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LETTER

Ocean acidification has a strong effect on communities living on plastic in mesocosms

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Scientific Significance Statement

Plastic waste in the ocean is an urgent environmental concern and has given rise to a novel habitat, known as the "plastisphere." Under ocean acidification (OA), changes in plastisphere community composition may alter plastic degradation, deposition, and passage through food webs, but these have not been studied yet. This is the first study about the effects of simulated high CO_2 on the plastisphere using a mesocosm. We discovered that after 1 month the beta diversity of prokaryotic communities living on single-use plastic drinking bottles was significantly different under different carbon dioxide concentrations, with more pathogens at high CO_2. Based on function prediction analysis, the relative abundance of bacterial taxa involved in nitrogen and nitrate respiration and ureolysis was significantly higher under simulated high CO_2. We conclude that OA has significant effects on the plastisphere and its predicted functions.

Abstract

We conducted a mesocosm experiment to examine how ocean acidification (OA) affects communities of prokaryotes and eukaryotes growing on single-use drinking bottles in subtropical eutrophic waters of the East China Sea. Based on 16S rDNA gene sequencing, simulated high CO_2 significantly altered the prokaryotic community, with the relative abundance of the phylum Planctomycetota increasing by 49%. Under high CO_2, prokaryotes in the plastisphere had enhanced nitrogen dissimilation and ureolysis, raising the possibility that OA may modify nutrient cycling in subtropical eutrophic waters. The relative abundance of pathogenic and animal parasite bacteria also increased under simulated high CO_2. Our results show that elevated CO_2 levels

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Xu Zhang and Ping Zhang contributed equally to this work.

Author Contribution Statement: XL and JH-S conceived and designed the experiments. KG, GG, and XL designed the mesocosm experiment. DZ, ZD, RH, XZ, YT, NW, HL, XW, XJ, JS, QF, XY, LQ, and YR performed the mesocosm experiment. ZD and HL fixed bottles on the tubes. XZ and PZ did DNA extractions. XL and XZ analyzed the data. XL wrote the manuscript. JH-S revised the manuscript. All authors reviewed the manuscript.

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significantly affected several animal taxa based on 18S rDNA gene sequencing. For example, *Mayorella amoebae* were highly resistant, whereas *Labyrinthula* were sensitive to OA. Thus, OA may alter plastisphere food chains in subtropical eutrophic waters.

Plastic pollution has become one of the most concerning environmental issues due to its persistence in the environment. Van Sebille et al. (2015) estimated that around 236,000 metric tons of plastics are dispersed throughout the ocean. These plastics often entangle or are ingested by marine animals of all sizes, from microbes to whales. Microplastic fragments can also be transferred through food webs along with chemicals and pathogens (Napper and Thompson 2020).

Plastics provide substrata for microbial attachment and are now a novel habitat for a variety of organisms. The term "plastisphere" was first used by Zettler et al. (2013) to describe an ecosystem of microbes living on floating plastic with communities that were distinct from the surrounding water. Plastics can carry organisms horizontally and vertically from the sea surface to the seafloor (Amaral-Zettler et al. 2020). Plastic types, environmental conditions, and biogeography all play significant roles in the development and diversity of the plastisphere (Amaral-Zettler et al. 2015; Toyofuku et al. 2016; Napper and Thompson 2020; Seeley et al. 2020). Core members of the plastisphere vary depending on the type of plastic as well as location (Dudek et al. 2020; Wu et al. 2020; Du et al. 2022). Nitrogen cycling can be affected by the plastisphere in sediments (Seeley et al. 2020) and different types of plastic may also influence biogeochemical cycles (Romera-Castillo et al. 2018; Sanz-Lázaro et al. 2021; Wang et al. 2021; Shen et al. 2022). Yet few have considered the effects of anthropogenic change (such as warming, acidification and eutrophication) on plastisphere composition and function.

Considering the high durability of plastics in the ocean, it is prudent to investigate the effects of future projected environmental conditions on plastisphere community composition and function. One of the most widespread anthropogenic changes on Earth is ocean acidification (OA) which happens as CO_2 from the atmosphere dissolves in seawater to produce carbonic acid, which lowers surface ocean pH. Since the beginning of the Industrial Revolution, surface ocean H^+ ion concentration has increased by 26% due to the increasing atmospheric CO_2. Under a high CO_2 emissions scenario (SSP3-7.0), average surface seawater pH is projected to drop another 0.3 units during this century (IPCC, 2021). There is a severe lack of knowledge about how the plastisphere will be affected by OA as only two studies have investigated this; both used drinking bottles placed along natural gradients in CO_2 at marine volcanic seeps in Japan (Harvey et al. 2020; Kerfahi et al. 2022). In these studies, OA greatly altered the composition of bacterial assemblages on plastic and decreased the prevalence of genes associated with cell-to-cell interactions and antibiotic resistance.

In this study, a mesocosm platform was used to investigate both eukaryotic and prokaryotic community composition and function on polyethylene terephthalate (PET) drinking bottles in subtropical coastal seawater in Wuyuan Bay, East China Sea. Seawater acidification is more severe in this bay than in the open ocean because nutrient inputs have caused eutrophication and stimulated microbial degradation of organic matter, lowering seawater pH (Cai et al. 2011; Wallace et al. 2014). Mesocosm experiments bridge the gap between laboratory experiments and CO_2 seep studies by simulating effects on pelagic ecosystems in miniature. We sequenced 16S rDNA and 18S rDNA to explore the effects of OA on the prokaryotic and eukaryotic community composition and predicted functions of the plastisphere.

Materials and methods

Mesocosm setup

We used the Facility for the Study of Ocean Acidification Impacts of Xiamen University (24°31′48″N, 118°10′47″E), starting 09 October 2019, for 32 d (Fig. 1). Nine cylindrical transparent thermoplastic polyurethane mesocosm bags, each 3 m deep × 1.5 m diameter, were fixed in steel frames and covered by cone lids. In situ seawater around the platform was filtered (pore size of 0.01 μm, MU801-4T, Midea, China) with prefiltration by 5-mm nylon net and then pumped simultaneously into the bags, filling them with 3000 liters within 36 h. Bags 1, 3, 5, 7, and 9 were acidified with CO_2-saturated seawater to adjust seawater to 1000 μatm CO_2 as a high CO_2 (HC) treatment corresponding to the year 2100 under a high Greenhouse Gas emissions scenario. Bags 2, 4, 6, and 8 were controls (ambient CO_2, AC). AC and HC bags were aerated with ambient air of ~ 410 ppmv CO_2 and premixed air-CO_2 of 1000 ppmv CO_2 (5 L min^{-1}), respectively. Three plastic bottles per mesocosm bag were attached inside the mesocosms (Fig. 1). Each bag was inoculated with 80 liters of in situ seawater containing a natural microbe community filtered by a 180-μm mesh for the main investigation of the effects of OA on the plankton community in the mesocosm bags as described by Huang et al. (2021). Seawater was collected from 0.5 m depth from each bag at 10 a.m. every 1–3 d to measure pHtotal using an Environmental Water Analyzer (iSEA, Ma et al. 2018) and total alkalinity measured using an Automated Spectrophotometric Analyzer (Li et al. 2013). Samples were filtered through a 0.45-μm cellulose acetate membrane for NO_3^-, NO_2^-, NH_4^+, and PO_4^{3-} measurements by an auto-analyzer (AA3, Seal).

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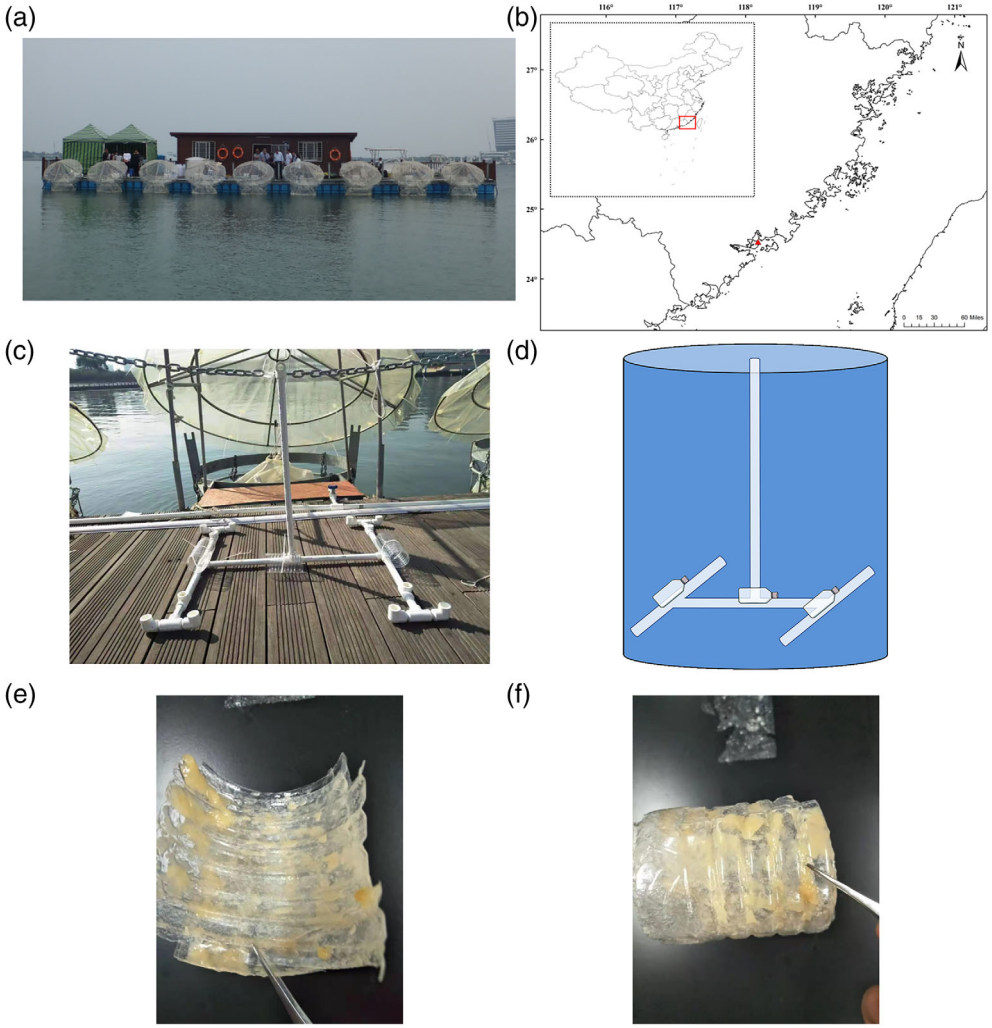

Fig. 1. Ocean acidification mesocosms in Wuyuan Bay, Xiamen, China (**a**). Location of mesocosms (**b**). Deployment of PET drinking bottles in the mesocosms (**c**,**d**). Biofilm on a drinking bottle after the 32-d experiment (**e**,**f**).

DNA extraction, amplification, and sequencing

After 32 d, the plastic bottles were removed. For the following analysis, two plastic bottles were chosen randomly from a pool of three bottles for each bag. Bottles were scratched for DNA extraction. A 70°C preheated lysis buffer (100 mM Tris, 40 mM ethylene diamine teraacetic acid, 100 mM NaCl, 1% sodiumdodecyl sulfate was used to extract DNA, followed by phenol–chloroform extraction and ethanol precipitation. DNA from samples was used to amplify the 16S V4–V5 and 18S V9 regions. The16S V4–V5 region was amplified using the primers 515AF (GTGYCAGCMGCCGCGGTAA) and 926R (CCGYCAATTYMTTTRAGTTT; Parada et al. 2016), while the 18S V9 region was amplified using the primer 1389F (TTGTACACACCGCCC) and 1510R (CCTTCYGCAGGTT CACCTAC; Amaral-Zettler et al. 2009). The amplification conditions were: initial denaturation at 95°C for 3 min, 29 cycles of denaturation for 16S V4–V5 and 30 cycles for 18S V9 at 95°C for 30s, annealing at 53°C for 30 s extension at 72°C for 45 s, and final extension at 72°C for 10 min. Polymerase chain reaction (PCR) products were purified using an AxyPrepDNA gel extraction kit (Axygen) from 2% agarose gel after electrophoresis. A DNA library was constructed following the MiSeq Reagent Kit guide (Illumina). The sequencing was conducted using an Illumina MiSeq PE300 platform (Majorbio Bio-pharm Technology Co. Ltd.) after the purification and quantification of PCR products.

Sequence assignment and data analysis

Our sequencing data have been uploaded to NCBI (project ID: PRJNA895187). Raw fastq sequences were adapters removed and quality filtered by fastp (v0.19.6) and merged by FLASH (v1.2.7) before analysis. The filtered reads were imported into QIIME 2, and DADA2 was used to de-noise sequences, resulting in high-resolution amplicon sequence

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variants (ASVs). A number of 1,130,309 sequences for 18S V9 and 952,158 sequences for 16S V4-V5 were obtained. For taxonomic classification, we used the SILVA 138 database and the TARA 18S V9 database (http://taraoceans.sb-roscoff.fr/EukDiv/index.html) for 16S V4-V5 and 18S V9 sequencing data, respectively. All samples were standardized by random subsampling using the "sub.sample" command in Mothur. The prokaryotic and eukaryotic sequences were rarified to 21,400 and 41,865 reads per sample, respectively. Alpha diversity was estimated using Mothur 1.30. Beta diversity was analyzed with QIIME 2. Alpha diversity describes the species diversity within a community. Beta diversity describes the species diversity between communities. Beta diversities were calculated by Bray–Curtis matrixes and visualized by non-metric multidimensional scaling (NMDS) analysis. We performed analysis of similarities (ANOSIM) for the significant difference test. We performed linear discriminant analysis effect size (LEfSe) analysis to identify taxa that were differentially abundant in different samples. The default setting of the Linear Discriminant Analysis (LDA) score was set to 2.0 and $p < 0.05$. Functional Annotation of Prokaryotic Taxa (FAPROTAX) was used to predict the functional profile of bacterial communities (Louca et al. 2016).

Statistical analyses

Differences in pH_{total} value between HC and AC at different time points were tested by a one-way ANOVA test in SPSS. We performed the non-parametric Kruskal–Wallis rank tests to detect statistical differences in alpha diversity between treatments. The Wilcoxon rank-sum test was used to test for significant differences in relative abundance of taxa and predicted functions of prokaryotic community between treatments. Statistical significance was determined at $p < 0.05$.

Results

Seawater in mesocosms

The in situ pHtotal in Wuyan Bay was 7.78, which is representative of eutrophic coastal seawater in the region. The pH_{total} varied in the AC and HC treatments in response to a growing then declining phytoplankton population. Under HC, pH decreased from 7.70 ± 0.02 on Day 0 (after CO_2-saturated water addition) to 7.60 ± 0.02 on Day 2, subsequently increased to a peak of 8.06 ± 0.05 under HC on Day 10. The pH value under AC decreased from 7.77 ± 0.01 on Day 0 to 7.67 ± 0.01 on Day 2, then peaked on Day 8 at 8.28 ± 0.12. The pH values under HC and AC then gradually declined (7.67 ± 0.05 under HC and 7.82 ± 0.06 under AC). From Day 0 to Day 2, an increase in bacteria concentration was responsible for the decrease in pH under HC and AC. As intended, the pH_{total} of the HC treatments was always lower than the AC treatment ($p < 0.05$; Fig. 2). On Day 0, the concentration of $NO_3^- + NO_2^-$ was $29.23 \pm 1.21\,\mu mol\,L^{-1}$ under HC and $26.05 \pm 6.48\,\mu mol\,L^{-1}$ under AC; NH_4^+ was

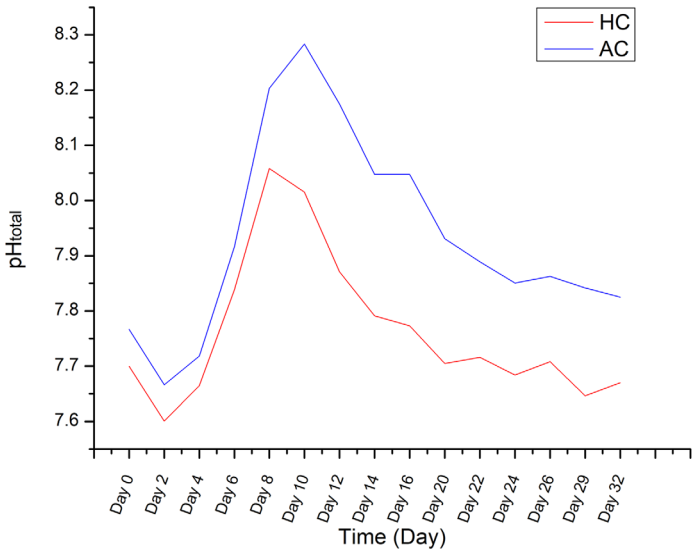

Fig. 2. Temporal variations mean pH_{total} in High CO_2 (1000 ppmv, "HC") and Ambient CO_2 ($\sim$ 410 ppmv, "AC") treatments over the 32-d mesocosm experiment in Wuyuan Bay.

$11.58 \pm 1.47\,\mu mol\,L^{-1}$ under HC and $12.62 \pm 1.23\,\mu mol\,L^{-1}$ under AC; and PO_4^- was $1.29 \pm 0.11\,\mu mol\,L^{-1}$ under HC and $1.24 \pm 0.24\,\mu mol\,L^{-1}$ under AC. The seawater was potentially moderately phosphorus-limited eutrophic (Guo et al. 1998).

Effects of OA on plastisphere prokaryotic communities

The 16S V4–V5 region was successfully amplified from DNA samples from 16 plastic bottles with 6 samples from AC and 10 samples from HC (AC_2_1, AC_2_2, AC_4_1, AC_4_2, AC_6_1, AC_8_3, HC_1_1, HC_1_2, HC_3_3, HC_5_1, HC_5_2, HC_5_3, HC_7_1, HC_7_2, HC_9_1, HC_9_3). Twenty-five phyla, 51 classes, 135 orders, 205 families, 435 genera, 866 species, and 2064 ASVs were identified based on 952,158 clean sequences after the elimination and rarefaction of raw sequences.

Proteobacteria (40%), Planctomycetota (35%), and Bacteroidota (19%) were the most abundant phyla, while Alphaproteobacteria (33.53%), Planctomycetes (26.53%), Bacteroidia (18.04%) were the most abundant taxa at the class level (Supplementary Fig. 1). There were 680 unique ASVs under HC and 554 unique ASVs under AC with 830 ASVs in common (Fig. 3). The alpha diversity indexed by Shannon was significantly higher under AC than HC ($p < 0.05$) (Supplementary Table 2). HC significantly affected the beta diversity of the prokaryotic community based on NMDS analysis ($R = 0.32$, $p = 0.023$) (Fig. 3; Supplementary Table 3). The relative abundance of Planctomycetota phylum was 49% higher ($p = 0.026$) under HC ($40\% \pm 11.06\%$) compared to AC ($26\% \pm 10.55\%$), which contributed substantially to the altered Beta diversity metric in HC compared to AC. In contrast, the phylum Myxococcota was significantly higher under AC ($0.55\% \pm$

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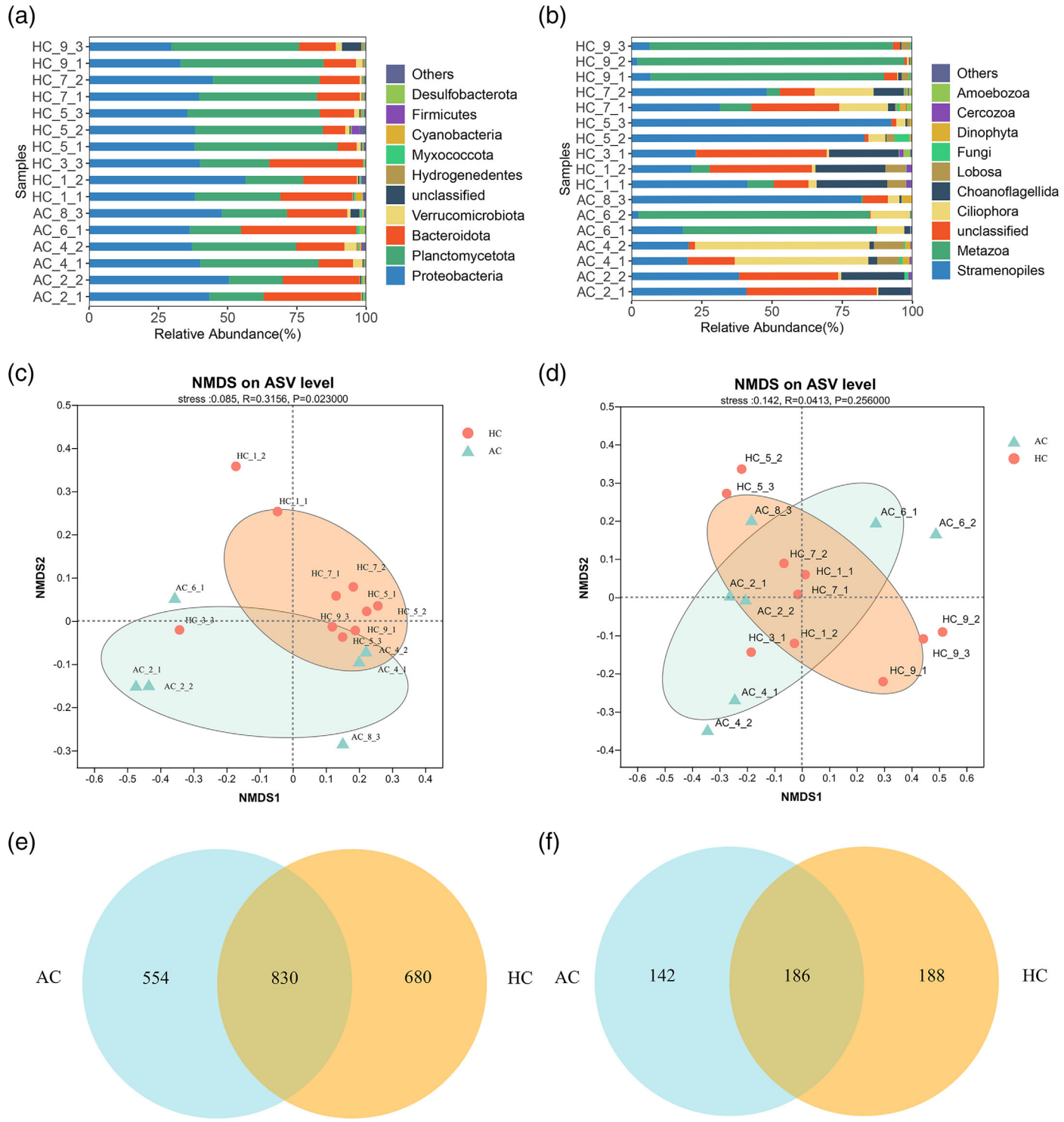

Fig. 3. The prokaryotic composition of different samples at the phylum level (**a**). The eukaryotic composition of different samples at the phylum level (**b**). NMDS analysis of the prokaryotic community under high carbon and ambient carbon at the ASV level (ANOSIM, $R = 0.32$, $p = 0.023$) (**c**). NMDS analysis of the eukaryotic community under HC and AC at the ASV level (ANOSIM, $R = 0.12$, $p = 0.10$) (**d**). Venn diagram of unique ASV and shared ASVs under HC and AC of the prokaryotic community (**e**). The Venn diagram of unique ASVs and shared ASVs under HC and AC of the eukaryotic community (**f**). The indication of the sample name: HC-1-1 represents the sample of plastic bottle replicate 1 from mesocosm bag 1 under HC.

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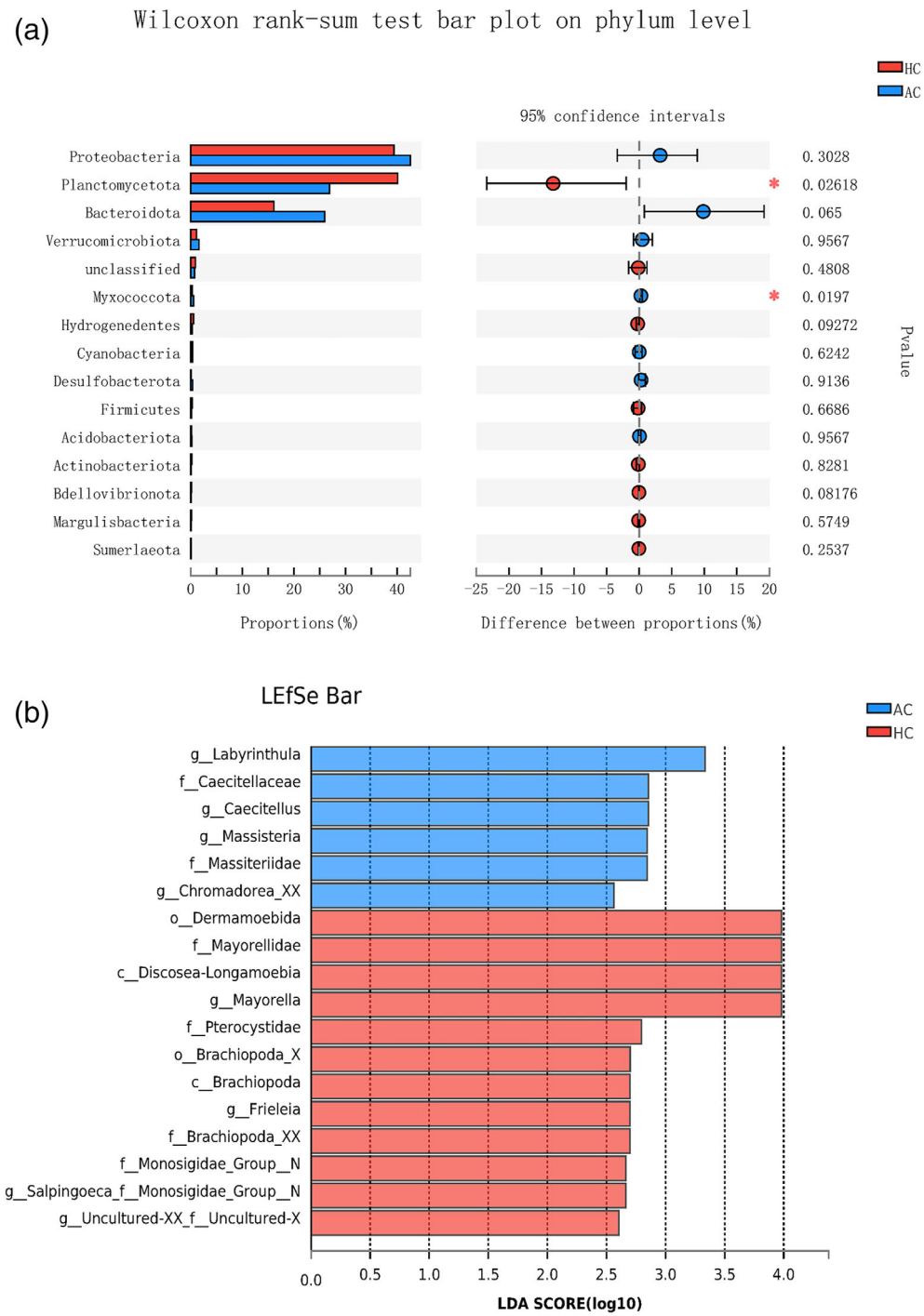

Fig. 4. Prokaryotic community under HC (red) and AC (blue) at the phylum level for the top 15 most abundant phyla (*$p < 0.05$) (**a**). Taxa are differentially abundant under HC (red) and AC (blue) based on LEfSe analysis for the eukaryotic community. LDA score was set to 2.0 and $p < 0.05$. ("p_" indicates the phylum level; "c_" indicates at order level; "f_" indicates the family level; "g_" indicates the genus level) (**b**).

0.23%) compared to HC (0.28% ± 0.11%; $p = 0.02$; Fig. 4). At the family level, the relative proportion of Phycisphaeraceae was significantly higher ($p = 0.006$) under HC (8.35% ± 2.74%) compared to AC (3.91% ± 1.63%) whereas the proportion of Cryomorphaceae was significantly higher ($p = 0.02$) under AC (3.22% ± 1.98%) compared to HC (1.09% ± 1.24%). The relative abundance of some species with low abundance showed significant differences between HC and AC. Phycisphaera ($p = 0.045$) and SM1A02 ($p = 0.015$) were significantly higher under HC than AC. In contrast, the relative abundance of

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Dinoroseobacter ($p = 0.014$) and Vicingus ($p = 0.005$) were significantly higher under AC (Supplementary Fig. 2).

LEfSe analysis of prokaryotes showed that 44 and 46 taxa were relevant to AC and HC respectively. At the phylum level, Planctomycetota (LDA = 4.84) and Bacteroidia (LDA = 4.79) were relevant to HC and AC, respectively. At the order level, Phycisphaerales (LDA = 4.92) and Flavobacteriales (LDA = 5.02) were relevant to HC and AC, respectively (Supplementary Table 4). Based on the FAPROTAX prediction, the functions of "animal_parasites_or_symbionts" (0.43% ± 0.20% under AC and 1.34% ± 0.73% under HC, $p = 0.01$), "human_pathogens_all" (0.41% ± 0.21% under AC and 1.17% ± 0.56% under HC, $p = 0.01$), "nitrogen_respiration" (0.30% ± 0.17% under AC and 0.82% ± 0.60% under HC, $p = 0.03$), "nitrate_respiration" (0.30% ± 0.17% under AC and 0.82% ± 0.6%, $p = 0.03$) and "ureolysis" (0.05% ± 0.04% under AC and 1.06% ± 2.91% under HC, $p = 0.01$) of the prokaryotic community were significantly more abundant under HC compared to AC (Fig. 5, Supplementary Tables 5 and 6).

Effects of OA on the eukaryotic community in the plastisphere

The 18S V9 region was successfully amplified from DNA samples from 17 plastic bottles with 7 samples from AC and 10 samples from HC (AC_2_1, 2_2, 4_1, 4_2, 6_1, 6_2, 8_3, HC_1_1, 1_2, 3_1, 5_2, 5_3, 7_1, 7_2, 9_1, 9_2, 9_3) with 26 phyla, 63 classes, 87 orders, 120 families, 435 genera, 215 species, 516 ASVs identified based on 1,130,309 clean sequences after sequence elimination and rarefaction.

Stramenopiles (34%), Metazoa (26%), Ciliophora (11%), Choanoflagellida (7%), and Lobosa (2%) were the most abundant phyla, while Bacillariophyta (29.41%) and Gastrotricha (26.29%) were the most abundant taxa ate the class level (Supplementary Fig. 1). There were unique 188 ASVs under HC and 142 ASVs under AC with 186 ASVs in common. No significant differences were detected between HC and AC in alpha diversity (Supplementary Table 2) and beta diversity (Fig. 3, NMDS, $R = 0.043$, $p = 0.25$). Significant differences in the relative abundance of taxa between HC and AC were detected in animals with relatively low abundance. The relative abundance of Centroheliozoa at phylum level ($p = 0.05$), Dermamoebida ($p = 0.001$) at order level, Discosea–Longamoebia at class level ($p = 0.01$), Mayorellidae ($p = 0.001$) at family level were all significantly higher under HC compared to AC (Supplementary Fig. 3). Based on LEfSe analysis, 18 and 6 taxa were relevant to HC and AC, respectively (Fig. 4). Discosea–Longamoebia class (LDA = 3.98), Dermamoebida order (LDA =3.98), Mayorellidae family

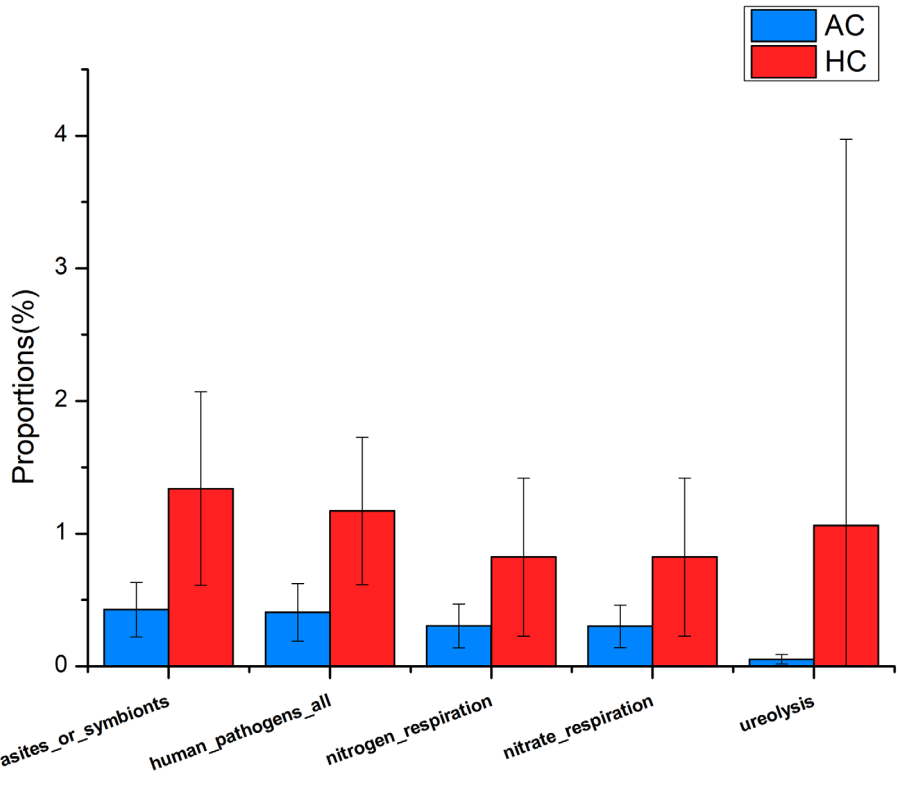

Fig. 5. The significantly different predicted functions (FAPROTAX) of the prokaryotic community under simulated HC and AC based on the Wilcoxon rank-sum test.

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(LDA = 3.98), *Mayorella* genus (LDA = 3.98), and *Brachiopoda* genus (LDA = 2.7002) were relevant to HC. At the genus level, Labyrinthula (LDA = 3.33), Massisteria (LDA = 2.85), Caecitellus (LDA = 2.85), and Chromadorea (LDA = 2.56) were relevant to AC.

Discussion

In just 32 d, 25 prokaryotic Phyla and 26 eukaryotic Phyla had colonized on single-use plastic drinking bottles, demonstrating that plastics can quickly support highly complex communities with a proliferation of protists and metazoan groups that are seldom studied (Du et al. 2022).

Our results demonstrate that the prokaryotic community was more significantly affected by HC compared to the eukaryotic community (Fig. 3). In our study, Planctomycetota which have unusual features, such as intracellular compartmentalization and a lack of peptidoglycan in their protein cell wall (Fuerst and Sagulenko 2011), had significantly higher relative abundance under HC (Fig. 4). It would be interesting to investigate whether the response of Planctomycetota to HC is consistent in other locations. At the late stage of the phytoplankton bloom in mesocosm bags, dinoflagellate concentrations were higher under HC (Wang et al. in prep.) which may result in varied dissolved organic matter in quality and quantity. This might explain the different prokaryotic compositions of the plastisphere under different CO_2 treatments (Buchan et al. 2014; Xu et al. 2022).

The dominant primary producers were Stramenopiles (mainly diatoms) and there were comparatively few Cyanobacteria in the plastisphere. Diatoms are widely found in the plastisphere in the photic zone due to their ability to secrete sticky exopolysaccharides (Amaral-Zettler et al. 2020). In the mesocosm bags, the high concentration of diatoms easily attached to plastic surfaces. Studies along CO_2 seep gradients in Italy and Japan have shown that elevated CO_2 significantly increases diatom abundance on plastic surfaces (Johnson et al. 2013; Kerfahi et al. 2022). However, no plastisphere primary producers were significantly affected by our CO_2 treatments in our study (Fig. 4), perhaps due to lower light intensity although a combination of many factors may be the cause such as differences in phytoplankton biogeography. In future, amplicon sequencing combined with microscope observation should be used to better understand the effects of OA on the plastisphere, because amplicon sequencing does not always distinguish statistically significant differences between the communities (Dudek et al. 2020).

Plastics are providing a novel and globally multiplying habitat for marine animals. We found a high proportion of Metazoa and Ciliphora in the plastisphere community (Supplementary Fig. 1). Some secondary producers with relatively low abundance were clearly affected by increases in CO_2, indicating that the organisms in higher trophic levels are sensitive to high CO_2 (Fig. 4). For example, *Mayorella* proliferated in simulated high CO_2 whereas *Labyrinthula* were more prevalent in ambient CO_2 conditions. *Mayorella* can engulf and digest microalgae (Laybourn-Parry et al. 1987). The Labyrinthulids feed saprotrophically, especially on marine algae (Finlay and Esteban 2019). Such changes may affect food chain dynamics.

In our study, stimulated high CO_2 significantly increased the relative abundance of bacterial taxa involved in nitrogen and nitrate respiration, as well as ureolysis (Fig. 5). Thus, OA may promote denitrification and organic nitrogen utilization in the plastisphere in subtropical eutrophic seawater. Until now, very few studies on the effects of climate change on nitrogen cycling in the plastisphere have been reported (Shen et al. 2022). Our findings are consistent with a recent study conducted in eutrophic seawater in Xiamen, which illustrated that the plastisphere had higher denitrifying activity and N_2O production compared to the surrounding seawater (Su et al. 2022).

Perhaps of most concern was our observation of enrichment of human pathogenic microorganisms and animal parasites in the plastisphere under simulated high CO_2. Plastic pollution is a transport vehicle that may accelerate the spread of infectious diseases (Zettler et al. 2013; Laverty et al. 2020; Du et al. 2022). Mariculture usually lowers seawater pH due to intense respiration (Gao et al. 2022) and produces plastic wastes from lost culture gear and packaging so that some seafood growing areas have a severe plastic pollution problem (Chen et al. 2018; Feng et al. 2020). The intersection of global change, plastic pollution and altered microbial communities is a major unstudied risk in mariculture. The higher relative abundances of the functions of prokaryotic communities under simulated high CO_2 in this study were based on FAPROTAX predictions, not direct measurements. We recommend that metagenomics and metatranscriptomics be used to identify modifications to plastisphere community functions induced by global change.

In summary, this first study of the effects of simulated high CO_2 on the plastisphere in eutrophic coastal conditions showed significant biological changes. To better prepare for the interactive effects of climate change and plastic pollution, investigations should target different types of plastics, regions, and seasons, especially in coastal areas that are relied upon to provide human food.

Data availability statement

Data generated in this manuscript are in NCBI (project ID: PRJNA895187) available in figshare (https://figshare.com/articles/dataset/Ocean_acidification_has_a_strong_effect_on_communities_living_on_plastic_in_mesocosms/22427992).

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