J., Champenois, W., Alliouane, S., Taillandier, V., Louis, F., Obolensky, G., Grisoni, J.M., Guieu, C., 2017. First mesocosm experiments to study the impacts of ocean acidification on plankton communities in the NW Mediterranean Sea (MedSeA project). Estuar. Coast Shelf Sci. 186, 11–29. https://doi.org/10.1016/j.ecss.2016.05.014.

Grossart, H.P., Allgaier, M., Passow, U., Riebesell, U., 2006. Testing the effect of CO_2 concentration on the dynamics of marine heterotrophic bacterioplankton. Limnol. Oceanogr. 51, 1–11. https://doi.org/10.4319/lo.2006.51.1.0001.

Grossart, H.P., Dziallas, C., Tang, K.W., 2009. Bacterial diversity associated with freshwater zooplankton. Environ. Microbiol. Rep. 1, 50–55. https://doi.org/10.1111/j.1758-2229.2008.00003.x.

Gruber, N., Clement, D., Carter, B.R., Feely, R.A., van Heuven, S., Hoppema, M., Ishii, M., Key, R.M., Kozyr, A., Lauvset, S.K., Monaco, C. Lo, Mathis, J.T., Murata, A., Olsen, A., Perez, F.F., Sabine, C.L., Tanhua, T., Wanninkhof, R., 2019. The oceanic sink for anthropogenic CO_2 from 1994 to 2007. Science 363, 1193–1199. https://doi.org/10.1126/science.aau5153.

Hale, R., Calosi, P., McNeill, L., Mieszkowska, N., Widdicombe, S., 2011. Predicted levels of future ocean acidification and temperature rise could alter community structure and biodiversity in marine benthic communities. Oikos 120, 661–674. https://doi.org/10.1111/J.1600-0706.2010.19469.X.

Hammill, E., Johnson, E., Atwood, T.B., Harianto, J., Hinchliffe, C., Calosi, P., Byrne, M., 2018. Ocean acidification alters zooplankton communities and increases top-down pressure of a cubozoan predator. Global Change Biol. 24, e128–e138. https://doi.org/10.1111/gcb.13849.

Harvey, B.P., Agostini, S., Kon, K., Wada, S., Hall-spencer, J.M., 2019. Diatoms dominate and alter marine food-webs when CO_2 rises. Diversity 11, 242. https://doi.org/10.3390/d11120242.

Huang, R., Sun, J., Yang, Y., Jiang, X., Wang, Z., Song, X., Wang, T., Zhang, D., Li, H., Yi, X., Chen, S., Bao, N., Qu, L., Zhang, R., Jiao, N., Gao, Y., Huang, B., Lin, X., Gao, G., Gao, K., 2021. Elevated pCO_2 impedes succession of phytoplankton community from diatoms to dinoflagellates along with increased abundance of viruses and bacteria. Front. Mar. Sci. 8, 1–14. https://doi.org/10.3389/fmars.2021.642208.

Hutchins, D.A., Sañudo-Wilhelmy, S.A., 2022. The Enzymology of ocean global change. Ann. Rev. Mar. Sci 14, 187–211. https://doi.org/10.1146/annurev-marine-032221-084230.

Hyun, B., Kim, J.M., Jang, P.G., Jang, M.C., Choi, K.H., Lee, K., Yang, E.J., Noh, J.H., Shin, K., 2020. The effects of ocean acidification and warming on growth of a natural community of coastal phytoplankton. J. Mar. Sci. Eng. 8, 1–15. https://doi.org/10.3390/jmse8100821.

Jeong, S.Y., Kim, T.G., 2021. Effects of plants on metacommunities and correlation networks of soil microbial groups in an ecologically restored wetland. Microb. Ecol. 81, 657–672. https://doi.org/10.1007/s00248-020-01625-3.

Kerfahi, D., Harvey, B.P., Kim, H., Yang, Y., Adams, J.M., Hall-Spencer, J.M., 2022. Whole community and functional gene changes of biofilms on marine plastic debris in response to ocean acidification. Microb. Ecol. 85, 1202–1214. https://doi.org/10.1007/s00248-022-01987-w.

Kim, J.-M., Lee, K., Shin, K., Kang, J.-H., Lee, H.-W., Kim, M., Jang, P.-G., Jang, M.-C., 2006. The effect of seawater CO_2 concentration on growth of a natural phytoplankton assemblage in a controlled mesocosm experiment. Limnol. Oceanogr. 51, 1629–1636. https://doi.org/10.4319/lo.2006.51.4.1629.

King, N., 2005. Choanoflagellates. Curr. Biol. 15, 113–114. https://doi.org/10.1016/j.cub.2005.02.004.

Kroeker, K.J., et al., 2011. Divergent ecosystem responses within a benthic marine community to ocean acidification. Proc. Natl. Acad. Sci. U.S.A. 108, 14515–14520. https://doi.org/10.1073/pnas.1107789108.

Lentendu, G., Dunthorn, M., 2021. Phylogenetic relatedness drives protist assembly in marine and terrestrial environments. Global Ecol. Biogeogr. 30, 1532–1544. https://doi.org/10.1111/geb.13317.

Li, Y., Xu, Z., Liu, H., 2021. Nutrient-imbalanced conditions shift the interplay between zooplankton and gut microbiota. BMC Genom. 22, 1–18. https://doi.org/10.1186/s12864-020-07333-z.

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., Raes, J., 2015. Determinants of community structure in the global plankton interactome. Science 348. https://doi.org/10.1126/science.1262073, 1262073–1262073.

Lin, X., Huang, R., Li, Y., Li, F., Wu, Y., Hutchins, D.A., Dai, M., 2018. Interactive network configuration maintains bacterioplankton community structure under elevated CO_2 in a eutrophic coastal mesocosm experiment. Biogeosciences 15, 551–565. https://doi.org/10.5194/bg-15-551-2018.

Lüthi, D., Le Floch, M., Bereiter, B., Blunier, T., Barnola, J.M., Siegenthaler, U., Raynaud, D., Jouzel, J., Fischer, H., Kawamura, K., Stocker, T.F., 2008. High-resolution carbon dioxide concentration record 650,000-800,000 years before present. Nature 453, 379–382. https://doi.org/10.1038/nature06949.

MacKe, E., Callens, M., De Meester, L., Decaestecker, E., 2017. Host-genotype dependent gut microbiota drives zooplankton tolerance to toxic cyanobacteria. Nat. Commun. 8 https://doi.org/10.1038/s41467-017-01714-x.

Maugendre, L., Gattuso, J.P., Poulton, A.J., Dellisanti, W., Gaubert, M., Guieu, C., Gazeau, F., 2017. No detectable effect of ocean acidification on plankton metabolism in the NW oligotrophic Mediterranean Sea: results from two mesocosm studies. Estuar. Coast Shelf Sci. 186, 89–99. https://doi.org/10.1016/j.ecss.2015.03.009.

Meakin, N.G., Wyman, M., 2011. Rapid shifts in picoeukaryote community structure in response to ocean acidification. ISME J. 5, 1397–1405. https://doi.org/10.1038/ismej.2011.18.

Mostofa, K.M.G., Liu, C.Q., Zhai, W., Minella, M., Vione, D., Gao, K., Minakata, D., Arakaki, T., Yoshioka, T., Hayakawa, K., Konohira, E., Tanoue, E., Akhand, A., Chanda, A., Wang, B., Sakugawa, H., 2016. Reviews and Syntheses: ocean acidification and its potential impacts on marine ecosystems. Biogeosciences 13, 1767–1786. https://doi.org/10.5194/bg-13-1767-2016.

Nagelkerken, I., Russell, B.D., Gillanders, B.M., Connell, S.D., 2016. Ocean acidification alters fish populations indirectly through habitat modification. Nat. Clim. Change 6, 89–93. https://doi.org/10.1038/nclimate2757.

O'Brien, P.A., Morrow, K.M., Willis, B.L., Bourne, D.G., 2016. Implications of ocean acidification for marine microorganisms from the free-living to the host-associated. Front. Mar. Sci. 3 https://doi.org/10.3389/fmars.2016.00047.

Pescador, D.S., Delgado-Baquerizo, M., Fiore-Donno, A.M., Singh, B.K., Bonkowski, M., Maestre, F.T., 2022. Ecological clusters of soil taxa within bipartite networks are highly sensitive to climatic conditions in global drylands. Philos. Trans. R. Soc. B Biol. Sci. 377.

Pörtner, H.-O., Roberts, D.C., Tignor, M., Poloczanska, E.S., Mintenbeck, K., Alegría, A., Craig, M., Langsdorf, S., Löschke, S., Möller, V., Okem, A., R, B., 2021. Climate change 2022: impacts, adaptation, and vulnerability. Contribution of working group II to the sixth assessment report of the intergovernmental panel on climate change. Climate Change 2021: The Physical Science Basis. IPCC, 2022.

Raven, J.A., et al., 2011. Algal and aquatic plant carbon concentrating mechanisms in relation to environmental change. Photosynth. Res. 109, 281–296. https://doi.org/10.1128/10.1007/s11120-011-9632-6.

Riebesell, U., Bach, L.T., Bellerby, R.G.J., Monsalve, J.R.B., Boxhammer, T., Czerny, J., Larsen, A., Ludwig, A., Schulz, K.G., 2017. Competitive fitness of a predominant pelagic calcifier impaired by ocean acidification. Nat. Geosci. 10, 19–23. https://doi.org/10.1038/ngeo2854.

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., Gilbert, J.a., 2013. Ocean acidification shows negligible impacts on high-latitude bacterial community structure in coastal pelagic mesocosms. Biogeosciences 10, 555–566. https://doi.org/10.5194/bg-10-555-2013.

Schulz, K.G., Bach, L.T., Bellerby, R.G.J., Bermúdez, R., Büdenbender, J., Boxhammer, T., Czerny, J., Engel, A., Ludwig, A., Meyerhöfer, M., Larsen, A., Paul, A.J., Sswat, M., Riebesell, U., 2017. Phytoplankton blooms at increasing levels of atmospheric carbon dioxide: experimental evidence for negative effects on prymnesiophytes and positive on small picoeukaryotes. Front. Mar. Sci. 4 https://doi.org/10.3389/fmars.2017.00064.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12, R60. https://doi.org/10.1186/gb-2011-12-6-r60.

Smith, M.W., Allen, L.Z., Allen, A.E., Herfort, L., Simon, H.M., 2013. Contrasting genomic properties of free-living and particle-attached microbial assemblages within a coastal ecosystem. Front. Microbiol. 4, 1–20. https://doi.org/10.3389/fmicb.2013.00120.

Spisla, C., Taucher, J., Bach, L.T., Haunost, M., Boxhammer, T., King, A.L., Jenkins, B.D., Wallace, J.R., Ludwig, A., Meyer, J., Stange, P., Minutolo, F., Lohbeck, K.T., Nauendorf, A., Kalter, V., Lischka, S., Sswat, M., Dörner, I., Ismar-Rebitz, S.M.H., Aberle, N., Yong, J.C., Bouquet, J.M., Lechtenbörger, A.K., Kohnert, P., Krudewig, M., Riebesell, U., 2021. Extreme levels of ocean acidification restructure the plankton community and biogeochemistry of a temperate coastal ecosystem: a mesocosm study. Front. Mar. Sci. 7, 1–24. https://doi.org/10.3389/fmars.2020.611157.

Sswat, M., Stiasny, M.H., Taucher, J., Algueró-Muñiz, M., Bach, L.T., Jutfelt, F., Riebesell, U., Clemmesen, C., 2018. Food web changes under ocean acidification promote herring larvae survival/631/158/2165/631/158/2446 article. Nat. Ecol. Evol. 2, 836–840. https://doi.org/10.1038/s41559-018-0514-6.

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., Amann, R., 2012. Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. Science 336, 608–611. https://doi.org/10.1126/science.1218344.

Teixidó, N., Gambi, M.C., Parravacini, V., Kroeker, K., Micheli, F., Villéger, S., Ballesteros, E., 2018. Functional biodiversity loss along natural CO_2 gradients. Nat. Commun. 9, 1–9. https://doi.org/10.1038/s41467-018-07592-1.

Thornton, D.C.O., 2014. Dissolved organic matter (DOM) release by phytoplankton in the contemporary and future ocean. Eur. J. Phycol. 49, 20–46. https://doi.org/10.1080/09670262.2013.875596.

Tu, Q., Yuan, M., He, Z., Deng, Y., Xue, K., Wu, L., Hobbie, S.E., Reich, P.B., Zhou, J., 2015. Fungal communities respond to long-term CO_2 elevation by community reassembly. Appl. Environ. Microbiol. 81, 2445–2454. https://doi.org/10.1128/AEM.04040-14.

Van de Waal, D.B., et al., 2019. Highest plasticity of carbon-concentrating mechanisms in earliest evolved phytoplankton. Limnol. Oceanogr. Lett. 4, 37–43. https://doi.org/10.5061/dryad.j9m4cm6.

Vincent, F., Bowler, C., 2020. Diatoms are selective segregators in global ocean planktonic communities. mSystems 5. https://doi.org/10.1128/msystems.00444-19.

Wallace, R.B., Baumann, H., Grear, J.S., Aller, R.C., Gobler, C.J., 2014. Coastal ocean acidification: the other eutrophication problem. Estuar. Coast Shelf Sci. 148, 1–13. https://doi.org/10.1016/j.ecss.2014.05.027.

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

Wang, P., Laws, E., Wang, Y., Chen, J., Song, X., Huang, R., Wang, T., Yi, X., Sun, J., Guo, X., Liu, X., Gao, K., Huang, B., 2022. Elevated pCO_2 changes community structure and function by affecting phytoplankton group-specific mortality. Mar. Pollut. Bull. 175, 113362 https://doi.org/10.1016/j.marpolbul.2022.113362.

Whiteley, N.M., 2011. Physiological and ecological responses of crustaceans to ocean acidification. Mar. Ecol. Prog. Ser. 430, 257–271. https://doi.org/10.3354/meps09185.

Widdicombe, S., Needham, H.R., 2007. Impact of CO_2-induced seawater acidification on the burrowing activity of Nereis virens and sediment nutrient flux. Mar. Ecol. Prog. Ser. 341, 111–122, 10.3354.meps341111.

Williams, T.J., Long, E., Evans, F., Demaere, M.Z., Lauro, F.M., Raftery, M.J., Ducklow, H., Grzymski, J.J., Murray, A.E., Cavicchioli, R., 2012. A metaproteomic assessment of winter and summer bacterioplankton from Antarctic Peninsula coastal surface waters. ISME J. 6, 1883–1900. https://doi.org/10.1038/ismej.2012.28.

Xu, R., et al., 2022. Response of soil protozoa to acid mine drainage in a contaminated terrace. J. Hazard Mater. 421, 126790 https://doi.org/10.1016/j.jhazmat.2021.126790.

Yuan, M.M., Guo, X., Wu, Linwei, Zhang, Y., Xiao, N., Ning, D., Shi, Z., Zhou, X., Wu, Liyou, Yang, Y., Tiedje, J.M., Zhou, J., 2021. Climate warming enhances

microbial network complexity and stability. Nat. Clim. Change 11, 343–348. https://doi.org/10.1038/s41558-021-00989-9.

Zhang, R., Xia, X., Lau, S.C.K., Motegi, C., Weinbauer, M.G., Jiao, N., 2013. Response of bacterioplankton community structure to an artificial gradient of pCO_2 in the Arctic Ocean. Biogeosciences 10, 3679–3689. https://doi.org/10.5194/bg-10-3679-2013.

Zhou, J., Deng, Y., Luo, F., He, Z., Yanga, Y., 2011. Phylogenetic molecular ecological network of soil microbial communities in response to elevated CO_2. mBio 2, 1–8. https://doi.org/10.1128/mBio.00122-11.