

Effects of lowered carbon supplies on two farmed red seaweeds, *Pyropia haitanensis* (Bangiales) and *Gracilaria lemaneiformis* (Gracilariales), grown under different sunlight conditions

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Abstract Growth and photosynthesis of cultivated seaweeds usually suffer carbon limitation during cultivation in the field. *Pyropia haitanensis* and *Gracilaria lemaneiformis*, collected from Nan'ao Island, Shantou, China, were cultured under ambient carbon and decreased carbon supply, with ambient sunlight and decreased sunlight conditions, aiming to investigate how the decreased carbon supply and sunlight conditions affect growth and photosynthesis in these two maricultured seaweed species. Decreased carbon supply significantly lowered the relative growth rate (RGR), quantum efficiency of open PS II (F_v'/F_m'), maximum photosynthetic electron transport rate ($rETR_m$), and NO_3^- uptake rate in both of the two seaweeds. Under ambient sunlight condition, the RGR of the *P. haitanensis* and *G. lemaneiformis* grown at decreased carbon supply was reduced about 83 and 95 %, respectively, compared with the algae grown at ambient carbon condition. The RGR, F_v'/F_m' , and NO_3^- uptake rate were higher in *P. haitanensis* but were lower in *G. lemaneiformis*, with under decreased sunlight compared to ambient sunlight. The results indicated that decreased carbon supply reduced growth and PS II activity in both of the seaweeds, with the reduction being greater in *G. lemaneiformis* than in *P. haitanensis*. Additionally, *G. lemaneiformis* was adapted to grow at relative higher light conditions than *P. haitanensis*.

Keywords Decreased carbon supply · Sunlight · Growth · Photosynthesis · *Pyropia haitanensis* · *Gracilaria lemaneiformis*

Introduction

Atmospheric CO₂ concentrations have been rising since the industrial revolution. It is anticipated that increasing atmospheric CO₂ will lead to a linear proportional increase in the dissolved CO₂ concentrations and a concomitant reduction of pH (seawater acidification) in near-shore areas, as a consequence of the continuous gas exchange between the air and seawater. Extensive attention has been paid to the influences of increasing atmospheric CO₂ concentrations and/or seawater acidification on the physiology and ecology of seaweeds (e.g., Zou and Gao 2010; Harley et al. 2012; Koch et al. 2013). However, the realistic situation is that low dissolved inorganic carbon (DIC) concentrations in seawater would be of frequent occurrence, especially under the conditions of slow water exchanges, high standing stock, and large seaweed density (Friedlander and Levy 1995; Israel and Friedlander 1998; Richards et al. 2011; Zou 2014). Seaweed cultivation beds are often subject to low carbon as intense photosynthetic activity depletes DIC in the water. The highest difference in DIC content between a seaweed farm and natural seaweed vegetation bed was more than five times (Chung et al. 2013). In some seaweed species, it has been reported that as carbon level was decreased in seawater, pigment contents (García-Sánchez et al. 1994; Mercado et al. 1999; Andría et al. 2001; Zou 2014), Rubisco content, and carbonic anhydrase (CA) activity were increased (García-Sánchez et al. 1994; Andría et al. 2001), and photosynthetic activity was reduced (Mercado et al. 1999; Zou 2014).

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Generally, there is usually an excess of light energy reaching the photosynthetic apparatus, and different mechanisms have evolved to protect the seaweeds from such excess energy (photoprotection). However, seaweed thalli also experience low irradiance as light is sharply attenuated by seawater and the sinking of thalli to deeper water and self-shading due to high stocking density. Seaweeds can improve the capability of acclimation to low-light growth condition through adjusting photosynthetic activity, biosynthetic processes, and absorption of nutrients (Lopes et al. 1997; Talarico and Maranzana 2000; Zou and Gao 2009). In general, the irradiance changes with the tide and weather, and the photosynthetic performance of seaweeds is related to these variations in light intensity. Diurnal variation of photosynthesis of algae has been extensively studied in the natural environment, with the main focus on diurnal variation of algal photosynthesis in natural light (Magnusson 1997; Beer et al. 2006; Serôdio et al. 2008), different light quality (Sagert et al. 1997; McMinn et al. 2003), and different growth depth (Sagert et al. 1997; Campbell et al. 2008). However, much less is understood on the diurnal variation of photosynthesis in seaweeds grown under different sunlight and carbon supply conditions.

Pyropia haitanensis (Bangiales, Rhodophyta) and *Gracilaria lemaneiformis* (Gracilariales, Rhodophyta) are the most two important species for seaweed cultivation in South China. *Pyropia haitanensis* is used for human food, and *G. lemaneiformis* is commonly used for high-quality raw material in the agar industry and also for human food. Moreover, the cultivation of those two species can be an effective bioremediation measure for eutrophication control in coastal waters (Fei 2004; Yang et al. 2005, 2015). The characteristics of photosynthetic inorganic carbon (Ci) utilization in these two algae have been investigated (Zou and Gao 2002; Zou et al. 2004). The results showed that *P. haitanensis* and *G. lemaneiformis* mainly used HCO_3^- as its carbon source via external CA. Increasing CO_2 concentration improved photosynthetic capacity of *P. haitanensis* when exposed to air during low tide. CO_2 concentration had a weaker effect than light levels on the photosynthesis performance and growth of *G. lemaneiformis* (Zou and Gao 2009).

Pyropia haitanensis and *G. lemaneiformis* when cultivated in the bay usually experience decreased CO_2 supply due to slow water exchange. Additionally, the two seaweed species also experience decreased irradiance due to self-shading and/or high tide. Therefore, it is interesting to examine how these two maricultured seaweeds respond to low carbon supply when growing at different sunlight levels. In the present study, we cultured *P. haitanensis* and *G. lemaneiformis* outdoor with different carbon supply and sunlight availability. We specially focused on (1) growth and biochemical composition, (2) photosynthetic characteristics, and (3) how N uptake respond to decreased carbon supplied on these two seaweeds under different sunlight conditions.

Materials and methods

Thalli of *P. haitanensis* and *G. lemaneiformis* were collected from a cultivation field at the Shen'ao Bay, Nan'ao Island, Shantou, China (23° 20' N, 116° 40' E) during December 2013 and March 2014, respectively. Thalli were artificially cultivated by means of the pole system. The thalli were gently rinsed to remove sediments and epiphytes. Only unwounded and healthy thalli were selected. Samples were transported to the laboratory in a plastic bucket with some seawater (temperature ca. 4 °C) and were maintained in plexiglass aquaria with filtered natural seawater (salinity ca. 33 PSU) at 20 ± 0.5 °C. The two seaweeds received an irradiance of about 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (PAR, LD cycle 12:12 h). The seawater was continuously aerated and renewed every day. The thalli were used for experimental treatments after 2 days of above laboratory maintenance (Zou 2014). The irradiance was quantified by means of a quantum sensor (QSL2100, USA).

Culture treatments

The thalli were cultured outdoors with two levels of inorganic carbon (Ci) availability and two levels of irradiance in the following four treatments: (1) ambient carbon supply + ambient sunlight, (2) decreased carbon supply + ambient sunlight, (3) ambient carbon supply + decreased sunlight, and (4) decreased carbon supply + decreased sunlight. For the ambient carbon (AC) treatment, the seawater was aerated continuously with ambient air (the CO_2 concentration in the air was ca. 390 ppm; containing ca. 11.5 $\mu\text{M CO}_2$, 1780 $\mu\text{M HCO}_3^-$, 210 $\mu\text{M CO}_3^{2-}$). For the low carbon (DC) treatment, the culture media had low Ci seawater (containing ca. 0.1 mM Ci) and were aerated continuously with air through a 5 M NaOH solution (the CO_2 concentration in the air was ca. 50 ppm; Zou 2014; containing ca. 1.6 $\mu\text{M CO}_2$, 800 $\mu\text{M HCO}_3^-$, 300 $\mu\text{M CO}_3^{2-}$). The low Ci seawater was prepared from Ci-free seawater as described below. Ci was removed from sterilized natural seawater by reducing pH to less than 4.0 with the addition of 0.5 M HCl and then sparging with high purity N_2 gas for at least 5 h. Finally, the pH in the seawater was adjusted to pH 8.1 with freshly prepared 0.5 M NaOH solution. For the treatment of decreased sunlight (DL), the culture vessels were wrapped with sunshade net, and for the treatment of ambient sunlight (AL), nothing wrapped around the culture vessel. The irradiance of DL treatment was about 20 % of ambient sunlight in the culture vessel during daytime. The irradiance under the algal thalli were grown was measured at 2-h intervals between 06:30 and 20:30 in a typical day during the culture. The irradiances were at their maximum about 12:30, and the maximum value for *P. haitanensis* and *G. lemaneiformis* was about 1400 and 1563 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively (Fig. 1). For all of the treatments, the culture seawater was supplemented 40 μM

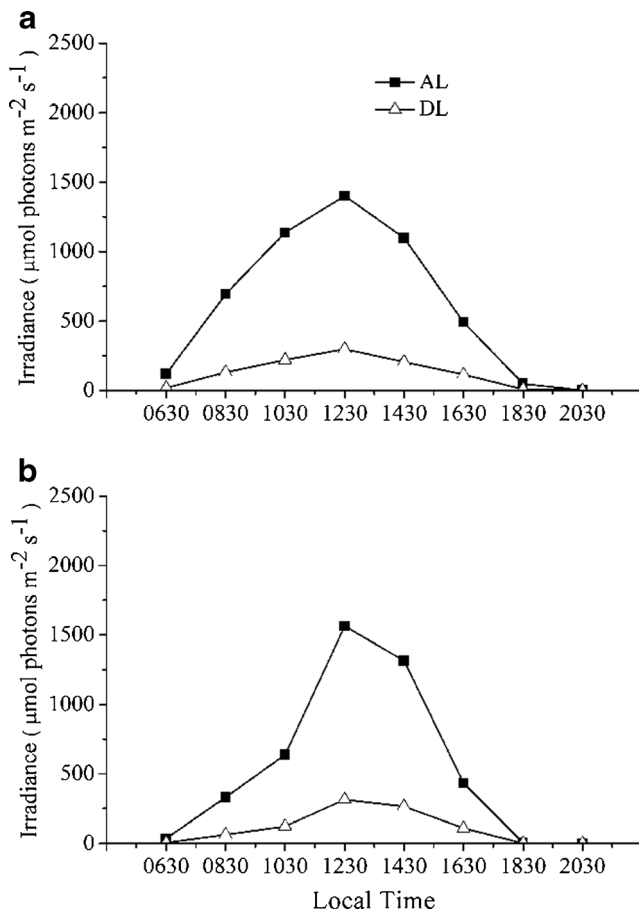


Fig. 1 Daily variation of irradiance throughout a type day during culture treatment for *Pyropia haitanensis* (a) and *Gracilaria lemaneiformis* (b). AL means the treatment of ambient sunlight. DL means the treatment of decreased sunlight

H₂PO₄⁻ and 150 μM NO₃⁻ to avoid the possible nutrient limitation. Three replicate cultures were maintained for each growth treatment.

Experimental treatments started when 20 g fresh weight (FW) algae were introduced into each of jars containing 10-L filtered seawater and cultured under outdoor conditions. The water motion resulting from the aeration allowed the algae to move gently without tumbling. The Ci concentrations of the culture media were examined periodically with a Total Organic Carbon Analyzer (TOC-5000A, Shimadzu, Japan), to ensure that the desired Ci level in the culture seawater for experimental treatment was right. The algal thalli were grown under these different carbon and irradiance regimes for 15 days and then were harvested to be used for experimental measurements.

Growth rates

Biomass was measured to estimate growth. The relative growth rate (RGR), expressed as percentage of increase in FW biomass per day (% day⁻¹), was estimated assuming

exponential growth during the culture period according to the formula: $RGR = [(\ln W_t - \ln W_0) / t] \times 100$, where W_0 is the initial and W_t the final FW of the algae, and t is the time of culture in days. The algal samples were softly blotted to remove excess water on filter papers before FW weighing.

Biochemical components

To determine pigment contents, about 0.1 g FW per sample was extracted in 100 % methanol. The concentration of chlorophyll *a* (Chl *a*) and carotenoids (Car) was calculated spectrophotometrically using the equations given by Porra (2005) and Parsons and Strickland (1963). To determine phycoerythrin (PE) and phycocyanin (PC), about 0.2 g FW per sample was extracted in 0.1 M phosphate buffer (pH 6.8). The extracts were then centrifuged at 10,000×*g* for 20 min. PE and PC contents in the supernatants were quantified according to Beer and Eshel (1985). For soluble protein (SP) determination, samples of about 0.2 g FW of algal biomass was homogenized with 0.1 M phosphate buffer (pH 6.8). The extracts were then centrifuged at 5000×*g* for 20 min. SP contents in the supernatants were quantified according to the method of Bradford (1976).

The diurnal variation of chlorophyll fluorescence

The chlorophyll fluorescence of the *P. haitanensis* and *G. lemaneiformis* was monitored at regular interval (2 h) during a day, using a portable pulse modulation fluorometer (Junior-PAM, Walz, Germany). The quantum efficiency of open PS II ($F_v'/F_m' = (F_m' - F_o') / F_m'$) of the algal thalli (dark-adapted for 10 s) was measured (Ralph et al. 1999). The rapid light curves (RLCs) consisted of the fluorescence response to eight different and increasing actinic irradiance levels as in our previous study (Zou 2014). The parameters of the RLCs were calculated following the model equation from Jassby and Platt (1976): $rETR = rETR_m \times \tanh(\alpha E / rETR_m)$, where $rETR_m$ is the saturated maximum rETR, \tanh is the hyperbolic tangent function, α is the initial slope of the RLCs, and E is the incident irradiance.

Uptake rates of nitrate

The uptake rate of nitrate was determined by the disappearance of nitrate from the culture medium over a given time interval and expressed by the following equation: Nitrate uptake rate = $(N_0 - N_t) \times V \times W_0^{-1} \times t^{-1}$, where N_0 is the initial concentration of culture medium without incubated algae, N_t the concentration of culture medium with incubated algae after t hours, V the volume of the culture medium, and W_0 the initial FW of the algae (Harrison 1988). NO₃⁻ concentration was measured according to Strickland and Parsons (1972).

Statistical analyses

The data are expressed as the means \pm standard deviation (SD, $n \geq 3$). Statistical significance of the data was tested with analysis of variance (ANOVA) or *t* test using SPSS for Window version 19.0. The significance level was set at $P < 0.05$.

Results

Growth rates

Generally, *G. lemaneiformis* exhibited much higher ($P < 0.01$) rate of growth than *P. haitanensis* (Fig. 2). The growth of *P. haitanensis* required a relative low sunlight irradiance. It showed a negative RGR at ambient sunlight and a positive RGR in decreased sunlight. The RGR of *P. haitanensis* thalli grown with a decreased carbon supply was reduced about 83 % (under ambient sunlight) and 37 % (under decreased sunlight) compared with the thalli grown at ambient carbon supply (Fig. 2a).

The mean RGR of *G. lemaneiformis* under the different conditions remained positive, with lower RGR ($P < 0.01$) of thalli grown under decreased carbon supply compared with ambient carbon availability. The RGR of the *G. lemaneiformis* thalli grown under decreased carbon supply was reduced about 95 % (under ambient sunlight) and 35 % (under decreased sunlight) compared with the thalli grown under ambient carbon supply (Fig. 2b).

Biochemical composition

Either decreased carbon supply or decreased sunlight increased the contents of chlorophyll *a* (Chl *a*; $P < 0.05$ for carbon levels; $P < 0.01$ for sunlight levels) and carotenoids (Car; $P < 0.05$ for carbon levels; $P < 0.01$ for sunlight levels) in

P. haitanensis. However, the Chl *a* ($P = 0.934$) and Car ($P = 0.278$) contents were similar in the *G. lemaneiformis* thalli grown under different carbon availability. The Chl *a* ($P < 0.01$) and Car ($P < 0.01$) contents were greater in the *G. lemaneiformis* grown at decreased sunlight than the algae grown at ambient sunlight (Table 1).

The contents of PE ($P = 0.195$), PC ($P = 0.490$), and SP ($P = 0.848$) were similar between the *P. haitanensis* thalli cultured in the two different carbon concentrations. However, PE ($P < 0.05$), PC ($P < 0.05$), and SP ($P < 0.01$) contents were greater in *G. lemaneiformis* grown with decreased carbon supply compared to the thalli grown at ambient carbon supply. In both *P. haitanensis* and *G. lemaneiformis*, growth under decreased sunlight significantly increased the contents of PE ($P < 0.01$ for *P. haitanensis*; $P < 0.01$ for *G. lemaneiformis*), PC ($P < 0.01$ for *P. haitanensis*; $P < 0.01$ for *G. lemaneiformis*), and SP ($P < 0.01$ for *P. haitanensis*; $P < 0.05$ for *G. lemaneiformis*) compared with ambient sunlight (Table 1).

Chlorophyll fluorescence

The daily variation of the quantum efficiency of open PS II (F_v'/F_m') was contrary to change of daily irradiance. The F_v'/F_m' values in both of *P. haitanensis* and *G. lemaneiformis* showed a decrease with gradually increasing sunlight, reached the lowest value at midday between 10:30 and 14:30, and thereafter increased with gradually decreasing sunlight (Figs. 3a, b and 4a, b). Values of the maximum photosynthetic electron transport rate ($rETR_m$) showed clear diurnal trends. The values of both *P. haitanensis* thalli and *G. lemaneiformis* thalli were at their maximum at midday and at their minimum at morning and/or night (Figs. 3c, d and 4c, d). It was seen that both the F_v'/F_m' ($P < 0.01$ for *P. haitanensis*; $P < 0.01$ for *G. lemaneiformis*) and $rETR_m$ ($P < 0.01$ for *P. haitanensis*; $P < 0.01$ for *G. lemaneiformis*) values in the two seaweeds grown under decreased carbon supply were lower than these

Fig. 2 The relative growth rate (RGR) of *Pyropia haitanensis* (a) and *Gracilaria lemaneiformis* (b) grown at different carbon and sunlight availability. Vertical bar represents \pm SD of the means ($n = 3$). AC means the treatment of ambient carbon supply. DC means the treatment of decreased carbon supply. AL means the treatment of ambient sunlight. DL means the treatment of decreased sunlight

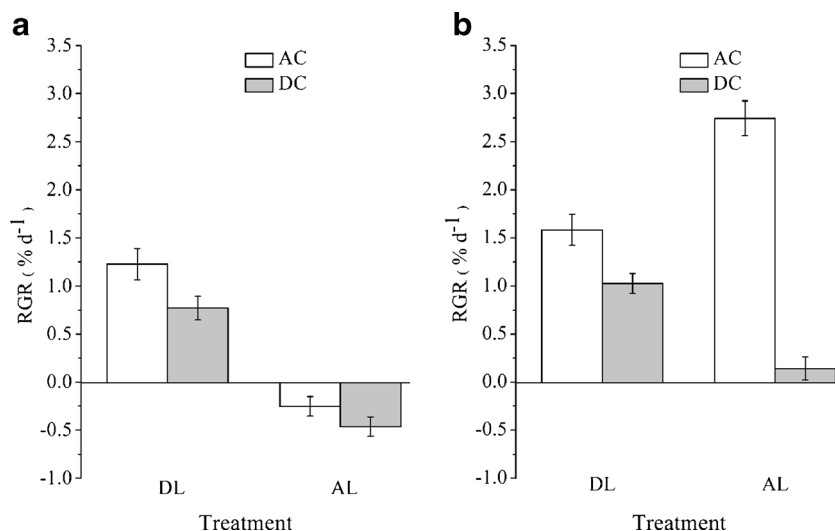


Table 1 Chlorophyll *a* (Chl *a*), carotenoids (Car), phycoerythrin (PE) and phycocyanin (PC), and soluble protein (SP) contents in *Pyropia haitanensis* and *Gracilaria lemaneiformis* grown at different carbon and sunlight availability

| | Growth conditions | | | |
|--------------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| | DL-AC | DL-DC | AL-AC | AL-DC |
| <i>P. haitanensis</i> | | | | |
| Chl <i>a</i> (mg g ⁻¹ FW) | 1.331 ± 0.033 ^a | 1.552 ± 0.061 ^b | 0.835 ± 0.074 ^c | 1.084 ± 0.085 ^d |
| Car (mg g ⁻¹ FW) | 0.602 ± 0.021 ^a | 0.711 ± 0.053 ^b | 0.463 ± 0.045 ^c | 0.588 ± 0.062 ^{ad} |
| PE (mg g ⁻¹ FW) | 1.611 ± 0.036 ^a | 1.583 ± 0.048 ^{ac} | 1.432 ± 0.035 ^{bc} | 1.207 ± 0.051 ^b |
| PC (mg g ⁻¹ FW) | 1.522 ± 0.050 ^a | 1.490 ± 0.049 ^a | 1.289 ± 0.055 ^b | 1.196 ± 0.036 ^b |
| SP (mg g ⁻¹ FW) | 16.950 ± 0.839 ^a | 16.850 ± 0.520 ^a | 13.566 ± 0.311 ^b | 13.210 ± 0.687 ^b |
| <i>G. lemaneiformis</i> | | | | |
| Chl <i>a</i> (mg g ⁻¹ FW) | 0.472 ± 0.015 ^a | 0.467 ± 0.033 ^a | 0.317 ± 0.042 ^b | 0.314 ± 0.033 ^b |
| Car (mg g ⁻¹ FW) | 0.162 ± 0.003 ^a | 0.151 ± 0.008 ^a | 0.124 ± 0.016 ^b | 0.099 ± 0.010 ^b |
| PE (mg g ⁻¹ FW) | 1.746 ± 0.091 ^a | 1.918 ± 0.083 ^b | 0.893 ± 0.043 ^c | 1.049 ± 0.077 ^d |
| PC (mg g ⁻¹ FW) | 0.370 ± 0.005 ^a | 0.420 ± 0.014 ^b | 0.293 ± 0.018 ^c | 0.340 ± 0.020 ^d |
| SP (mg g ⁻¹ FW) | 0.880 ± 0.033 ^a | 0.986 ± 0.051 ^b | 0.601 ± 0.036 ^c | 0.795 ± 0.068 ^{ad} |

Data are means ± SD (*n* = 3); different superscripts indicate significant difference (*P* < 0.05). AC means the treatment of ambient carbon supply. DC means the treatment of decreased carbon supply. AL means the treatment of ambient sunlight. DL means the treatment of decreased sunlight

grown with ambient carbon supply. The F_v'/F_m' (*P* < 0.01) of *P. haitanensis* grown at ambient sunlight were lower than those grown with decreased sunlight, and for the $rETR_m$ (*P* = 0.951) in *P. haitanensis* thalli, there was no significant difference between the thalli grown under the two sunlight conditions. However, the F_v'/F_m' (*P* < 0.05) and $rETR_m$ (*P* < 0.01) of *G. lemaneiformis* grown under ambient sunlight were higher than for the algae grown under decreased sunlight.

Nitrogen uptake rate

Figure 5a illustrates that the NO₃⁻ uptake rates were lower (*P* < 0.01) for *P. haitanensis* thalli cultured at decreased carbon supply compared to those grown at ambient carbon supply. Under ambient carbon growth condition, the uptake rate of nitrate in *P. haitanensis* thalli grown under decreased sunlight was higher (*P* < 0.01) than for the thalli grown at ambient

Fig. 3 The diurnal variation of the quantum efficiency of open PS II (F_v'/F_m') (a, b) and the maximum photosynthetic electron transport rate ($rETR_m$) (c, d) in *Pyropia haitanensis* grown at different carbon and sunlight availability. Vertical bar represents ±SD of the means (*n* = 5). Significance levels are ***P* < 0.01 and **P* < 0.05. AC means the treatment of ambient carbon supply. DC means the treatment of decreased carbon supply. AL means the treatment of ambient sunlight. DL means the treatment of decreased sunlight

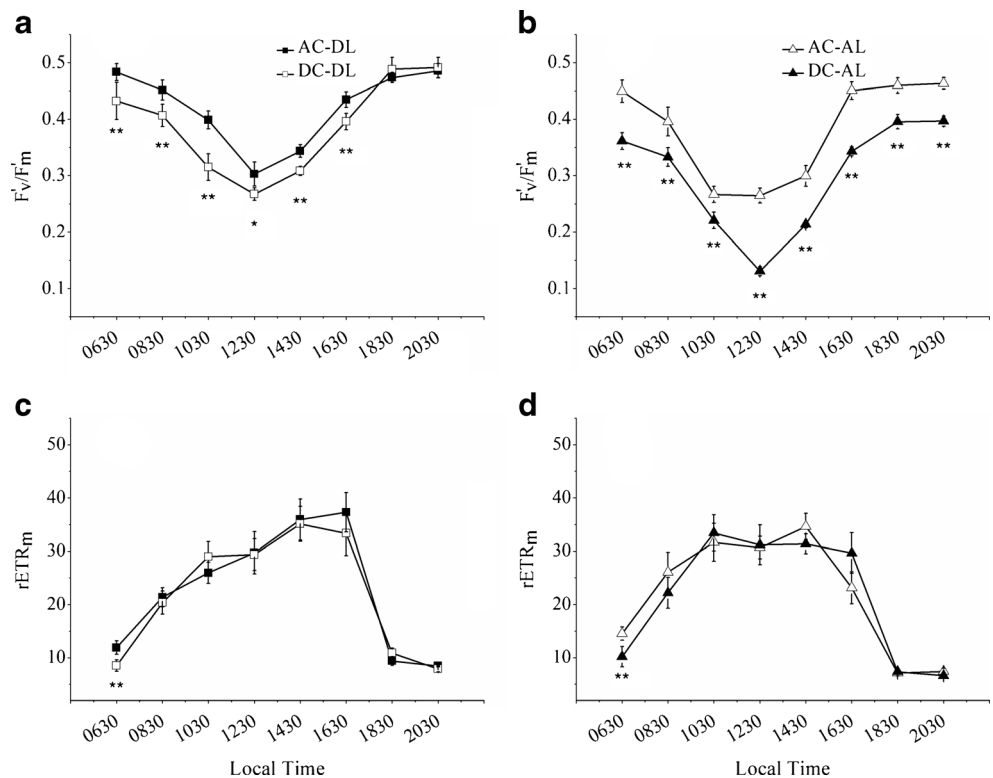
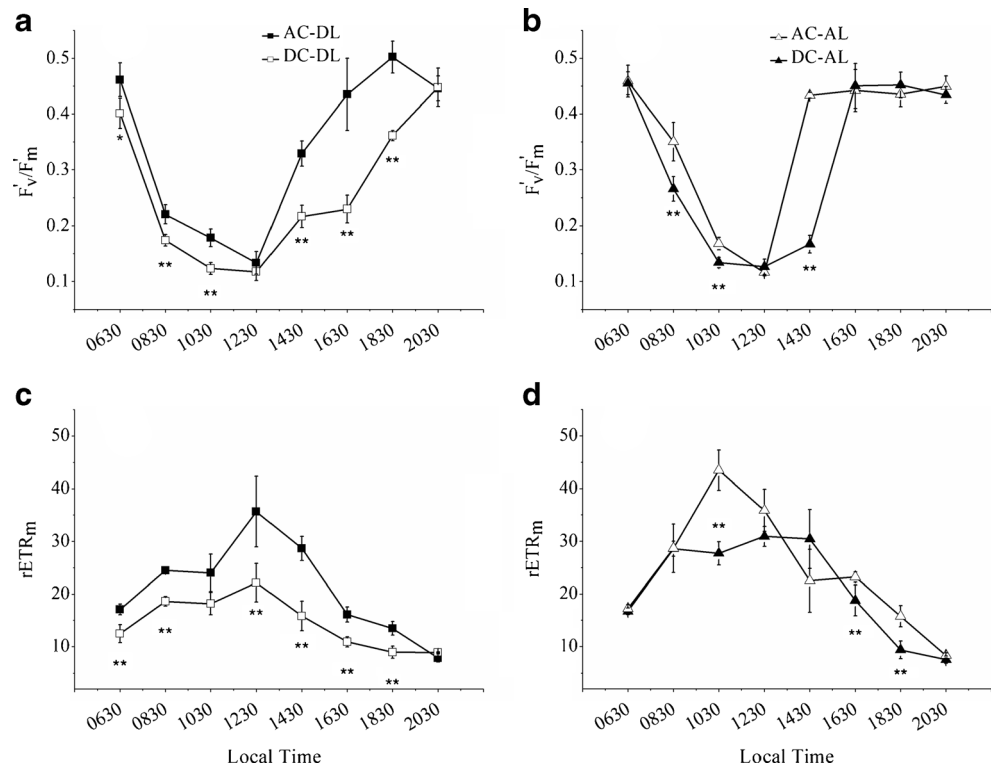


Fig. 4 The diurnal variation of the quantum efficiency of open PS II (F_v/F_m') (**a**, **b**) and the maximum photosynthetic electron transport rate ($rETR_m$) (**c**, **d**) in *Gracilaria lemaneiformis* grown at different carbon and sunlight availability. Vertical bar represents \pm SD of the means ($n=5$). Significance levels are $**P<0.01$ and $*P<0.05$. AC means the treatment of ambient carbon supply. DC means the treatment of decreased carbon supply. AL means the treatment of ambient sunlight. DL means the treatment of decreased sunlight



sunlight. However, under decreased carbon growth condition, the NO_3^- rates were similar ($P=0.687$) between the thalli grown at the two sunlight conditions.

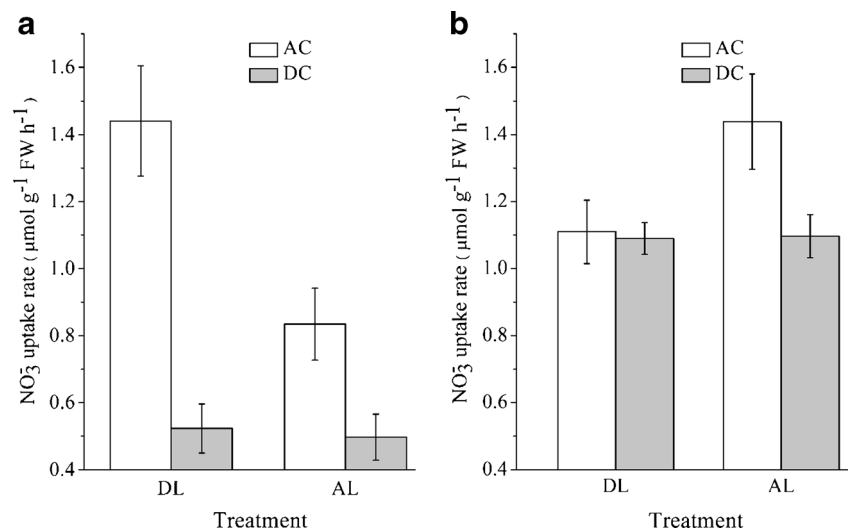
Under ambient sunlight growth condition, the uptake rate of nitrate was decreased ($P<0.05$) in the *G. lemaneiformis* thalli grown with decreased carbon supply than for the algae grown with ambient carbon supply. However, under decreased sunlight growth condition, the different carbon levels had no effect on the NO_3^- uptake rates of the thalli ($P=0.996$). Additionally, under ambient carbon growth condition, the uptake rate of nitrate of ambient sunlight-grown *G. lemaneiformis* thalli was increased ($P<0.05$) relative to the decreased

sunlight-grown thalli. However, under decreased carbon growth condition, the NO_3^- rates showed no significant differences ($P=0.881$) between the thalli grown under the different sunlight conditions (Fig. 5b).

Discussion

Growth and biochemical components The RGR of *P. haitanensis* and *G. lemaneiformis* cultured under low carbon supply were lower than the algae grown under ambient carbon supply. Similar results have been described for

Fig. 5 The nitrogen uptake rate during the sunlight period in *Pyropia haitanensis* (**a**) and *Gracilaria lemaneiformis* (**b**) grown at different carbon and sunlight availability. Vertical bar represents \pm SD of the means ($n=3$). AC means the treatment of ambient carbon supply. DC means the treatment of decreased carbon supply. AL means the treatment of ambient sunlight. DL means the treatment of decreased sunlight



Gracilaria tenuistipitata (García-Sánchez et al. 1994) and *Ulva conglobata* (Zou 2014). The effect of decreased carbon supply on the RGR of *G. lemaneiformis* was greater than on *P. haitanensis*. This implies that *P. haitanensis* has more effective strategies for decreased carbon supply compared to *G. lemaneiformis*. In our previous study (Zou and Gao 2002; Zou et al. 2004), we found that the pH compensation point of *P. haitanensis* (pH 9.9) was greater than *G. lemaneiformis* (pH 9.58). Such a relative high pH compensation point suggested that *P. haitanensis* was more capable of abstracting inorganic carbon from surrounding seawater to drive photosynthesis than *G. lemaneiformis*. However, under carbon starvation, seaweeds cannot gain sufficient photosynthetic carbon to compensate for respiratory Ci consumption, and this can result in negative growth of the seaweeds.

The cellular components were affected by the low carbon levels in seawater. The present results showed that the decreased carbon supply increased the Chl *a* and Car contents of *P. haitanensis* thalli and increased the phycoerythrin and phycocyanin contents of *G. lemaneiformis* thalli. These results agree with those shown in previous studies, where the pigment contents were increased by the low carbon growth conditions in *Gracilaria* sp., *Ulva intestinalis* (Andría et al. 2001, as *Enteromorpha*), and *U. conglobata* (Zou 2014). Contrasting results have been described for *G. tenuistipitata* (García-Sánchez et al. 1994) and *Porphyra leucosticta* (Mercado et al. 1999), in which the phycobiliproteins decreased in low carbon-grown thalli compared with high carbon-grown thalli. Consequently, it appears that the response to lowered carbon in seaweeds is species specific. The electron flow between PS II and PS I and carbon fixation pathway are related to the concentration of photosynthetic units and their minimal turnover time (Falkowski and Raven 1997). In this study, the increased pigment (Chl *a*, Car, PE, and PC) contents in the low carbon condition could compensate for the decreased electron transport rates. Thereby, both of these two economic red algae increased their capacity to acclimate low carbon. The SP content of *G. lemaneiformis* thalli grown at decreased carbon was higher than at ambient carbon, but low carbon growth conditions had no significant effect on SP content of *P. haitanensis* thalli. Additionally, the SP content was also increased by the low carbon growth conditions in *G. tenuistipitata* (García-Sánchez et al. 1994) and *U. conglobata* (Zou 2014). These results might imply a higher capacity of N assimilation in some species under low carbon supply.

The present results showed that the pigments and SP contents of *P. haitanensis* and *G. lemaneiformis* thalli grown at low sunlight were higher than in the algae grown at ambient sunlight. This would be conducive to light absorption and energy conduction as stated by Talarico and Maranzana (2000). Our results also showed that the carbon levels had no effect on the acclimation to low sunlight.

Photosynthetic characteristics The present results showed a clear diurnal pattern of chlorophyll fluorescence. The maximum value of F_v'/F_m' occurred close to morning and/or night, and minimum values occurred in the middle of the day, but the $rETR_m$ reached maximum values at the middle of the day and the $rETR_m$ increased with the weakening of illumination. The results agree with many other studies (e.g., Sagert et al. 1997; Beer et al. 2006; Campbell et al. 2008). With irradiance intensity increasing, photon flux is in excess of that required for photosynthesis and PS II activity is decreased reversibly to protect the algae from excess light energy. As irradiance decreased, F_v'/F_m' and $rETR_m$ began to recover. The recovery might be related to the production of D_1 protein and non-photochemical quenching which dissipates excess energy as heat (Magnusson 1997; Serôdio et al. 2008). In this study, both of the two seaweeds grown at different culture conditions could adjust chlorophyll fluorescence with sunlight changes, implying that the photoprotective capacity of the two seaweeds has not been destroyed.

Both *P. haitanensis* and *G. lemaneiformis* thalli grown at decreased carbon supply had reduced F_v'/F_m' and $rETR_m$. These results are consistent with the observations of Zou (2014), where the F_v'/F_m' and $rETR_m$ were decreased under the decreased carbon growth conditions in *U. conglobata*. In the course of carbon starvation, the endogenous electron acceptors reduce and oxygen becomes the main available electron acceptor. At the same time, oxygen also can act as electron acceptor in photorespiration and Mehler reaction (Durchan et al. 2001); therefore, the activity of photorespiration and Mehler reaction may be enhanced or steady. However, the activity of PS II will be inhibited by active oxygen radicals (e.g., superoxide, hydroxyl radicals, and hydrogen peroxide) which are produced in the Mehler reaction. When carbon limitation occurs in seawater, more photosynthetic pigments absorb more light energy than can be utilized for carbon assimilation. The excess energy may result in photoinhibition. The non-photochemical quenching (NPQ) in algae can dissipate excess energy as heat, but this was not tested and requires further experimentation. Additionally, two processes can contribute to adjust the electron transport rate: the Mehler reaction and the rate of cyclic electron transport around PS I (Mercado et al. 1999). Therefore, the decreased electron transport rates might be associated with a decreased rate of cyclic electron transport around PS I.

Growth irradiance modulates photosynthetic activity of seaweeds to prevent possible photodamage. Furthermore, previous reports have indicated that irreversible photoinhibition was induced by photooxidative damage to PS II which may lead to significant decrease in plant productivity (Baker 1991). This could explain why F_v'/F_m' and RGR of *P. haitanensis* grown at low irradiance were greater than in the thalli grown at ambient irradiance and why *P. haitanensis* cultured under ambient sunlight had a negative RGR. The high RGR of algae in

low sunlight might be also related to the CCMs, which facilitate energy dissipation at high irradiance and downregulate energy consumption under low irradiance. Additionally, the F_v'/F_m' , $rETR_m$, and RGR of *G. lemaneiformis* grown at low irradiance were lower than in the algae grown at ambient irradiance. These results indicate that short-term relative higher sunlight probably caused photodamage and decreased PS II capacity in *P. haitanensis*, whereas such photodamage did not occur in *G. lemaneiformis*. This suggests that *G. lemaneiformis* is more able to adapt to relatively higher growth sunlight conditions than *P. haitanensis*.

Nitrogen metabolism The results showed that decreased carbon supply in culture reduced the NO_3^- uptake, and this possibly explains the lower growth rate of the two algae under low carbon supply. The low NO_3^- uptake rate may be caused by the decreased activity of nitrate reductase (NR) at low levels of CO_2 , as shown by Mercado et al. (1999). The opposite pattern was obtained regarding the high levels of CO_2 . For example, the activity of NR and NO_3^- uptake were increased in *P. leucosticta* (Mercado et al. 1999), *Ulva rigida* (Gordillo et al. 2001), and *Hizikia fusiforme* (Zou 2005) grown at high levels of CO_2 compared with the algae grown at normal CO_2 level.

Under ambient carbon, the NO_3^- uptake rate of *P. haitanensis* cultured at ambient sunlight was higher than for the algae cultured at decreased sunlight, whereas it was lower in *G. lemaneiformis* grown at ambient sunlight compared with the thalli grown under decreased sunlight. Light is an important factor involved in NR regulation (Lillo 1994). In general, light can stimulate nitrate reduction in algae (Lopes et al. 1997). Additionally, nitrate uptake demands carbon skeletons, reducing agent, and energy (Chow et al. 2004). Consequently, the response of NO_3^- uptake rate to light in seaweeds is species-specific.

In conclusion, *P. haitanensis* and *G. lemaneiformis* grown at low carbon supplement condition showed lower RGR, nitrogen uptake rate, F_v'/F_m' , and $rETR_m$ compared with the algae grown with ambient carbon supply, and the effect of decreased carbon supply on the RGR of *G. lemaneiformis* was greater than for *P. haitanensis*. Low sunlight-grown *P. haitanensis* had increased RGR, nitrogen uptake rate, and F_v'/F_m' compared with the algae grown at ambient sunlight. However, these above three physiological indicators of *G. lemaneiformis* reflected contrary results. We therefore suggest that the photosynthetic activity and aquaculture production of these economically important seaweeds would be lessened in seawater with low carbon supply, which usually occurs in cultivation sites with high biomass density and lowered seawater exchange. Additionally, the data suggest that *P. haitanensis* has a more effective strategy for decreased carbon supply compared with *G. lemaneiformis*, whereas *G. lemaneiformis* is better able to adapt to grow at higher sunlight conditions than *P. haitanensis*.

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