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RESEARCH ARTICLE

Comparative physiological behaviors of *Ulva lactuca* and *Gracilariopsis lemaneiformis* in responses to elevated atmospheric CO_2 and temperature

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Abstract

Physiological metabolisms of seaweeds usually suffered climate changes in the field. *Gracilariopsis lemaneiformis* and *Ulva lactuca*, collected from Nan'ao Island, Shantou, China, were cultured under ambient and elevated CO_2 supply (390 and 800 μl L^{-1}), with low and high temperatures (15 °C and 25 °C) for 2 weeks, aiming to compare the difference of the main physiological metabolism between two seaweed species in response to the elevated CO_2 and high temperature. At 15 °C, the pH reduction in the culture medium caused by elevated CO_2 was larger in *G. lemaneiformis* than in *U. lactuca*. At 25 °C, elevated CO_2 significantly increased photosynthetic rates (P_n or P_g) and maintained constant respiratory rates (R_d) in *G. lemaneiformis*. However, for 25 °C-grown *U. lactuca*, the increment of CO_2 did not enhance the P_n (P_g) rates but rapidly decreased the R_d rates itself. With the higher R_d/P_g ratios in *G. lemaneiformis* than *U. lactuca*, the warming thereby promoted more allocation of photosynthetic products to respiratory consumption in *G. lemaneiformis*. Both P_g and R_d rates exhibited lower temperature acclimation in two seaweeds. In addition, elevated CO_2 markedly increased the relative growth rate (RGR) and phycobiliprotein (PB) contents at 25 °C, but exhibited no enhancement of chlorophyll *a* (Chl *a*), carotenoids (Car), soluble carbohydrate (SC), and soluble protein (SP) contents in *G. lemaneiformis*, with the reduction of SC when temperature increased only. We suggested that climate changes were probably a more benefit to *U. lactuca* than to *G. lemaneiformis*, inherently justifying the metabolism during *G. lemaneiformis* maricultivation.

Keywords Elevated CO_2 · Temperature · Photosynthesis · Respiration · *Ulva lactuca* · *Gracilariopsis lemaneiformis*

Introduction

The red seaweed *Gracilariopsis lemaneiformis* (Gracilariales, Rhodophyta) had been commonly used as a food delicacy in abalone aquaculture and raw material for agar acquisition, and it was an important species for seaweed cultivation in China (Tseng 2001). Due to the ever-increasing demand for Gracilariopsis as a source of agar, much more focus had been fixed on this algal species. As a result, artificial cultivation of *G. lemaneiformis* had been strongly prompted along the coastlines in China (Li et al. 1984). Unfortunately, some accompanying algae (e.g., epiphytes such as *Ulva* spp.) with *Gracilariopsis* sp. rapidly developed in the cultivation sites, which always became one of the main problems regarding *Gracilariopsis* sp. cultivation. These accompanying algae were mainly thread- or sheet-like and therefore had a high surface area/volume ratio (Littler and Littler 1980). Such algae were reported to exhibit high rates of nitrogen uptake and photosynthesis, resulting in high growth rates (Svirski et al. 1993). Moreover, the growth rates and photosynthesis of *Gracilariopsis* sp. were often inhibited in the presence of *Ulva lactuca* (Friedlander et al. 1996; Li et al. 2008; Chen et al. 2015). Therefore, the associated technique would be improved to determine the potential productivity of *Gracilariopsis* in raft cultivation.

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Recently, *G. lemaneiformis* had been cultivated on large scales in both the southern and the northern parts of China, covering a large latitudinal gradient in ocean temperature. In addition, the maricultivation of *G. lemaneiformis* at Nan'ao Island, Shantou, China (south coast of China), was from January to June, being subjected to strong variation in temperature and irradiance throughout the cultivation period due to the seasonal change. The cultivation of this species can be an effective bioremediation measure for eutrophication control waters (Zou et al. 2004). Furthermore, mariculture species such as *G. lemaneiformis* had a major impact on coastal carbon fluxes and sequestration (Zou and Gao 2013b), in response to global climate changes (mainly increased atmospheric CO_2 levels and global warming). An increasing amount of attention was being devoted to the elevated CO_2 and temperature responses of this alga. Oxygen evolution characteristics of *G. lemaneiformis* were examined to establish the mechanism of photosynthetic use of exogenous inorganic carbon (Ci), that was the alga was able to use HCO_3^- as a source of Ci for photosynthesis (Zou et al. 2004). Moreover, the elevation of CO_2 significantly increased relative growth rates (RGRs) of *G. lemaneiformis* with an increase of irradiance, but it did not mostly alter the parameters for photosynthetic responses to Ci in culture (Zou and Gao 2009). In addition, photosynthetic rates of *G. lemaneiformis* increased with growth temperature while the respiration maintained constant among different temperatures, resulting in lower temperature sensitivities at higher growth temperature (Kingsolver 2009; Zou and Gao 2013b). However, how the interaction of multiple variables, such as temperature, CO_2 levels, and irradiance, affected the acclimation of photosynthesis and respiration of *G. lemaneiformis* was scarce to know. Moreover, whether this acclimation style of *G. lemaneiformis* was affected by epiphytes (such as *Ulva lactuca*) still needed to be further studied.

This study focused on the maricultured alga *G. lemaneiformis* and the native macroalgae *U. lactuca*. The two algae were grown with monoculture under two CO_2 levels (i.e., ambient air and elevated CO_2 levels) and temperatures (15 °C and 25 °C). The growth, biochemical components, and photosynthetic and respiratory rates of the two species were determined in this study. The responses of respiration and photosynthesis to short- and long-term increasing CO_2 and temperature were also assayed under shading condition. Our objectives were (1) to examine whether or not the thermal acclimation of photosynthetic and respiratory rates changed with elevated CO_2, (2) to compare the photosynthetic benefit resulted from the elevated CO_2 or temperature between *G. lemaneiformis* and *U. lactuca*, (3) and to evaluate the potential influence on the main physiological metabolisms (photosynthesis and respiration) between species in response to climate changes.

Materials and methods

Algal material and sampling site

Gracilariopsis lemaneiformis and *Ulva lactuca* were collected from a cultivation field at Shenao Bay, Nan'ao Island, Shantou, in the southern of China (23°20′ N, 116° 55′ E) in March 2013. *G. lemaneiformis* was subjected to a wide range of in situ temperature from 11–13 °C in January to 25–28 °C in June over the period of sea cultivation (Zou and Gao 2013b), and always grew symbiotically with *U. lactuca* during the cultivation. The thalli of the two algae were gently rinsed of any accumulated sediments and cleared of visible epiphytes, then placed into a plastic barrel containing natural seawater, kept cool, and kept in darkness during the transportation to the laboratory. In the lab, the samples were maintained in filtered natural seawater (salinity 30) enriched with 100 μM $NaNO_3$ and 10 μM NaH_2PO_4 (final concentration) in a 5-L plexiglass aquarium at 15 °C for 3 days prior to further treatment. The two algae received an irradiance of 150 μmol photons m^{-2} s^{-1} (PAR) illuminated by a bank of fluorescent lamps with 12L:12D period. The seawater was changed every 2 days and was continuously aerated by a filter pump to keep air equilibrium of the dissolved inorganic carbon.

For the experimental treatments, *G. lemaneiformis* and *U. lactuca* were cultured at two levels of temperature, i.e., 15 °C and 25 °C (low- and high-cultured temperature), and CO_2 concentrations, i.e., 390 and 800 μl L^{-1} (ambient and elevated CO_2 level). Our previous study had revealed the CO_2 system in seawater depending on the culture condition applied (Liu and Zou 2015b). Experimental treatments were started when 5-g fresh weight (FW) algae were introduced into each of 12 Erlenmeyer flasks containing 5 L filtered seawater. The flasks were placed into two illumination chambers (HP 1000 G, Ruihua Instrument company, Wuhan, China), the temperature conditions of which were controlled at 15 °C and 25 °C. The light conditions (light intensity and light period) for all treatments were the same as indicated above. In each chamber, three flasks were aerated with ambient CO_2, and the remainder flasks were aerated with elevated CO_2. Replicate cultures (n = 3) were maintained at each treatment condition to avoid pseudo-replication. For practical reason, the 12L:12D light cycle was set from 08:00 to 20:00 (local time). Changes in pH values of culture media were measured and recorded using a pH meter (CyberScan pH 510). The two algae were cultured with the above conditions for 2 weeks and then harvested in the effort to determine physiological and biochemical responses under four CO_2 and temperature conditions.

Measurements of respiration and photosynthesis

Before the measurement of net photosynthetic (P_n) and respiratory rates (R_d), all treated *G. lemaneiformis* and *U. lactuca*

were sampled (about 0.5 g FW) and put into 0.5-L flasks filled with seawater, then we wrapped thick towels around the flasks for 2 h during light cycle (local time 12:00, with the irradiance of 30 μmol photons m^{-2} s^{-1}). In situ conditions, such as low irradiances, could occur on cloudy days as well as an effect of overshadowing by the algal community itself. Measurements of the P_n and R_d rates conducted with samples without shadowed acclimation served as the control; others were considered as the shading treatment. Secondly, the P_n and R_d rates of the two algae were measured using a Clark-type oxygen electrode (YSI 5300, USA) that was held in a circulating water bath to keep the desired measurement temperature. Aliquots of 0.2 g FW of algae samples were introduced into the chamber with 8 mL seawater, which was magnetically stirred. The R_d measurements were carried out at 100% air-equilibrium oxygen concentrations in seawater. The samples were allowed to equilibrate in the darkness until the rate of oxygen consumption was constant, usually for 4–6 min, then the rate of R_d was monitored. The R_d rates were recorded to examine the instantaneous effect of elevated CO_2 (measured media: natural and CO_2-enriched seawater) and temperature (measured temperatures: 15 and 25 °C) on the two algae. Immediately, following the respiration measurement, irradiance-saturated P_n rates were determined at the irradiance of 800 μmol photons m^{-2} s^{-1} derived from a halogen lamp. Moreover, the R_d and P_n rates were measured with the same algal samples.

Growth rates and biochemical components

The RGRs were measured in all treatments, which was expressed as percentage increase in FW biomass per day. It was estimated according to the exponential formula: $RGR = \ln(W_t/W_0) \times t^{-1} \times 100\%$, where W_0 referred to the initial and W_t the final FW of seaweeds, and t was the time of cultivation in days.

Chlorophyll *a* (Chl *a*) were extracted in 100% methanol from 0.1 g FW per sample. The concentrations of Chl *a* were determined spectrophotometrically according to the method of Wellburn (1994). To determine phycobiliprotein (PB), about 0.2 g FW of algal biomass were placed in 8 mL phosphate buffer (0.1 mol L^{-1}, pH 6.8), homogenized at 4 °C using a mortar and pestle. The extracts were then centrifuged at 5000*g* for 20 min. The concentrations of PB in the supernatant were determined spectrophotometrically according to Beer and Eshel (1985).

In the extraction of soluble carbohydrates (SCs) and soluble proteins (SPs), six fresh thalli from each culture were ground in a mortar with distilled water and extraction buffers (0.1 M phosphate buffer, pH = 6.8). SCs (determined as sucrose equivalents, a phenol-sulfuric acid method) and SPs were estimated from the supernatant Bradford (1976).

Statistics

The data were expressed at the mean values ± SD ($n = 3$) for the three independent replicates. Multi-factorial ANOVAs were performed including short- and long-term temperature fluctuation, CO_2 level, and shading. When significant differences were detected, post hoc tests were performed using a Duncan test. The significance level was set at 0.05.

Results

pH fluctuation

For 15 °C- or 25 °C-grown *Gracilariopsis lemaneiformis* and *Ulva lactuca*, the pH values in the culture media fluctuated with the day time for the cultures bubbled with ambient air or with elevated atmospheric CO_2 level (Fig. 1(a, b)). The elevated pH values occurred during the light period and the lowered values happened in the darkness. Compared with *U. lactuca* (Fig. 1(b)), the daily fluctuations were generally larger in the culture of *G. lemaneiformis* (Fig. 1(a)). For 15 °C-grown (or 25 °C-grown) *G. lemaneiformis*, the pH of the culture medium aerated with elevated CO_2 was up to 0.53 units (0.20 units) lower than that of the culture medium aerated with ambient air, at the end of the light period. However, the pH fluctuation was up to 0.30 units (0.23 units) with the elevation of CO_2 in the culture for *U. lactuca* grown at 15 °C (or 25 °C) at the same cultural period.

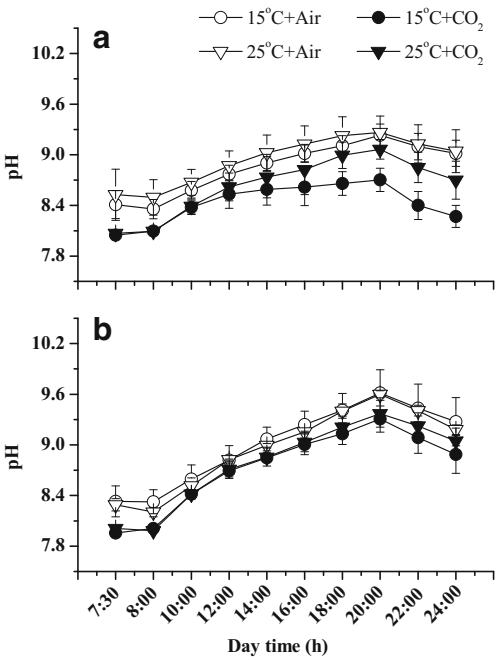

Fig. 1 Daily fluctuations of seawater pH in the culture of *Gracilariopsis lemaneiformis* (a) and *Ulva lactuca* (b) grown at two temperatures (15, 25 °C) and ambient and elevated CO_2 levels (390, 800 μl L^{-1}). Data were means ± SD ($n = 3$)

Photosynthesis

In the initial samples after 2-h shading treatment, different responses of P_n or P_g rates were shown in *G. lemaneiformis* and *U. lactuca*. As the short-term elevation of CO_2 had no significant effect on the P_g as well as the R_d ($P > 0.05$), it was not considered a main factor following the analysis of ANOVA. The ANOVA results were displayed in Table 1. Species (S), growth temperature (T), short-time shading (S^*), and measured temperature (MT) had significant effects on the P_n or P_g, while no obvious effect on the P_n or P_g was found with the action of CO_2 levels (Table 1). The most double and triple or four factor interactions on P_n (or P_g) were significant.

In *G. lemaneiformis*, the P_n rates were significantly increased when measured at 25 °C ($P < 0.05$, Fig. 2(a)), with the photosynthetic Q_{10} value being 2.59 under the ambient CO_2 condition (the Q_{10} value of 2.00 at elevated CO_2) in the 15–25 °C interval and 2.30 (2.83) between 25 and 35 °C (Table 2). Once the shading treatment is done, the P_n rates of 15 °C-grown algae significantly lowered compared with those of the control ($P < 0.05$, Fig. 2(b)). However, the P_n rates of 25 °C-grown algae remained constant ($P > 0.05$, Fig. 2(b)). Regardless of irradiation conditions, 15 °C-grown *G. lemaneiformis* exactly showed higher values of P_n rates than 25 °C-grown algae ($P < 0.05$, Fig. 2(a, b)). Likewise, the higher P_n rates were observed in *U. lactuca* grown at 15 °C compared

Table 1 ANOVA results for experiment with *Gracilariopsis lemaneiformis* and *Ulva lactuca* (macroalgae species, S) after being exposed to combined conditions of growth temperatures (15 and 25 °C, T), CO_2 levels (390 and 800 μl L^{-1}, C), short-time shading (S^*), and measured temperature (15 and 25 °C, MT) on gross photosynthetic rates (P_g), respiratory rates (R_d), and ratios between them (R_d/P_g)

		P_g			R_d			R_d/P_g		
Factors	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
S	1	144.928	2192.679	0.000	9446.679	3550.724	0.000	0.223	353.414	0.000
T	1	51.705	782.266	0.000	1184.746	445.311	0.000	0.000	0.517	0.473
C	1	0.022	0.339	0.561	.809	0.304	0.582	0.003	3.994	0.047
S^*	2	1.816	27.472	0.000	29.016	10.906	0.000	0.013	21.024	0.000
MT	1	163.852	2478.989	0.000	1594.966	599.500	0.000	0.077	122.030	0.000
S × T	1	14.442	218.502	0.000	424.312	159.486	0.000	0.001	1.688	0.195
S × C	1	0.002	0.023	0.879	3.344	1.257	0.263	0.006	9.644	0.002
S × S^*	2	0.783	11.845	0.000	113.925	42.821	0.000	0.003	4.454	0.013
S × MT	1	26.791	405.330	0.000	97.286	36.567	0.000	0.057	89.993	0.000
T × C	1	3.270	49.468	0.000	14.829	5.574	0.019	0.024	38.615	0.000
T × S^*	2	1.120	16.947	0.000	18.584	6.985	0.001	0.015	24.223	0.000
T × MT	1	14.573	220.477	0.000	51.143	19.223	0.000	0.007	10.446	0.001
C × S^*	2	0.844	12.768	0.000	44.473	16.716	0.000	0.009	14.055	0.000
C × MT	1	0.004	0.066	0.798	4.539	1.706	0.193	9.304E-6	0.015	0.903
S^* × MT	2	2.253	34.093	0.000	44.713	16.806	0.000	0.007	11.678	0.000
S × T × C	1	2.570	38.886	0.000	17.689	6.649	0.011	0.010	15.531	0.000
S × T × S^*	2	1.654	25.026	0.000	12.818	4.818	0.009	0.001	2.082	0.127
S × T × MT	1	3.576	54.104	0.000	6.791	2.552	0.111	0.000	0.641	0.424
S × C × S^*	2	0.757	11.449	0.000	42.339	15.914	0.000	0.012	18.665	0.000
S × C × MT	1	0.012	0.176	0.675	0.931	0.350	0.555	0.001	1.394	0.239
S × S^* × MT	2	0.778	11.777	0.000	109.489	41.154	0.000	0.028	43.622	0.000
T × C × S^*	2	0.384	5.816	0.003	4.023	1.512	0.222	0.001	1.701	0.185
T × C × MT	1	0.191	2.884	0.091	0.130	0.049	0.825	0.000	0.676	0.412
T × S^* × MT	2	0.941	14.233	0.000	9.200	3.458	0.033	0.003	5.350	0.005
C × S^* × MT	2	0.000	18.329	0.000	6.952	2.613	0.075	0.013	21.251	0.000
S × T × C × S^*	2	0.955	14.444	0.000	6.642	2.496	0.085	0.009	13.967	0.000
S × T × C × MT	1	1.059	16.016	0.000	1.704	0.640	0.424	0.006	10.200	0.002
S × T × S^* × MT	2	0.719	10.878	0.000	6.737	2.532	0.082	0.011	17.004	0.000
S × C × S^* × MT	1	0.288	4.362	0.014	9.486	3.565	0.030	0.002	2.630	0.074
T × C × S^* × MT	2	0.136	2.053	0.131	0.252	0.095	0.910	0.000	0.470	0.625
S × T × C × S^* × MT	2	0.889	13.450	0.000	0.117	0.044	0.957	0.005	7.969	0.000

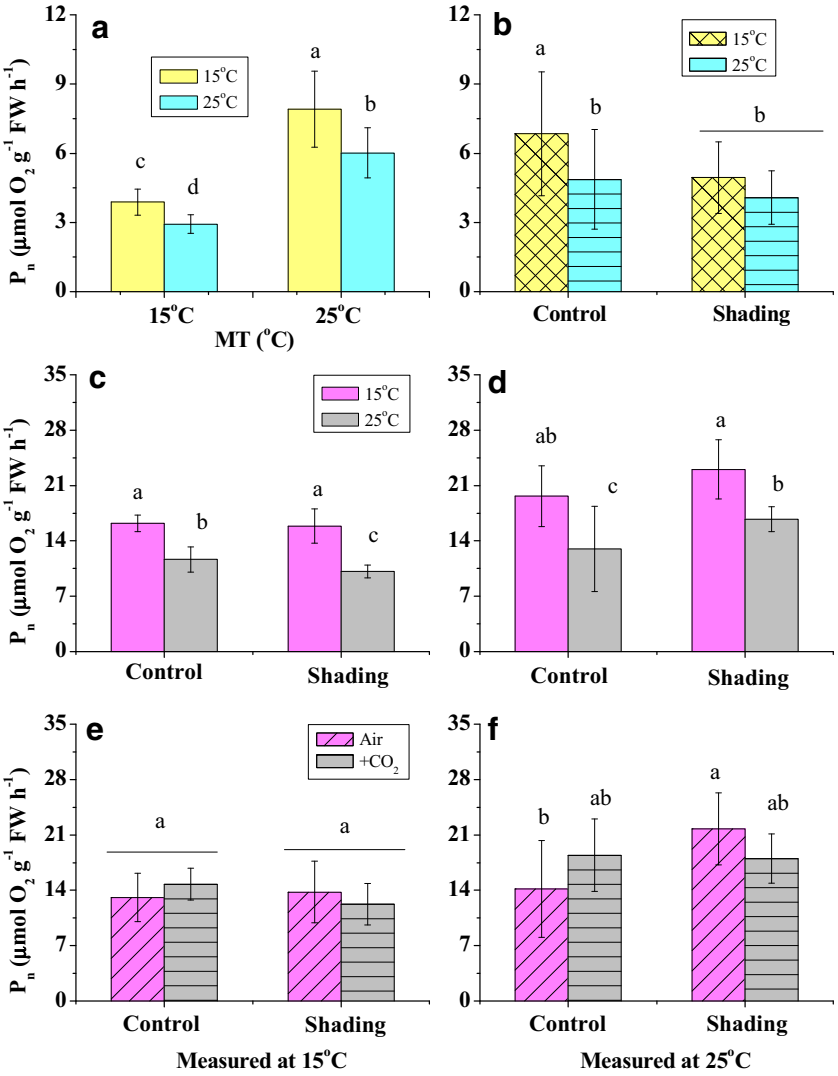

Fig. 2 Net photosynthetic rates (P_n) of *Gracilariopsis lemaneiformis* (a, b) and *Ulva lactuca* (c–f) exposure to combined conditions of growth temperatures (15 and 25 °C, T), CO_2 levels (390 and 800 μl L^{-1}, C), shading (S^*), and measured temperatures (15 and 25 °C, MT). The approaches were conducted with samples for measuring at two temperatures (15 and 25 °C) and CO_2 levels (natural and CO_2-enriched seawater). Measurements without shadowed acclimation served as the control; others were considered as the shading treatment. Data were pooled means ± SD, in accordance with significant effects obtained by ANOVA: *G. lemaneiformis*, interactive effects of T and MT (a, $n = 24$) and interactive effects of T and S^* (b, n = 24); *U. lactuca*, interactive effects of T, MT, and S^* (c, d, $n = 12$) and interactive effects of C, MT, and S^* (e, f, $n = 12$). Different letters above the histograms indicate significant differences ($P < 0.05$, Duncan post hoc test)

Table 2 Values of Q_{10} (the rate increase caused by raising temperature 10 °C) for photosynthesis (P_n) and respiration (R_d) of *Gracilariopsis lemaneiformis* and *Ulva lactuca* grown at 15 °C and 25 °C under two CO_2 levels (390 and 800 μl L^{-1}). Values were means ± SD ($n = 3$). Different superscripts indicated significant difference ($P < 0.05$)

Treatments	P_n (Q_{10})	R_d (Q_{10})
G. lemaneiformis		
15 °C + Air	2.59 ± 0.27^{ab}	3.90 ± 0.49^{a}
15 °C + CO_2	2.00 ± 0.30^{bc}	1.76 ± 0.38^{bc}
25 °C + Air	2.30 ± 0.23^{bc}	1.40 ± 0.34^{cde}
25 °C + CO_2	2.83 ± 0.33^{a}	2.06 ± 0.23^{b}
U. lactuca		
15 °C + Air	1.36 ± 0.17^{c}	3.37 ± 0.38^{b}
15 °C + CO_2	1.16 ± 0.21^{c}	4.88 ± 0.74^{a}
25 °C + Air	1.45 ± 0.15^{ab}	3.33 ± 0.37^{b}
25 °C + CO_2	0.88 ± 0.03^{d}	1.12 ± 0.18^{d}

with 25 °C-grown algae ($P < 0.05$, Fig. 2(c, d)), which were independent on measuring temperature and/or irradiation conditions. In addition, the elevation of CO_2 in the culture had no significant effect on P_n rates of *U. lactuca* ($P > 0.05$, Fig. 2(e, f)). Without CO_2 elevation in culture, an increase of measurement temperature from 15 to 25 °C (or from 25 to 35 °C) had significant effect on P_n, with a Q_{10} of approximately 1.5 for 15 °C- and 25 °C-grown algae (Table 2).

Respiration

The ANOVA results of R_d rates were also shown in Table 1. The single effect of species (S), growth temperature (T), measured temperature (MT), and shading (S^*) were significant, whereas elevated CO_2 (C) had no effect on R_d (Table 1). Compared with P_n (or P_g), R_d presented a similar pattern with the most double and triple interactions among variables maintained above.

In *G. lemaneiformis*, 15 °C-grown algae showed higher R_d rates than algae grown at 25 °C, and the lowest values of R_d were observed in higher-temperature-grown algae (25 °C) with the elevation of CO_2 (Fig. 3(a b)). Under all the growth conditions, the Q_{10} values of R_d were generally within 2.0, with an exception of the increased value ($Q_{10} = 3.90$) under 15 °C and ambient CO_2 condition (Table 2). In *U. lactuca*, the R_d rates increased with an increment of measuring temperature from 15 to 25 °C (Fig. 3(c, d)). Meanwhile, the R_d rates of *U. lactuca* presented a similar pattern with the P_n rates of *G. lemaneiformis*, that was lower growth temperature maintained a high level of R_d in *U. lactuca* (Fig. 3(c, d)). The R_d rates were independent of the short-term irradiation change and CO_2 elevation in the culture (Fig. 3(e, f)). Additionally, the highest values of R_d were observed with the elevation of CO_2 at 15 °C (the growth temperature) (Fig. 3(g, h)), along with significantly increased Q_{10} values of R_d at the same condition ($P < 0.05$, Table 2).

The ratio of respiration to photosynthesis

The ANOVA results of the ratios of respiration to photosynthesis (R_d/P_g) were displayed in Table 1. The single effect of species (S), elevated CO_2 (C), measured temperature (MT), and shading (S^*) were significant, whereas growth temperature (T) had no effect on R_d/P_g ratios (Table 1). The most double and triple or four factors interactive effects on the ratios of R_d/P_g were significant. In *G. lemaneiformis*, dark respiration was generally between 0.14 and 0.35 of gross photosynthesis, which was larger than that of *U. lactuca*. When grown at 25 °C, short-term exposure to 15 °C markedly decreased the ratios of R_d to P_g (i.e., P_n plus R_d) with lower irradiation in *G. lemaneiformis* ($P < 0.05$, Fig. 4(a)). Likewise, the R_d/P_g ratios of algae slightly decreased at 25 °C with the same light condition (Fig. 4(b)). For *U. lactuca* grown at 25 °C, the R_d/P_g ratios over all measured temperature dramatically reduced with lower light condition ($P < 0.05$, Fig. 4(c, d)). However, the R_d/P_g values of 15 °C-grown algae did not show significant alteration measured at growth temperature ($P > 0.05$, Fig. 5(c, d)).

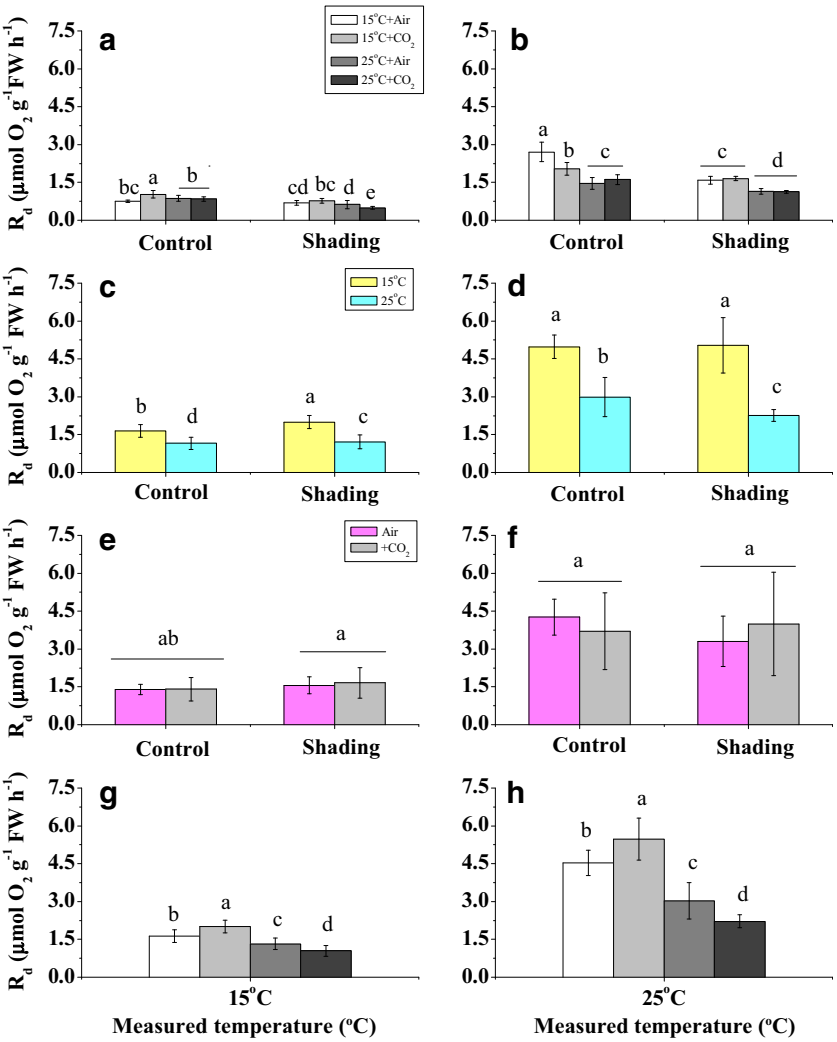

Fig. 3 Respiratory rates (R_d) of *Gracilariopsis lemaneiformis* (a b) and *Ulva lactuca* (c–h) exposure to combined conditions of growth temperature (15 and 25 °C, T), CO_2 levels (390 and 800 μl L^{-1}, C), shading (S^*), and measured temperature (15 and 25 °C, MT). The approaches were conducted with samples for measuring at two temperatures (15 and 25 °C) and CO_2 levels (natural and CO_2-enriched seawater). Measurements without shadowed acclimation served as the control; others were considered as the shading treatment. Data were pooled means ± SD, in accordance with significant effects obtained by ANOVA: *G. lemaneiformis*, interactive effects of T, C, MT, and S^* (a, b, $n = 6$); *U. lactuca*, interactive effects of T, MT, and S^* (c, d, $n = 12$); interactive effects of C, MT, and S^* (e, f, $n = 12$); and interactive effects of T, C, and MT (g, h, $n = 12$). Different letters above the histograms indicate significant differences ($P < 0.05$, Duncan post hoc test)

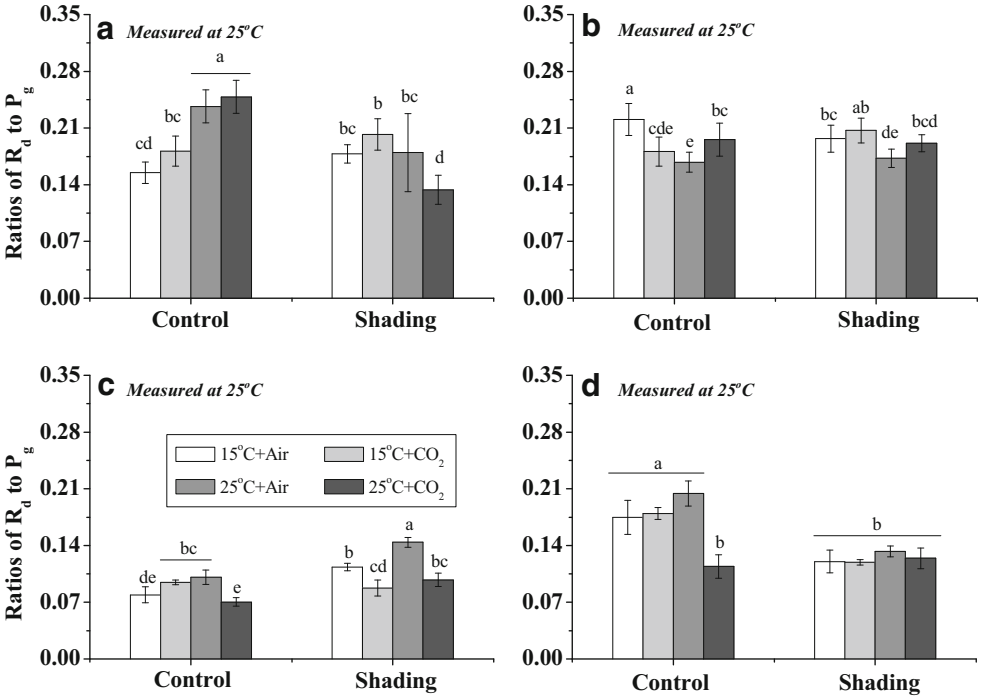

Fig. 4 The dark respiration (R_d) to gross photosynthesis (P_g) ratios of *Gracilariopsis lemaneiformis* (a, b) and *Ulva lactuca* (c, d) exposed to combined conditions of growth temperatures (15 and 25 °C, T), CO_2 levels (390 and 800 μl L^{-1}, C), shading (S^*), and measured temperature (15 and 25 °C, MT). The approaches were conducted with samples for measuring at two temperatures (15 and 25 °C) and CO_2 levels (natural and CO_2-enriched seawater). Measurements without shadowed acclimation served as the control; others were considered as the shading treatment. Vertical bars represented ± SD of the means ($n = 3$)

Biochemical components

For *G. lemaneiformis*, CO_2 availability exerted significant effect on the RGR at 25 °C ($P < 0.05$, Fig. 5(a)). Higher temperature slightly reduced the SC contents (Fig. 5(b)), but had no effects on the contents of SP, Chl *a*, and Car ($P > 0.05$, Fig. 5(c–e)). Elevated CO_2 caused a strong increase in PB contents at 25 °C ($P < 0.05$, Fig. 5(f)), while exhibited no significant variation in the contents of SC, SP, and any pigment ($P > 0.05$, Fig. 5(b–e)). The alteration about biochemical components of *U. lactuca* had been already reported by Liu and Zou (2015b) (data not shown). According to our previous report, the RGR and photosynthetic pigment contents (mainly for Chl *a* and Car) were affected by neither growth temperature nor CO_2 availability. An increase of the SP and SC contents was observed in 15 °C-grown algae compared with 25 °C-grown algae.

Discussion

In this study, the results of short-term and long-term effects of CO_2 levels and temperature increases on the physiology of two macroalgae (*Gracilariopsis lemaneiformis* and *U. lactuca*) were presented, which were different from morphological, bio-optical, and physiological characteristics. Combined effects of CO_2 (ambient CO_2, Air; elevated CO_2, $+CO_2$) and temperature (growth temperature, T; measured temperature, MT), together with short-term shade (S^*), on photosynthesis and respiration were tested. Most studies about the effect of global climate changes on aquatic organisms had been conducted with one or two experimental variables, while studies on the interaction of multiple factors were very scarce (Yildiz et al. 2013; Schoenrock et al. 2016; Sampaio et al. 2017).

According to our previous study (Liu and Zou 2015b), the light-saturated photosynthesis of *U. lactuca* represented a positive trend with CO_2 elevation at 25 °C. The effects of elevated CO_2 on photosynthesis were probably temperature-dependent when temperature was high enough for CO_2 or DIC to be rate-limited, as this effect on photosynthesis was not distinct at 15 °C. By comparing the data of *U. lactuca*, the photosynthesis of *G. lemaneiformis* was differently affected by increased ocean temperature and self-shading of light. When co-occurring, the impacts of those environmental conditions were additive; hence, the interactive climate factors were found on photosynthesis, such as the interaction of $T \times S^*$ as well as $T \times S^* \times MT$. Due to its preference of light, the maximum photosynthesis of *G. lemaneiformis* was statistically reduced with the increasing mat density (Jiang et al. 2016; Jiang et al. 2017). In the present study, compared with 25 °C-grown *Gracilariopsis* seedlings, the algae cultured at 15 °C could be disadvantaged under adverse conditions such as temporary weakened light. This point was basically consistent with the previous study (Jiang et al. 2017). When light was sufficient in surroundings, elevated CO_2 markedly enhanced the growth and photosynthesis of *G. lemaneiformis* at 25 °C, together with much higher temperature sensitivity (Q_{10}) than *U. lactuca* in response to short-term temperature increments. Therefore, this suggested that the regulation of carbon

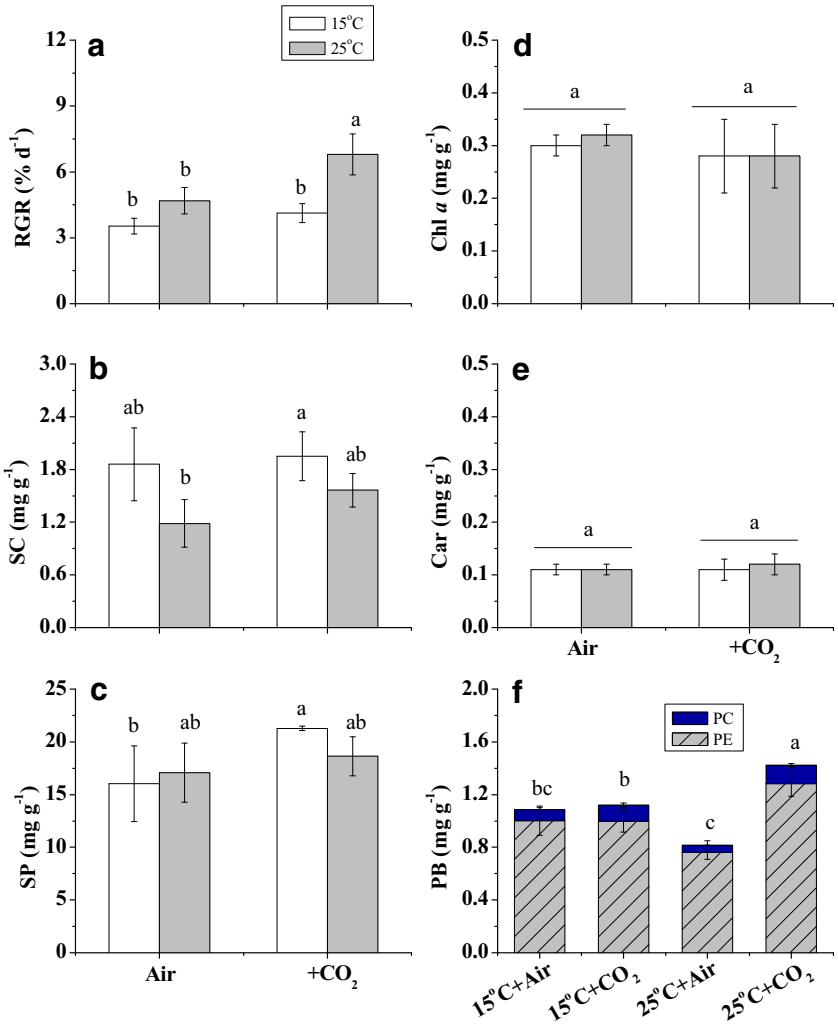

Fig. 5 The RGR (a), soluble carbohydrates (SC, b), soluble protein (SP, c), Chl *a* (d), Car (e), and phycobiliprotein (PB, f) contents in *Gracilariopsis lemaneiformis* grown at two temperatures (15, 25 °C) and ambient and elevated CO_2 levels (390, 800 μl L^{-1}). As the parameters maintained above in *Ulva lactuca* were published in Liu and Zou (2015), they are not displayed in the graphs

assimilation of *G. lemaneiformis* could be different from that of *U. lactuca*, probably occurring through an indirect action on the expression of photosynthetic enzymes.

An acclimation potential of photosynthesis to temperature could be seen when comparing instantaneous with long-term temperature effects on *G. lemaneiformis* and *U. lactuca*. It demonstrated that the two algae were able to photosynthetically acclimate to the lower growth temperature (15 °C), as their photosynthetic rates displayed a significant increase at 15 °C compared with the instantaneous responses. For example, the photosynthetic rates of 15 °C-grown *G. lemaneiformis* were much higher than those of the 25 °C-grown algae measured at 15 °C, or the photosynthetic rates of 25 °C-grown *G. lemaneiformis* measured with a temperature of 25 °C were much lower than the rates of 15 °C-grown algae measured at 25 °C. If the optimal temperature range of photosynthesis in *G. lemaneiformis* became lower with high temperature like *Pyropia haitanensis* (Liu and Zou 2015a), this partial photosynthetic thermal acclimation of *Gracilariopsis* seedlings could probably counteract the instantaneous effect of lower temperature (Zou and Gao 2013a), through the acceleration of photosynthetic enzymatic reactions, involving an increase in the amount, or activity, of enzymes that limit photosynthesis (Sage and Kubien 2007; Zou and Gao 2014b; Machalek et al. 1996). Hence, it could work to the advantages of *G. lemaneiformis* in competition with *U. lactuca* in cultivation. We proposed that appropriate measurements were needed to identify the role of temperature optima, in the effort to maintain a relative high photosynthesis during *G. lemaneiformis* maricultivation. Additionally, it was worthy to note that increased CO_2, i.e., hypercapnia-linked, variance of the potential of photosynthetic acclimation did not occur in both the two algae, mainly due to the metabolic suppression in cells (Kurihara et al. 2008).

Furthermore, the respiratory analysis revealed striking similarities between *G. lemaneiformis* and *U. lactuca*. The acclimation potential of respiration to low temperature (15 °C) was demonstrated in both the two algae. It was considered that, for *Gracilariopsis* and *Ulva* seedlings, an increase of soluble carbohydrates was suggested to be directly responsible for the subsequent recovery of respiration associated with this acclimation characteristics through increased

substrate consumption. The distributive tendency of photosynthetic substrates was in line with previous studies reported (Atkin and Tjoelker 2003; Staehr and Wernberg 2009; Zou and Gao 2013b). On the other hand, from a view of inner energy conversion, an increase in respiration in cold-acclimated algae might accelerate the rates of ATP turnover, resulting in an elevation of ADP concentrations and/or uncoupling of electron transport from proton translocation across the inner mitochondrial membrane (Campbell et al. 2007; Zou and Gao 2014a).

Besides the acclimation potential of respiration, there existed special responses to environmental factors in both the two algae. When transferred to 25 °C, elevated CO_2 decreased the respiration of *G. lemaneiformis* under lower irradiance conditions, whereas the respiration of 15 °C-grown *U. lactuca* was independent of light and CO_2 levels and higher than that of the algae grown at 25 °C. Therefore, the respiratory metabolisms of *U. lactuca* were typically considered more sensitive to warming than those of *G. lemaneiformis*. Due to naturally higher basal metabolism, the respiration of *U. lactuca* might be closer to its metabolic peak than that of *G. lemaneiformis*, easily reaching or overcoming the optimal metabolic threshold with short increases of temperature. Simultaneously, high temperature produced positive effects on the energy consumption of *U. lactuca*, benefiting itself by accelerating metabolisms, as predicted by the metabolic theory of ecology (Kingsolver 2009). Finally, it was predicted that the metabolism of *G. lemaneiformis* was mathematically justified by *U. lactuca* inherently higher biomass. In addition, an increase in soluble proteins and a decrease in fatty acids were found in 15 °C-grown *U. lactuca*, which were consistent with the low-temperature acclimation itself (Liu and Zou 2015b). At the large extent, a relative increase of antenna complexes in *G. lemaneiformis* logically induced an enhancement of the effective quantum yields at low temperature (Liu et al. 2016). However, the elevation of CO_2 had no remarkable effect on soluble carbohydrates in both *G. lemaneiformis* and *U. lactuca*, which was not associated with responses of terrestrial plants and microalgae species to elevated CO_2 (Jia et al. 2016; Li et al. 2016). Therefore, further research was needed to establish the biochemical underpinnings of physiological acclimation in the two algae.

Generally, photosynthetic and respiratory metabolisms were inseparable from each other. With an accurate understanding of the R_d/P_g ratio in response to short- and long-term environmental changes, the alteration of algal carbon balance and flux throughout coastal ecosystems were typically determined. In our study, different patterns of the temperature sensitivity (Q_{10}) between photosynthesis and respiration were expressed in both *G. lemaneiformis* and *U. lactuca*, finally resulting in different variations of the R_d/P_g ratios between them. The respiration of *G. lemaneiformis* was significantly affected by the instantaneous change in situ conditions, such as a reduction in irradiance (occurring on cloudy days or overshadowing by the algal community itself), and high respiration of the algae always run by increasing consumption of their photosynthetic products. In contrary, *U. lactuca* always maintained higher photosynthesis than *G. lemaneiformis*. It was thereby to compete growth resource surroundings with *G. lemaneiformis* in maricultivation.

Consequently, under sufficient light condition, elevated CO_2 markedly increased the growth and photosynthesis with the increased temperature in *G. lemaneiformis*, together with the higher Q_{10} values than that of *U. lactuca*. With the higher R_d/P_g ratios in *G. lemaneiformis* than *U. lactuca*, the warming thereby promoted more allocation of photosynthetic product to respiratory consumption for the former alga. Besides, both photosynthesis and respiration all displayed a low-temperature acclimation in *G. lemaneiformis* and *U. lactuca*. We concluded that increases in warming might be more beneficial to the respiration of *U. lactuca* than of *G. lemaneiformis*, mathematically justifying the metabolism of *G. lemaneiformis* by striving for the growth resource in surroundings.

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