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Effects of ocean acidification and nitrogen limitation on the growth and photophysiological performances of marine macroalgae *Gracilariopsis lemaneiformis*

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To investigate the effects of ocean acidification (OA) and nitrogen limitation on macroalgae growth and photophysiological responses, *Gracilariopsis lemaneiformis* was cultured under two main conditions: ambient (Low CO_2, LC, 390 μatm) and CO_2 enriched (High CO_2, HC, 1000 μatm), with low (LN, 7 μmol L^{-1}) and high (HN, 56 μmol L^{-1}) nitrate. High CO_2 levels decreased growth under both LN and HN treatments. HC reduced Chl *a*, carotenoids, phycoerythrin (PE), and phycocyanin (PC) under HN conditions, while only Chl *a* decreased under LN conditions. NO_3^- uptake rate was restricted under LN compared to HN, while HC enhanced it under HN. Net photosynthetic O_2 evolution rates did not differ between CO_2 and nitrate treatments. Dark respiration rates were higher under HN, further boosted by HC. The stimulated effective quantum yield (Y(II)) corresponded to decreased non-photochemical quenching (NPQ) under HN conditions. Nitrate, not CO_2, showed significant effects on the relative electron transport rate ($rETR_{max}$), light use efficiency (α) and saturation light intensity (I_k) that with lowered $rETR_{max}$ and α under LN culture. Our results indicate that OA may negatively affect *Gracilariopsis lemaneiformis* growth and alter its photophysiological performance under different nutrient conditions.

KEYWORDS

***Gracilariopsis lemaneiformis*, culture, ocean acidification, growth, nitrate, photosynthetic performance**

1 Introduction

As the impact of human activities intensifies, the combustion of fossil fuels and deforestation activities have led to a continuous and rapid increase in atmospheric CO_2 concentration. Since the industrial revolution, atmospheric CO_2 levels have risen from 280 ppmv to about 422.8 ppmv, as recorded by the Mauna Loa Observatory (MLO) in Hawaii (NOAA) as of January 2024 (www.CO2.earth). If the energy structure remains constant, these levels are projected to keep increasing and expected to reach 800 - 1000 ppmv according to the Intergovernmental Panel on Climate Change report (IPCC, 2021). As the main sink of anthropogenic CO_2, the ocean absorbs about 2.5 ± 0.6 Pg C per year according to Global Carbon Budget 2020 by Friedlingstein et al. (2020) which greatly offsets global warming (Häder and Gao, 2023). However, the increase in dissolved CO_2 in seawater also brings about changes in the marine carbonate system, mainly reflected by a decrease in seawater pH and carbonate ion concentration (CO_3^{2-}), and an increase in hydrogen ion (H^+), dissolved CO_2 (pCO_2) and bicarbonate concentration (HCO_3^-), which is termed as ocean acidification (OA) (Doney et al., 2009). OA as a widespread stressor, has attracted significant attention in recent years and has been suggested to affect marine organisms differently owing to variations in their sensitivity, leading to significant ecological changes (Kroeker et al., 2013). Recent studies have further shown that the ecological effects of OA are not limited to the individual organisms, affecting both the broader community and the entire marine ecosystem (Guinotte and Fabry, 2008; Riebesell and Gattuso, 2015; Nagelkerken and Connell, 2022).

Macroalgae are widely distributed in the intertidal zone and play an important role in carbon flux and sequestration, biogeochemical cycles, and the stability of coastal ecosystems (Falkowski and Raven, 2013; Zhang et al., 2017). Anthropogenic CO_2 dissolution induced OA may superimpose a lower pH caused by coastal eutrophication on a more acidic condition (Cai et al., 2011; Capone and Hutchins, 2013), which means that physical and chemical environment of aquaculture water will change both globally and regionally. Changing seawater carbonate composition may have divergent effects on marine macroalgae inhabiting in different intertidal zones, because the utilization mode and capacity of dissolved inorganic carbon (DIC) are significantly different (Giordano et al., 2005; Raven et al., 2008). Most macroalgae can use both CO_2 and HCO_3^- to counteract the photosynthetic substrate limitation of lowered dissolved CO_2 in seawater (Hepburn et al., 2011), and possess different CO_2 concentration mechanisms (CCMs) to overcome the reliance on a lowered rate of HCO_3^- dehydration to CO_2 by using intracellular and/or extracellular carbonic anhydrase (Beer et al., 2002; Dudgeon and Kübler, 2020). Therefore, enhanced pCO_2 and HCO_3^- may benefit species with lowered CCM capacity, however the neutral and negative effects have also been observed, which are sometimes regulated by other co-varied environmental factors, such as light intensity, nutrient status and temperature (Raven et al., 2011; Celis-Plá et al., 2015; Yang et al., 2021; Zhou et al., 2022). An *in-situ* study of the ocean natural CO_2 gradients (CO_2 seeps) has proven that high CO_2/low pH will disproportionately changes the species abundance of macroalgae and induces the shifts in community structure, subsequently leading to altered coastal habitats and ecosystem services (Hall-Spencer and Harvey, 2019).

In recent years, numerous studies have focused on the response of marine macroalgae to OA, which has greatly improved our understanding of the biological repercussions of OA (Koch et al., 2013). However, insufficient attention has been paid to non-calcareous species, particularly fleshy species, which are of significant ecological and economic value in coastal aquaculture. Among the OA-related studies on macroalgae, only a few have focused on photosynthesis and the physiology of aquaculture species (Tan et al., 2023). Several studies have shown that the effects of OA on marine organisms can be regulated by other covariate environmental drivers (Riebesell and Gattuso, 2015; Häder and Gao, 2023). Considering the multiple co-varied environmental factors in the context of climate change and local variations, the effect of OA in synergy with these environmental drivers on the growth, photosynthesis, yield, and quality of aquaculture species in coastal areas remains relatively unexplored.

Nutrient status is more diverse in coastal areas and aquaculture ponds due to global changes and aquaculture activity. Seawater warming may lead to enhanced stratification and a shallower upper mixed layer, which limits nutrient mixing from the bottom (Hutchins and Fu, 2017). In the context of global change, algal blooms caused by microalgae and macroalgae (green and golden tides) frequently occur in coastal waters, which also induce nutrient limitations and negatively affect nutrient availability (Feng et al., 2024). Therefore, the growth and productivity of seaweed in mariculture systems may be significantly affected by nutrient deficiency. Nitrogen limitation as a result of high biomass density cultures has been suggested to negatively influence the productivity of *G. lemaneiformis*, and seasonal fluctuations in N supplementation may further aggravate it, leading to a disconnection between the culture and the availability of dissolved nutrients in the seawater (Du et al., 2013; Martín et al., 2013). The marine macroalga *Gracilariopsis lemaneiformis* grown under nitrogen-limited conditions, exhibits significantly decreased growth and subsequently induces disease outbreaks (Wang et al., 2018). Variations in nutrient supply have significant implications for the phenotype, production, and quality of macroalgal cultivation (Celis-Plá et al., 2015; Ober and Thornber, 2017; Bao et al., 2019; Zhou et al., 2022; Li P. et al., 2023; Li T. et al., 2023).

Gracilariopsis lemaneiformis, a red alga (Rhodophyta) with high ecological and economic value, is widely recognized for its high nutritional value as a food or the main feed source for aquaculture animals (Zou and Gao, 2013). In addition, it serves as an important raw material in the agar industry owing to its high agar content (McHugh, 1991). Consequently, the yield of *G. lemaneiformis* cultivated offshore in China has been increasing annually, and some varieties have special characteristics such as high temperature resistance, high productivity, and high concentration of agar (Meng et al., 2009; Lu et al., 2012; Wang et al., 2016). However, considering the anticipated future ocean changes in coastal areas, understanding how *G. lemaneiformis* will respond to OA at different nitrate levels is of interest and requires further research. In this study, *G. lemaneiformis* was used to investigate the effects of OA on its growth, pigmentation, and photosynthetic

performance at low and high nitrate concentrations. The findings of this study offer fundamental insights and theoretical guidance for the cultivation of this algal species in different nutrient environments under conditions of seawater acidification.

2 Materials and methods

2.1 Algae culture condition

Gracilariopsis lemaneiformis thalli was collected from Nanao Island of Guangzhou Province, China, and was pre-cultured in laboratory condition with seawater collected from Southern China Sea of Southeast Asia Time-series Station (SEATS) (salinity 33 ppt, with almost undetectable phosphate and nitrate concentration and can be used as background nutrients) (Lu et al., 2020) enriched with Aquil medium (Morel et al., 1979) at 20°C and irradiance with fluorescent tube under 100 μmol $m^{-2}s^{-1}$ (L:D cycle of 12:12). For the experimental setup, thalli of ca. 0.1g in a 500 mL balloon flask were continuous bubbled with low (LC, 390 μatm) and high CO_2 (HC, 1000 μatm) air at flow rate of ca. 300 mL min^{-1} in respective CO_2 chamber (HP1000G-D, Ruihua, China) under modified Aquil Medium (Morel et al., 1979) that with low (ca. 7 μmol L^{-1}) and high (ca, 56 μmol L^{-1}) nitrate enrichment. Culture light and temperature were the same as those used in the pre-culture conditions. The LC and HC culture media were replaced with freshly prepared medium saturated with low and high CO_2 bubbling every other day. To maintain a consistent pH, the biomass of the thalli was regulated to not exceed 0.2 g following medium replacement in each treatment, ensuring that the pH fluctuation remained below 0.05 throughout each treatment. All relevant measurements were performed after 12 days of incubation, and each treatment had three biological replicates.

2.2 pH measurement

During the culture period, the pH values of the different CO_2 and nitrate treatments were measured every other day using a pH meter (Mettler Toledo DL15 Titrator, Sweden). Before pH measurement, 3 points calibration (pH 4.01, 7.01, and 10.01) was performed with NBS buffer solution (National Bureau of Standards, Hanna).

2.3 Relative growth rate measurement and determination of dry weight-fresh weight relationship

The relative growth rate (RGR) of the different treatments was determined by changes in fresh weight (FW) and was calculated using the following equation:

$$RGR = (\ln A - \ln B)/t$$

where, A and B indicate the fresh weights at the end and beginning of the culture period (t), respectively.

To determine the relationship between dry and fresh weight, ca. 0.03g to 0.14g thalli of each treatment were randomly collected during the culture and dried in an oven for 24 hours under 80°C. Dry weight change as a function of fresh weight was built with liner regression and the slopes of different treatments were obtained accordingly.

2.4 Pigmentation measurement

For the chlorophyll *a* (Chl *a*) and carotenoid measurement, ca. 0.05 g thalli were selected and extracted with methanol at 4°C overnight, and the supernatant were determined spectrophotometrically (DU800, Beckman, Fullerton, California, USA). Chl *a* and carotenoids were calculated following the methods described by Porra (2002) and Parsons and Strickland (1963). Phycoerythrin (PE) and phycocyanin (PC) determination, ca. 0.05 g thalli were randomly selected and ground into powder with 6 mL phosphate buffer (pH 6.80), and the supernatant was scanned with a spectrophotometer at wavelengths of 455 nm, 564 nm, 592 nm, 618 nm, and 645 nm; PE and PC were calculated using the equation from Beer and Eshel (1985).

2.5 NO_3^- uptake rate measurement

For the NO_3^- uptake rate measurement, 15 ml culture medium from different treatments were sampled at the beginning and after 24h of culture. NO_3^- concentration was determined using a nutrient autoanalyzer (QuAAtro, Bran-Luebbe Corporation, Germany).

2.6 Net photosynthesis and dark respiration measurements

Net photosynthetic oxygen evolution rates and dark respiration were measured using a Clark-type oxygen electrode (YSI 5300A, USA), and the methods which were detailed by Yang et al. (2021). Two points calibration (zero and 100%) of the oxygen electrode was performed prior to the measurements. Excess superfluous sodium sulfite (Na_2SO_3) added in distilled water to remove O_2 was used for zero-point correction, and distilled water bubbled with air until saturation was used for 100%-point correction. For the photosynthesis measurement, oxygen evolution rate was measured under culture light of 100 μmol m^{-2} s^{-1} and high light of 400 μmol m^{-2} s^{-1} (near saturation light intensity, according to the rapid light curve).

2.7 Induction cure and rapid light curve measurements

The induction curves of the different treatments were measured after 15 min of dark adaptation using an XE-PAM (Walz, Germany). The actinic light was set at 156 μmol m^{-2} s^{-1} and the saturation pulse intensity was 5000 μmol m^{-2} s^{-1}. For the rapid light curve measurement, thalli exposure under gradually increased actinic light of 156, 226, 337, 533, 781, 1077, 1593 and 2130 μmol m^{-2} s^{-1} for 10s in each light level, and subsequently with a saturation pulse. Maximum

quantum yield (F_V/F_M), was acquired from the first point of induction curve and effective quantum yield (Y(II)) and non-photochemical quenching (NPQ) were derived from the average of last 3 points of induction curve, and calculated using the following equation:

$$F_V/F_M = (F_M - F_0)/F_M$$

$$Y(II) = (F_M{}' - F_t)/F_M{}'$$

$$NPQ = (F_M - F_M{}')/F_M{}'$$

F_M represents the peak fluorescence attained after a 15-minute dark adaptation period. F_M' denotes the light-adapted maximum chlorophyll fluorescence observed under actinic light conditions. F_0 signifies the baseline fluorescence, while F_t indicates the stable fluorescence level maintained during the light exposures.

For the rapid light curve fitting, maximum relative electron transport rate ($rETR_{max}$), light use efficiency (α) and saturation light intensity (I_k) were detailed in Xu and Gao (2012) which was following the equation from Webb et al. (1974):

$$Y = rETR_{max} \times (1 - e^{-\alpha \times x/rETR_{max}})$$

$$I_k = rETR_{max}/\alpha$$

Y denote the relative electron transport rate (rETR), x represents the photon flux density, $rETR_{max}$ signify the peak electron transport rate, α symbolize the efficiency of light utilization, and I_k stand for the saturation light intensity.

2.8 Statistical analysis

Two-way or three-way analysis of variance (ANOVA) was used to determine the individual or interactive effects of CO_2, nitrate and light intensity on the observed parameters at a confidence level of 95% (P = 0.05), and Tukey's multiple comparisons were used to establish differences among treatments.

3 Results

3.1 pH variation during culture

The pH of different CO_2 and nitrate treatments were all kept in stable during the culture period, with averaged pH values of 8.20 ± 0.03, 7.84 ± 0.03, 8.22 ± 0.04 and 7.85 ± 0.04 under LC-LN, HC-LN, LC-HN and HC-HN condition (Figure 1).

3.2 Growth rate and dry weight-fresh weight relationship

The growth rate was significantly affected by both CO_2 (Two Way ANOVA, P< 0.0001) and nitrate concentrations (Two-Way ANOVA, P< 0.05) (Table 1). High CO_2 levels significantly decreased growth rate by 30.56% (P< 0.05) and 39.36% (P< 0.05) in the LN and HN treatments, respectively (Figure 2). No significant difference in growth rate was observed between LN and HN under HC conditions (P > 0.05); however, a significant decrease in growth rate of 25.86% was observed under LN conditions (P< 0.05) (Figure 2).

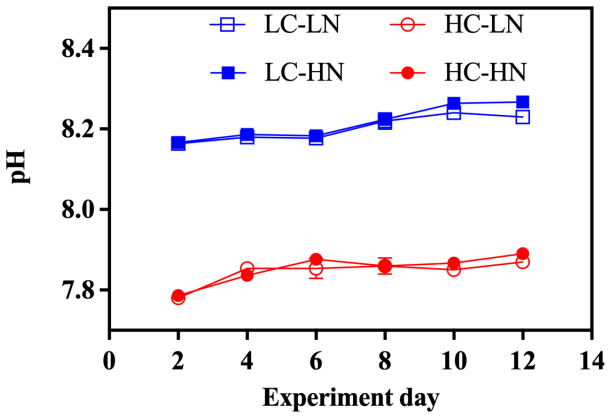

FIGURE 1
The measured pH of low CO_2 (LC) and high CO_2 (HC) culture under low nitrate (LN) and high nitrate (HN) conditions during the culture (n=3).

All treatments showed a significant linear relationship between dry and fresh weights (P< 0.0001) (Figure 3). Linear regression curves of dry weight as a function of fresh weight showed different slopes under different CO_2 and nitrate treatments (Figures 3A–D). Generally, HC (Figures 3C, D) showed higher slopes than LC cultures (Figures 3A, B), and LN (Figures 3A, C)-grown algae showed higher slopes than the HN treatments (Figures 3B, D), indicating that high CO_2 and low nitrate treatments increased the dry matter mass per unit fresh weight.

3.3 Pigmentations

After being grown under LN conditions for more than 12 days, the color of the algal body changed from reddish-brown to pale-yellow in both LC and HC, indicating that pigmentation was affected by different nitrate treatments (Figure 4). Chl *a*, carotenoids, PE, and PC were all significantly affected by CO_2 and nitrate concentrations (all P< 0.05, Two-Way ANOVA), except for Chl *a* which was not affected by nitrate (P > 0.05) (Table 1). Compared with the LC culture, HC significantly decreased Chl *a* by 15.07% (P< 0.05) and 17.18% (P< 0.05) under LN and HN conditions, respectively (Figure 5A). There were no significant changes in carotenoid, PE, and PC between LC and HC in LN cultures (all P > 0.05); however, HC decreased carotenoid, PE, and PC levels by 8.74% (P< 0.05), 28.59% (P< 0.05), and 21.99% (P< 0.05), respectively, in HN cultures (Figures 5B–D). No significant changes in Chl *a* and carotenoids were observed between LN and HN in both the LC and HC treatments (P > 0.05) (Figures 5A, B), except for a slight increase in carotenoid content in HN under LC conditions (P< 0.05) (Figure 5B). Compared with the HN culture, LN significantly decreased PE by 46.22% and 38.90% (P< 0.05)

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TABLE 1 Two- or three-way ANOVA analysis of individual and interactive effects of CO_2 (C), nitrate level (N), and irradiance treatments (L) on the studied parameters with confidence interval of 95% (P< 0.05).

Parameters	Treatment	F	P value	Significant
Growth	C*N	F (1, 8) = 4.24	0.07	N
	N	F (1, 8) = 12.62	0.01	Y
	C	F (1, 8) = 75.55	<0.0001	Y
Chl *a*	C*N	F (1, 8) = 0.37	0.56	N
	N	F (1, 8) = 5.04	0.06	N
	C	F (1, 8) = 39.01	<0.001	Y
Carotenoid	C*N	F (1, 8) = 1.01	0.34	N
	N	F (1, 8) = 19.48	<0.01	Y
	C	F (1, 8) = 28.98	<0.001	Y
PE	C*N	F (1, 8) = 5.73	0.04	Y
	N	F (1, 8) = 92.19	<0.0001	Y
	C	F (1, 8) = 25.24	0.001	Y
PC	C*N	F (1, 8) = 1.26	0.29	N
	N	F (1, 8) = 34.53	<0.001	Y
	C	F (1, 8) = 21.00	<0.01	Y
NO_3^-uptake rate	C*N	F (1, 8) = 31.79	<0.001	Y
	N	F (1, 8) = 68.27	<0.0001	Y
	C	F (1, 8) = 31.90	<0.001	Y
Dark respiration	C*N	F (1, 8) = 1.82	0.21	N
	N	F (1, 8) = 71.23	<0.0001	Y
	C	F (1, 8) = 12.11	0.01	Y
Photosynthesis	L	F (1, 16) = 244.7	<0.0001	Y
	C	F (1, 16) = 8.73	0.01	Y
	N	F (1, 16) = 0.45	0.51	N
	L*N	F (1, 16) = 0.01	0.92	N
	L*C	F (1, 16) = 0.02	0.89	N
	C*N	F (1, 16) = 6.10	0.03	Y
	L*C*N	F (1, 16) = 6.33	0.02	Y
F_V/F_M	C*N	F (1, 8) = 0.03	0.86	N
	N	F (1, 8) = 2.34	0.16	N
	C	F (1, 8) = 0.12	0.73	N
Y(II)	C*N	F (1, 32) = 4.08	0.05	N
	N	F (1, 32) = 190.5	<0.0001	Y
	C	F (1, 32) = 0.23	0.64	N
NPQ	C*N	F (1, 32) = 3.11	0.09	N
	N	F (1, 32) = 413.0	<0.0001	Y
	C	F (1, 32) = 6.05	0.02	Y
$rETR_{max}$	C*N	F (1, 8) = 0.04	0.84	N

(Continued)

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TABLE 1 Continued

Parameters	Treatment	F	P value	Significant
	N	F (1, 8) = 55.45	<0.0001	Y
	C	F (1, 8) = 0.003	0.96	N
α	C*N	F (1, 8) = 0.20	0.67	N
	N	F (1, 8) = 11.22	0.01	Y
	C	F (1, 8) = 0.86	0.38	N
I_k	C*N	F (1, 8) = 0.05	0.83	N
	N	F (1, 8) = 6.81	0.03	Y
	C	F (1, 8) = 0.47	0.51	N

"N" (not) indicates not significant, whereas "Y" (yes) indicates significant.

(Figure 5C), and PC by 26.97% and 23.47% (P< 0.05) under LC and HC conditions (Figure 5D).

3.4 NO_3^- uptake rate

The nutrient uptake rate showed different responses in CO_2 and NO_3^- treatments. Both the individual and interactive effects of CO_2 and nitrate concentrations on the NO_3^- uptake rate were observed (Two-Way ANOVA, all P< 0.05) (Table 1). Under the LN treatment, NO_3^- was consumed in both LC and HC culture after 24h of culture, and no significant difference in the NO_3^- uptake rate was observed between the CO_2 treatments (Figure 6). High CO_2 levels significantly stimulated the NO_3^- uptake rate under HN culture conditions (P< 0.05). Under HN treatments, a significant stimulation of the NO_3^- uptake rate by 259.44% (P< 0.05) was detected under HC compared with the LC treatment (Figure 6).

3.5 Net photosynthesis and dark respiration

High-light exposure significantly stimulated the net photosynthetic evolution rate of O_2 (Figures 7A, B). There was no significant change in the net photosynthetic evolution rate of O_2 between the CO_2 and nitrate treatments, either with exposure to low or high light (P > 0.05) (Figures 7A, B). Both the CO_2 and nitrate concentrations significantly affected the dark respiration rate, with a 24.23% increase (P< 0.05) (Table 1) under HC compared to the LC treatment under HN (Figure 7C). HN enhanced dark respiration rates by 55.18% (P< 0.05) and 65.38% (P< 0.05) under LC and HC conditions, respectively, compared to the LN culture (Figure 7C).

3.6 Fluorescence from rapid light cure and induction curve

Based on the measured rapid light curves (Figure 8A), the fitted parameters of the maximum relative electron transport rate ($rETR_{max}$), light-use efficiency (α), and saturation light intensity (I_k) were obtained (Table 2). There were significant effects of nitrate concentration (all P< 0.05), but not CO_2 (all P > 0.05) on the maximum of relative electron transport rate ($rETR_{max}$), light use efficiency (α), and saturation light intensity (I_k) (Table 1). In the LN culture, $rETR_{max}$ decreased by 26.24% (P< 0.05) and 24.95% (P< 0.05), and α by 16.72% (P< 0.05) and 12.53% (P< 0.05) in the LC and HC treatments, respectively, compared to the HN culture (Table 1). There was a slight but not significant increase in I_k under HN compared with the LN treatment (P > 0.05). No interactive effects of nitrate and CO_2 were detected on $rETR_{max}$, α or I_k (Two-Way ANOVA, P > 0.05) (Table 1).

According to the measured induction curve (Figures 8B, C), the F_V/F_M, Y(II), and NPQ values were acquired (Figures 8D–F). No significant changes in F_V/F_M were detected among the treatments (P > 0.05) (Figure 8D). Y(II) and NPQ were significantly affected by nitrate concentration (P< 0.05) (Table 1), with 29.83% (P< 0.05) and 38.55% (P< 0.05) decrease in Y(II) and 133.33% (P< 0.05) and 227.72% (P< 0.05) increase in NPQ in the LC and HC treatments, respectively, in comparison with the HN culture (Figures 8D, E). Significant effects of CO_2 on NPQ were observed in the HN

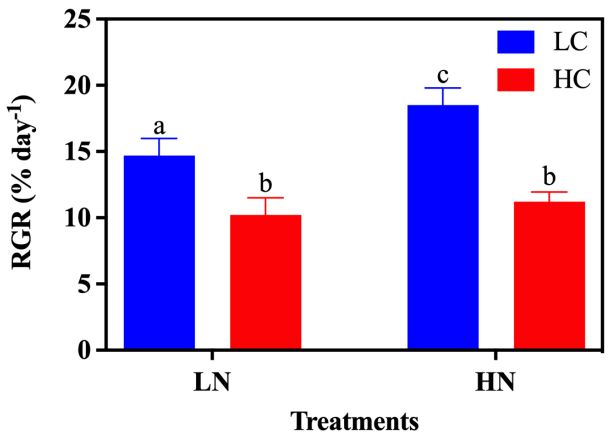

FIGURE 2
The relative growth rate (RGR) of *Gracilariopsis lemaneiformis* grown under different CO_2 and nitrate conditions (n = 3). Different lowercase letters above the bar indicate significant differences between treatments.

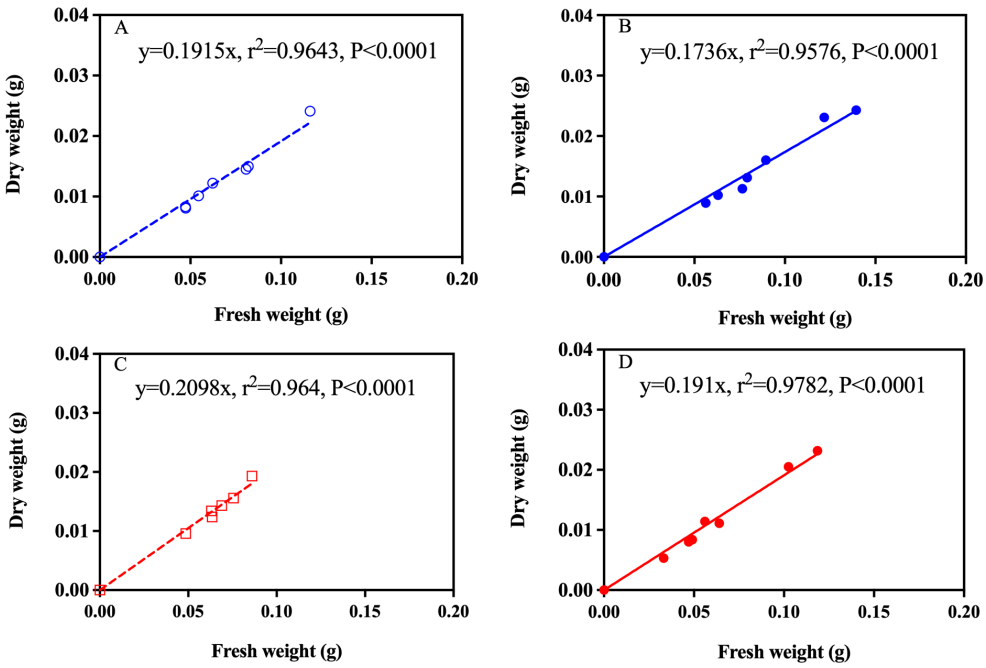

FIGURE 3
The linear regression of dry and fresh weights of *Gracilariopsis lemaneiformis* grown under **(A)** LC-LN, **(B)** LC-HN, **(C)** HC-LN, and **(D)** HC-HN conditions (n = 3).

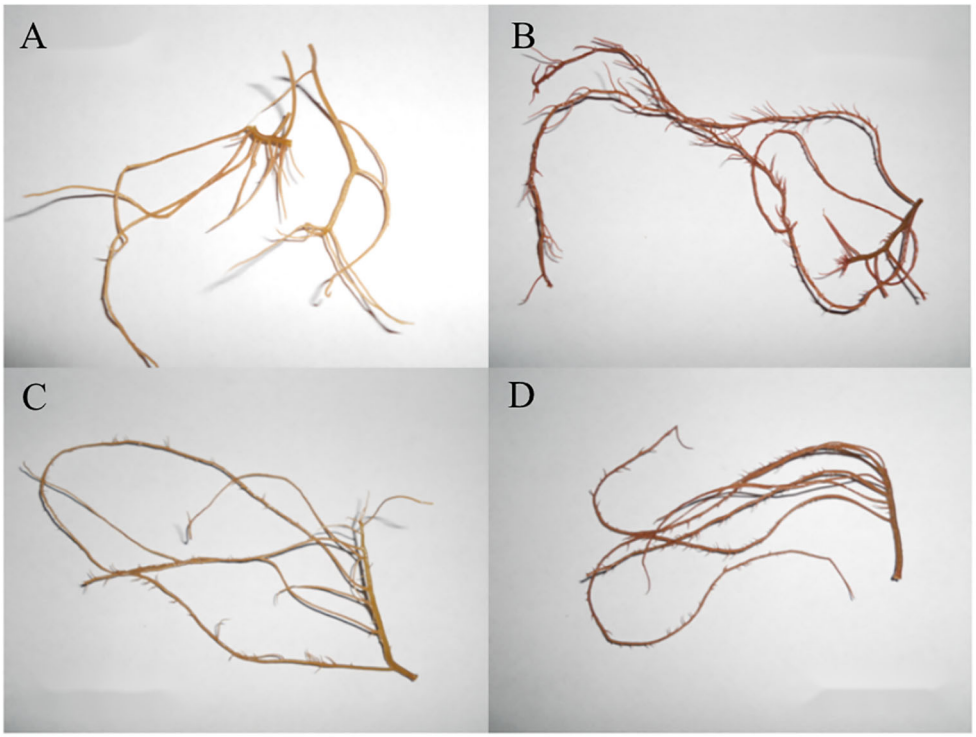

FIGURE 4
Photograph of *Gracilariopsis lemaneiformis* thalli grown under LC-LN **(A)**, LC-HN **(B)**, HC-LN **(C)**, and HC-HN **(D)** conditions for 12 days.

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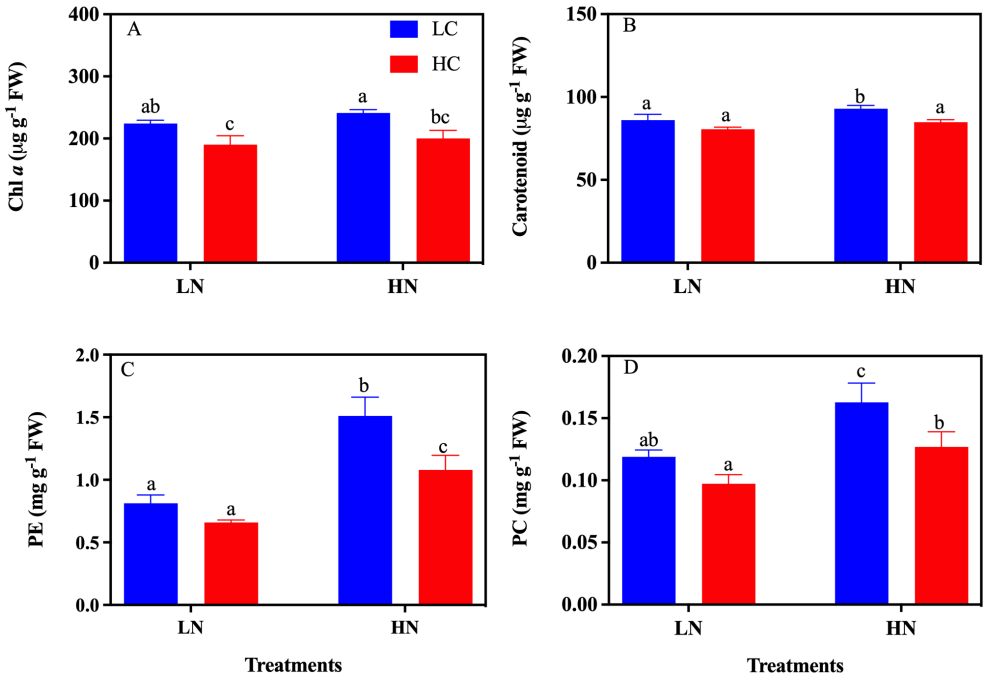

FIGURE 5
The pigment contents of *Gracilariopsis lemaneiformis* grown under different CO_2 and nitrate conditions (n = 3). **(A)** Chl *a*, **(B)** carotenoid, **(C)** phycoerythrin (PE), and **(D)** phycocyanin (PC). Different lowercase letters above the bar indicate significant differences between treatments.

treatment, with a 30.33% ($P< 0.05$) (Table 1) lower NPQ under HC than under LC culture (Figure 8F).

4 Discussion

Macroalgae are the major contributors to nearshore primary productivity, accounting for approximately 10% of global marine primary productivity (Smith, 1981). The large-scale cultivation of macroalgae is regarded as an important means of enhancing ocean carbon sink capacity, improving the eutrophication level in coastal waters, and mitigating climate change (Gao and McKinley, 1994; Duarte et al., 2017; Gao and Beardall, 2022). Macroalgae are subject to global challenges like ocean warming and elevated ultraviolet radiation, as well as regional influences including nutrient levels, salinity fluctuations, and light changes (Boyd et al., 2018; Ji and Gao, 2021). Therefore, investigating intricate coastal environmental shifts at a regional scale under global change may have significant implications on macroalgal cultivation.

Our results showed that high CO_2 levels had significant negative effects on the growth of *G. lemaneiformis* regardless of LN or HN conditions. Both high CO_2 and low nitrate treatments enhanced the dry mass of *G. lemaneiformis* relative to the fresh weight. Nitrate limitation lowered phycoerythrin (PE) and phycocyanin (PC) content and had significant effects on photosynthetic performance, including decreased dark respiration rate, effective quantum yield (Y(II)), maximum relative electron transport rate ($rETR_{max}$), and light use efficiency rate (α), and increased non-photochemical quenching (NPQ). High CO_2 synergy with high nitrate significantly decreased the pigmentation of *G. lemaneiformis* and NPQ, which stimulated the NO_3^- uptake rate and enhanced dark respiration.

Previous studies have shown that OA can have profound and differential effects on different types of marine macroalgae (Ji and Gao, 2021). For example, seawater acidification can significantly affect the calcification process and the abundance of most calcified algae (Gao et al., 1993; Kuffner et al., 2008), although other findings have indicated that the calcification rate of some calcifying species is not affected by the protective external layer or the creation of a microenvironment (Ji and Gao, 2021). Non-calcifying species, owing to the difference in inorganic carbon use among different types of

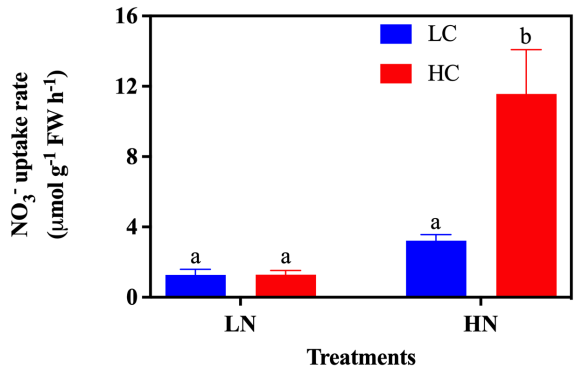

FIGURE 6
The NO_3^- uptake rate of *Gracilariopsis lemaneiformis* grown under different CO_2 and nitrate conditions (n = 3). Different lowercase letters above the bar indicate significant differences between treatments.

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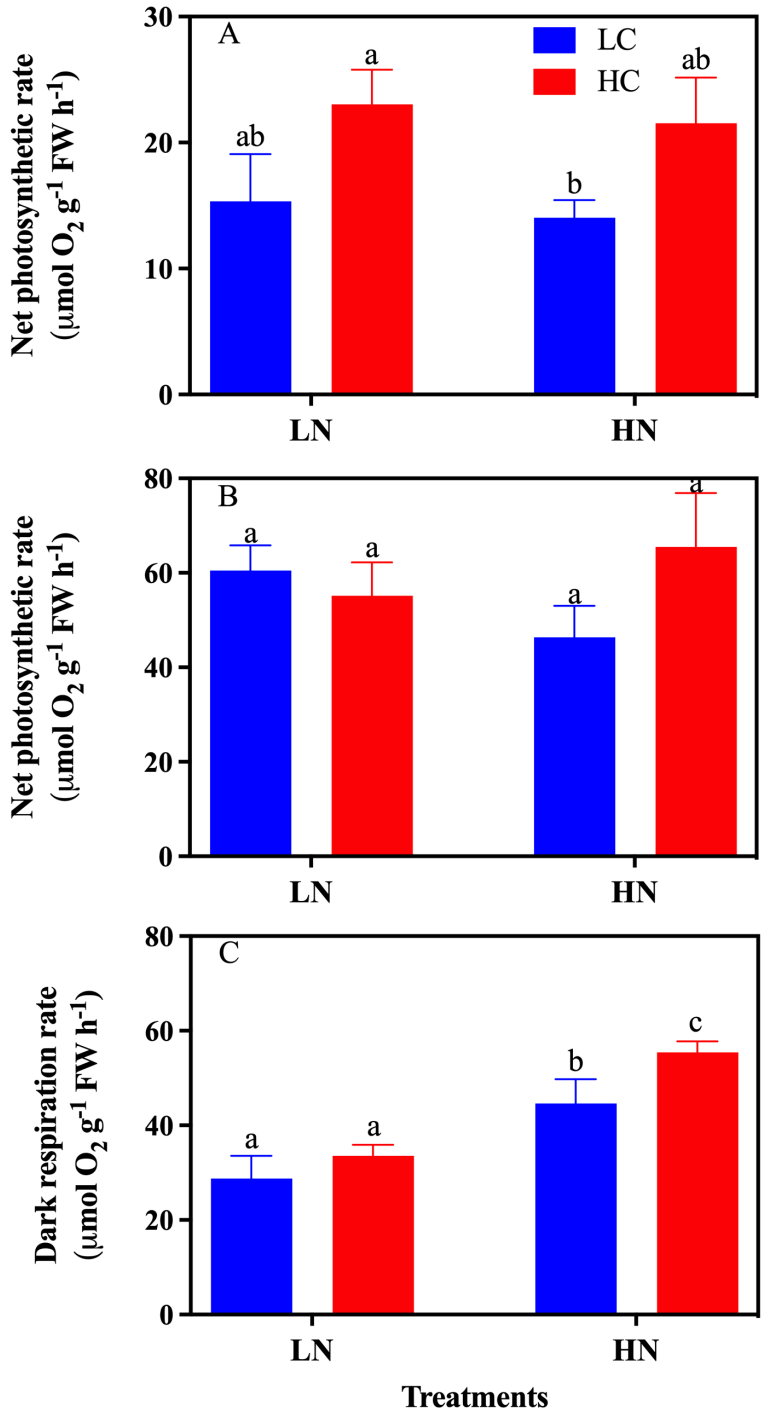

FIGURE 7
The net photosynthetic O_2 evolution rate and dark respiration of *Gracilariopsis lemaneiformis* grown under different CO_2 and nitrate conditions (n = 3). **(A)** 100 μmol $m^{-2}s^{-1}$, **(B)** 400 μmol $m^{-2}s^{-1}$, and **(C)** dark respiration rate. Different lowercase letters above the bar indicate significant differences between treatments.

macroalgae, exhibit diverse growth and physiological responses (Koch et al., 2013) which usually depend on the energy balance between synthesis and consumption (Häder and Gao, 2023). Considering the complex marine environment caused by global changes and local environmental variations, the responses of macroalgal growth and photosynthesis to OA exhibit interspecific differences (Ji and Gao, 2021). Even within the same species, different responses may arise when plants are exposed to different growth environments or cultivation systems. Farming of *G. lemaneiformis* in southern China usually begins from November to May of the following year. During the cultivation period, a series of environmental changes will be experienced, such as the change of seawater temperature from low to high (from 11°C to above 26°C in Nanao, Shantou, China), and the decrease of water nutrient

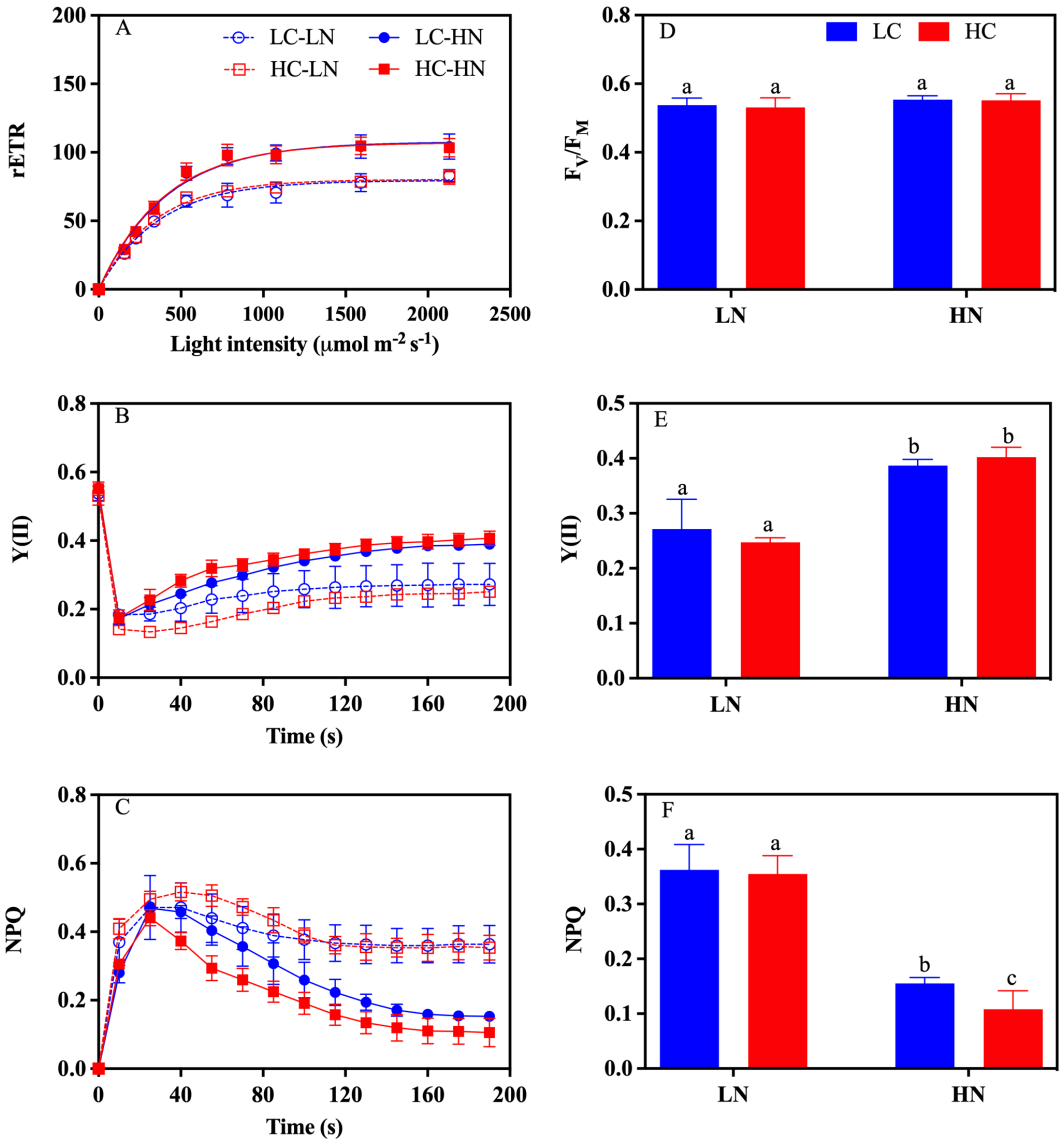

FIGURE 8
The chlorophyll fluorescence parameters of *Gracilariopsis lemaneiformis* under different CO_2 and nitrate conditions. **(A)** The rapid light curve, changes of **(B)** Y(II), and **(C)** NPQ from the induction curve and the **(D)** F_V/F_M, **(E)** Y(II), and **(F)** NPQ derived from the induction curve. Except for **(E)** Y (II) and **(F)** NPQ, where n=9, all other parameters have a sample size of 3 (n=3).

concentration and light intensity with the increase of cultivation density (Zou and Gao, 2009; 2014). Therefore, for the *G. lemaneiformis*, growth and photosynthesis are modulated and may show divergent responses to OA under co-varied environmental conditions. High CO_2 did not change the growth rate of *G. lemaneiformis* under both low and high nitrate condition when grown under natural solar light with average PAR level of 107.3 kJ m^{-2} (Zhou et al., 2022). Stimulated growth rates were observed in high CO_2 cultures under either low or high light conditions at a high culture temperature of 28°C (Chen et al., 2017). In the present study, high CO_2 levels had significant negative effects on the growth of *G. lemaneiformis* regardless of LN or HN conditions. There was no significant difference in photosynthetic oxygen evolution among the treatments, indicating saturation of the photosynthetic rate under the current seawater DIC and light levels (Yang et al., 2021). The respiration rate showed an increasing trend under both LC and HC compared to the LN treatment, with the increase being more pronounced under HC and HN conditions. This suggests that the energy fixed by algal photosynthesis is consumed at a higher rate

TABLE 2 The calculated maximum of relative electron transport rate ($rETR_{max}$), light use efficiency (α), and saturation light intensity (I_k) from the rapid light curve of Figure 8 (n = 3).

	$rETR_{max}$	α	I_k
LC-LN	79.52 ± 6.75^{a}	0.22 ± 0.01 ^{a}	361.51 ± 27.67 ^{a}
HC-LN	80.47 ± 2.15 ^{a}	0.24 ± 0.03 ^{a}	344.06 ± 48.24 ^{a}
LC-HN	107.80 ± 8.63 ^{b}	0.26 ± 0.01 ^{b}	407.34 ± 25.08 ^{a}
HC-HN	107.23 ± 6.25 ^{b}	0.27 ± 0.03 ^{b}	398.51 ± 26.59 ^{a}

Different superscript letters indicate significant difference between treatments.

under high CO_2/low pH conditions, potentially being exacerbated by stimulated photorespiration and other methods of energy consumption (Gao et al., 2012), thereby resulting in growth inhibition under the current culture conditions.

Under LN conditions, the absorption rate of NO_3^- was inhibited and exhibited no significant differences between the LC and HC treatments, suggesting that reduced nitrogen concentration in the aquatic environment has a more profound impact than elevated CO_2 levels and is the primary driving factor. Although HC treatment significantly enhanced the absorption rate of NO_3^- under HN conditions, it did not increase growth assimilation. This phenomenon is potentially associated with the partitioning of energy within the photosynthetic carbon and nitrogen metabolism, as the accumulation of carbon and nitrogen can be affected by OA (Chen et al., 2018).

The dry weight-fresh weight relationship in macroalgae varied among species (Wickham et al., 2019) and may differed when the environmental condition changed (Manns et al., 2017). The increased ratio of dry mass to fresh weight in *G. lemaneiformis*, induced by elevated CO_2 levels or diminished nitrate availability, signified a reduction in water content, suggesting an alteration in water retention capacity. This could be associated with modifications in the chemical composition of both soluble and insoluble organic substances, potentially including changes in the carbohydrate profiles and protein concentrations (Manns et al., 2017). A previous study showed that HC culture could increase the soluble carbohydrate content of *G. lemaneiformis* but decrease the soluble protein or AA contents (Chen et al., 2017, 2018). Where *Gracilariopsis* sp. grown in an extremely high inorganic C concentrations (5% CO_2) showed enhanced soluble carbohydrate concentration (Andria et al., 1999). Thus the composition and content of organic substances will change under different culture conditions, and subsequently determine the accumulation of organic matter (C and N) (Andria et al., 1999). Given that the chemical composition of *G. lemaneiformis* was not determined in this study, the effects of OA and low nitrogen on carbohydrates, proteins, and minerals remain to be explored in future research.

The pigment composition and content of macroalgae can be regulated by complex environmental changes, such as CO_2, light intensity, nutrients, and temperature, both individually and interactively (Häder and Figueroa, 1997; Zou and Gao, 2009; Yang et al., 2021; Zhou et al., 2022). Decreased photosynthetic pigments of Chl *a*, carotenoids, phycoerythrin (PE), and phycocyanin (PC) content due to high CO_2 under nitrogen-replete conditions in this study were in accordance with numerous previous studies (García-Sánchez et al., 1994; Andria et al., 1999). Synergistic effects of nutrient deficiency with high CO_2 have also been reported in previous studies; for example, Chl *a*, carotenoids, and PE were reduced by elevated CO_2 under N-and P deficiency condition (Zhou et al., 2022). In the present study, the transition of thalli color from reddish-brown to pale-yellow under nitrogen-limited conditions indicated that pigment composition and synthesis are regulated by nitrogen availability. In contrast to the general decline in pigment content observed under the HN treatment in HC conditions, the modest decrease in Chl *a* and the unchanged levels of carotenoids, PE, and PC under the LN treatment imply that nitrogen concentration predominantly influences the effect of OA on pigments. Specifically, the influence of low-nitrogen conditions on pigments appears to be greater than the effect of OA. Compared to the accessory light-harvesting pigments of carotenoids, PE, and PC, Chl *a* was not affected under the present LN conditions in comparison with HN, which suggests that energy allocation is preferential to the main light-harvesting pigment synthesis.

In the present study, high CO_2 levels did not change F_V/F_M and Y(II). The unchanged F_V/F_M under LN treatment suggests that the open Photosystem II is unaffected. However, when the actinic light intensity was close to the culture conditions, the decrease in photochemical efficiency indicated that actual photochemical capacity was significantly inhibited by LN. Simultaneously, the reduction in $rETR_{max}$ showed that nitrogen limitation had a significant effect on the electron transport chain of Photosystem II. An increase in NPQ generally characterizes the ability of algal photosynthetic mechanisms to dissipate excess light energy in the form of heat, which is an important photoprotective mechanism (Muller et al., 2001; Ruban, 2016; Stadnichuk and Krasilnikov, 2023). Under low nitrogen conditions, a higher NPQ indicates that the excitation energy generated by light quanta absorbed by the photosynthetic mechanism is in an excess state under the current cultivation light intensity.

5 Conclusions

Our findings suggest that OA may negatively affect the growth of *G. lemaneiformis*, with photophysiological performance differentially modulated by nutrient status. These results contribute to a deeper understanding of the potential effects of ongoing oceanic changes in coastal zones, caused by regional and global changes in marine primary producers, particularly within the macroeconomic context of the seaweed farming industry. Our study highlights the need for further research, both controlled indoor experiments and field investigations, to unravel the intricate interplay between OA and nutrient limitation in macroalgae in the context of global change.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

YY: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. WL: Writing – review & editing, Visualization, Resources, Methodology, Formal analysis,

Conceptualization. YL: Writing – review & editing, Visualization, Methodology. NX: Writing – review & editing, Visualization, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Publisher's note

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