Scientific Correspondence

Future CO_2-Induced Ocean Acidification Mediates the Physiological Performance of a Green Tide Alga[1]

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The oceans take up more than 1 million tons of CO_2 from the air per hour, about one-quarter of the anthropogenically released amount, leading to disrupted seawater chemistry due to increasing CO_2 emissions. Based on the fossil fuel-intensive CO_2 emission scenario (A1F1; Houghton et al., 2001), the H^+ concentration or acidity of surface seawater will increase by about 150% (pH drop by 0.4) by the end of this century, the process known as ocean acidification (OA; Sabine et al., 2004; Doney et al., 2009; Gruber et al., 2012). Seawater pH is suggested to decrease faster in the coastal waters than in the pelagic oceans due to the interactions of hypoxia, respiration, and OA (Cai et al., 2011). Therefore, responses of coastal algae to OA are of general concern, considering the economic and social services provided by the coastal ecosystem that is adjacent to human living areas and that is dependent on coastal primary productivity. On the other hand, dynamic environmental changes in the coastal waters can interact with OA (Beardall et al., 2009).

Macroalgae have diversified strategies in terms of inorganic carbon (Ci) acquisition, with different carboxylation efficiencies associated with different photosynthetic affinities for Ci (Johnston and Raven, 1990; Giordano et al., 2005; Zou and Gao, 2010). Most macroalgae can actively use bicarbonate or directly take up CO_2 to provide a CO_2 source for Rubisco, a mechanism known as the carbon-concentrating mechanism (CCM), while a few red and green macroalgae, known as "non bicarbonate users," acquire Ci solely by diffusion of dissolved CO_2 (Raven et al., 1995; Kübler et al., 1999). Therefore, macroalgae may respond differentially to increasing pCO_2 (for partial pressure of CO_2 in seawater) and the changing chemistry of seawater. Increasing atmospheric CO_2 concentrations have been

demonstrated to enhance the photosynthesis of intertidal macroalgae at low tide during emersion (Gao et al., 1999; Zou and Gao, 2005) and enhance the growth of the red algae *Porphyra yezoensis*, *Gracilaria* spp., and *Lomentaria articulata* (Gao et al., 1991, 1993; Kübler et al., 1999) and the brown alga *Hizikia fusiforme* (Zou, 2005). However, decreased growth rates under elevated CO_2 concentrations are observed in *Gracilaria tenuistipitata* (Garćía-Sánchez et al., 1994), *Porphyra leucostica* (Mercado et al., 1999), and *Porphyra linearis* (Israel et al., 1999). Neutral effects of elevated CO_2 levels (750 μatm) on the growth of several macroalgae are also reported (Israel and Hophy, 2002). Recent research shows that meiospore germination in the brown macroalga *Macrocystis pyrifera* benefits from the increased availability of CO_2 (820 μatm; Roleda et al., 2012). Despite these differential responses, OA is notorious for reducing calcification of the red coralline algae (Gao et al., 1993; Gao and Zheng, 2010), green *Halimeda* spp. (Sinutok et al., 2011), and brown *Padina* spp. (Johnson et al., 2012). Furthermore, elevated CO_2 has the potential to influence competition between noncalcareous macroalgae and coralline species (Hepburn et al., 2011).

Marine green algae represent a large paraphyletic group of green plants from which the higher plants (the embryophytes) developed (Douglas et al., 2004). They share the same photosynthetic pigments with, but live in a quite different environment from, terrestrial higher plants. These plants, mostly distributed in coastal waters, where biological production is high, experience dynamic environmental changes associated with reciprocal tides and human activities. In most coastal waters, because of the photosynthetic carbon removal from and respiratory carbon release to the ambient environment, fluctuation of pH usually shows a day-night (high to low) reversion pattern. Therefore, marine green algae are usually tolerant to acid-base perturbations (Larsson et al., 1997), although the mechanisms involved are not understood yet. From a physiological point of view, marine green algae may show fairly different responses from terrestrial plants to increasing CO_2 concentration, if acidity change acts to affect their physiology. However, to our knowledge, little has been documented on this aspect.

Increased availability of ambient CO_2 to about 1,000 μatm based on the projected future atmospheric CO_2 rise may not be large enough to affect the influx of CO_2
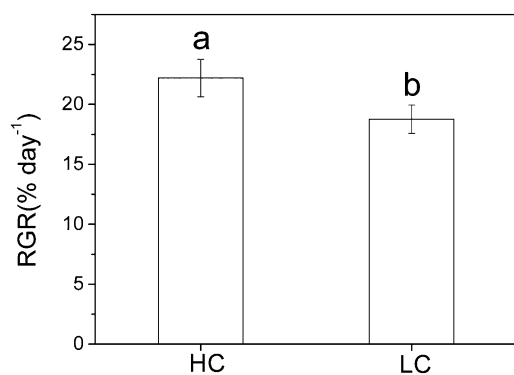
Figure 1. The relative growth rate (RGR) of *U. prolifera* plants grown at ambient (LC; 390 μatm) and elevated (HC; 1,000 μatm) CO$_2$ levels. Different letters above the columns indicate significant ($P = 0.038$) differences.

into cells because of the operation of CCMs, which will result in concentrated CO$_2$ within the cells (Raven et al., 2011). Although CCMs can be down-regulated under elevated CO$_2$ levels, they are supposed not to have been switched off (Wu et al., 2012), and intracellular CO$_2$ concentration and energetics for algal growth can be altered (Hopkinson et al., 2011; Raven et al., 2011). On the other hand, increased external acidity associated with the enrichment of CO$_2$ would either exert a stress that influences intracellular acid-base stability (Gao et al., 2012) or ease the acid-base regulation, since most algae maintain an average pH across the cell of between 7 and 7.5 (Smith and Raven, 1979). Therefore, energetics can also be affected due to changes of pH in the ambient environment. At the same time, the oxygenation process might be enhanced, since the CO$_2$-to-oxygen ratio surrounding Rubisco may be changed due to the down-regulation of CCMs. Based on the above theoretical assumptions, we hypothesize that the effects of future CO$_2$-induced OA on marine green algae will depend on light levels due to affected energetics associated with the down-regulation of CCMs as well as acid-base regulation; therefore, dark- and light-dependent respiration would be enhanced under stressful light levels due to changes in the algae's energetics to cope with the increased acidity. We chose the green macroalga *Ulva prolifera* (Zhang et al., 2011), which is commonly found in the coastal waters of the Pacific Ocean, to test this hypothesis. This species is also notorious for forming
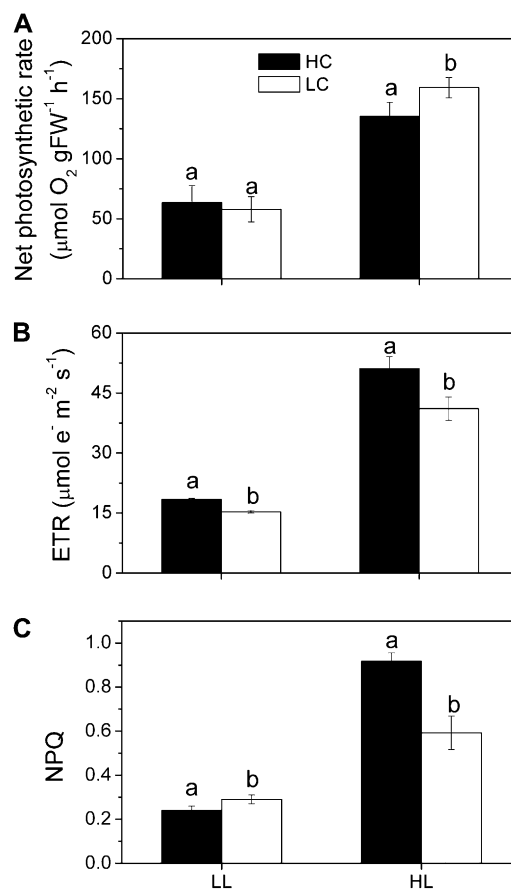

Figure 2. The net photosynthetic rate (A), ETR (B), and NPQ (C) of *U. prolifera* plants grown for 2 months at ambient (LC; 390 μatm) and elevated (HC; 1,000 μatm) CO$_2$ levels measured at growth light (LL; 100 μmol m^{-2} s^{-1}) and high light (HL; 600 μmol m^{-2} s^{-1}). The reaction medium (sterilized seawater) was equilibrated with the ambient or elevated CO$_2$ air before use. FW, Fresh weight.

large-scale green tides (Lu and Qiao, 2008) in recent years.

PHOTOSYNTHETIC PERFORMANCE

U. prolifera plants, developed from zoospores under different CO$_2$ and pH levels, showed significant differences in growth and photosynthetic performance. The relative growth rate was significantly ($P = 0.038$) enhanced (Fig. 1) under elevated CO$_2$ level. Under the

Table I. *Photosynthetic parameters of relationships of photosynthetic oxygen evolution (Fig. 3A) and ETR (Fig 3B) as a function of PAR in U. prolifera plants grown at ambient (LC; 390 μatm) and elevated (HC; 1,000 μatm) CO$_2$ levels*

Data are means $\pm$ SD of three measurements; different letters represent significant differences between the CO$_2$ levels (a/b, $P < 0.05$; a/a, $P > 0.05$). I_K, Light saturation perimeter.

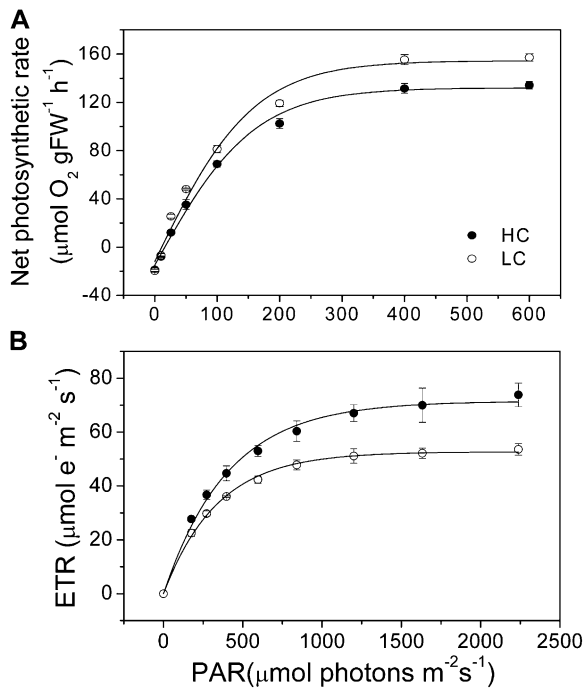| Condition	P_{max}	$\alpha_{(P)}$	$I_{K(P)}$	ETR_{max}	$\alpha_{(ETR)}$	$I_{K(ETR)}$
HC	147.0 $\pm$ 4.3a	0.93 $\pm$ 0.03a	158.4 $\pm$ 8.0a	71.6 $\pm$ 5.5a	0.18 $\pm$ 0.01a	404.3 $\pm$ 43.0a
LC	166.5 $\pm$ 4.2b	1.06 $\pm$ 0.06a	157.0 $\pm$ 6.2a	51.1 $\pm$ 4.4b	0.15 $\pm$ 0.02b	349.0 $\pm$ 27.2a

Figure 3. Photosynthetic oxygen evolution (A) and relative ETR (B) as a function of PAR in the *U. prolifera* plants grown for 2 months at ambient (LC; 390 μatm) and elevated (HC; 1,000 μatm) CO_2 levels. The reaction medium (sterilized seawater) was equilibrated with ambient air before use. FW, Fresh weight.

Figure 4. Changes in ETR for *U. prolifera* plants grown for 2 months at ambient (LC; 390 μatm) and elevated (HC; 1,000 μatm) CO_2 levels when measured in CO_2- or DIC-free reaction medium (buffered with 20 mM Tris, pH 8.2) under a PAR of 600 μmol photons m^{-2} s^{-1} and 20°C.

growth light level (100 μmol photons m^{-2} s^{-1}), electron transport rate (ETR) was higher ($P = 0.001$), while nonphotochemical quenching (NPQ) was significantly lower ($P = 0.04$), and no significant difference ($P = 0.6$) was found in the net photosynthetic (oxygen evolution) rate between the high-CO_2 (HC)- and low-CO_2 (LC)-grown plants. Under the high light levels (600 μmol photons m^{-2} s^{-1}), both the ETR and NPQ were higher ($P = 0.01$), while the net photosynthetic oxygen evolution rate was significantly lower ($P = 0.046$) in the HC-grown plants (Fig. 2). When measured at the ambient CO_2 level (pH 8.2), the light-saturated photosynthetic oxygen evolution rate (P_{max}) and the apparent photosynthetic light use efficiency [$\alpha_{(P)}$] were significantly ($P = 0.005$ and 0.023, respectively) lower (Fig. 3A; Table I). However, the light-saturated ETR (ETR$_{max}$) and electron transport efficiency [$\alpha_{(ETR)}$] were higher ($P = 0.01$ and 0.038, respectively; Fig. 3B; Table
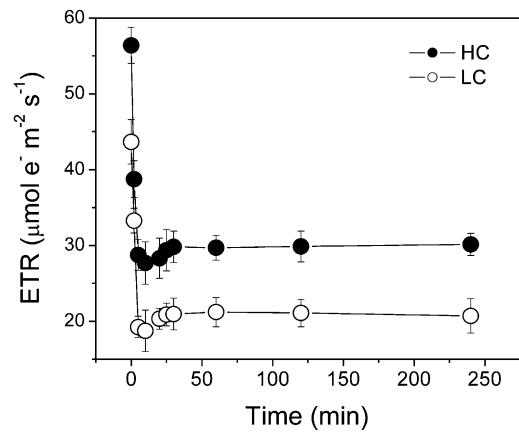
I) in HC-grown than in LC-grown individuals. When examined in a CO_2-free medium (pH 8.2), the ETR decreased immediately, with the depletion of the intracellular Ci pool within 5 min, and was then sustained for the following 4 h, with the rate of the HC-grown plants being significantly ($P = 0.01$) higher by 45% than that in the LC-grown ones (Fig. 4). The HC-grown alga increased their NPQ to a much higher extent (about twice) compared with the LC-grown ones when exposed to stressful high-light levels (Fig. 5), reflecting that the HC condition led to additional light stress.

RESPIRATORY RESPONSE, CCM ACTIVITY, AND PIGMENTS

When light-dependent (photorespiration) and dark respirations were compared, the HC-grown thalli showed a higher (by 52%; $P = 0.017$) photorespiration rate than the LC-grown ones, but no significant ($P = 0.41$) difference was found in the dark respiration (Fig. 6).

The relationship of photosynthetic oxygen evolution versus dissolved Ci concentration (Fig. 7A) showed higher $K_{1/2DIC}$ or $K_{1/2CO2}$, reflecting reduced photosynthetic dissolved Ci (DIC) affinity (Fig. 7B), in the

Table II. *Parameters of the seawater carbonate system under the ambient (LC; 390 μatm) and elevated (HC; 1,000 μatm) CO_2 concentrations*

DIC, pH, salinity, nutrient concentration, and temperature were used to derive all other parameters using a CO_2 system-analyzing software (CO2SYS). Data are means ± SD of six measurements; different letters represent significant differences between the CO_2 levels (a/b, $P < 0.05$; a/a, $P > 0.05$). NBS, National Bureau of Standards.

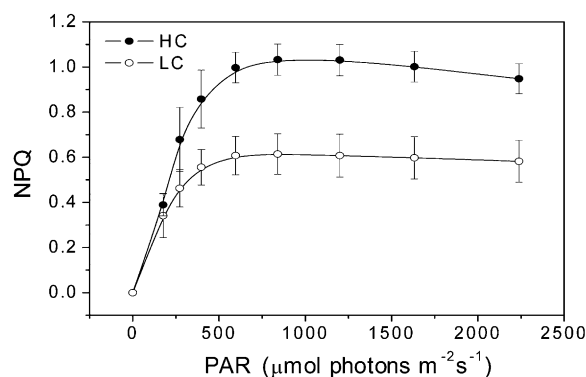| Condition	pCO$_2$	pH$_{NBS}$	DIC	HCO$_3^-$	CO$_3^{2-}$	CO$_2$	Total Alkalinity
	μatm		*μmol kg^{-1}*				
LC	390 ± 11a	8.18 ± 0.01a	1,903.2 ± 14.7a	1,738.1 ± 10.0a	152.2 ± 5.5a	13.0 ± 0.4a	2,123.8 ± 22.0a
HC	975 ± 28b	7.82 ± 0.02b	2,043.3 ± 36.6b	1,935.6 ± 33.9b	75.4 ± 3.6b	32.4 ± 0.9b	2,131.4 ± 40.5a

Figure 5. Changes in NPQ as a function of PAR in the *U. prolifera* plants grown for 2 months at ambient (LC; 390 μatm) and elevated (HC; 1,000 μatm) CO$_2$. The reaction medium (sterilized seawater) was equilibrated with the ambient air before use.

HC-grown plants. Such a reduced photosynthetic affinity for DIC and/or CO$_2$ indicated a down-regulated CCM or a decrease in CCM activity. The efficiency of Ci acquisition, reflected by the ratio of V_{max} to half-maximal photosynthetic rate ($K_{1/2}$) for both DIC and CO$_2$, decreased significantly ($P = 0.0001$) in the HC-grown plants, by up to 31%.

The contents of chlorophyll (Chl) *a* and Chl *b* decreased ($P = 0.039$ and 0.004, respectively), while that of carotenoids increased ($P = 0.032$) in the HC-grown thalli (Fig. 8A). The Chl *a/b* ratio in the thalli grown in the LC condition was significantly lower than that in the HC condition ($P = 0.001$; Fig. 8B).

POSSIBLE MECHANISMS AND IMPLICATIONS

The green alga *U. prolifera*, when grown under the elevated CO$_2$ concentration in a controlled seawater carbonate system, showed enhanced growth (Fig. 1) and reduced its maximal photosynthetic oxygen evolution rate and light use efficiency (Figs. 2A and 3A; Table I) as well as its efficiency to dissipate excessive light energy (Figs. 2C and 5), although acclimation to the elevated CO$_2$ level led to a higher electron transfer rate under the growth light level (Figs. 2B and 3B), which is consistent with that previously reported by Liu et al. (2012) for the same species grown under the same high CO$_2$ level but for a longer period (80 d). Along with the decreased photosynthetic affinity for HCO$_3^-$ and/or CO$_2$ (Fig. 7), photorespiration increased (Fig. 6), Chl *a* and *b* contents decreased, and carotenoid contents increased remarkably under the elevated CO$_2$ (Fig. 8). The increased photorespiration and carotenoid contents, together with the down-regulated CCM, implied that the green alga increased its defensive strategy against CO$_2$-driven seawater acidification at the cost of a reduced ratio of carboxylation to oxygenation.

Although light use efficiency increased due to saved energy from down-regulated CCM or easing acid-base regulation under the OA condition, decreased contents

of photosynthetic pigments (Fig. 8) and/or enhanced photorespiration in the HC-grown thalli did lead to decreased oxygen evolution at the subsaturating and saturating light levels (Fig. 7), although their ETR increased either at deprived (Fig. 4) or elevated (Liu et al., 2012) DIC levels. Since the operation of CCMs demands energy and concentrated intracellular CO$_2$ can acidify the thylakoid lumen, an essential component of the CCM (Raven, 1997), down-regulation of CCM in the HC-grown thalli might have decreased the intracellular CO$_2$ level and have contributed to lower the NPQ at the same time. HC-grown red alga *P. yezoensis* showed decreased oxygen evolution rate (K. Gao, unpublished data), although its growth rate was stimulated (Gao et al., 1991). A cyanobacterium grown at an elevated DIC level also decreased its net photosynthetic reductant production (Mackenzie et al., 2004). The enhanced growth rate of *U. prolifera* might be attributed to the energy saved due to the down-regulated CCM operation as well as nitrogen metabolism. Elevated CO$_2$ concentrations stimulated the uptake of NO$_3^-$ in another *Ulva* species, *Ulva rigida* (Gordillo et al., 2001), and enhanced the activity of nitrate reductase in *U. rigida* (Gordillo et al., 2001, 2003) and *Ulva linza* (data not shown).

The persistence of photosynthetic electron transport without supply of ambient Ci from the ambient environment (Fig. 4) indicated the involvement of some pathways that continuously drained the electrons when the plants were exposed to oversaturating light levels. Consumption or reduction of O$_2$ by both photorespiration and the Mehler reaction must have played a critical role in photoprotection, sustaining the electron flow (Ort and Baker, 2002). The fact that the sustained ETR was much higher in the HC-grown than in the LC-grown plants, even in the CO$_2$-free medium, reflected that these pathways were enhanced under the HC condition. Photorespiration was indeed much higher in the HC-grown than in the LC-grown plants (Fig. 6). On the other hand, light-stimulated synthesis
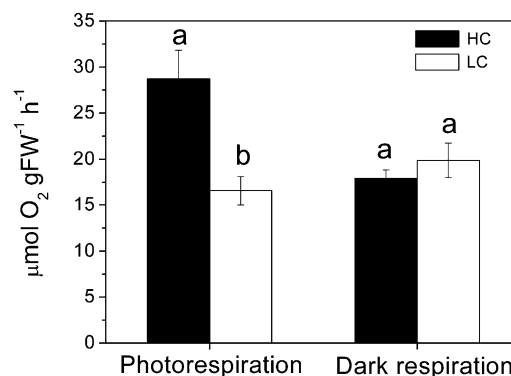

Figure 6. Changes in photorespiration (measured at 600 μmol photons m^{-2} s^{-1}) and dark respiration for young *U. prolifera* grown for 2 months at ambient (LC; 390 μatm) and elevated (HC; 1,000 μatm) CO$_2$ levels. Different letters above the bars represent significant ($P < 0.05$) differences between the CO$_2$ levels. FW, Fresh weight.
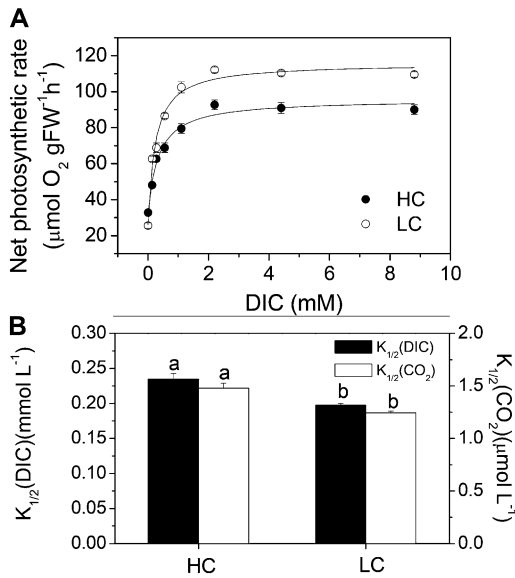
Figure 7. A, Changes in net photosynthetic rate for *U. prolifera* plants grown for 2 months at ambient (LC; 390 μatm) and elevated (HC; 1,000 μatm) CO_2 levels, measured at 600 μmol photons m^{-2} s^{-1} and 20°C, as a function of DIC concentration. B, DIC and CO_2 concentrations for the $K_{1/2}$, derived from A. Different letters above the bars represent significant ($P < 0.05$) differences between the CO_2 levels. FW, Fresh weight.

of fatty acids might also play a role in additional electron drainage (Willms et al., 1999).

The elevated CO_2 concentrations, projected for the end of this century, are known to down-regulate algal CCMs (Rost et al., 2003; Wu et al., 2010), but they may not be adequate to supply enough CO_2 by diffusion to sustain intracellular CO_2 concentrations. While intracellular Ci pools might be reduced in HC-grown algae to be lower than that in the LC-grown ones (Spijkerman, 2011; Raven et al., 2012), intracellular CO_2 availability around Rubisco in *U. prolifera* would have decreased under OA, so that the photosynthetic oxygen evolution rate was reduced (Figs. 2A and 3A) and photorespiration was enhanced (Fig. 6). On the other hand, the enhancement of ETR in the HC-grown plants (Figs. 2B, 3B, and 4) indirectly indicated that the enhancement of photorespiration or other metabolic pathways, such as the water-water cycle (Asada, 1999), led to additional electron drainage while playing the photoprotective role under excessive light conditions (Crawley et al., 2010). At the same time, NPQ increased faster in the HC-grown plants (Figs. 2C and 5). Stimulated NPQ and photorespiration under OA were recently noted in diatoms and phytoplankton assemblages when exposed to high light levels (Gao et al., 2012).

Under growth subsaturating light, down-regulated CCM due to increased external CO_2 availability might have saved some energy for its operation, and this saved energy, such as ATP generated by transmembrane H^+-ATPase, could be used for carboxylation, leading to decreased energy-dependent nonphotochemical

quenching (the key component of NPQ; Kanazawa and Kramer, 2002) and increased ETR (Fig. 2B; Liu et al., 2012) and growth (Fig. 1). However, when the alga was exposed to excessive light levels, enhanced defensive pathways under the OA condition, such as photorespiration and NPQ, as well as the synthesis of carotenoids would have taken their toll on the energetics (Figs. 6 and 8A). At the same time, the alga decreased its light-capture pigments (Fig. 8A). Such a "pigment economy" phenomenon could avoid the overexcitation of electron transport as a sign of adaptation, and reduction of the antenna size and an increase in the Chl a/b ratio (Fig. 8B) provided further evidence to support this.

For terrestrial green plants, short-term exposures to increased CO_2 levels stimulate the net photosynthesis of both C_3 and even some C_4 plants (Ziska and Bunce, 2006), while long-term exposures to elevated CO_2 concentration often lead to a declined photosynthetic rate in many plant species (Thomas and Strain, 1991). Dark respiration and photorespiration are also reduced at the leaf level, while plant growth is stimulated (Bunce, 2004). In addition, intracellular acid-base regulation is affected by elevated CO_2 levels in C_3 higher plants (Yin et al., 1990). In contrast, the green alga *U. prolifera* has to cope with both decreasing pH and increasing partial pressure of CO_2 in the marine environment because of the atmospheric CO_2 rise. Such an acid-based perturbation would affect ion channels across the cell membrane and the associated
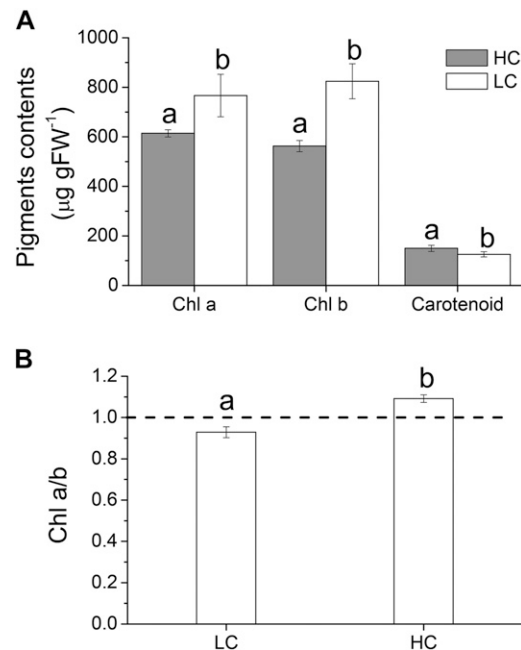

Figure 8. Pigment contents (Chl *a*, Chl *b*, and carotenoid; A) and Chl *a/b* ratio (B) of *U. prolifera* plants grown for 2 months at ambient (LC; 390 μatm) and elevated (HC; 1,000 μatm) CO_2 levels. The different letters above the bars represent significant ($P < 0.05$) differences between the LC and HC conditions. FW, Fresh weight.

energetic cost in order to maintain intracellular pH stability; therefore, OA can be a stressor for marine plants, although it may favor their energy budget when light energy is limited (Gao et al., 2012). When other environmental stressors, such as excessive light, coexisted with OA, the green alga's defensive strategy was enhanced (Figs. 2B, 5, and 6). From an evolutionary point of view, of advantage to the green plants evolving to terrestrial environments might have been avoidance of the stress of OA, which is known to have occurred at the end of the Ordovician period (Veron, 2008). Green plants have been suggested to have evolved about 400 million years ago (Leliaert et al., 2011), when atmospheric CO$_2$ was as high as 3,000 μL L^{-1} and the extinction of marine organisms occurred with an ancient OA event (Berner, 2006; Veron, 2008).

In the natural environment, although *U. prolifera* can tolerate some extent of pH change, increasing seawater acidity due to the ongoing atmospheric CO$_2$ rise would lower the baseline of coastal pH and challenge most organisms' ability to acclimate or adapt to coastal OA, which is suggested to proceed faster than in the pelagic waters (Cai et al., 2011). Our study obviously demonstrates that OA mediated the photochemical and photorespiratory pathways of the green alga grown under OA conditions, but how this affects its life cycle (interchange of sporophyte and gametophyte life stages) remains to be investigated.

MATERIALS AND METHODS

Thalli and Culture Conditions

Thalli of *Ulva prolifera* were collected in June 2009 from the coastal water of Lianyungang (119.3°E, 34.5°N), Jiangsu province of China, where the alga caused a green tide (Keesing et al., 2011). Selected thalli were cleaned of epiphytes and cultured in the laboratory at 100 μmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR; 12/12 h of light/dark) and at 20°C before the released spores were collected and allowed to settle on glass slides in darkness. After adhesion, the spores were cultured under ambient (390 μatm; LC) and elevated (1,000 μatm; HC) CO$_2$ levels and at the above light and temperature conditions. The HC level was obtained in a CO$_2$ plant chamber, which automatically controlled the CO$_2$ concentration in it with variation of less than 5%. The LC level was obtained by pumping the ambient air from outside (top of the building) of the laboratory. The pH levels were maintained at 8.18 and 7.82 in the LC and HC cultures, respectively, with variations of less than 0.04, by constantly removing increased algal biomass and renewing the medium every 24 h. The dissolved pCO$_2$ and HCO$_3^-$ levels were 32.4 and 1,936 μM, respectively, increased by 149% and 113%, and CO$_3^{2-}$ concentration was 75 μM, decreased by 50%, in the HC compared with the LC treatment (Table II), representing typical chemical changes in the carbonate system associated with OA. Culture under the two CO$_2$ levels lasted for about 2 months before the physiological performances were investigated during the last week.

Measurement of Growth

The relative growth rate (RGR) of the young sporophytes was estimated after the plants had acclimated to the CO$_2$ levels for more than 50 d as follows: RGR = 100 × (lnN_t − lnN_0)/t, where N_0 is the initial fresh weight and N_t is that after t days.

Measurements of Photosynthetic Oxygen Evolution

Photosynthetic oxygen evolution was measured by using a Clark-type oxygen electrode (YSI model 5300A). About 1-cm-long segments of *U. prolifera* were prepared with scissors (the cutting damage was minimized by incubating the segments in natural seawater for 1 h). About 0.05-g samples (about 0.03–0.04 mg of Chl *a*) were transferred to the cuvette containing 8 mL of filtered fresh seawater (30% salinity, pH 8.2, 1.9 mM DIC) for determining the net photosynthetic rate as a function of light (P-E curve), and the temperature was controlled at 20°C. The net photosynthetic rate was also measured in seawater medium equilibrated with either high (1,000 μatm) or low (390 μatm) CO$_2$ levels under growth light (100 μmol photons m^{-2} s^{-1}) and high light (600 μmol photons m^{-2} s^{-1}). The photosynthesis-versus-DIC concentrations curve was measured by adding sodium bicarbonate solution into DIC-free seawater medium buffered with 20 mM Tris (pH 8.2) at final DIC concentrations within a range of 0 to 8.8 mmol L^{-1}. Parameters for the P-E curves were analyzed as follows (Jassby and Platt 1976): P = P$_{max}$ × tanh(α × E/P$_{max}$) + Rd, where P is the photosynthetic rate, tanh is the hyperbolic tangent, E is the irradiance, and Rd is the dark respiration rate. The $K_{1/2}$ (reciprocal of photosynthetic affinity) values for DIC or CO$_2$ were calculated using the Michaelis-Menten equation.

Determination of Photorespiration

Photorespiration was estimated as the difference in net photosynthetic oxygen evolution of the thalli at reduced (2%) and ambient (21%) O$_2$ levels (Björk et al., 1993). The Tris-buffered seawater (pH 8.2) was flushed with either pure N$_2$ or air to establish the low (2%) or air-saturated (21%) levels of dissolved O$_2$. Oxygen concentration was measured as above.

Chl Fluorescence Measurements

After 10 min of dark adaptation, fluorescence induction curves were measured with a xenon-pulse amplitude-modulated fluorometer (Walz) for examining the photochemical performance of *U. prolifera* grown at different CO$_2$ levels. The actinic light was set at 100 and 600 μmol photons m^{-2} s^{-1}, respectively, and the saturating pulse was 5,000 μmol photons m^{-2} s^{-1} (0.8 s). The NPQ was attained as follows: NPQ = (F_m − F_m')/F_m', where F_m is the maximum fluorescence yield after dark adaptation and F_m' is the maximum fluorescence yield under actinic light. The ETR was calculated as follows (Genty et al., 1989): ETR (μmol e$^-$ m^{-2} s^{-1}) = F_v'/F_m' × 0.5 × PFD × A, where F_v'/F_m' represents the effective PSII quantum yield, PFD is the photosynthetically active photon flux density, and A is the fraction of incident photons absorbed by *U. prolifera* thallus (Franklin and Badger, 2001). The rapid light curves for ETR and NPQ were measured under eight different PAR levels (every measurement lasted for 10 s), and the reaction medium was the same as that used for the measurement of the P-E curve. The parameters of the ETR curves were analyzed according to Webb et al. (1974): ETR = ETR$_{max}$ × (1 − e$^{(-\alpha*E/ETRmax)}$), where α is the efficiency of electron transport and E is the irradiance. To investigate the ETR in CO$_2$-free seawater, DIC-free seawater medium was obtained by adding 1.0 M HCl to lower the pH to less than 3.0, then sparging for at least 1 h with high-purity N$_2$ gas, and buffering with 20 mM Tris (pH 8.2).

Determination of Photosynthetic Pigments

About 100 mg fresh weight of thalli was extracted with 10 mL of absolute methanol at 4°C for 24 h in darkness, and Chl *a*, Chl *b*, and carotenoid concentrations were estimated according to Wellburn (1994).

Data Analysis

The data are shown as means ± SD of three measurements. Statistical analysis was performed with one-way ANOVA (Tukey's) or Student's *t* test. The 95% confidence level was used in all analyses.

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