



Ocean warming interacts with elevated nitrogen to modulate carbon storage-excretion balance in *Gracilariopsis lemaneiformis*

Jichen Chen^{a,b}, Chi Song^a, Siwei Tang^a, Sofia Delira^c, Hongmei Chen^{a,*}, Guang Gao^{a,b,**} 

^a State Key Laboratory of Marine Environmental Science, College of Ocean and Earth Sciences, Xiamen University, Xiamen, 361005, China

^b Ocean Decade International Cooperation Center, Qingdao, 266000, China

^c School of Arts and Sciences, Tufts University, Medford, MA, USA

ARTICLE INFO

Keywords:

Cellular carbon allocation
Dissolved organic carbon
Eutrophication
Carbon dioxide removal
Global warming
Seaweed farming

ABSTRACT

Macroalgae farming represents a proposed approach for carbon dioxide removal (CDR), as macroalgae exhibit distinct advantages over other blue carbon taxa in cultivation scalability and cost effectiveness. Yet how future warming and coastal nitrogen enrichment will jointly affect macroalgal carbon fixation and sequestration remains poorly understood. Here, we investigated physiological and biochemical responses (growth, photosynthesis, pigment content, and carbon accumulation and excretion) of the commercially important seaweed *Gracilariopsis lemaneiformis* under a cross-factorial design of two temperatures (LT, 20°C; HT, 23°C) and two nitrate concentrations (LN, 8 µM; HN, 200 µM). By day 30, HN alone significantly increased growth, photosynthetic activity, pigments, phycobiliproteins, and particulate organic carbon/nitrogen (POC/PON) accumulation, but did not alter dissolved organic carbon (DOC) production rates or DOC/refractory DOC (RDOC) accumulation. Notably, HT alone had no significant effect on growth, POC accumulation or DOC/RDOC accumulation by day 30, but markedly promoted POC accumulation when combined with HN. These findings highlight a shift in carbon partitioning towards cellular storage (POC) rather than secretion (DOC) under future scenarios of combined ocean warming and elevated nitrogen levels. This study provides critical insights into using macroalgae farming as a potential CDR strategy through POC export to the deep ocean under concurrent climate change and coastal eutrophication.

1. Introduction

Anthropogenic CO₂ emissions have totaled 2400 ± 240 Gt CO₂ between 1850 and 2019, with 42% released in the past three decades (IPCC, 2023). These emissions have driven global warming to 1.1°C above past levels (1850–1900) by 2011–2020, exacerbating ocean acidification, extreme weather events, and urban extreme heat risk (Baker et al., 2018; Harrould-Kolieb and Herr, 2012; IPCC, 2023; Yang et al., 2021b). Since 1950, the upper 2000 m of the global ocean has warmed significantly, accumulating 351 ± 59.8 ZJ of heat, with projections of a 2–6 fold increase by 2100 (Cheng et al., 2022). Continuous warming is also projected to increase the frequency of ENSO and La Niña events (Cai et al., 2015). To address climate change, the international community has reached a landmark agreement through joint efforts. The Paris Agreement establishes a central target of limiting global warming to 2°C above

pre-industrial levels by the end of the century, with an aspirational goal of capping warming at 1.5°C.

To meet these targets, carbon dioxide removal (CDR) has become essential (Chiquier et al., 2022). The ocean, Earth's largest carbon sink, absorbs 25% of anthropogenic CO₂ emissions (Le Quéré et al., 2010). Blue carbon ecosystems (e.g., mangroves, salt marshes, and seagrasses) sequester 25.8–40.6 Tg C yr⁻¹ globally, but their potential is limited by restricted distribution (Gao et al., 2022a). In contrast, macroalgae cultivation offers a more feasible and impactful approach to blue carbon sequestration, with higher capacity and broader distribution in suitable regions (Gao et al., 2022a). Notably, macroalgae contribute an estimated 173 Tg C yr⁻¹ to global carbon sequestration, with RDOC and sinking POC accounting for 68% and 32%, respectively (Krause-Jensen and Duarte, 2016). Labile DOC fuels the microbial loop, while its refractory fraction (RDOC) functions as a long-term carbon reservoir (>100 years). In contrast, POC represents a pool with sequestration

* Corresponding author.

** Corresponding author. State Key Laboratory of Marine Environmental Science, College of Ocean and Earth Sciences, Xiamen University, Xiamen, 361005, China.

E-mail addresses: chenhm@xmu.edu.cn (H. Chen), guang.gao@xmu.edu.cn (G. Gao).

<https://doi.org/10.1016/j.marenvres.2026.108341>

Received 30 May 2026; Received in revised form 30 July 2026; Accepted 8 August 2026

Available online 10 August 2026

0141-1136/© 2026 Elsevier Ltd. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

potential, primarily through gravitational export to the deep sea or burial in algal beds and shelf sediments though its effect remains uncertain (Gao et al., 2022b; Hurd et al., 2023).

In addition to CO₂ emission, consistent nutrient inputs since the late 1970s have caused coastal eutrophication, notably an increasing N:P ratio (Wang et al., 2018). Coastal eutrophication is mainly driven by agricultural runoff, aquaculture, and urban discharge (Lan et al., 2024), resulting in significant economic losses to public health, commercial fisheries, recreation, and ecosystem management (Gao et al., 2025; Wurtsbaugh et al., 2019). For example, in the EU, eutrophication triggered toxic phytoplankton blooms have been causing negative impacts on fisheries, coastal ecosystems, and public health, leading to economic losses of approximately US\$ 1 billion annually (Malone and Newton, 2020). A global assessment using Google Earth Engine found that approximately 1.15 million km² of coastal waters (depth ≤ 200 m) have eutrophic potential (Maure et al., 2021). Rising dissolved inorganic nitrate concentrations further increase the risk of harmful algal blooms in the East China Sea, the Changjiang Diluted Water, and Bohai Sea (Wang et al., 2021; Zhou et al., 2022). Macroalgae farming synergistically addresses these challenges by serving as a marine CDR strategy, mitigating coastal eutrophication, and thereby promoting overall ecosystem health (Gao et al., 2022a; Tian et al., 2023).

Temperature critically influences carbon fixation by modulating enzyme activity. For example, in the red alga *Grateloupia turuturu*, photosynthetic carbon fixation efficiency shows a unimodal response to temperature, peaking at 20°C and declining toward 30°C (Shen et al., 2025). Beyond its effect on fixation, warming also exacerbates respiratory carbon loss (Cavan and Boyd, 2018), and can stimulate DOC release (Bennett et al., 2024). Regarding the interactive effects of warming and nitrogen enrichment, although physiological responses have been investigated (Fernández et al., 2020; Ma et al., 2021; Wu et al., 2022a), it remains poorly understood how the interaction of future warming and eutrophication reshapes the balance between carbon release and biomass storage in seaweeds, ultimately governing the pathways via which seaweeds function as a marine carbon dioxide removal (mCDR) strategy.

China leads global seaweed mariculture, with the proportion of *Gracilariopsis lemaneiformis* increasing from 4% in 2003 to 20% in 2023, contributing the second largest production now (just behind *Saccharina japonica*) (Yang et al., 2026). Among main mariculture seaweeds, *G. lemaneiformis* exhibits the highest carbon removal capacity and sequestration capacity, thus requiring the smallest cultivation area to achieve China's carbon neutrality goal by 2060 (Gao et al., 2022b; Yang et al., 2026). Additionally, *G. lemaneiformis* has the highest nitrogen removal capacity among farmed seaweeds, making it particularly relevant for mitigating coastal eutrophication, a pressing environmental issue in southern China (Gao et al., 2022b). Considering the warming trend during the cultivation period, whether seasonal warming will amplify or mitigate the impact of nitrogen on carbon storage remains incompletely understood. We hypothesize that ocean warming and high nitrogen levels will individually affect carbon fixation and sequestration, with their interaction modifying these effects. To test this, we cultured *G. lemaneiformis* under factorial temperature (20°C, 23°C) and nitrate (8 μM, 200 μM) treatments and investigated its physiological and biochemical responses. Notably, this study focuses on farmed *G. lemaneiformis* rather than wild populations because farmed *G. lemaneiformis* has a wider distribution and greater biomass, conferring greater ecological significance for marine CDR.

2. Materials and methods

2.1. Seaweed collection and culture conditions

Gracilariopsis lemaneiformis was collected in April 2023 from a farming area in Ningde, Fujian Province, China (119.31°E, 26.39°N). In this region, *G. lemaneiformis* in Ningde undergoes two annual cultivation

cycles: from fall to spring (September to May in next year). Previous studies have reported an average seawater temperature of 20°C from fall to spring in the farming area (Jiang et al., 2022; Wu et al., 2015; Yang et al., 2025; Zhang et al., 2024). Prior to experimentation, thalli were rinsed three times with sterile seawater to remove epiphytes and debris, following a previously described protocol (Chen et al., 2025; Jiang et al., 2024). Natural seawater collected from coastal waters near Jinjiang was used as the culture medium. The seawater was aged in darkness at 20°C for 30 days and then filtered through a 0.1 μm pore-size filter to deplete background labile DOC, remove particulate matter, and reduce microbial load. No antibiotic sterilization was performed, allowing the algae to retain their associated microbial community as found in the natural environment. To standardize physiological baselines and enhance sensitivity to nitrate concentration changes, thalli were subjected to a 7-day nitrogen starvation period prior to the experiment (Chen et al., 2025; Kamer and Fong, 2001). For the experiment, thalli (0.5 g per bottle) were cultured in 1 L sterilized polycarbonate bottles containing aged, sterile seawater. Temperature was maintained at 20°C, and light intensity was set to 100 μmol photons m⁻² s⁻¹ under a white light spectrum, with a 12:12 h light-dark cycle. This light intensity is widely used as the ambient PAR setting in *G. lemaneiformis* cultivation experiments and is generally sufficient to achieve light-saturated or near-saturated photosynthetic rates, whereas higher irradiance may lead to photoinhibition (Gao et al., 2024; Jiang et al., 2020; Ma et al., 2021; Wang et al., 2025; Yang et al., 2021a).

A 2×2 factorial design was used to test the effects of two independent variables: temperature and nitrate concentration (Table 1), with three replicates per treatment. Each replicate consisted of one culture bottle containing about three individuals, each approximately 10 cm in length. The lower temperature and nitrate represent ambient conditions in seaweed farming from fall to spring. This higher temperature (+3°C) corresponds to the model projection for the year 2100, based on the intermediate Shared Socio-economic Pathway (SSP2-4.5) for greenhouse gas emissions (IPCC, 2023). The higher nitrate condition falls within the range of eutrophic coastal waters (Teichberg et al., 2010). Phosphorus was set to 50 μM to avoid phosphorus limitation during the experiment, ensuring that all physiological responses of *G. lemaneiformis* were driven solely by nitrate. Notably, the aim of this study was not to propose fertilization strategies for macroalgae cultivation, but to investigate the potential effects of progressively worsening eutrophication scenarios on cellular carbon storage and excretion of *G. lemaneiformis*. To maintain sufficient nutrient supply while maximally preserving the phycosphere bacterial community, half of the culture medium was replaced with fresh and sterile seawater containing equivalent nutrients every two days (on days 2, 4, 6, ..., 10, 12, ..., 20, 22, ..., 30) (Jiang et al., 2024).

2.2. Measurement of specific growth rate (SGR)

Before weighing, all thalli were blotted with tissue paper to remove the surface water on the tissue. Fresh weight (FW) of *G. lemaneiformis* was measured on days 0, 7, 10, 17, 20, 27, and 30 using an analytical balance (BSA124S, Sartorius, Germany). To minimize disturbance from tissue harvesting, FW was measured prior to other analyses at each time

Table 1
Experimental groups design.

Groups	Temperature (°C)	NO ₃ -N (μM)
LTLN	20	8
LTHN	20	200
HTLN	23	8
HTHN	23	200

Abbreviations: LT, low temperature (ambient); HT, high temperature (projected for 2100 (IPCC, 2023)); LN, low nitrate concentration (ambient); HN, high nitrate concentration (eutrophic).

point, and the harvested tissue was excluded from subsequent growth rate calculations.

Specific growth rate (SGR) was calculated using the following equation:

$$\text{SGR} (\% \text{ d}^{-1}) = \ln(W_2 / W_1) / (t_2 - t_1) \times 100. \quad (1)$$

W_1 and W_2 represent the FW in t_1 (day 1) and t_2 (day 2), respectively.

2.3. Measurement of chlorophyll fluorescence of photosystem II (PSII)

Apical tips (2 cm in length, 0.05 g FW) of *G. lemaneiformis* were collected on days 0, 10, 20, and 30 for photosynthetic analysis using a multi-color pulse amplitude modulation chlorophyll fluorometer (Multi-Color-PAM; Walz, Germany). The measurement protocol was consistent with our previous study (Chen et al., 2025). Prior to measurement of the maximum quantum yield of photosystem II (Fv/Fm) and non-photochemical quenching (NPQ), samples were dark-adapted for 15 min to allow full relaxation of NPQ, following the protocol of Maxwell and Johnson (2000). Fv/Fm was calculated as $(F_m - F_o)/F_m$, and NPQ as $(F_m - F_m')/F_m$, where F_m and F_o denote the maximum and minimum chlorophyll fluorescence of dark-adapted samples, respectively, and F_m' represents the maximum fluorescence yield under actinic light. The actinic light intensity was set to match the culture conditions ($100 \mu\text{mol photons m}^{-2}\text{s}^{-1}$), and a saturating pulse ($2500 \mu\text{mol photons m}^{-2}\text{s}^{-1}$; 0.8 s duration) was applied to ensure the complete reduction of all PSII reaction centers for accurate determination of maximal fluorescence (F_m) (Maxwell and Johnson, 2000). After measurement, samples (approximately 0.1 g FW) were collected and immediately frozen for subsequent analysis of pigments and phycobiliproteins.

2.4. Measurement of pigments and phycobiliproteins

The samples collected from the chlorophyll fluorescence measurements described above were used for the determination of pigment and phycobiliprotein contents. Pigments were extracted by immersing each fresh sample (0.05 g FW) in 10 mL of 95% ethanol (a ratio of 200 mL g⁻¹ FW) and storing it in complete darkness at 4°C for 24 h to ensure complete pigment leaching (Liu et al., 2016). The extracts were then centrifuged, and the supernatant was analyzed spectrophotometrically for pigment concentrations following the equations of Wellburn (1994).

$$\text{Chl } a (\text{mg g}^{-1} \text{ FW}) = 13.36 \times A_{665} - 5.19 \times A_{649} \quad (2)$$

$$\text{Carotenoids} (\text{mg g}^{-1} \text{ FW}) = (1000 \times A_{470} + 904.788 \times A_{665} - 3138.3245 \times A_{649}) / 245 \quad (3)$$

Phycocyanin (PC) and phycoerythrin (PE) were extracted and quantified as described by Beer and Eshel (1985). Each sample (0.05 g FW) was homogenized in 100 mL of phosphate buffer (pH 6.8) on ice. Following centrifugation at $6000 \times g$ for 10 min at 4°C, the supernatant was collected for absorbance readings at 455, 564, 592, 618, and 645 nm. The respective concentrations were calculated using the following equations:

$$\text{PC} (\text{mg g}^{-1} \text{ FW}) = [(A_{618} - A_{645}) - (A_{592} - A_{645}) \times 0.51] / 0.15 \quad (4)$$

$$\text{PE} (\text{mg g}^{-1} \text{ FW}) = [(A_{564} - A_{592}) - (A_{455} - A_{592}) \times 0.2] / 0.12 \quad (5)$$

2.5. Measurement of algal POC, PON contents, and C: N molar ratio

Thallus samples (0.02 g FW) were collected on days 0, 10, 20, and 30, washed with sterilized seawater, and oven dried at 60°C to constant weight. After initial drying, the samples were subjected to acid fumigation with 36% HCl in a nearly sealed environment for 24 h at room temperature (approximately 22°C) to remove inorganic carbon, and

then re-dried at 60°C till constant weight. Subsequently, the samples were stored in a desiccator at room temperature until measurement. For elemental analysis, approximately 0.8 mg of each sample (taken from the tip) was packed into a tin capsule. POC and PON contents were measured with an elemental analyzer (Vario EL Cube, Elementar, Germany), and the C:N ratio was derived from their molar values. POC and PON refers to the organic carbon/nitrogen content of the harvested seaweed thallus.

The following equation calculates the POC and PON accumulation contents:

$$\Delta\text{POC} (\text{mg C g}^{-1} \text{ DW}) = (W_t \times C_t - W_0 \times C_0) / W_0 \quad (6)$$

$$\Delta\text{PON} (\text{mg N g}^{-1} \text{ DW}) = (W_t \times N_t - W_0 \times N_0) / W_0 \quad (7)$$

The variables W_t and W_0 designate the DW (dry weight) at time t (days 10, 20, 30) and at day 0, respectively. Similarly, C_t , N_t , C_0 and N_0 represent the POC or PON content at time t and at day 0.

2.6. Measurement of DOC and RDOC concentration

Water samples (30 mL) were filtered through pre-combusted GF/F filters, and the filtrate was preserved in pre-combusted amber bottles at -20°C. Dissolved organic carbon (DOC) concentration was determined using a Shimadzu TOC-LCPH analyzer by catalytic oxidation at 680°C. Recalcitrant DOC (RDOC) was operationally defined as the DOC fraction remaining after 60 days of dark incubation at respective cultivation temperatures, a duration sufficient to achieve full degradation of bioavailable DOC fractions (Kristensen et al., 1992; Liu et al., 2025; Wada et al., 2008). Previous studies reported that the labile DOC released by the red alga *Chondrus crispus*, the brown alga *Ecklonia cava*, and open ocean water can be completely consumed within 60 days (Kristensen et al., 1992; Liu et al., 2025; Wada et al., 2008). To verify this, a dark incubation experiment was conducted using DOC derived from *G. lemaneiformis* at 17°C, 20°C, and 23°C, in which the labile DOC fraction was completely depleted by day 60 (Fig. S1).

The DOC production rate was calculated using the following equation:

$$\text{DOC production rate} (\text{mg C g}^{-1} \text{ DW d}^{-1}) = (\text{DOC}_{t1} - \text{DOC}_{t0}) \times V \times 12 / (W_{t0} \times 1) \quad (8)$$

The DOC production rate over a 24-h period was calculated for each phase. DOC_{t0} and W_{t0} represent the DOC concentration (mol L^{-1}) and initial DW measured on days 0, 10, 20, and 30, respectively. DOC_{t1} corresponds to the DOC concentration measured on the following day (days 1, 11, 21, and 31). V represents the culture volume (1 L); 1 represents in one day. No water exchange was conducted during the 24-h period.

The calculation equation of RDOC transformation rate is:

$$\text{RDOC transformation rate} (\%) = (\text{DOC}_{t2} - \text{DOC}_{s2}) / (\text{DOC}_{t1} - \text{DOC}_{s1}) \times 100 \% \quad (9)$$

DOC_{t1} and DOC_{t2} represent the DOC concentration in the culture liquid prior to and following the dark treatment, respectively. Similarly, DOC_{s1} and DOC_{s2} represent the corresponding DOC concentrations in the seawater.

The calculation equation of DOC accumulation (ΔDOC) and RDOC accumulation (ΔRDOC) contents is shown as follows:

$$\Delta\text{DOC} = \sum_1^t \Delta\text{DOC}_t \quad (10)$$

$$\Delta\text{RDOC} = \sum_1^t (\Delta\text{DOC}_t \times [R_{t0} + (R_{t10} - R_{t0}) \times (t - t_0) / 10]) \quad (11)$$

$$\Delta\text{DOC}_t (\text{mg C g}^{-1} \text{ DW} \cdot \text{d}^{-1}) = P_{t0} + (P_{t10} - P_{t0}) \times (t - t_0) / 10 \quad (12)$$

Here, t represents the cultivation time in days. P_{t0} and P_{t10} are the DOC production rates measured at the beginning and the end of the time interval containing day t , respectively. Similarly, R_{t0} and R_{t10} represent the RDOC transformation rates at the beginning and end of that interval.

2.7. Measurement of bacterial abundance

To ensure that the phycosphere bacteria of *G. lemaneiformis* constituted the sole microbial source, rigorous aseptic techniques were conducted throughout the experiment. Seawater, glassware, and all consumables, such as centrifuge tubes and pipette tips, were sterilized before use. Bacterial samples were collected on days 11, 21, and 31. Each sample was divided into two aliquots: one was used to determine the real-time bacterial abundance during the culture period, whereas the other was stored in darkness at respective cultivation temperatures for 60 days for subsequent analysis of bacterial abundance in the RDOC phase. Bacterial abundance (cells mL^{-1}) was quantified by flow cytometry (BD Accuri C6, Accuri Cytometers, USA). Briefly, 1.6 mL of culture solution was collected at each time point and fixed with 400 μL of 2.5% glutaraldehyde, yielding a final concentration of 0.5% glutaraldehyde, which is widely recognized to preserve the structural integrity of bacterial cells (Gasol and Giorgio, 2000; Kepner and Pratt, 1994; Marie et al., 2001). Fixed samples were preserved at -80°C . For analysis, 10 μL of the fixed sample was diluted with 990 μL of water, stained with 5 μL of SYBR Green I working solution ($50\times$) in the dark for 20 min, and measured at an excitation wavelength of 488 nm. To ensure the accuracy, the fluorescence of unstained samples was calculated and subtracted to avoid autofluorescence interference. The measured total bacterial abundance, though inclusive of non-viable cells, suggests enhanced availability of labile organic matter (such as macroalgal DOC). Total bacterial abundance includes non-viable cells and should be interpreted as a proxy for potential microbial biomass supported by labile DOC, rather than as a direct measure of metabolic activity or remineralization. Thus, these data are consistent with the presence of a larger potential microbial biomass supported by algal secretions.

2.8. Statistical analysis

All data are expressed as mean $\pm$ SD of three independent replicates. Statistical significance was analyzed with SPSS 25.0 software (IBM, USA). Repeated-measures ANOVA (RM-ANOVA) was used to evaluate the effects of time and its interaction with temperature and nitrate concentration on following parameters: SGR, Fv/Fm, NPQ, Chl a , carotenoid, PC, PE, POC, PON, C: N molar ratio, ΔPOC , DOC, DOC production rate, ΔDOC , RDOC, RDOC transformation rate, ΔRDOC , and bacterial abundance. Two-way ANOVA (TW-ANOVA) was conducted to analyze the effects of temperature and nitrate concentration on these parameters at each time point. Post-hoc comparisons were conducted using the LSD test. The significance level was set at $p < 0.05$ under homogeneity of variance (Levene's test is met); otherwise, a $p < 0.01$ threshold was adopted if Levene's test is not met (Gao et al., 2017; Pallant, 2010). All figures were created using the software Origin 8.5 and PowerPoint.

3. Result

3.1. Specific growth rate and photosynthesis response

The SGR of *G. lemaneiformis* declined throughout the experiment, though the declines were greatest when nitrate concentrations were low (Table S1, Fig. 1A). Temperature had little effect on SGR except for a significant interaction on day 17 when a negative effect was detected with increased nitrate (Table S2). Increased nitrate significantly increased the SGR at both temperatures, except on day 7.

Time had a significant interactive effect with nitrogen concentration on the Fv/Fm during cultivation period (Table S1). The Fv/Fm of

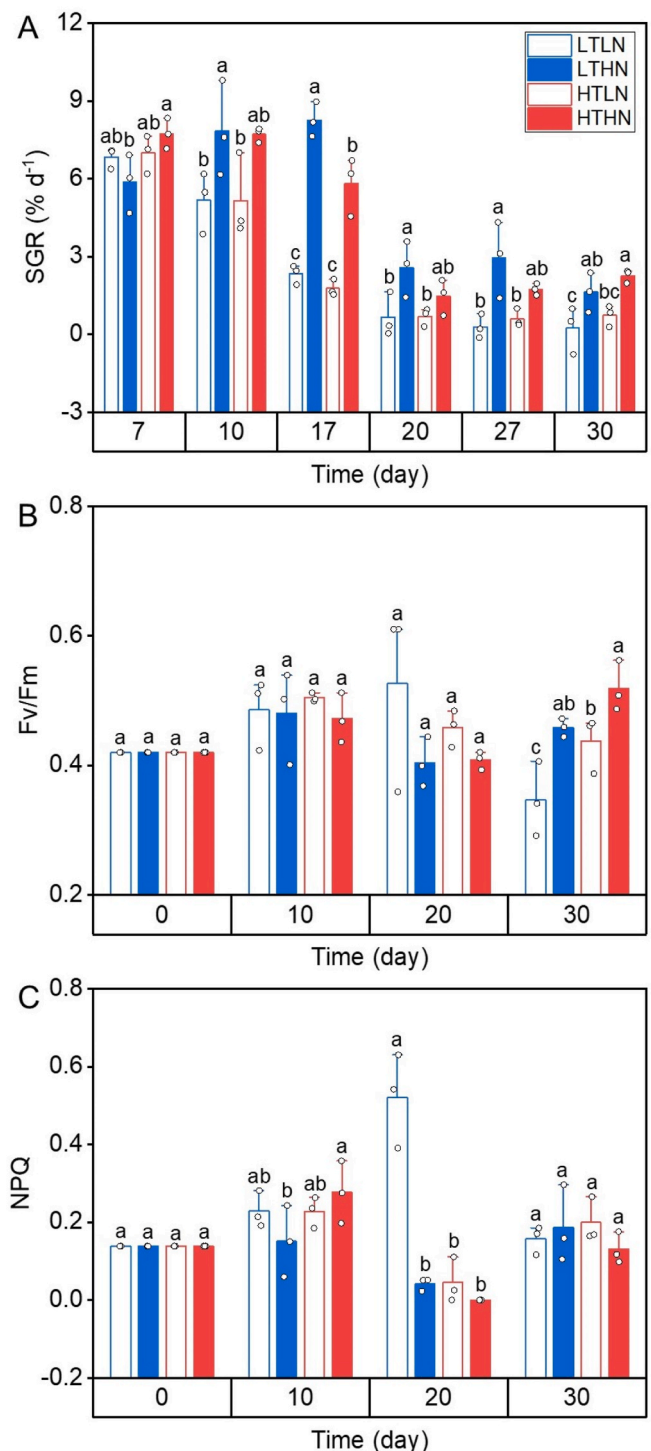


Fig. 1. Specific growth rate (SGR) (A), Fv/Fm (B), and NPQ (C) of *G. lemaneiformis* grown under different temperature and nitrate conditions. Abbreviations: LT, 20°C treatment; HT, 23°C treatment; LN, low nitrate level; HN, high nitrate level; Different letters indicate that there are significant differences among different treatment groups at corresponding time point ($p < 0.05$ if Levene's test is met, otherwise $p < 0.01$).

G. lemaneiformis remained relatively stable across the culture period, with the control group exhibiting the greatest fluctuation (Fig. 1B). By day 30, both high temperature and nitrate promoted Fv/Fm, with their combination resulting in the highest value.

Over the 30 days period, the NPQ of *G. lemaneiformis* was significantly influenced by the interaction of time with temperature and

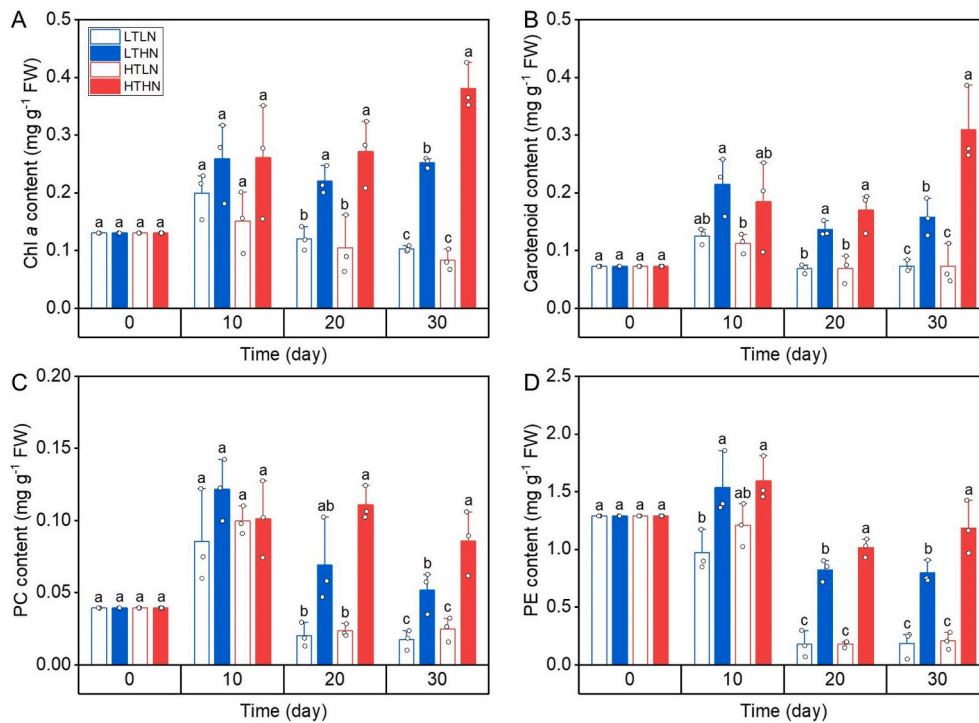


Fig. 2. Chl *a* content (A), carotenoid content (B), PC content (C), and PE content (D) of *G. lemaneiformis* grown under different temperature and nitrate conditions. Abbreviations: LT, 20°C treatment; HT, 23°C treatment; LN, low nitrate level; HN, high nitrate level; Different letters indicate that there are significant differences among different treatment groups at corresponding time point ($p < 0.05$ if Levene's test is met, otherwise $p < 0.01$).

nitrogen (Table S1), showing a common rise-then-fall trend across all groups, albeit with lower fluctuation in three treatments (Fig. 1C). A distinct temperature $\times$ nitrogen interaction emerged on day 20, where increased nitrate suppressed NPQ specifically under low temperature (Table S2).

3.2. Pigments and phycobilin protein contents response

The Chl *a* contents of *G. lemaneiformis* under different treatments exhibited distinct trajectories: a sustained increasing trend under high nitrate, compared to an initial rise followed by a decline under low nitrate (Fig. 2A). The combination of high temperature and high nitrogen showed largest accumulation on day 30. Moreover, nitrogen exerted a significant positive effect on Chl *a* content on day 20, which was further enhanced by high temperature by day 30 (Table S2). Similar to Chl *a*, carotenoid content was significantly increased by elevated nitrogen throughout the experiment (Fig. 2B–Table S2). Under high nitrate conditions, high temperature provided a further boost to carotenoid accumulation by day 30.

The PC content of *G. lemaneiformis* experienced an initial increase followed by a decrease over the cultivation period, with the largest fluctuation observed under low nitrate and warming condition (Fig. 2C). Nitrogen had a significantly promoting effect on the later two-thirds period, especially under warming condition on day 30 (Table S2). In contrast to PC content, time had a significant interactive effect with nitrogen on PE content in the cultivation period (Table S1). The PE content of *G. lemaneiformis* showed a decreasing trend with time under low nitrate while a first increase followed by a decrease under high nitrate (Fig. 2D). Nitrogen had a stimulative effect on the PE content throughout the cultivation period (Table S2). Compared to high nitrate alone, its combination with warming further promoted PE accumulation during the final two-thirds of the period.

3.3. POC, PON content and C: N molar ratio

Time had a significant interactive effect with temperature or nitrogen concentration on the POC content (Table S1). All treatments had rising trends over time, with the most pronounced and consistent accumulation observed under high temperature low nitrate condition (Fig. 3A). The POC content was inhibited by increased nitrate on day 20 while it was promoted by warming on day 30 (Table S2).

The PON content of *G. lemaneiformis* declined throughout the experiment under low nitrate, while it showed an initial increase followed by a decrease under high nitrate (Table S1, Fig. 3B). Nitrogen had a dramatically stimulative effect on PON content throughout the culture period (Table S2). Temperature had no significant effect on the PON content.

Time had significant interactive effects with nitrogen concentration on the C: N molar ratio (Table S1): The C: N molar ratio under low nitrogen showed a rising trend with time but remained stable under high nitrogen (Fig. 3C). High nitrogen reduced the C: N molar ratio regardless of temperature in the whole period (Table S2), and this difference widened over time.

3.4. POC and PON accumulation

The POC accumulation in *G. lemaneiformis* increased throughout the cultivation period, with the greatest accumulation occurring under high nitrate, particularly in combination with low temperature (Table S1, Fig. 4A). High nitrogen significantly promoted POC accumulation on days 20 and 30 (Table S2). Similarly, PON accumulation increased throughout the experiment under high nitrate (Table S1, Fig. 4B). Temperature had opposite effects on PON accumulation under high nitrate on days 10 and 30 (Table S2): Warming increased it on day 10 but inhibited it on day 30.

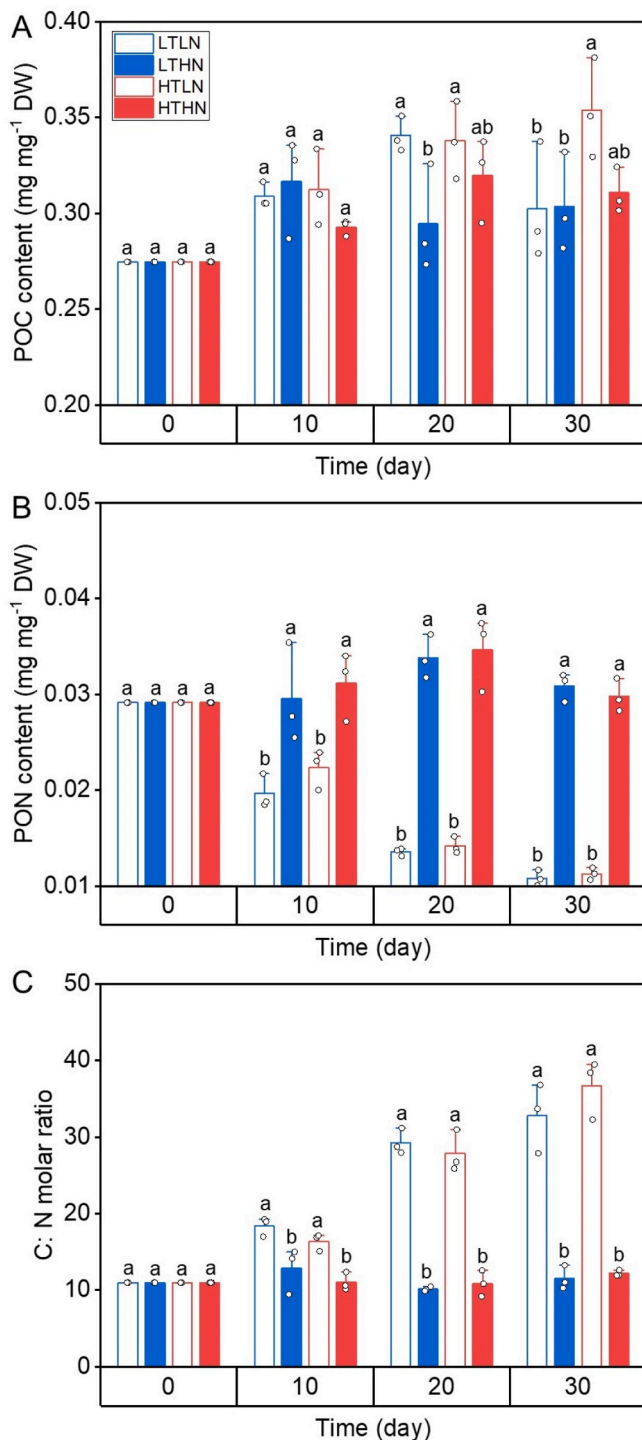


Fig. 3. POC content (A), PON content (B), and C: N molar ratio (C) of *G. lemaneiformis* grown under different temperature and nitrate conditions. Abbreviations: LT, 20°C treatment; HT, 23°C treatment; LN, low nitrate level; HN, high nitrate level; Different letters indicate that there are significant differences among different treatment groups at corresponding time point ($p < 0.05$ if Levene's test is met, otherwise $p < 0.01$).

3.5. DOC and RDOC accumulation

The DOC production rate of *G. lemaneiformis* showed a trend of first decreasing and then increasing over time. The decline in DOC production rate observed during the rapid growth phase could be attributable to enhanced metabolic demand for fixed carbon to fuel proliferation,

which suppresses extracellular carbon exudation. In contrast, the increase in DOC production on day 31 is associated with the algal mature stage, characterized by elevated carbon release. The lowest rates were observed under low temperature (Table S1, Fig. 5A). Temperature had a significant effect on the DOC production rate on days 11 and 21 (Table S2). Warming reduced the rate, especially when combined with high nitrate on day 11, but had the completely opposite effect on day 21.

DOC and RDOC accumulation both increased with time (Fig. 5B–C). During the culture period, temperature or nitrogen did not show significant effects on DOC or RDOC accumulation.

3.6. Bacterial abundance

Bacterial abundance during the culture period increased over time, except under the combined high nitrate and low temperature condition (Table S1, Fig. 6A). On day 31, high nitrate significantly inhibited bacterial abundance at low temperature, but did not cause any significant decrease at high temperature (Table S2). In the dark incubation, bacterial abundance showed a continuous increasing trend under high nitrate, but an initial increase followed by a decrease under low nitrate. Nitrogen significantly influenced bacterial abundance on days 81 and 91, with high nitrate increasing proliferation under low temperature.

4. Discussion

4.1. Response of growth and photosynthesis in *G. lemaneiformis* to warming and nitrogen availability

The present study revealed that elevated temperature exerted limited individual effects on the physiology of *G. lemaneiformis*. Temperature significantly influenced the SGR on day 17, the Fv/Fm (day 30), and NPQ (day 20), while it had no significant effects on pigment or phyco-biliprotein contents in the whole period.

This general lack of a strong warming effect aligns with findings by Yang et al. (2021a), who reported that ocean warming had little impact on the SGR, Fv/Fm, or NPQ of *G. lemaneiformis*. According to the Metabolic Theory of Ecology, temperature exerts fundamental control over biological rates, including metabolism and growth (Brown, 2004). Thermal performance curves for growth and metabolism in seaweeds typically follow a unimodal pattern, increasing with temperature up to a physiological optimum and declining thereafter. The natural temperature range experienced by cultivated *G. lemaneiformis* in the local area is 16.0–21.5°C (Yang et al., 2025). Our experimental temperatures of 20°C and 23°C appear to symmetrically flank the species' thermal optimum; thus, the 3°C warming did not yield a significant effect on growth relative to the 20°C control. Similarly, a 5°C increase from 20°C to 25°C did not significantly affect growth of *Ulva prolifera* (Gao et al., 2016). In contrast, Zou et al. (2014) observed that a 2°C temperature increase (to 26°C) could negatively affect growth of *G. lemaneiformis*, suggesting that thermal responses may be context dependent, potentially influenced by the different higher temperatures or acclimation status. It is worth noting that SGR of *G. lemaneiformis* went down during the culture period. This phenomenon has been commonly documented for both laboratory and field culture of *G. lemaneiformis* due mainly to increased biomass and decreased available light (Duan et al., 2019; Jiang et al., 2024; Yang et al., 2006; Yu and Yang, 2008). The decreased SGR led to the slowdown of biomass accumulation (Fig. S2).

In comparison, nitrogen availability was a dominant factor regulating growth and photosynthesis. The SGR of *G. lemaneiformis* was significantly enhanced under high nitrate throughout the entire period, except on day 7. Elevated nitrate consistently enhanced the growth of both *G. blodgettii* and *G. lemaneiformis* under both 5°C and 10°C warming scenarios (Ma et al., 2021), implying that eutrophic waters may confer a physiological advantage to these species under future warming. In contrast, increased nitrate decreased the growth rate of *Sargassum horneri* under both 20°C and 24°C (Wu et al., 2022b). Furthermore, while

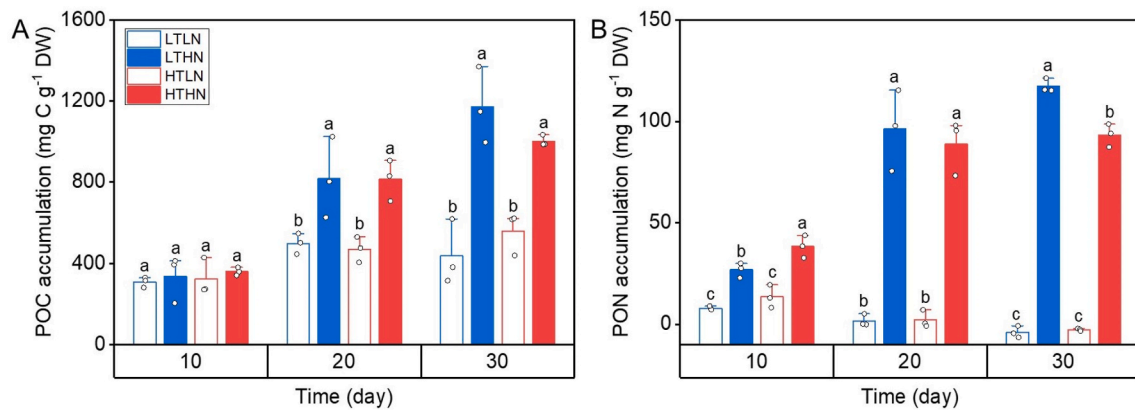


Fig. 4. POC accumulation (A) and PON accumulation (B) of *G. lemaneiformis* grown under different temperature and nitrate conditions. Abbreviations: LT, 20°C treatment; HT, 23°C treatment; LN, low nitrate level; HN, high nitrate level; Different letters indicate that there are significant differences among different treatment groups at corresponding time point ($p < 0.05$ if Levene's test is met, otherwise $p < 0.01$).

nitrate enrichment typically promotes growth, the response direction is contingent upon whether the temperature surpasses the species' thermal tolerance limit (Gouvêa et al., 2017). By day 30, Fv/Fm was also increased under high nitrate, consistent with prior work (Chen et al., 2025). Conversely, NPQ was suppressed on day 20, indicating a reduced demand for photoprotection. Furthermore, high nitrate stimulated the accumulation of pigments and phycobiliproteins in *G. lemaneiformis*. Nitrogen enrichment was reported to promote the SGR in *G. lemaneiformis* under various pCO_2 levels (20, 400, and 1000 μatm) (Jiang et al., 2020). Moreover, increased nitrogen availability also enhanced the SGR of *G. lemaneiformis* under various temperatures (20°C, 25°C, and 30°C) (Ma et al., 2021). These studies suggested the promotion of high nitrogen in the growth of *G. lemaneiformis*. Enhanced nitrogen also improved the photosynthetic capacity in *G. lemaneiformis*, as evidenced by increased Fv/Fm and pigment content (Ma et al., 2021). In *Laurencia catarinensis*, Chl *a* content was similarly increased under high nitrogen across a range of temperatures (20°C, 24°C, and 28°C) (Gouvêa et al., 2017). The PC and PE contents of red alga *Hypnea cervicornis* increased under high nitrate treatments (100 and 200 μM) but decreased at 400 and 500 μM of nitrate treatment (Ribeiro et al., 2012). Jiang et al. (2024) reported that enhanced nitrogen availability upregulated the expression level of genes associated with the photosynthetic light reaction and antenna systems. These findings suggest that an appropriate nitrate concentration can promote photosynthetic capacity.

Although ocean warming alone had no significant effect on the SGR, pigments, or phycobiliprotein contents in *G. lemaneiformis*, its combined effect with high nitrogen promoted all these parameters. The combination of high temperature and high nitrate led to the greatest accumulation of pigments and phycobiliproteins in *G. lemaneiformis* on days 20 and 30. On day 30, carotenoid, PC, and PE contents under the combined treatment were significantly higher than those under high nitrate alone. This interactive stimulation is supported by findings in *L. catarinensis*, where high temperature inhibited growth under low nitrogen but enhanced it under high nitrogen (Gouvêa et al., 2017). The activation of photosynthesis-related genes under high nitrate (Jiang et al., 2024), combined with elevated temperature, may further enhance photosynthetic performance via increased activity of carbon fixation enzymes (e.g., Rubisco) and improved light energy utilization. These results collectively suggest that the co-occurrence of elevated temperature and nitrogen enrichment can exert a stronger positive effect on the photosynthetic performance and growth of *G. lemaneiformis* than either factor in isolation, emphasizing the importance of considering multiple environmental drivers simultaneously.

The interactive effects of temperature and nitrate availability varied dynamically across the cultivation period. For example, on day 20, the combined low temperature and low nitrate (LTLN) treatment yielded the

maximum NPQ value relative to all other experimental groups, indicative of the most severe physiological stress imposed by this regime. Consistently, the LTLN group exhibited the lowest dissolved organic carbon (DOC) production rate on day 21; this observation implies that algal cells diverted greater amounts of cellular substrates and energy reserves toward stress mitigation, consequently diminishing the efflux of extracellular carbon compounds. Nevertheless, the pronounced NPQ enhancement triggered by LTLN was no longer detectable by day 30, a phenomenon attributable to gradual physiological acclimation of the algal population. Overall, NPQ under LTLN condition followed a unimodal pattern characterized by an initial rise followed by a subsequent decline. In contrast, the specific growth rate declined progressively up to day 20, demonstrating that successful acclimation was established in the algae after this time point. Such sequential acclimation and physiological recovery trajectories have previously been documented in *G. lemaneiformis* exposed to marine heatwave stress (Jiang et al., 2024). Notably, LTLN was the only treatment that displayed significantly elevated NPQ on day 20 throughout the entire culture period, reflecting asynchronous acclimation progress among the four treatments.

4.2. Response of POC and PON accumulation in *G. lemaneiformis* to warming and nitrogen availability

Elevated temperature alone promoted the cellular POC content on day 30 but had no significant influence on cellular PON content, the C: N molar ratio, or POC and PON accumulation. The observed rise in POC content under high temperature may be linked to the concurrent enhancement in photosynthetic efficiency (Fv/Fm) on day 30, suggesting a stimulation of carbon fixation. This is consistent with findings in *Phaeocystis pouchetii*, where elevated temperature increased cellular POC content under varying light and CO_2 conditions (Rokitta et al., 2023), potentially due to the upregulation of carbon fixation enzymes. However, the increase in cellular POC content did not result in POC accumulation, possibly due to only a marginal stimulation of SGR. Alternatively, warming may enhance respiratory carbon loss, counteracting gains in gross carbon fixation (Cavan and Boyd, 2018), which aligns with reports that marine heatwaves did not significantly affect POC accumulation in *G. lemaneiformis* (Jiang et al., 2024).

In contrast, aligning with ecological stoichiometry theory (Sterner and Elser, 2002), nitrogen availability exerted a stronger promotion on POC and PON accumulation than warming. High nitrate promoted cellular PON content while suppressing cellular POC content on day 20 and reducing the C: N ratio. This reflects a well-established response where nitrogen enrichment elevates the cellular nitrogen quota, as observed in small planktonic organisms (Olsen et al., 2014), and the

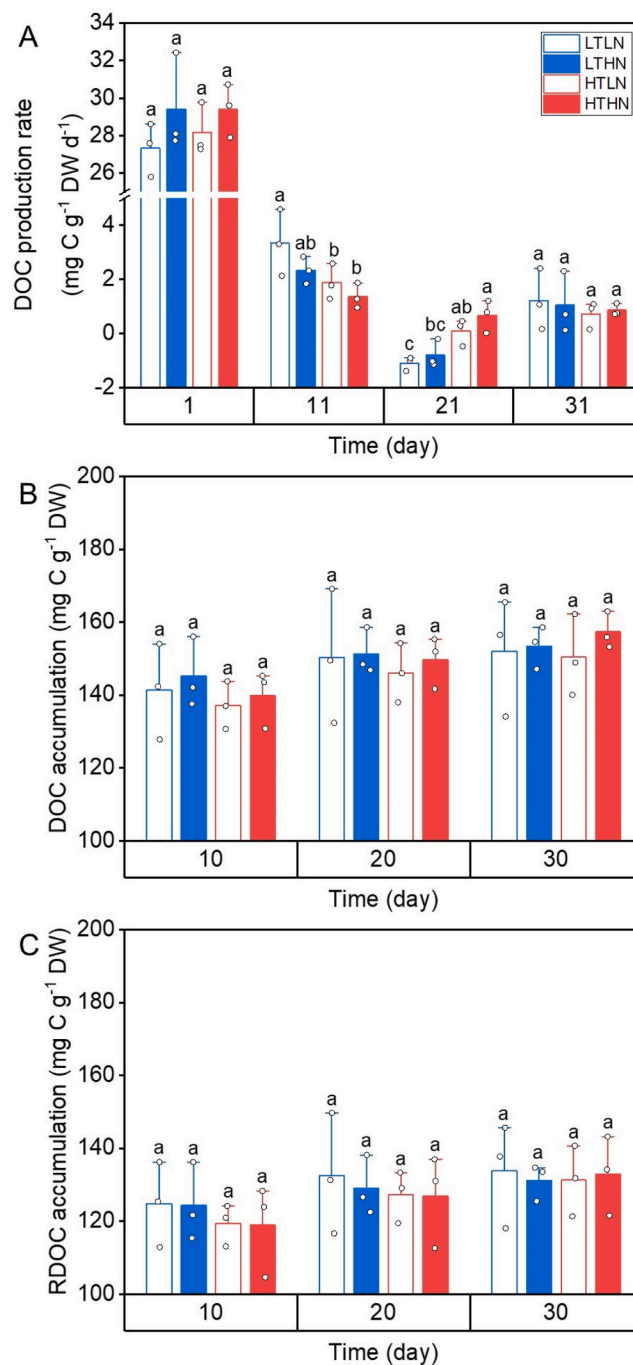


Fig. 5. DOC production rate (A), DOC accumulation (B), and RDOC accumulation (C) of *G. lemaneiformis* grown under different temperature and nitrate conditions. Abbreviations: LT, 20°C treatment; HT, 23°C treatment; LN, low nitrate level; HN, high nitrate level; Different letters indicate that there are significant differences among different treatment groups at corresponding time point ($p < 0.05$ if Levene's test is met, otherwise $p < 0.01$).

diatom *Thalassiosira punctigera* (Li et al., 2018). Contrary to its inhibitory effect on day 20, increased nitrate did not influence POC content on day 30. This discrepancy may be attributed to the fact that on day 20, high nitrate promoted the assimilation of inorganic nitrogen into amino acids, proteins, and other nitrogen containing compounds, a process that consumes a substantial proportion of energy and fixed carbon. In contrast, on day 30, the Fv/Fm was promoted by increased nitrate, whereas it was inhibited on day 20. The combined effects of carbon allocation and photosynthetic capacity likely contributed to the distinct patterns of cellular POC content observed between the two time points.

High nitrate enhanced nitrogen assimilation capacity by activating key genes encoding nitrite reductase (NIR), glutamine synthetase (GLUL), and glutamate synthase (GLT), thereby resulting in increased PON content (Chen et al., 2025). More importantly, enhanced POC and PON accumulation under high nitrate was linked to improved SGR, photosynthesis, and pigment synthesis, which support carbon-nitrogen reallocation for stoichiometric homeostasis. Consistent with our data, sufficient nitrogen optimizes C:N stoichiometry and boosts POC accumulation in *G. lemaneiformis* (Jiang et al., 2024). In *G. lemaneiformis*, high nitrogen availability upregulated key carbon fixation genes (e.g.,

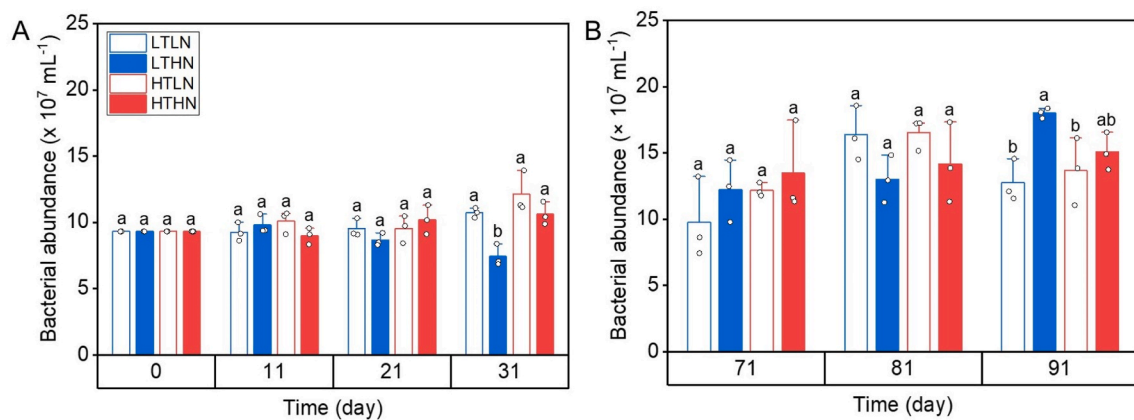


Fig. 6. Bacterial abundance in culture period (A) and in dark incubation (B) of *G. lemaneiformis* grown under different temperature and nitrate conditions. Abbreviations: LT, 20°C treatment; HT, 23°C treatment; LN, low nitrate level; HN, high nitrate level; Different letters indicate that there are significant differences among different treatment groups at corresponding time point ($p < 0.05$ if Levene's test is met, otherwise $p < 0.01$).

carbonic anhydrase, phosphoglycerate kinase, and fructose-1,6-bisphosphatase (Chen et al., 2025). This enhanced the metabolic potential for POC accumulation, which, in conjunction with net biomass increase, contributed to greater overall carbon storage. However, under increased nitrate, warming enhanced respiration and inhibited growth, thereby reducing POC accumulation. Ecologically, this stoichiometric plasticity enables *G. lemaneiformis* cultivation to absorb excess nitrogen and sequester CO₂, aiding in restoring stoichiometric balance in eutrophic coastal waters. High temperature alone promoted the cellular POC content by increasing photosynthesis and carbon fixation related enzymes (e.g. antenna complex and Rubisco), while its combined effect with high nitrate offset the promotion—likely due to the maintenance of a balanced cellular C: N ratio. High nitrate led to the increase of cellular PON content, POC and PON accumulation. The combination of high temperature and high nitrate did not significantly affect cellular POC content, suggesting that high temperature offset the stimulatory effect of high nitrate on cellular POC quota in the combined treatment. High temperature did not further promote POC accumulation under high nitrate, as its positive effect on carbon fixation capacity was offset by limited growth stimulation. Our finding that elevated nitrate promotes POC accumulation is consistent with the observation that nutrient enrichment can enhance the carbon sink capacity of macroalgae *Pterocladia capillacea* and *Turbinaria ornata* by promoting their biomass and subsequent deposition in deep-sea sediments (Hung et al., 2024). On day 10, the combined treatment further enhanced PON accumulation compared to high nitrate alone, attributable to the significant biomass accumulation (day 7) and slight enhancement of cellular PON content under combined treatment (Fig. S2). However, the PON accumulation under the combined treatment on day 30 was significantly attenuated relative to high nitrate alone, likely due to the decrease in SGR on days 20 and 27. These results indicate that *G. lemaneiformis* exhibits stronger nitrogen removal capacity under combined warming and high nitrogen in the early growth phase, whereas future warming may partially compromise this ability in later stages. Overall, the promoted accumulation of POC and PON under the combined treatment was primarily driven by the effect of high nitrate, a pattern corroborated by studies on marine heatwaves and nitrogen enrichment (Jiang et al., 2024).

4.3. Response of carbon secretion and sequestration

High temperature exerted time-dependent effects on the DOC production rate, showing significant inhibition on day 11 but promotion on day 21 (Table S2). Previous studies show that DOC production is positively related to photosynthesis (Weigel and Pfister, 2021). Although

high temperature did not affect Fv/Fm on days 11 or 21, it significantly reduced NPQ on day 20, indicating an alleviation of photosynthetic stress. In the initial phase, warming increased the respiratory rate and basal maintenance metabolism, which exacerbated carbon demand and consequently reduced carbon secretion. In contrast, during the later phase, when macroalgae reached the plateau stage, carbon demand decreased, while carbon release increased under the adverse effects of warming. Zhao et al. (2024) reported that warming significantly enhanced the photosynthetic performance and DOC production in cyanobacterium *Synechococcus*, suggesting that the increased photosynthetic capacity may contribute to DOC production. However, warming did not significantly affect the POC, DOC or RDOC accumulation, nor did it affect bacterial abundance during the culture period in the present study. Therefore, even though no net DOC accumulation was observed for *G. lemaneiformis* under warming, the enhanced DOC release and the concurrent rapid bacterial transformation could compensate for each other, such that the overall DOC concentration remained unchanged. The discrepancy in DOC accumulation patterns between *G. lemaneiformis* and *Synechococcus* could be attributed to the absence of normalization to biomass for *Synechococcus* (Zhao et al., 2024). Carbon accumulation and secretion in *G. lemaneiformis* are not substantially influenced by future warming (Fig. S3).

In contrast, high nitrate showed no significant effect on DOC production rate, DOC accumulation or RDOC accumulation but significantly influenced bacterial abundance both during the culture period (day 31) and in dark incubation (days 91). High nitrate significantly promoted SGR, favoring the POC accumulation over DOC release. The reduced bacterial abundance on day 31 likely reflected diminished energy availability. Jiang et al. (2024) reported that under sufficient nitrate availability, *G. lemaneiformis* preferentially allocated carbon to POC accumulation rather than to DOC secretion. Mueller et al. (2016) reported that increased nutrient availability did not activate the DOC release of turf algae under full light. This suggests that high nitrate regulates carbon partitioning by diverting carbon toward cellular storage rather than secretion (Fig. S3).

On day 21, high nitrate alone had no significant effect on the DOC production rate, but exhibited significant promotion (the highest value) when combined with warming. This enhancement coincided with peak levels of photosynthetic pigments and phycobiliproteins under combine treatment, indicating superior photosynthetic activity likely drove increased DOC exudation. The trend was consistent with significant promotion of POC and PON accumulation. This indicates that DOC secretion scaled with biomass accumulation, such that surplus carbon was released. Moreover, the combination of warming and high nitrate

promoted the accumulation of DOC or RDOC, although not significantly, which may be due to interference from high initial values. High temperature alone increased energy costs, triggering a reprioritization of carbon allocation toward core metabolic processes and thereby limiting carbon accumulation and secretion—an effect that was alleviated by high nitrate. Furthermore, warming stimulated bacterial respiration, potentially accelerating labile DOC consumption and thus constraining the observed DOC accumulation. High temperature significantly inhibited the PON accumulation under high nitrate while it did not show significant effects on POC accumulation. This finding implies that organic carbon was preferentially stored intracellularly within *G. lemaneiformis* under the combination of warming and eutrophication. Both warming and the combined treatment also enhanced bacterial abundance after two months of dark incubation. Warming likely stimulated the release of structurally simple fixed carbon, making it more susceptible to microbial consumption. The microbial utilization and fate of DOC are fundamentally governed by its molecular composition. DOC is commonly classified into LDOC, semi-labile (SLDOC), semi-refractory (SRDOC), RDOC, and ultra-refractory (URDOC) fractions based on its turnover time and reactivity in the water column (Paine et al., 2021). Ocean warming may promote microbial consumption that speeded up the conversion of LDOC to DIC (Paine et al., 2021; Zhao et al., 2024). In this study, bacterial abundance was used as a relative indicator of labile DOC availability and total microbial biomass potential. However, it does not distinguish metabolic activity and thus cannot directly quantify rates of DOC remineralization to CO₂ or transformation to RDOC. This represents a key avenue for future mechanistic research.

In future warming scenarios and eutrophication, seaweed farming seems unlikely to trigger elevated carbon release; instead, it facilitates greater POC production. Therefore, the incremental carbon sequestration by seaweed farming in future oceans may predominantly stem from two pathways: sedimentation of POC onto algal beds and export to deep ocean (Gao et al., 2022b). According to Filbee-Dexter et al. (2024), 15% POC of seaweeds can be exported to deep oceans. In addition, to harness the carbon sequestration potential of macroalgae (via RDOC and POC) effectively, farming strategies should be designed to simultaneously deliver ecological benefits, helping to mitigate the interconnected challenges of climate change and coastal eutrophication.

A limitation of this study is the use of single treatment levels for temperature and nitrate. Future investigations should incorporate gradients of these stressors (e.g., intermediate temperatures and nitrate concentrations) to elucidate potential non-linear or threshold responses in macroalgal carbon metabolism and secretion. Furthermore, a wider range of macroalgal species should be explored to accurately estimate carbon accumulation and secretion in coastal macroalgal farming. Likewise, a longer RDOC incubation may be required for more accurate quantification of DOC to RDOC transformation. To construct a complete carbon budget, future work should incorporate measurements of respiratory carbon losses and characterize the chemical composition of the dissolved exudates.

5. Conclusion

In this study, a comprehensive series of physiological and biochemical analyses was performed to elucidate the response of cellular carbon storage and excretion in the widely cultivated seaweed, *G. lemaneiformis*, to ocean warming and elevated nitrogen conditions. These findings suggest that elevated nitrogen promotes growth, photosynthesis, and POC accumulation while having no influence on DOC accumulation, thereby facilitating cellular carbon storage. By contrast, ocean warming alone had no significant effect on POC or PON accumulation, while both were enhanced when ocean warming is combined with elevated nitrate. Although warming, alone or combined with high nitrate, stimulated the DOC production rate, this stimulation did not ultimately lead to an increase in DOC or RDOC accumulation. This study, conducted under controlled laboratory conditions using a single macroalgal species (*G. lemaneiformis*), elucidates the mechanisms governing carbon fixation

and secretion in response to elevated temperature and nitrate enrichment. Within the constraints of this experimental setup (such as limited light intensity and temperature fluctuation), our results suggest a preferential allocation of fixed carbon toward POC accumulation rather than DOC secretion under the simulated co-occurring warming and eutrophication scenarios during the cultivation period. This species has been incorporated into regional blue carbon accounting frameworks, including Fujian Province's fishery carbon sink standards and Guangdong Province's carbon footprint accounting standard for farmed macroalgae (DB44/T 2729—2025). The POC-favored partitioning observed under combined warming and eutrophication supports the use of *G. lemaneiformis* aquaculture as a dual-purpose strategy for carbon sequestration and nutrient bioremediation in eutrophic coastal zones, and aligns with China's Marine Environment Protection Law and the Beautiful China initiative. However, these findings should be interpreted with caution, as they may not fully capture the complexity of field conditions. Future studies incorporating a broader range of macroalgal species, extended temporal scales, and field validation experiments are necessary to assess the generalizability of these conclusions.

CRedit authorship contribution statement

Jichen Chen: Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Chi Song:** Data curation, Formal analysis, Investigation, Validation. **Siwei Tang:** Methodology, Validation. **Sofia Delira:** Writing – review & editing. **Hongmei Chen:** Funding acquisition, Methodology, Validation, Writing – review & editing. **Guang Gao:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Supervision, Validation, Writing – review & editing.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the International Cooperation Seed Funding Project for China's Ocean Decade Actions (GHZZ3702840002024020000020), the National Key Research and Development Program of China (2024YFF0507003), the Open Project of Key Laboratory of Marine Environmental Survey Technology and Application, Ministry of Natural Resources, P.R. China (MESTA-2024-A001), the National Natural Science Foundation of China under contract No. 42476035, the Fundamental Research Funds for the Central Universities of China under contract No. 20720210077 to H. M. C., and the MEL Internal Research Program (MELRI2304). The authors also appreciate the support from Google Climate Action Research Awards and the PhD Fellowship of the State Key Laboratory of Marine Environmental Science at Xiamen University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2026.108341>.

Data availability

Data will be made available on request.

References

- Baker, H.S., Millar, R.J., Karoly, D.J., Beyerle, U., Guillod, B.P., Mitchell, D., Shiogama, H., Sparrow, S., Woollings, T., Allen, M.R., 2018. Higher CO₂ concentrations increase extreme event risk in a 1.5°C world. *Nat. Clim. Change* 8 (7), 604–608.

- Beer, S., Eshel, A., 1985. Determining phycoerythrin and phycocyanin concentrations in aqueous crude extracts of red algae. *Mar. Freshw. Res.* 36 (6), 785–792.
- Bennett, E., Paine, E.R., Britton, D., Schwoerbel, J., Hurd, C.L., 2024. The effect of temperature on rates of dissolved organic carbon (DOC) release by the kelp *Ecklonia radiata* (phylum Ochrophyta): implications for the future coastal ocean carbon cycle. *J. Phycol.* 60 (6), 1471–1484.
- Brown, J.H., 2004. Toward a metabolic theory of ecology. *Ecology* 85 (7), 1771–1789.
- Cai, W., Santos, A., Wang, G., Yeh, S.-W., An, S.-L., Cobb, K.M., Collins, M., Guiliardi, E., Jin, F.-F., Kug, J.-S., Lengaigne, M., McPhaden, M.J., Takahashi, K., Timmermann, A., Vecchi, G., Watanabe, M., Wu, L., 2015. ENSO and greenhouse warming. *Nat. Clim. Change* 5 (9), 849–859.
- Cavan, E.L., Boyd, P.W., 2018. Effect of anthropogenic warming on microbial respiration and particulate organic carbon export rates in the sub-Antarctic southern ocean. *Aquat. Microb. Ecol.* 82 (2), 111–127.
- Chen, J., Song, C., Ke, Y., Wang, M., Qiu, J., Nie, L., Li, G., Gao, G., 2025. Nitrate modulates high CO₂ effects on carbon partitioning in *Gracilariopsis lemaneiformis*: Trade-offs between particulate and dissolved organic carbon sequestration. *Environ. Res.* 286, 122897.
- Cheng, L., von Schuckmann, K., Abraham, J.P., Trenberth, K.E., Mann, M.E., Zanna, L., England, M.H., Zika, J.D., Fasullo, J.T., Yu, Y., Pan, Y., Zhu, J., Newsom, E.R., Bronselaer, B., Lin, X., 2022. Past and future ocean warming. *Nat. Rev. Earth Environ.* 3 (11), 776–794.
- Chiquier, S., Patrizio, P., Bui, M., Sunny, N., Mac Dowell, N., 2022. A comparative analysis of the efficiency, timing, and permanence of CO₂ removal pathways. *Energy Environ. Sci.* 15 (10), 4389–4403.
- Duan, Y., Yang, N., Hu, M., Wei, Z., Bi, H., Huo, Y., He, P., 2019. Growth and nutrient uptake of *Gracilaria lemaneiformis* under different nutrient conditions with implications for ecosystem services: a case study in the laboratory and in an enclosed mariculture area in the East China Sea. *Aquat. Bot.* 153, 73–80.
- Fernández, P.A., Gaitán-Espitia, J.D., Leal, P.P., Schmid, M., Revill, A.T., Hurd, C.L., 2020. Nitrogen sufficiency enhances thermal tolerance in habitat-forming kelp: implications for acclimation under thermal stress. *Sci. Rep.* 10 (1), 3186.
- Filbee-Dexter, K., Pessarrodona, A., Pedersen, M.F., Wernberg, T., Duarte, C.M., Assis, J., Bekkby, T., Burrows, M.T., Carlson, D.F., Gattuso, J., Gundersen, H., Hancke, K., Krumhansl, K.A., Kuwae, T., Middelburg, J.J., Moore, P.J., Queirós, A.M., Smale, D. A., Sousa-Pinto, I., Suzuki, N., Krause-Jensen, D., 2024. Carbon export from seaweed forests to deep ocean sinks. *Nat. Geosci.* 1–14.
- Gao, G., Beardall, J., Jin, P., Gao, L., Xie, S., Gao, K., 2022a. A review of existing and potential blue carbon contributions to climate change mitigation in the Anthropocene. *J. Appl. Ecol.* 59 (7), 1686–1699.
- Gao, G., Clare, A.S., Rose, C., Caldwell, G.S., 2017. Eutrophication and warming-driven green tides (*Ulva rigida*) are predicted to increase under future climate change scenarios. *Mar. Pollut. Bull.* 114 (1), 439–447.
- Gao, G., Gao, L., Jiang, M., Jian, A., He, L., 2022b. The potential of seaweed cultivation to achieve carbon neutrality and mitigate deoxygenation and eutrophication. *Environ. Res. Lett.* 17 (1), 014018.
- Gao, G., Li, G., Xu, J., Feng, Y., Hall-Spencer, J.M., 2025. Coastal restoration policy needs to consider seaweed diversity. *Nat. Ecol. Evol.* 9, 742–745.
- Gao, G., Zhong, Z., Zhou, X., Xu, J., 2016. Changes in morphological plasticity of *Ulva prolifera* under different environmental conditions: a laboratory experiment. *Harmful Algae* 59, 51–58.
- Gao, L., Xiong, Y., Fu, F.-X., Hutchins, D.A., Gao, K., Gao, G., 2024. Marine heatwaves alter competition between the kelp macroalgae *Gracilariopsis lemaneiformis* and the harmful bloom alga *Skeletonema costatum*. *Sci. Total Environ.* 947, 1–13.
- Gasol, J.M., Giorgio, P.A.D., 2000. Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. *Sci. Mar.* 64 (2), 197–224.
- Gouvêa, L.P., Schubert, N., Martins, C.D.L., Sissini, M., Ramlov, F., Rodrigues, E.R.d.O., Bastos, E.O., Freire, V.C., Maraschin, M., Carlos Simonassi, J., Varella, D.A., Franco, D., Cassano, V., Fonesca, A.L., Barufi, J.B., Horta, P.A., 2017. Interactive effects of marine heatwaves and eutrophication on the ecophysiology of a widespread and ecologically important macroalga. *Limnol. Oceanogr.* 62 (5), 2056–2075.
- Harrould-Kolieb, E.R., Herr, D., 2012. Ocean acidification and climate change: synergies and challenges of addressing both under the UNFCCC. *Clim. Policy* 12 (3), 378–389.
- Hung, C.-C., Chang, J.-S., Liao, C.-H., Lee, T.-M., 2024. Exploring the impact of ocean warming and nutrient overload on macroalgal blooms and carbon sequestration in deep-sea sediments of the subtropical western North Pacific. *Mar. Pollut. Bull.* 208, 116918.
- Hurd, C.L., Gattuso, J.P., Boyd, P.W., 2023. Air-sea carbon dioxide equilibrium: will it be possible to use seaweeds for carbon removal offsets? *J. Phycol.* 60 (1), 4–14.
- IPCC, 2023. IPCC, 2023: summary for policymakers. In: *Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*, pp. 1–34.
- Jiang, H., Liao, X., Zou, D., Huang, B., Liu, Z., 2020. The regulations of varied carbon-nitrogen supplies to physiology and amino acid contents in *Gracilariopsis lemaneiformis* (Gracilariaceae, Rhodophyta). *Algal Res.* 47, 1–8.
- Jiang, M., Gao, L., Huang, R., Lin, X., Gao, G., 2022. Differential responses of bloom-forming *Ulva intestinalis* and economically important *Gracilariopsis lemaneiformis* to marine heatwaves under changing nitrate conditions. *Sci. Total Environ.* 840, 1–13.
- Jiang, M., Hall-Spencer, J.M., Gao, L., Ma, Z., Gao, G., 2024. Nitrogen availability regulates the effects of a simulated marine heatwave on carbon sequestration and phycosphere bacteria of a marine crop. *Limnol. Oceanogr.* 69 (2), 339–354.
- Kamer, K., Fong, P., 2001. Nitrogen enrichment ameliorates the negative effects of reduced salinity on the green macroalga *Enteromorpha intestinalis*. *Mar. Ecol. Prog. Ser.* 218, 87–93.
- Kepner, R.L., Pratt, J.R., 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. *Microbiol. Rev.* 58 (4), 603–615.
- Krause-Jensen, D., Duarte, C.M., 2016. Substantial role of macroalgae in marine carbon sequestration. *Nat. Geosci.* 9 (10), 737–742.
- Kristensen, E., Andersen, F.S., Blackburn, T.H., 1992. Effects of benthic macrofauna and temperature on degradation of macroalgal detritus: the fate of organic carbon. *Limnol. Oceanogr.* 37, 1404–1419.
- Lan, J., Liu, P., Hu, X., Zhu, S., 2024. Harmful algal blooms in eutrophic marine environments: causes, monitoring, and treatment. *Water* 16 (17), 1–64.
- Le Quéré, C., Takahashi, T., Buitenhuis, E.T., Rödenbeck, C., Sutherland, S.C., 2010. Impact of climate change and variability on the global oceanic sink of CO₂. *Glob. Biogeochem. Cycles* 24 (4), 1–10.
- Li, Z., Wu, Y., Beardall, J., 2018. Physiological and biochemical responses of *Thalassiosira punctigerata* nitrate limitation. *Diatom Res.* 33 (2), 135–143.
- Liu, T., Li, Y., Shen, Y., 2025. Limited microbial degradation of elevated concentrations of dissolved organic carbon in the deep ocean. *Limnol. Oceanogr.* 70 (4), 989–1000.
- Liu, X., Jing, M., Liu, L., Li, Y., Li, R., Wang, Z., Zhang, X., 2016. A comparative study of the different extraction techniques about chlorophyll concentration of *Ulva prolifera* (in Chinese version). *Mar. Environ. Sci.* 35 (1), 144–148.
- Ma, C., Qin, S., Cui, H., Liu, Z., Zhuang, L., Wang, Y., Zhong, Z., 2021. Nitrogen enrichment mediates the effects of high temperature on the growth, photosynthesis, and biochemical constituents of *Gracilaria blodgettii* and *Gracilaria lemaneiformis*. *Environ. Sci. Pollut. Control Ser.* 28 (17), 21256–21265.
- Malone, T.C., Newton, A., 2020. The globalization of cultural eutrophication in the coastal ocean: causes and consequences. *Front. Mar. Sci.* 7, 670.
- Marie, D., Partensky, F., Vaulot, D., Brussaard, C., 2001. Enumeration of phytoplankton, bacteria, and viruses in marine samples. *Current Protocols in Cytometry* 10 (1), 11.11.11–11.11.15.
- Maúre, E.D.R., Terauchi, G., Ishizaka, J., Clinton, N., DeWitt, M., 2021. Globally consistent assessment of coastal eutrophication. *Nat. Commun.* 12 (1), 1–9.
- Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence—a practical guide. *J. Exp. Bot.* 51 (345), 659–668.
- Mueller, B., den Haan, J., Visser, P.M., Vermeij, M.J.A., van Duyl, F.C., 2016. Effect of light and nutrient availability on the release of dissolved organic carbon (DOC) by Caribbean turf algae. *Sci. Rep.* 6 (1), 1–10.
- Olsen, Y., Reinertsen, H., Sommer, U., Vadstein, O., 2014. Responses of biological and chemical components in North East Atlantic coastal water to experimental nitrogen and phosphorus addition – a full scale ecosystem study and its relevance for management. *Sci. Total Environ.* 473–474, 262–274.
- Paine, E.R., Schmid, M., Boyd, P.W., Diaz-Pulido, G., Hurd, C.L., 2021. Rate and fate of dissolved organic carbon release by seaweeds: a missing link in the coastal ocean carbon cycle. *J. Phycol.* 57 (5), 1375–1391.
- Pallant, J., 2010. *SPSS Survival Manual: a Step by Step Guide to Data Analysis Using IBM SPSS*. Open University Press, Buckingham, UK.
- Ribeiro, A.L.N.L., Tesima, K.E., Souza, J.M.C., Yokoya, N.S., 2012. Effects of nitrogen and phosphorus availabilities on growth, pigment, and protein contents in *Hypnea cervicornis* J. Agardh (Gigartinales, Rhodophyta). *J. Appl. Phycol.* 25 (4), 1151–1157.
- Rokitta, S.D., Grossmann, C.H., Werner, E., Moye, J., Castellani, G., Nöthig, E.M., Rost, B., 2023. Future warming stimulates growth and photosynthesis in an Arctic microalga more strongly than changes in light intensity or pCO₂. *Limnol. Oceanogr.* 68 (12), 2789–2799.
- Shen, Y., Jiang, R., Chang, J., Cai, L., Zhu, Y., Yin, Y., Shao, L., Wu, M., Zhang, J., He, P., 2025. The effect of temperature on the photosynthetic carbon fixation efficiency of sessile macroalgae in the mussel farming area of Gouqi Island through stable isotope. *Mar. Environ. Res.* 209, 107190.
- Sterner, R.W., Elser, J.J., 2002. *Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere*. Princeton University Press.
- Teichberg, M., Fox, S.E., Olsen, Y.S., Valiela, I., Martinetto, P., Iribarne, O., Muto, E.Y., Petti, M.A.V., Corbisier, T.N., Soto-Jiménez, M., Páez-Osuna, F., Castro, P., Freitas, H., Zitelli, A., Cardinale, M., Tagliapietra, D., 2010. Eutrophication and macroalgal blooms in temperate and tropical coastal waters: nutrient enrichment experiments with *Ulva* spp. *Glob. Change Biol.* 16 (9), 2624–2637.
- Tian, S., Chen, B., Wu, M., Cao, C., Gu, Z., Zheng, T., Zou, D., Ma, Z., 2023. Are there environmental benefits derived from coastal aquaculture of *Sargassum fusiforme*? *Aquaculture* 563, 738909.
- Wada, S., Aoki, M.N., Mikami, A., Komatsu, T., Tsuchiya, Y., Sato, T., Shinagawa, H., Hama, T., 2008. Bioavailability of macroalgal dissolved organic matter in seawater. *Mar. Ecol. Prog. Ser.* 370, 33–44.
- Wang, B., Xin, M., Wei, Q., Xie, L., 2018. A historical overview of coastal eutrophication in the China Seas. *Mar. Pollut. Bull.* 136, 394–400.
- Wang, J., Wu, H., Jia, L., Xu, J., Chen, Z., Lu, W., Cen, Y., Zhou, W., 2025. Ocean acidification interacts with low salinity and phosphorus limitation to modulate growth, photosynthesis, and physiology of mass-cultivated *Gracilariopsis lemaneiformis*. *J. Appl. Phycol.* 37 (5), 3017–3030.
- Wang, Y., Liu, D., Xiao, W., Zhou, P., Tian, C., Zhang, C., Du, J., Guo, H., Wang, B., 2021. Coastal eutrophication in China: trend, sources, and ecological effects. *Harmful Algae* 107, 102058.
- Weigel, B.L., Pfister, C.A., 2021. The dynamics and stoichiometry of dissolved organic carbon release by kelp. *Ecology* 102 (2), e03221.
- Wellburn, A.R., 1994. The spectral determination of Chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol.* 144 (3), 307–313.

- Wu, H., Chen, J., Feng, J., Liu, Y., Li, X., Chen, R., Xu, J., 2022a. Thermal fluctuations and nitrogen enrichment synergistically accelerate biomass yield of *Pyropia haitanensis*. *Aquat. Bot.* 179, 103501.
- Wu, H., Huo, Y., Hu, M., Wei, Z., He, P., 2015. Eutrophication assessment and bioremediation strategy using seaweeds co-cultured with aquatic animals in an enclosed bay in China. *Mar. Pollut. Bull.* 95 (1), 342–349.
- Wu, H., Li, X., Liu, Y., Wang, C., Ji, C., Xu, J., 2022b. Increased temperature and nitrogen enrichment inhibit the growth of the golden tide blooming Macroalgae *Sargassum horneri* in the yellow Sea, China. *J. Mar. Sci. Eng.* 10 (11), 1692.
- Wurtsbaugh, W.A., Paerl, H.W., Dodds, W.K., 2019. Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. *WIREs Water* 6 (5), e1373.
- Yang, W., Wu, Y., Liu, Y., Zhuang, P., Li, C., Zhang, J., Chen, Y., Zhuang, Y., Lin, H., Qiu, H., Huang, Y., Qiu, W., Cai, W.-J., Chen, L., Qi, D., 2025. Massive carbon inputs from fish farming reduce carbon sequestration capacity in a macroalgae mariculture area. *Mar. Environ. Res.* 212, 107515.
- Yang, Y., Li, W., Li, Y., Xu, N., 2021a. Photophysiological responses of the marine macroalga *Gracilariopsis lemaneiformis* to ocean acidification and warming. *Mar. Environ. Res.* 163, 105204.
- Yang, Y., Xiong, Y., Wang, T., Zhang, J., Feng, L., Gao, G., 2026. Seaweed and shellfish mariculture as nature-based solutions: mitigating nutrient pollution from coastal fish mariculture and sewage. *Mar. Pollut. Bull.* 222, 118793.
- Yang, Y.-F., Fei, X.-G., Song, J.-M., Hu, H.-Y., Wang, G.-C., Chung, I.K., 2006. Growth of *Gracilaria lemaneiformis* under different cultivation conditions and its effects on nutrient removal in Chinese coastal waters. *Aquaculture* 254 (1–4), 248–255.
- Yang, Z., Kagawa, S., Li, J., 2021b. Do greenhouse gas emissions drive extreme weather conditions at the city level in China? Evidence from spatial effects analysis. *Urban Clim.* 37, 100812.
- Yu, J., Yang, Y.-F., 2008. Physiological and biochemical response of seaweed *Gracilaria lemaneiformis* to concentration changes of N and P. *J. Exp. Mar. Biol. Ecol.* 367 (2), 142–148.
- Zhang, D., Sun, J.-Z., Fu, M.-H., Li, C.-J., 2024. Photosynthetic performance and antioxidant activity of *Gracilariopsis lemaneiformis* are sensitive to phosphorus deficiency in elevated temperatures. *Front. Mar. Sci.* 11, 1432937.
- Zhao, H., Zhang, Z., Nair, S., Li, H., He, C., Shi, Q., Zheng, Q., Cai, R., Luo, G., Xie, S., Jiao, N., Zhang, Y., 2024. Overlooked vital role of persistent algae-bacteria interaction in ocean recalcitrant carbon sequestration and its response to ocean warming. *Glob. Change Biol.* 30 (11), e17570.
- Zhou, Z.-X., Yu, R.-C., Zhou, M.-J., 2022. Evolution of harmful algal blooms in the East China Sea under eutrophication and warming scenarios. *Water Res.* 221, 118807.
- Zou, D., Gao, K., Harley, C., 2014. Temperature response of photosynthetic light- and carbon-use characteristics in the red seaweed *Gracilariopsis lemaneiformis* (Gracilariales, Rhodophyta). *J. Phycol.* 50 (2), 366–375.