Ocean acidification alters phytoplankton diversity and community structure in the coastal water of the East China Sea

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Abstract

Anthropogenic CO_2 emissions and their continuous dissolution into seawater lead to seawater pCO_2 rise and ocean acidification (OA). Phytoplankton groups are known to be differentially affected by carbonate chemistry changes associated with OA in different regions of contrasting physical and chemical features. To explore responses of phytoplankton to OA in the Chinese coastal waters, we conducted a mesocosm experiment in a eutrophic bay of the southern East China Sea under ambient (410 µatm, AC) and elevated (1000 µatm, HC) pCO_2 levels. The HC condition stimulated phytoplankton growth and primary production during the initial nutrient-replete stage, while the community diversity and evenness in both pCO_2 treatments were reduced during this stage due to the rapid nutrient consumption and diatom blooms, and the subsequent shift from diatoms to hetero-dinoflagellates led to a decline in primary production during the mid and later phases under nutrient depletion. HC treatment suppressed the diatom-to-dinoflagellate succession and enhanced the subsequent remineralization of organic matter, thereby facilitating smaller phytoplankton to dominant and sustaining primary production. Our findings indicate that, the impacts of OA on

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phytoplankton diversity in the coastal water of the southern East China Sea depend on availability of nutrients, with primary productivity and biodiversity of phytoplankton reduced in the eutrophicated coastal water.

1 Introduction

It is commonly known that sequestration of CO_2 in coastal waters play important roles against global warming due to their high primary productivity (Rogelj et al., 2022), which resulted in faster CO_2 removal due to photosynthesis than dissolution of CO_2 from the air (Stukel et al., 2023). It has been assessed that, with the increasing anthropogenic CO_2 emissions, the oceanic CO_2 sink increased from 1.7 ± 0.4 pg C yr^{-1} in the 1980s to 2.5 ± 0.6 pg C yr^{-1} in the 2010s (Friedlingstein et al., 2025). Nevertheless, such apparent oceanic CO_2 uptake is altering carbonate chemistry in surface oceans, leading to a pH drop of by 0.017−0.027 units per decade, with a potential further drop by 0.3−0.4 units at the end of this century (Canadell et al., 2023; Gattuso et al., 2015). Such progressive ocean acidification (OA) has been shown to impact many marine organisms (Gattuso et al., 2015), including primary producers (Gao et al., 2020), subsequently feeding back on the CO_2 sequestration efficiency in marine systems including coastal waters.

OA in eutrophic coastal waters are suggested to progress faster than in open oceans by roughly 20 % due to CO_2 dissolution and enhanced remineralization of organic maters (Cai et al., 2011). The subsequent changes in carbonate chemistry may thus drive shifts in phytoplankton community structures/diversity and affect primary productions in differential ways due to regional environmental traits and species-specific physiology (Feng et al., 2024; Gao et al., 2012). While the effects of elevated pCO_2 on different phytoplankton assemblages have been demonstrated, positive, neutral and negative effects have been reported, reflecting differences in experimental approaches and/or phytoplankton compositions (Gao et al., 2020). Among the different approaches, field mesocosm experiments under elevated pCO_2 projected for future OA scenario have been employed to investigate the effects of OA on ecological processes, including primary production. For example, mesocosm experiments showed that growth of coccolithophores was reduced under 710 µatm pCO_2 during early summer in 2001 (Engel et al., 2005) and their ability to form blooms disappeared under 1000−3000 µatm pCO_2 during early summer in 2011 (Riebesell et al., 2017). Under elevated pCO_2, phytoplankton communities in Norwegian coastal mesocosms shifted from *Bathycoccus* to

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Micromonas (Meakin and Wyman, 2011). In oligotrophic or mesotrophic conditions, diatoms were insensitive to OA, but responded positively under nutrient enrichment (Bach et al., 2019). Furthermore, nutrients enrichment also increased Chl *a* concentration under high pCO_2 condition (Riebesell et al., 2017; Tanaka et al., 2013; Schulz et al., 2013). By contrast, mesocosm experiments conducted in highly eutrophic water showed that high pCO_2 did not affect Chl *a* concentration (Liu et al., 2017). Previously, we showed, by running a mesocosm experiment during spring of 2018 in the southern coastal water of the East China Sea, that elevated pCO_2 of 1000 μatm suppressed the succession from diatoms to dinoflagellates and increased the abundance of viruses and heterotrophic bacteria, promoting refueling of nutrients for phytoplankton growth (Huang et al., 2021). These findings indicate that plankton communities supported by nutrients from remineralization are more sensitive to OA than those having access to higher availability of inorganic nutrients (Bach et al., 2016; Bach et al., 2019). Overall, it is likely that the effects of OA on community structure can vary temporally and spatially, and the availabilities or levels of eutrophication can modulate the effects of OA, alongside regional chemical and physical differences (Paul and Bach, 2020).

In coastal regions, changes in seawater carbonate chemistry influence primary production which in reverse affect the pH change due to faster photosynthetic CO_2 removal than its dissolution (Gao, 2021), resulting in increased pH during daytime and declined pH during nighttime. Such large diel pH fluctuation may require phytoplankton to invest more energy to maintain cellular homeostasis in response to the negative effects of increased hydrogen ion concentration (the acidic stress), thereby affecting the functioning of planktonic ecosystem (Raven and Beardall, 2020; Rokitta et al., 2012; Taylor et al., 2017). The positive effects of increased inorganic carbon substrates (e.g., CO_2 and HCO_3^-) and the negative effect of increased H^+ concentration may shape the responses of coastal ecosystems to OA, leading to controversial results (Wu et al., 2017; Vázquez et al., 2023; Chauhan et al., 2024). To better understand the consequences of OA in Chinese eutrophic coastal regions, we conducted a mesocosm experiment in the eutrophic coastal water of Wuyuan Bay, Xiamen, China, using *in situ* plankton communities during October-December, 2019 and investigated how OA shapes the diversity of phytoplankton community and affects primary production processes. Our results show that in the eutrophic coastal water of East China Sea, with the natural decrease of temperature, elevated pCO_2 increased primary production by promoting the

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phytoplankton biomass (indicated by Chl *a* concentration) under nutrient-replete condition, and promoted smaller phytoplankton's growth to sustain the primary production after the nutrient depletion and diatom bloom collapsed, though suppressed the emergence of dinoflagellates.

2 Materials and methods

2.1 Mesocosms setup and sampling

The *in situ* mesocosm experiment was conducted at the Facility for the Study of Ocean Acidification Impacts of Xiamen University (FOANIC-XMU) located in the subtropical coastal region Wuyuan Bay of southern East China Sea (24.52°N, 117.18°E) from 9th October (day 0 relative to algal inoculation) to 14th November, 2019. Nine cylindrical and transparent thermoplastic polyurethane (TPU) mesocosm bags, each 3 m deep and 1.5 m in diameter, were filled with approximately 3000 L of *in situ* seawater that had been prefiltered (MU801-4T, Midea, China, pore size of 0.01 μm). The mesocosms were hooked to and secured within steel frames. Two pCO_2 treatments were established to investigate the effects of ocean acidification on the *in situ* phytoplankton community: an ambient pCO_2 treatment (AC, 410 μatm; 4 numbered bags) and a high pCO_2 treatment representative of end-of-century projections (HC, 1,000 μatm; 5 numbered bags). To adjust CO_2 in seawater in the HC bags to the projected 1000 μatm in 2100s, approximately 11 L of CO_2-saturated seawater was added to each HC bag. The AC and HC pCO_2 levels were maintained by bubbling with ambient air and pre-mixed air-CO_2 (1000 μatm CO_2), respectively, at a rate of 5 L min^{-1} using a CO_2 Enricher (CE-100B, Wuhan Ruihua Instrument & Equipment Ltd, China). After the carbonate system had been stabilized (leveled pH), 720 L of *in situ* seawater were filtered by a 180 μm mash to exclude large zooplankton, and each mesocosm bag was inoculated with 80 L of it to initiate the coastal microbial community. Samples were taken from each bag at a depth of 0.5m at 9:00 a.m. by niskin bottles every 1−3 days for physical, chemical and biological analysis.

2.2 Measurement of environmental factors

Solar light intensity was monitored every minute throughout the experimental period using a real-time solar irradiance monitoring device (EKO, Japan). Salinity, temperature and pH in each mesocosm were measured with a salinometer, a digital thermometer and a pH meter (Orino 2 STAR, Thermo Scientific, U. S. A, calibrated with standard NBS buffer), respectively. Dissolved inorganic

carbon (DIC) was sampled and measured using an Environmental Water Analyzer (Ma et al., 2018), and total alkalinity (TA) measured using an Automated Spectrophotometric Analyzer (Li et al., 2013). Other seawater carbonate chemistry parameters were calculated with CO2SYS software with known parameters of pCO_2, salinity, pH, temperature, and nutrient concentration.

Nutrient samples of each bag were filtered through cellulose acetate membrane (0.22 μm, 47 mm), and the filtrate was divided into 2 subsamples; one was stored at −20 ℃ for the measurement of NO_3^-+NO_2^-, PO_4^{3-}, and NH_4^+; another stored at 4℃ for the measurement of SiO_3^{2-}. Measurement of NO_3^-+NO_2^-, PO_4^{3-}, and SiO_3^{2-} concentration was conducted using an auto-analyzer (AA3, Seal, Germany), NH_4^+ was measured with indophenol blue spectrophotometry using a spectrophotometer (Tri-223, Spectrum, China) at 25 ℃. NO_2^- and NO_3^- were analyzed using the copper-cadmium reduction method, the standard concentrations used for the NO_2^-+NO_3^- calibration curve were 0, 1.04, 2.08, 4.16, 10.4, 20.8, and 41.6 μM, and those for NO_2^- calibration curve were 0, 0.04, 0.08, 0.16, 0.4, 0.8, and 1.6 μM (Dai et al., 2008). PO_4^{3-} and SiO_3^{2-} were measured using typical spectrophotometric method (Knap et al., 1994), and the calibration curves of both parameters were prepared using standard concentrations of 0, 0.08, 0.16, 0.32, 0.8, 1.6, 3.2 μM and 0, 4, 8, 16, 40, 80, 160 μM, respectively.

2.3 Measurement of chlorophyll *a* and particle organic matters

Water samples of each mesocosm (100 − 1000 mL, depending on the biomass in the mesocosms) were filtered onto GF/F filter (Whatman, United States) by suction filter with low vacuum pressure (≤0.02 Mpa) and soaked in 5 mL pure methanol overnight. The extracts were centrifuged at 8000 × g and 4 ℃ for 10 min, then the absorption spectra of supernatants from 400 to 800 nm were measured using a UV-VIS spectrophotometer (DU 800, Beckman, U. S. A). The Chl *a* concentration was calculated according to the following equation (Ritchie, 2006):

$$\text{Chl } a\ (\mu g\ L^{-1}) = 16.29 \times (A665 - A750) - 8.54 \times (A652 - A750)$$

where $A750$, $A665$, and $A652$ represents the absorbance of Chl *a* at wave length 750, 665, and 652 nm, respectively.

For the analysis of particulate organic carbon (POC) and nitrogen (PON) across two particle size fractions, water samples of known volume were first filtered through a 20 μm mash to obtain subsamples containing particle organic matters smaller than 20 μm. Particles larger than 20 μm

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(retained on 20 μm mesh) were backwashed using an equal volume of prefiltered (0.22 μm) *in situ* seawater, yielding in subsamples containing particulate organic matters larger than 20 μm. All subsamples were then filtered on pre-combusted (450 ℃, 6 h) GF/F filter (Whatman) and stored at −20 ℃ until analysis. Before analyses, all filters were fumed over pure HCl for 12 h and dried at 60 ℃ to remove inorganic carbon, then packed in tin cups and measured with a CHNS elemental analyzer (Vario EL cube Elementar, Germany).

2.4 Net primary production and dark respiration

Before sunrise, 120 mL of water samples from each mesocosm were collected and dispensed into six 25 mL borosilicate bottles (three bottles for 12 h incubations, and three for 24 h incubations). For each culture duration of each mesocosm, two bottles were illuminated under natural light and one bottle was wrapped tightly in aluminum foil as a dark control. After incubation, cells were filtered onto the GF/F filters (Whatman) under dim light and stored at −20 ℃. Before measurement, filters were placed individually in 20 mL scintillation vials and exposed to HCl fumes overnight, dried at 60 ℃ for over 6 h to remove any unincorporated $H^{14}CO_3^-$. The incorporated ^{14}C by algae was counted with a liquid scintillation counter (Beckman, LS6500, Germany) in the presence of 5 mL scintillation cocktail (Hisafe 3, Perkin-Elmer, United States). Nighttime respiratory carbon loss was calculated as the difference between carbon fixation over 12 h (daytime primary production) and 24 h (daily net primary production).

2.5 Determination of phytoplankton biomass and community structure

Water samples (500−2000 mL) from each mesocosm were collected into polyethylene bottles and fixed with 1.5 % acidic lugol's iodine. The samples were statically placed for 2-3 days and concentrated into 50 mL subsamples in the centrifuge tube using siphons within 3 days, then examined with a microscopy (Nikon Eclipse Ns2) and a plankton counting chamber to assess phytoplankton abundance and diversity based on the morphological characteristics as previous described (Hasle and Syvertsen, 1997; Steidinger and Jangen, 1997; Yang and Liu, 2018). To distinguish whether dinoflagellates are autotrophic or heterotrophic, we observed living algal cells in the unstained water samples under the microscope for cell transparency and the presence of chloroplasts.

An aliquot of 100 μL for each mesocosm was loaded onto a counting chamber for microscopic

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enumeration. In each aliquot, the count was deemed valid only when the total number of cells exceeded 200; otherwise, the subsample volume for microscopy was increased to achieve sufficient counts. For samples collected during the exponential growth phase that exhibited excessively high cell densities, appropriate dilution with 0.22 μm–filtered, sterilized seawater was performed prior to counting (State Oceanic Administration 2005).

2.6 Statistical analyses

The data were all expressed by the mean and standard deviation (means ± SD) and plotted by Origin 2024. Independent-samples *t*-test was conducted to check the significant effects of increased pCO_2 at the level of $p < 0.05$ using SPSS 19. To evaluate α-diversity, Shannon diversity index was calculated based on the relative abundances of phytoplankton taxa using the estimate R and diversity functions from the vegan package (version 2.6-4) in R (Version 4.2.2). Shannon index incorporates both species diversity and evenness. Patterns of physiological parameters over time were emphasizing using generalized additive models (GAMs) and constructed using the 'mgcv' package in R to analyze changes in physiology through the experiment.

3 Results

3.1 Environmental changes in the mesocosms

Throughout the experiment, most days were sunny, with daytime mean PAR (12h-average photosynthetic active radiation) ranging from 200 to 850 μmol photons m^{-2} s^{-1} (Fig. S1). The environmental temperatures decreased gradually from 26.7 ± 0.05 ℃ at day 0 (9th October) to 21.1 ± 0.28 ℃ at day 38 (14th November) (Fig. 1 a). Significant differences in pH_{NBS} and pCO_2 between HC and AC were maintained throughout most of the experimental period, while there's no significant difference in total alkalinity (TA) between the two pCO_2 treatments ($p = 4.02 \times 10^{-8}$, 2.87×10^{-12}, 0.549, respectively. Figs. 1 b-d, S5 a-c).

Following a sharp increase in phytoplankton biomass from day 4 to day 8 (Fig. 3 a), the pH_{NBS} in the HC and AC mesocosms increased and peaked at 8.24 ± 0.16 and 8.56 ± 0.14, respectively (Fig. 1 b). Correspondingly, the pCO_2 value dropped to the lowest points of 238.48 ± 49.02 μatm (HC) and 82.82 ± 32.88 μatm (AC) (Fig. 1 c). Then, as the phytoplankton biomass decreased after day 8, pH_{NBS} gradually declined and pCO_2 increased, both stabilizing at relatively constant levels from day 18 until the end of experiment. There were no obvious temporal changes observed in total

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alkalinity (TA) throughout the experiment (Fig. 1 d).

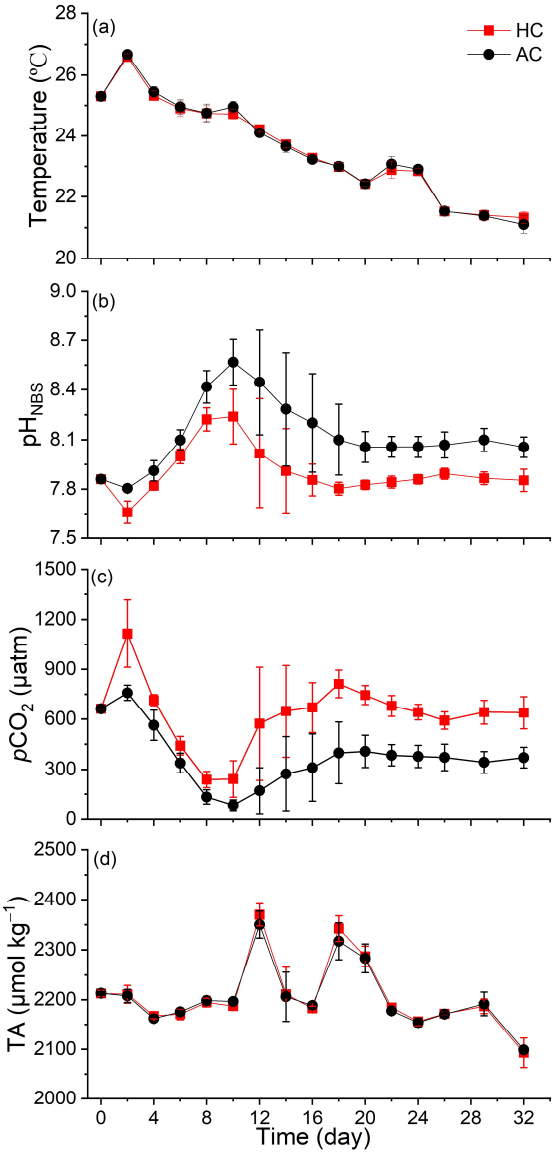

Figure 1. Temporal variation of seawater temperature (a), pH_{NBS} (b), pCO_2 (c) and TA (d) in HC (1000 µatm) and AC (410 µatm) mesocosms. The pCO_2 was estimated from the measured pH_{NBS} and DIC concentration using the CO2SYS program. Data are means ± SD of 5 replicates for HC and 4 replicates for AC mesocosms.

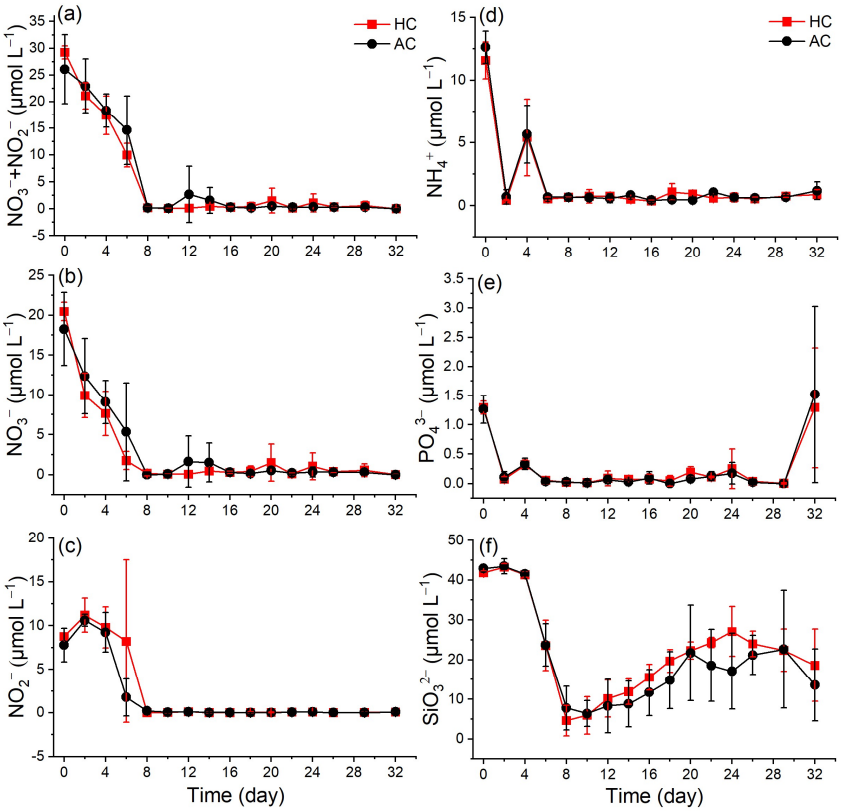

Figure 2. Temporal variation of nutrients (NO_3^-, NO_2^-, NO_3^-+NO_2^-, NH_4^+, PO_4^{3-}, and SiO_3^{2-}) in HC (1000 μatm) and AC (410 μatm) mesocosms. Data are means ± SD of 5 replicates for HC and 4 replicates for AC mesocosms.

The initial nutrient concentrations reflected the eutrophic condition in the coastal seawater (NO_3^-+NO_2^-: 27 μM, PO_4^{3-}: 1.4 μM). In the mesocosm bags, nutrient concentrations declined dramatically in the early phase (up to day 8, Fig. 2). The NO_3^-+NO_2^- and NO_3^- concentrations decreased sharply to nearly 0 by day 8 (Fig. 2 a, b). In contrast, the NO_2^- concentration experienced a slight increase on day 2, then declined to nearly 0 by day 8 and remained at a low level until the end of experiment in both HC and AC mesocosms (Fig. 2 c). Under HC condition, the NO_3^-

concentration decreased more rapidly than that under the AC until day 20, although the difference was not significant (p = 0.423, Fig. S5 d). Both NH_4^+ and PO_4^{3-} concentrations dropped to nearly 0 after 4 days and remain relatively stable thereafter. There were no significant difference observed between HC and AC mesocosms (p = 0.579 and 0.631, respectively, Figs. 2 d, e, S5 e, f). The SiO_3^{2-} concentration decreased from 41.81 ± 0.48 μM in HC and 42.88 ± 0.91 μM in AC on day 0 to a minimum of 4.62 ± 3.82 μM and 7.79 ± 5.52 μM by day 8, respectively. Thereafter, SiO_3^{2-} concentrations in both HC and AC mesocosms gradually increased until the end of experiment (p = 0.343, Figs. 2 f, S5 g).

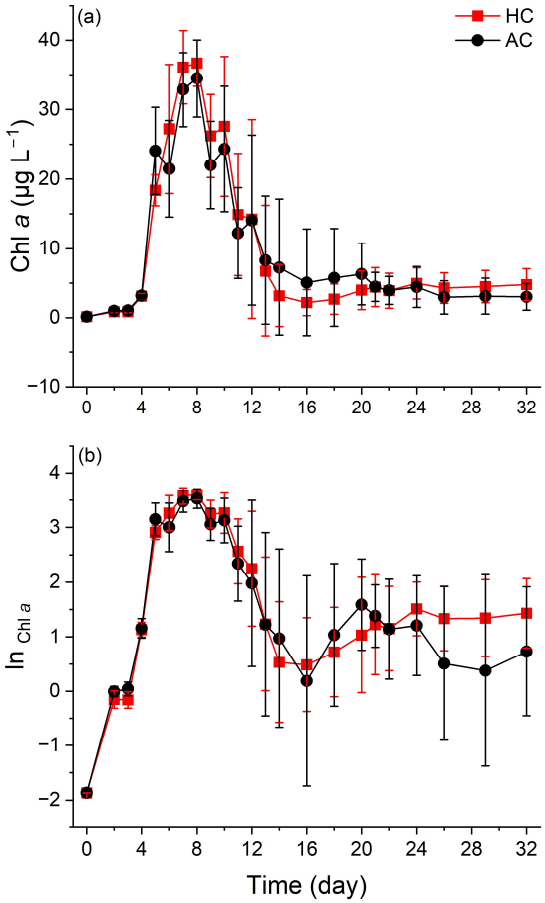

Figure 3. Temporal variations of Chl *a* concentration (a) and the LN scale of Chl *a* concentration (b) in HC (1000 μatm) and AC (410 μatm) mesocosms. Data are means ± SD of 5 replicates for HC and

4 replicates for AC mesocosms.

3.2 Chlorophyll *a* concentration

Phytoplankton biomass, indirectly indicated by Chl *a* concentration, increased to peak at 35.88 ± 3.25 μg L^{-1} in the HC and 36.54 ± 4.88 in the AC mesocosms on day 8, and then gradually decreased to 1.12 ± 0.43 μg L^{-1} in HC by day 14 and 0.58 ± 0.31 μg L^{-1} in AC by day 16, followed by a slight increase by the end of experiment (Fig. 3 a). Based on the natural logarithm (ln) scale of Chl *a* concentration, the phytoplankton growth kinetics under the two pCO_2 treatments showed the following phases (Fig. 3 b): the exponential phase (from day 0 to day 5), the stationary phase (from day 6 to day 10), the decline phase (from day 11 to day 16), and a second exponential phase from day 17 to day 24 in the HC and to day 20 in the AC mesocosms. Then, phytoplankton assemblages in the HC mesocosms entered a second stationary phase until the end of experiment, while in the AC ones, they entered a decline phase until day 29, followed by a slight increase on day 32.

Throughout the experiment, the elevated pCO_2 resulted in higher average value of Chl *a* concentration at most sampling times, although the differences were not statistically significant (p = 0.142, Fig. S5 h).

3.3 Primary production and dark respiration

The primary production and night-respiratory per water volume showed patterns similar to those of phytoplankton biomass (indicated by Chl *a* concentration) (Fig. 4, a, b and c). They reached their maximal values on day 6, which corresponded to the end of exponential phase. As the phytoplankton communities entered the stationary phase, daytime (12 h) primary production, daily (24 h) net primary production and nighttime respiration per water volume progressively decreased, and then slightly increased again when the phytoplankton communities underwent the second exponential phase. The elevated pCO_2 increased both daytime and daily net primary production during the middle phase of the experiment, although the positive effect on 24 h primary production tended to decline by the end of experiment (p = 0.038 and 0.012, Fig. S6 a, b). The nighttime respiration of phytoplankton was suppressed before day 8 and enhanced thereafter under the elevated pCO_2, though no significant difference was observed (p = 0.444, Fig. S6 c).

Primary productivity per Chl *a* increased sharply on day 4, and decreased to the lowest values on day 8. On day 12, both daytime and 24 h primary productivity in the HC increased drastically

and then remained relatively stable until the end of experiment (Fig. 4 d, e). In contrast, two additional peaks were observed in the AC mosocosms on days 16 and 26. The elevated pCO_2 appeared to have enhanced primary productivity from day 2 to day 20, though these effects were not statistically significant ($p = 0.946$ for daytime and $p = 0.985$ for 24 h, Figs. 4 d, e, S6 d, e).

Nighttime respiration per µg Chl *a* initially increased on day 4, then decreased to nearly zero in both the HC and AC mesocosms on day 8 and remained relatively stable till the end of experiment. The elevated pCO_2 had a negative effect on phytoplankton respiration before day 12, but increased it thereafter, though no significant difference was observed between the HC and AC treatments ($p = 0.834$, Figs. 4 f, S6 f).

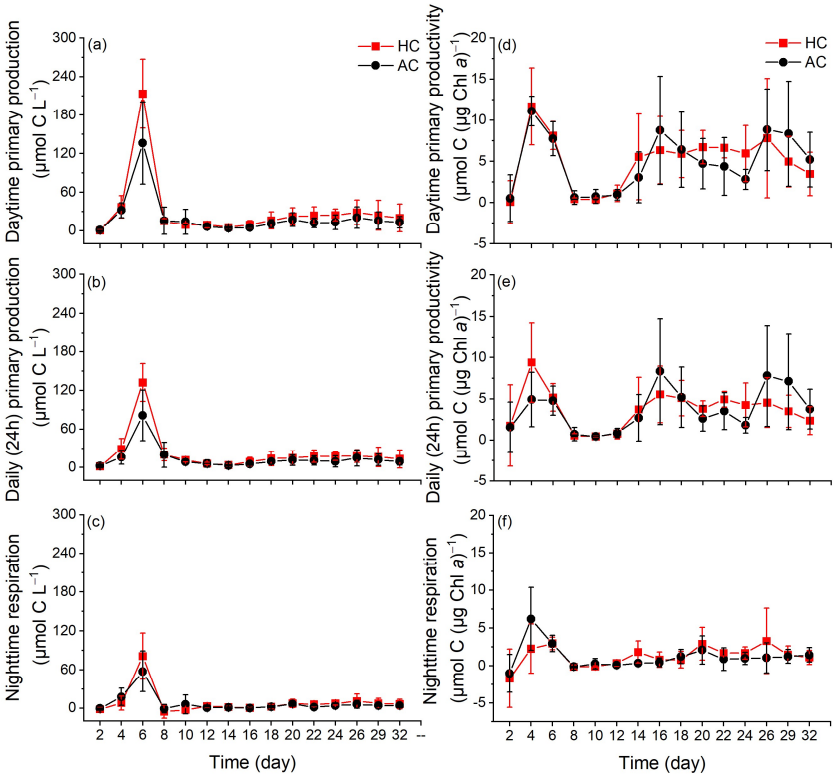

Figure 4. The changes of daytime primary production (a) and primary productivity (b), daily (24h) primary production (c) and primary productivity (d), nighttime respiration per water volume (e) and per Chl *a* (f) in HC (1000 µatm) and AC (410 µatm) mesocosms. Data are means ± SD of 5 replicates

for HC and 4 replicates for AC mesocosms.

3.4 Changes in Phytoplankton community and diversity

A total of 47 genera identified microscopically include 33 genera of diatoms, 7 of dinoflagellates, 2 of cyanobacteria, 2 of chlorophyta, 2 of cryptophyta and 1 of euglenophyta. In all mesocosms, the dominant species included *Cerataulina pelagica*, *Eucampia cornuta*, *Guinardia delicatula*, *Leptocylindrus danicus*, *Skeletonema costatum*, *Protoperidinium* sp., *Gyrodinium spirale*, *Cryptophyta* sp. and *Pyramimonas* sp. (Fig. S4).

Phytoplankton communities underwent dynamic succession in the mesocosms (Fig. 5). Diatoms (mainly *Cerataulina pelagica*) dominated the phytoplankton communities during the early and middle stages of the experiment, as indicated by the similar temporal trends in total phytoplankton and diatom cell counts compared with Chl *a* concentration (Figs. 5 a, b, S4 a). There was no significant difference in diatom density between the HC and AC mesocosms ($p = 0.259$, Fig. S7a), although the average value was lower in the former than in the latter treatment. Autotrophic dinoflagellates began to emerge on day 8 and rapidly declined on day 12 in both HC and AC enclosures (Fig. 5 c). Except for days 6 to 18, the elevated pCO_2 increased the biomass of autotrophic dinoflagellates, though the difference was insignificant ($p = 0.505$, Fig. S7 b). Hetero-dinoflagellates began to emerge on day 6, with their abundance peaked on day 12 in the AC and on day 14 in the HC mesocosms, then decreased by day 22. The elevated pCO_2 did not result in any significant change in terms of their cell numbers ($p = 0.785$, Figs. 5 d, S7 c). On day 26, the biomass of hetero-dinoflagellates increased again in the HC treatment, while it remained constant in the AC treatment ($p = 0.729$, Independent-samples *t*-test).

The biomass of small taxa (Cyanobacteria, Chlorophyta, Cryptophyta and Euglenophyta) started to increase on day 8, the HC treatment significantly increased the total biomass of these small phytoplankton species thereafter ($p = 0.019$, Figs. 5 e, S4 h, i, S7 d). From day 22, when diatoms biomass decreased to the lowest level, the temporal variation in small taxa biomass became the main factor controlling overall phytoplankton dynamics (Figs. 5 e, S4 h, i). Accordingly, the positive effect of HC on the small phytoplankton species led to an earlier transition of phytoplankton from the large diatoms and dinoflagellate (mainly fall within the micro size fraction) to the smaller

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ones (Fig. 6 a, b). This accelerated transition in the HC treatment was also evidenced by higher concentration of POC and PON in the <20 μm fraction and lower concentration in the >20 μm fraction (Figs. S2, S3).

In both HC and AC mesocosms, Shannon diversity index decreased sharply from day 2, reaching the lowest values on day 8 in AC mesocosms and on day 10 in HC mesocosms (Fig. S8 a). Before day 22, Shannon diversity index increased under elevated pCO_2, whereas it is lowered under elevated pCO_2 level since day 24, although the differences were not statistically significant (p = 0.161, Fig. S8 b).

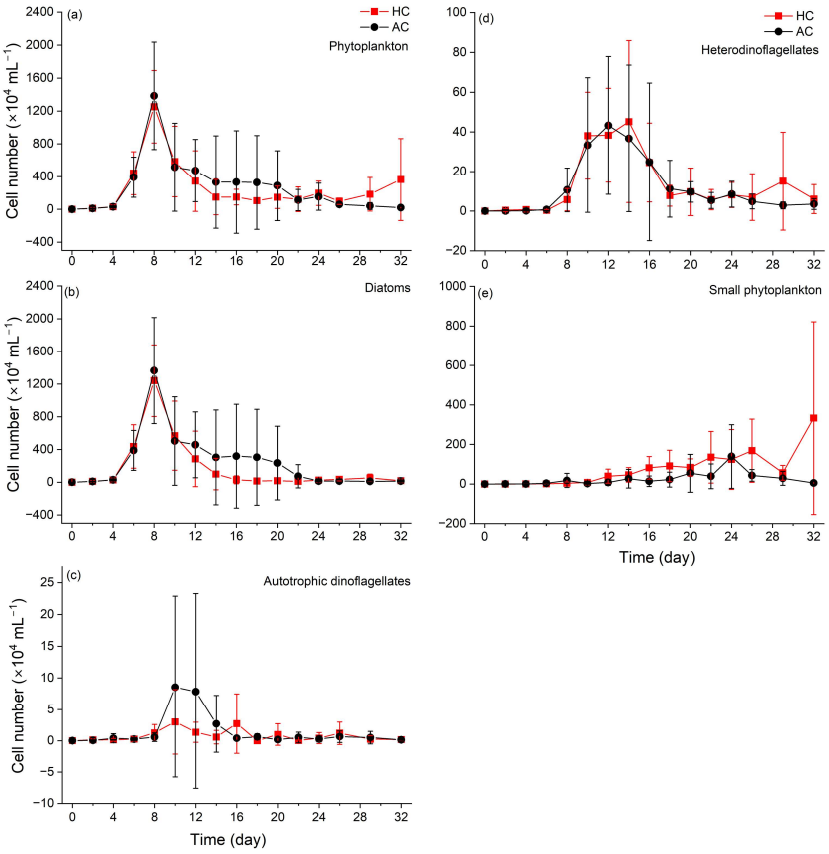

Figure 5. Temporal variations of phytoplankton (a), diatoms (b), autotrophic dinoflagellates (c), heterodinoflagellates (d) and (e) small phytoplankton (Cyanobacteria, Chlorophyta, Cryptophyta

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and Euglenophyta) cell numbers in HC (1000 µatm) and AC (410 µatm) mesocosms. Data are means ± SD of 5 replicates for HC and 4 replicates for AC mesocosms.

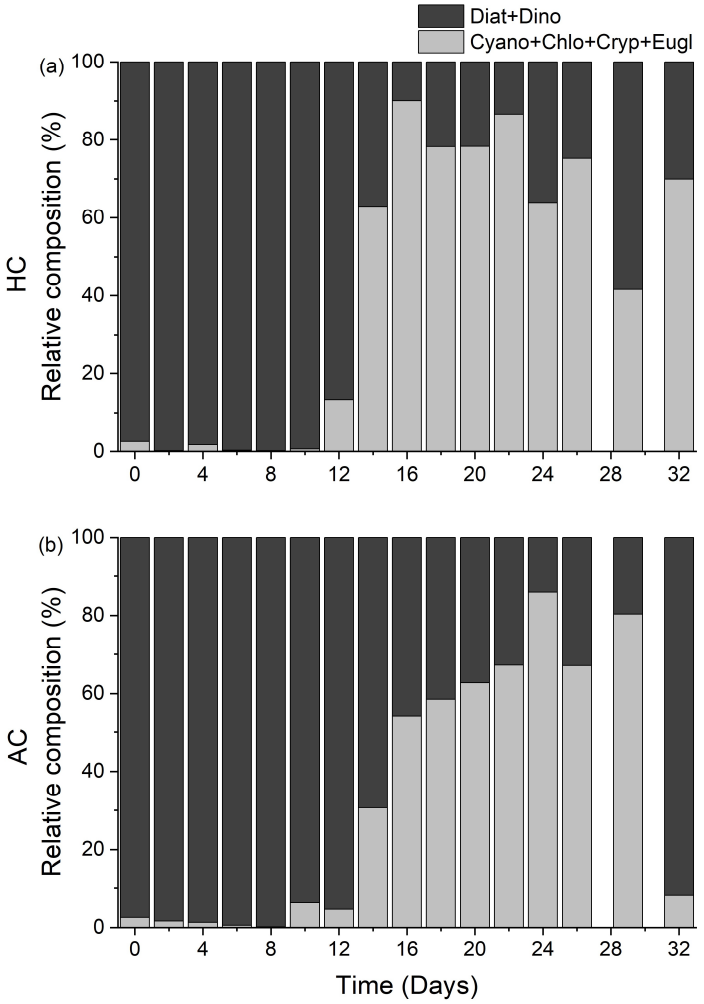

Figure 6. Temporal variations of the relative composition of diatoms + dinoflagellates (Diat + Dino, black), Cyanobacteria + Chlorophyta + Cryptophytes + Euglenophyta (Cyano + Chlo + Cryp + Eugl, grey) in HC (1000 µatm, a) and AC (410 µatm, b) mesocosms. Data are means of 5 replicates for HC and 4 replicates for AC mesocosms.

4 Discussion

Ocean global changes have been suggested to alter community structure and reduce the phytoplankton diversity due to physicochemical environmental changes (Henson et al., 2021; Yuan et al., 2020). Specifically, there appears a growing trend of increasing dinoflagellates abundance relative to diatoms (Carreto et al., 2018). Our mesocosm experiment, conducted in the highly eutrophic Wuyuan Bay in the southern East China Sea during late autumn, also indicated that elevated pCO_2, along with the natural decrease of surface water temperature and declined nutrient availability, altered the structure and diversity of phytoplankton community. The diatom dominance corresponded to the decreased diversity and evenness of phytoplankton community, while these were recovered when the diatom dominance was replaced by dinoflagellates. However, this shift from diatoms to autotrophic dinoflagellates was relatively suppressed under elevated pCO_2 conditions. In our mesocosms, the dinoflagellates that emerged during the mid-phase (e.g., *Protoperidinium* sp., *Pentapharsodinium dalei* and *Heterocapsa* sp., Fig. S9 a) were predominantly small (<20 μm, Fig. S4 g) (Gu et al., 2013; Hanifah et al., 2022), but these dinoflagellates were soon replaced by an even smaller size fraction, including Cyanobacteria, Chlorophyta, Cryptophytes, and Euglenophyta (Figs. 5, 6, S4). Ultimately, these smaller taxa maintained the primary production of phytoplankton communities after nutrient depletion (Fig. 4).

When diatoms dominated the phytoplankton community (before day 8), primary production per water volume and per Chl *a* did not change in the same pattern with increased diatom biomass (Fig. 4). This is likely attributable to the larger size of photosynthetic unit (PSU) and lower reaction center-to-Chl *a* ratio in diatoms, which could result in relatively lower photosynthetic efficiency (Wu et al., 2014; Malerba et al., 2018). Meanwhile, The competitive advantages conferred by CO_2-concentrating mechanisms (CCMs) in diatoms led to insignificant lower biomass (Figs. 5 b, S7 a) but higher primary production per water volume and per μg Chl *a* (Fig. 4 a, b, d and e) under elevated pCO_2: their higher CO_2 affinity and CCMs plasticity may help diatoms gain a competitive advantage in DIC uptake under ocean acidification scenarios (Huang et al., 2021; Raven and Beardall, 2020). Furthermore, the down-regulated of CCMs in diatoms can save energy for other physiology processes and thereby fuel their primary production (see the review by Gao and Campbell, 2014 and the references therein). These benefits resulting from elevated pCO_2 also led to higher diversity

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and evenness (Fig. S8 a, b), suggesting that more diatom species were benefited from the elevated pCO_2. While previous works have demonstrated a positive effect of elevated pCO_2 on the photosynthetic carbon fixation by diatoms grown under low light and phytoplankton assemblages in waters of higher nutrient availability (Gao et al., 2022), our results (Figs. 5 b, S1) indicate that nutrient limitation can override or even reverse to the positive effects of elevated pCO_2 on diatoms (Boyd et al., 2016; Li et al., 2018).

It appeared that dinoflagellates were less sensitive to the depletion of nutrients compared to diatoms, with autotrophic dinoflagellates were more sensitive to elevated pCO_2 (Figs. 5 c, S7 b). These suppressed transition from diatoms to autotrophic dinoflagellates under the elevated pCO_2 was consistent with our previous works, though conducted in different seasons (Huang et al., 2021), which is likely due to the different CCMs efficiency and/or acidic resilience between dinoflagellates and diatoms. Since the affinity of ribulose 1, 5-diphosphate carboxylase/oxidase (Rubisco) for CO_2 is much lower in autotrophic dinoflagellates than in diatoms (Reinfelder, 2011), elevated pCO_2 must have benefitted the former more compared the latter, though invisible growth advantage was observed on the autotrophic dinoflagellates between days 8 and 16. The heterodinoflagellates can utilize organic matters (Glibert and Legrand, 2006) and prey on microbes including bacteria and smaller microalgae (Jeong et al., 2010). This versatile nutrition strategy supported their rapid bloom starting from day 8, leading to the replacement of autotrophic ones from day 12 onward (Fig. 5 c, d). Although they were shown to be insensitive to ocean acidification (Meunier et al., 2017), their respiration was depressed due to the acidic stress, raising their resilience in terms of energetic cost (Wang and Gao, 2024). These mechanisms likely explain the observed insignificant effects of HC on hetero-dinoflagellates.

The gradual increase in SiO_3^{2-} concentration, a nutrient exclusively required by diatoms, coincided with their decline, confirming the low abundance of diatoms in the mid and late phase of experiment, while the slightly increases in NO_3^-+NO_2^-, PO_4^{3-} and SiO_3^{2-} concentrations from day 10 onward (Fig. 2 a, e, f) should be attributed to remineralization by heterotrophic bacteria (Arístegui et al., 2009; Bunse and Pinhassi, 2017). These regenerated NO_3^-+NO_2^- and PO_4^{3-} subsequently refueled the growth of small phytoplankton taxa, recover the diversity and evenness in the phytoplankton communities (Figs. 5 e, S8 a, b) (Thingstad and Rassoulzadegan, 1995).

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Alternatively, it is plausible that grazing activity by zooplankton, which was not quantifited in this study, also contributed to the apparent rise in diversity and evenness, as grazers tend to consume dominant phytoplankton taxa (Thingstad and Rassoulzadegan, 1999; Calbet and Landry, 2004).

The dominant small taxa, such as *Cryptophyta* sp. and green microalga *Pyramimonas* sp. (Fig. S4 h, i) during days 16−24, achieved primary productivity (per μg Chl *a*) comparable to the diatom-dominated community observed on days 4–6 (Fig. 4 b, d). The success of these small taxa after nutrient depletion can be attributed to their small size and larger surface-to-volume ratio (Finkel et al., 2009; Giordano et al., 2005), which might enable them with higher efficiency in nutrients uptake and CO_2 diffusion. Furthermore, the higher abundance of viruses and heterotrophic bacteria in the HC mesocosms (Huang et al., 2021; Lin et al., 2018) intensified nutrient remineralization, subsidizing these small, fast-growing phototrophs and leading to their earlier emergence on day 16 compared to day 24 in the AC mesocosms (Fig. 6). While it's possible that picophytoplankton originally present in this region (Zhong et al., 2020) were missed by microscope-based identification, it is reasonable to infer that they also contributed to the late-phase primary production. Previous studies indicated that, after diatom/dinoflagella blooms and nutrient depletion, remineralized nutrients in the seawater may also favor the growth of picophytoplankton (Nishibe et al., 2015; Fu et al., 2009) and elevated pCO_2 would further benefit their growth. Thus, it is likely that, picophytoplankton also dominated the phytoplankton communities in the HC mesocosms.

Though previous studies have suggested the changing temperatures influenced the phytoplankton growth and community structure individually or interactively with OA and other environmental factors (Bénard et al., 2018; Courboulès et al., 2018; Li et al., 2018), the results from the present autumn mesocosm experiment revealed the same pattern with our previous spring mesocosm experiment (Huang et al., 2021), suggesting that it is not the seasonal temperature trajectories but the availability of nutrients that controlled the shift from diatom to dinoflagellate dominance, leading to declines in primary productivity (Huang et al., 2021; Cloern, 1996). Such consistency underscores that nutrient availability and stoichiometry are the primary determinants of phytoplankton community composition, usually exerting stronger and more immediate effects on taxonomic and functional group dominance (Karl et al., 1996; Paerl and Paul, 2012; Ptacnik et al., 2008; Meyer et al., 2016), though thermal and acidic stresses can impact phytoplankton

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photosynthesis and respiration to greater extent under nutrient limitation (Li et al., 2018; Gao et al., 2022).

Reduced nutrient availability usually decreases phytoplankton community richness (Gazeau et al., 2017), although ocean acidification appeared to partly offset such effects (Fig. S8). However, these compensatory effects diminished once both the initial and regenerated nitrogen sources were exhausted (after day 24, Fig. S8 b). At that point, only a few small phytoplankton taxa tolerant to low pH remained dominant, indicating a loss of diversity in the community and less stable ecosystems (Mccann, 2000) under combination of acidic stress and nutrient limitation. In summary, beyond compensating previous works, our study further demonstrated that progressive ocean acidification is likely to reduce primary production and phytoplankton diversity in the eutrophicated coastal water of the southern East China Sea.

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Data availability Statement

All relevant data are presented in the papr and its Supporting Information file, and will be available upon request to the corresponding author Kunshan Gao.

Conflict of Interest

The authors declare no competing interests.

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Author's Contributions

Kunshan Gao and Guang Gao designed the mesocosm experiment; Yuming Rao, Na Wang, Jiazhen Sun, Xiaowen Jiang, Di Zhang, Liming Qu, He Li, Qianqian Fu, Xuyang Wang, Cong Zhou, Zichao Deng, Yang Tian, Xiangqi Yi, Ruiping Huang performed the mesocosm experiment; Yuming Rao analyzed the data and wrote up the manuscript; Na Wang performed microscopy observation; Kunshan Gao edited the manuscript; All authors reviewed and contributed to revision of the

manuscript.

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