J. Phycol. **47,** 87–97 (2011)

DOI: 10.1111/j.1529-8817.2010.00929.x

SHORT- AND LONG-TERM EFFECTS OF ELEVATED CO_2 ON PHOTOSYNTHESIS AND RESPIRATION IN THE MARINE MACROALGA *HIZIKIA FUSIFORMIS* (SARGASSACEAE, PHAEOPHYTA) GROWN AT LOW AND HIGH N SUPPLIES[1]

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The short-term and long-term effects of elevated CO_2 on photosynthesis and respiration were examined in cultures of the marine brown macroalga *Hizikia fusiformis* (Harv.) Okamura grown under ambient (375 $\mu L \cdot L^{-1}$) and elevated (700 $\mu L \cdot L^{-1}$) CO_2 concentrations and at low and high N availability. Short-term exposure to CO_2 enrichment stimulated photosynthesis, and this stimulation was maintained with prolonged growth at elevated CO_2, regardless of the N levels in culture, indicating no down-regulation of photosynthesis with prolonged growth at elevated CO_2. However, the photosynthetic rate of low-N-grown *H. fusiformis* was more responsive to CO_2 enrichment than that of high-N-grown algae. Elevation of CO_2 concentration increased the value of $K_{1/2}$(Ci) (the half-saturation constant) for photosynthesis, whereas high N supply lowered it. Neither short-term nor long-term CO_2 enrichment had inhibitory effects on respiration rate, irrespective of the N supply, under which the algae were grown. Under high-N growth, the Q_{10} value of respiration was higher in the elevated-CO_2-grown algae than the ambient-CO_2-grown algae. Either short- or long-term exposure to CO_2 enrichment decreased respiration as a proportion of gross photosynthesis (*Pg*) in low-N-grown *H. fusiformis*. It was proposed that in a future world of higher atmospheric CO_2 concentration and simultaneous coastal eutrophication, the respiratory carbon flux would be more sensitive to changing temperature.

Key index words: **brown algae; CO_2; *Hizikia fusiformis*; marine macroalgae; photosynthesis; respiration**

Abbreviations: **Ci, inorganic carbon; fwt, fresh weight; *Pg*, gross photosynthesis; *Pn*, net photosynthesis; *Rd*, dark respiration**

The atmospheric CO_2 concentration has been rising since the Industrial Revolution and will continue to rise from the present 375 $\mu L \cdot L^{-1}$ to 500–900 $\mu L \cdot L^{-1}$ by the end of the century (Joos et al. 1999). In the oceans, CO_2 dissolved in seawater exists in three main inorganic forms collectively known as dissolved inorganic carbon (Ci): aqueous CO_2, HCO_3^-, and CO_3^{2-}, which interconvert according to the equations

$$CO_2 + H_2O \longleftrightarrow H^+ + HCO_3^- \longleftrightarrow CO_3^{2-} + 2H^+ \quad (1)$$

In normal natural seawater (pH 8.2, salinity 35 psu), HCO_3^- is the majority (~90%) of total dissolved Ci (2.0–2.2 mM), followed by CO_3^{2-} (~9%), and then aqueous CO_2 (~1%, 10–15 μM). An increase of CO_2 concentration in the atmosphere will increase proportionally the concentration of dissolved CO_2 in the surface oceans, covarying with a decrease of pH in the seawater (Riebesell et al. 2007). Under this scenario, growing interest has been paid to the photosynthetic response of marine macroaglae to CO_2 enrichment in seawater (Beer and Koch 1996, Andría et al. 1999, Mercado et al. 1999, Israel and Hophy 2002, Zou 2005).

Photosynthesis of marine macroalgae is frequently fully or nearly saturated with the current availability of dissolved Ci in seawater (Johnston et al. 1992, Beer 1994, Beer and Koch 1996, Raven 1997), which is primarily attributed to the photosynthetic HCO_3^- utilization acting as carbon-concentrating mechanism (CCM) like terrestrial C_4 plants. In contrast, some macroalgal species seem to depend on the passive diffusion of CO_2 as their only source of Ci to drive photosynthesis, and their photosynthesis would be severely limited with the current levels of dissolved Ci in seawater. Some other species also exhibit nonsaturated photosynthesis in current ambient seawater, although they possess the ability of using HCO_3^- (Johnston et al. 1992, Mercado and Niell 1999, Zou et al. 2003). However, the short-term response of photosynthesis to CO_2 enrichment in seawater does

[1]Received 4 December 2009. Accepted 20 July 2010.
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not necessarily reflect the long-term response (acclimation). Moreover, the effect of CO_2 on algal metabolism may extend beyond simply acting as the photosynthetic substrate, mainly due to the interaction between carbon and nitrogen metabolism (Turpin 1991, Huppe and Turpin 1994, Magnusson et al. 1996). The concentration of inorganic nitrogen (N) is one of the factors that most frequently limit productivity of marine macroalgae in natural seawater (Smith 1984, Lobban and Harrison 1997). However, urbanization and increasing human agricultural and industrial activities in coastal areas have greatly increased the inputs of inorganic nutrients (such as nitrogen and phosphorus) into many coastal waters, which lead to eutrophication of these coastal ecosystems. Such increased anthropogenic nutrient loading to nutrient-poor coastal ecosystems is anticipated to physiologically stimulate the photosynthesis and growth of the marine algae. It is also expected that increasing N availability in seawater may affect the response to Ci availability in marine algae (Andría et al. 1999, Gordillo et al. 2001, 2003).

Since marine macroalgae are important components of primary productivity in coastal ecosystems, changes in photosynthesis as well as respiration have the potential to significantly affect carbon flux and sequestration. There are many reports that have investigated the effects of elevated CO_2 on the growth, photosynthesis, and cell biochemical components in marine macroaglae (Johnston and Raven 1990, Björk et al. 1993, Gao et al. 1993, García-Sânchez et al. 1994, Andría et al. 1999, Kübler et al. 1999, Mercado et al. 1999, Gordillo et al. 2001, 2003, Israel and Hophy 2002, Zou 2005). However, relatively few studies have simultaneously examined both short-term and long-term effects of elevated CO_2 on photosynthesis and respiration in marine macroalgal species.

H. fusiformis is one of the most common brown macroalgal species endemic to northwest coasts of the Pacific Ocean, growing on lower intertidal rocks. This alga is now becoming one of the most important species for macroalgae mariculture in China, owing to its high economic value and increasing market demand, where it is used as a food delicacy and for industrial materials. *H. fusiformis* has been reported to be photosynthetically limited under current ambient Ci concentration of seawater, although it possesses the capacity of HCO_3^- utilization during photosynthesis facilitated by the extracellular carbonic anhydrase activity (Zou et al. 2003). Our previous study also showed that the growth and uptake and assimilation of nitrate were enhanced, but the photosynthetic characteristics, measured in common ambient seawater, were not significant altered in *H. fusiformis* following the culture at elevated CO_2 (Zou 2005). Nevertheless, no further information is available on the comparison of short-term direct and long-term indirect effects of CO_2 enrichment on gas exchange properties (including both photosynthesis and respiration), and how these responses may vary with other important environmental factors, such as N availability and temperature. In this study, we cultured *H. fusiformis* at ambient (375 $\mu L \cdot L^{-1}$) and elevated (700 $\mu L \cdot L^{-1}$) CO_2, and at low and high N supply for 2 weeks and have examined the short- and long-term effects of elevated CO_2 on the photosynthesis and respiration of this alga. The objectives of the present study were to investigate: (i) whether there is a down-regulation (acclimation) of photosynthesis in response to elevated CO_2, and if so, whether the degree of such down-regulation is affected by N availability; (ii) whether short-term (or long-term) exposure to CO_2 enrichment inhibits respiration rate, and if so, whether this direct (or indirect) effect varies with the growth, N availability, and changing temperature; and (iii) whether the ratio of respiration to *Pg* is affected by potential changes in growth CO_2 concentration and N availability.

MATERIALS AND METHODS

Plant materials and experimental treatments. *H. fusiformis* was collected from the lower intertidal rocky shore along the coast of Nanao Island, Shantou, China (23°20′ N, 116°55′ E), during March 2006. Healthy thalli free from any visible epiphytes and accumulated sediments were selected and were transported to the laboratory. The algae were maintained in glass aquaria tanks containing filtered natural seawater (salinity ~33 psu) under 180 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (PAR; 400–700 nm) illuminated by fluorescent tubes for 12:12 light:dark photoperiod and at 20°C in CO_2 chambers (Conviron, EF7; Winnipeg, Manitoba, Canada). The seawater for maintenance was aerated vigorously with ambient air and was renewed every day. After 3 d of laboratory maintenance, the healthy individuals (3–5 cm in length) were selected for use in subsequent culture treatments.

We used a fully factorial design with two levels of Ci concentration and two levels of N supply as follows: (i) ambient CO_2 + low N; (ii) ambient CO_2 + high N; (iii) elevated CO_2 + low N; and (iv) elevated CO_2 + high N. For experimentation, ~3 g fresh weight (fwt) of algae was introduced into each of the Erlenmeyer flasks that contained 5 L of filtered natural seawater. Six flasks were placed into each of two CO_2 chambers. One of the CO_2 chambers was programed to supply 375 $\mu L \cdot L^{-1}$ CO_2 (ambient-CO_2 treatment) in aeration for culture, and the other was programed to supply a CO_2 concentration of 700 $\mu L \cdot L^{-1}$ (elevated-CO_2 treatment). The water agitation caused by the aeration allowed the thalli to move gently without tumbling. In each CO_2 chamber, half of the flasks (three) contained nitrogen (400 μM NO_3^-)-supplemented filtered natural seawater (high-N-supply treatment), and the other three flasks contained non-N-supplemented filtered natural seawater (low-N-supply treatment, total inorganic N concentration <8 μM). For all of the treatments, the culture seawater was supplemented with 100 μM $H_2PO_4^-$ in an effort to avoid possible phosphate limitation and thereby to avoid unnecessary complications in interpreting the results. The medium in each flask was completely renewed every 3 d of culture. The inorganic N concentration of the seawater media in low-N treatments after 3 d of cultivation was nearly undetectable, while that in high-N treatment was >250 μM. The concentrations of the total dissolved Ci, measured by using a Total Organic Carbon Analyzer (TOC-5000A; Shimadzu, Kyoto, Japan), were 2.01 and 2.09 mM, and the pH values were 8.21 and 7.92, in the seawater in equilibrium with ambient air and

CO_2-enriched air, respectively. The measured total alkalinity (~2,226 $\mu eq \cdot L^{-1}$) was similar between the seawater equilibrated with ambient air and the CO_2-enriched air. The estimated concentrations of dissolved CO_2 and HCO_3^-, according to Strickland and Parsons (1972), were respectively 12.1 and 1,795.3 μM in ambient seawater and 25.6 and 1,951.5 μM in CO_2-enriched seawater. An increase of CO_2 levels in aeration lowered the pH value of medium ~0.3–0.6 units during culture. The light and temperature conditions of the CO_2 chambers, under which the algae were grown, were the same as for preincubation described above. The algae were cultured under the above four treatments (three replicates per treatment) for 15 d and then were harvested for further measurements. The culture duration would be enough for acclimation in seaweeds (Björk et al. 1993, Mercado et al. 1999, Zou 2005).

Photosynthetic measurements. Photosynthetic rates were measured as oxygen evolution using a Clark-type oxygen electrode (YSI Model 5300, Yellow Spring, OH, USA). The oxygen electrode was held in a temperature-controlled cuvette. The fronds of *H. fusiformis* were cut into small segments with a sharp razor blade and incubated in seawater medium identical to the culture treatment under 180 $\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$ and 20°C for at least 2 h. This pretreatment aimed to minimize the possible effect of cutting damage of fronds cells (wound respiration) on the photosynthetic determination. Segments of about 0.2 g fwt were transferred to the O_2 electrode cuvette containing 8 mL reaction medium, which was magnetically stirred. Photon irradiance was supplied from a halogen lamp. Temperature was maintained at the desired level using a circulating water bath (Cooling Circulator; Cole Parmer, Chicago, IL, USA).

Net photosynthetic (*Pn*) rates as a function of photon irradiance (*P–I* curves) were measured at growth temperature (20°C). Irradiance varied between 0 and 600 $\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$ (PAR), measured with a PAR quantum sensor (SKP 200; ELE International, Leighton Buzzard, UK). The different levels of irradiance were achieved by altering the distance between the light source and the photosynthetic cuvette.

P–I curves of *H. fusiformis* grown under different treatment combinations were measured in ambient and CO_2-enriched seawater, respectively, in order to distinguish the short-term and long-term effects of CO_2 enrichment in seawater on the *P–I* curves. The ambient and CO_2-enriched seawater here mean the seawater equilibrated with the ambient air (~375 $\mu L \cdot L^{-1}$ CO_2) and CO_2-enriched air (700 $\mu L \cdot L^{-1}$ CO_2), respectively. The expression of the short-term effect of CO_2 enrichment denotes the effect observed on algae grown in current ambient-CO_2 seawater when variables are measured at both ambient and elevated CO_2 (amb_{375} vs. amb_{700}, data collected on the same algae). The long-term effect was used to compare variables measured on algae grown and measured at ambient CO_2 to that of algae grown and measured at elevated CO_2 (amb_{375} vs. $elev_{700}$, data collected on different algae). Down-regulation was considered to have occurred when plants grown at elevated CO_2 had lower photosynthetic rates than plants grown at ambient CO_2 when both were measured at a high-CO_2 level. The degree of down-regulation was estimated by the $Pn_{elev700}$:Pn_{amb700} ratio, called the assimilation ratio (Sage 1994).

Pn rates as a function of external Ci concentration in seawater (*P–C* curves) of *H. fusiformis* grown under different treatment combinations were also measured at growth temperature (20°C) and 600 $\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$. Before measurement, Ci-free buffered seawater was prepared. Ci was removed from the natural seawater by reducing pH to <4.0 with the addition of 0.5 M HCl, and then sparging for at least 3 h with high purity N_2 gas. A known amount of Tris (Sigma, St. Louis, MO, USA) was added to give a final concentration of 20 mM, and the pH was adjusted to 8.2 with freshly prepared 0.5 M NaOH and 0.5 M HCl. All the manipulations were under N_2. The Tris buffer per se had no effects on the photosynthesis (Zou et al. 2003). Segments of *H. fusiformis* of ~0.2 g fwt were incubated in the photosynthetic cuvette with 10 mL of buffered Ci-free seawater. The algae were left to photosynthesize to deplete the Ci present in the medium and in the algal cells till no further O_2 evolved, which took ~20 min. Aliquots of $NaHCO_3$ stock solution were then injected into the cuvette in order to create increasing Ci concentration in the reaction medium. O_2 evolution was recorded within 4–8 min after addition of $NaHCO_3$.

Respiratory measurements. Rates of dark respiration (*Rd*) of *H. fusiformis* grown under different treatment combinations were determined in ambient and CO_2-enriched seawater, respectively, using the electrode cuvette as described above. The seawater for measurements was achieved through equilibrating with ambient air (375 $\mu L \cdot L^{-1}$ CO_2) or CO_2-enriched (700 $\mu L \cdot L^{-1}$ CO_2) air. The seawater was sealed and stirred magnetically during measurement to prevent CO_2 exchanges with the atmosphere and to prevent the boundary layer depletion of O_2 and CO_2. Three measurement temperatures (12, 20, and 28°C) were adopted to establish whether the response of *Rd* to the elevation of CO_2 varied with changing temperature. The order of temperature treatments (20, 12, and 28°C) was randomly assigned.

Measurements of *Rd* were initiated after at least 3 h of photosynthesis in the growth chambers. Algal samples were left in darkness for 45–60 min following transfer to the electrode cuvette in an effort to avoid any possible postillumination transients of gas exchange, which was reported in terrestrial higher plants (Atkin et al. 1998). Then the algal samples were allowed to acclimate to the electrode cuvette environment (i.e., desired measurement temperature, ambient or CO_2-enriched seawater) for a minimum of 20 min before commencement of respiration measurement. Respiration was recorded when the O_2 uptake stabilized, usually within 5–10 min. The short-term and long-term effects were then analyzed from the relationship between respirations versus CO_2 levels in seawater, which were measured at different temperatures. In addition, immediately following the respiration measurement, the irradiance-saturated *Pn* rates were also determined at the irradiance of 600 $\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$ in order to examine how *Rd* as a proportion of photosynthesis varied with growth treatment and changing temperature.

Calculations and statistics. The apparent photosynthetic efficiency (α) was estimated as the irradiance-limited slope of the *P–I* curve. Irradiance-saturated maximum photosynthetic rate (*Pm*) was calculated as the mean in the asymptote region of the *P–I* curve. The apparent carboxylating efficiency (ACE) (i.e., the initial slope of *P–C* curve) was calculated by linear regression over the range of 0–0.55 mM Ci. This value was used to indicate how effectively algae use low concentrations of Ci, as discussed by Johnston et al. (1992). The apparent half-saturation values [$K_{1/2}$(Ci), indicating the affinity constant for Ci] was estimated using a double-reciprocal plot of the rates of O_2 evolution and the Ci concentrations.

Q_{10} values (i.e., the proportional change in respiration per 10°C rise) were calculated for respiration over the measured temperature interval of 12°C to 28°C using the following equation according to Atkin et al. (2000): $Q_{10} = 10^{(\text{slope} \cdot 10)}$, where the slope is the regression slope of $\log_{10}$-transformed respiration rates versus temperatures plot.

The data were expressed as mean values ± SD (n = 4–6) for the three independent replicate cultures. Statistical significance of the data was analyzed with *t*-test and analysis of variance (ANOVA) followed by the Student–Newman–Keuls post hoc procedure at a significance level of $P < 0.05$ by using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Photosynthesis. Figure 1 presents the *P–I* curves, which were measured in ambient and CO_2-enriched seawater respectively, for *H. fusiformis* grown under the four different treatments. Photosynthesis was saturated with the photon irradiance of 150–200 μmol photons $\cdot m^{-2} \cdot s^{-1}$, and no photoinhibition was observed at the maximum measuring irradiance of 600 μmol photons $\cdot m^{-2} \cdot s^{-1}$. Under low-N-supply growth condition, the irradiance-saturated rate of photosynthesis (*Pm*) in ambient-CO_2-grown *H. fusiformis* was increased by 47.5% (paired-samples *t*-test, $t = -11.445$, df = 5, $P < 0.001$) with the short-term exposure to CO_2 enrichment of seawater (*Pm* of ambient-CO_2-grown algae measured at ambient CO_2 vs. *Pm* of the same algae measured at elevated CO_2, Pm_{amb375} vs. Pm_{amb700}; Table 1). This simulation persisted fully in the long term (Pm_{amb375} vs. $Pm_{elev700}$), with the assimilation ratio ($Pm_{elev700}$: Pm_{amb700}) being 1.2, indicating no evidence of a down-regulation of photosynthesis after long-term exposure to CO_2 enrichment.

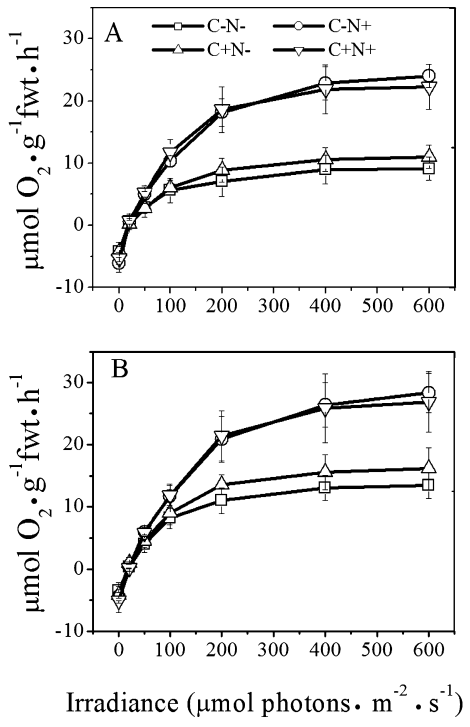

FIG. 1. Net photosynthesis versus irradiance curves (*P–I* curves) of *Hizikia fusiformis* grown under different CO_2 and N-supply conditions. Aeration with ambient air and low N supply (375 μL $\cdot L^{-1}$ CO_2 and non-N-supplemented seawater, C–N–). Aeration with ambient air and high N supply (375 μL $\cdot L^{-1}$ CO_2 and NO_3^--supplemented seawater, C–N+). Aeration with CO_2-enriched air and low N supply (700 μL $\cdot L^{-1}$ CO_2 and non-N-supplemented seawater, C+N–). Aeration with CO_2-enriched air and high N supply (700 μL $\cdot L^{-1}$ CO_2 and NO_3^--supplemented seawater, C+N+). Curves were measured in (A) current ambient and (B) CO_2-enriched seawater, respectively. CO_2-enriched seawater was achieved from natural ambient seawater in equilibrium with 700 μL $\cdot L^{-1}$ CO_2 air. Photosynthetic rates were measured at growth temperature (20°C). Values are means (±SD), $n = 4–6$.

The similar trend was observed under high-N growth condition (Table 1). *Pm* of ambient-CO_2-grown *H. fusiformis* was increased by 17.7% (paired-samples *t*-test, $t = -4.622$, df = 4, $P = 0.01$) with the short-term exposure to CO_2 enrichment of seawater. The prolonged growth at CO_2 enrichment did not evoke a down-regulation of such photosynthetic enhancement, because there was no difference between $Pm_{elev700}$ and Pm_{amb700} (Table 1). The assimilation ratio at high N supply ($Pm_{elev700}$:Pm_{amb700}) was 0.95. It appeared that the extent of the photosynthetic response to CO_2 enrichment was less in the high-N growth condition than in the low-N growth condition.

Short-term exposure to CO_2-enriched seawater increased ($P < 0.05$) the apparent photosynthetic efficiency (α) in *H. fusiformis* grown at low N supply but had no significant effect on α in *H. fusiformis* grown at high N supply. The value of α was not significantly changed with prolonged growth at elevated CO_2; however, it was markedly increased ($P < 0.01$) with the high-N growth condition compared to the low-N condition (Table 1).

Figure 2 illustrates the *P–C* curves for *H. fusiformis* grown under the four different treatments. It appeared that under low N supply, *H. fusiformis* grown at ambient CO_2 exhibited a higher *Pn* rate, plotted against Ci concentrations in seawater, than the algae grown at elevated CO_2 (Fig. 2). However, under high-N growth conditions, *H. fusiformis* grown in ambient and elevated CO_2 displayed similar *Pn* for a given Ci concentration. *H. fusiformis* appeared to have a relatively high value of $K_{1/2}$(Ci) (i.e., a relatively low photosynthetic affinity for Ci). While extra CO_2 in culture increased the value of $K_{1/2}$(Ci), high N supply in culture decreased it (Table 2). The ACE was reduced ($P < 0.01$) by the CO_2 enrichment in low-N-grown algae, whereas there was a pronounced increase ($P < 0.01$) under high-N in comparison with the low-N growth condition (Table 2).

Respiration. Figure 3 displays the *Rd* versus temperature relationships, which were measured in ambient and CO_2-enriched seawater, respectively, for *H. fusiformis* grown under the four different treatments. There were no significant differences in *Rd* between measurements in normal seawater and in CO_2-enriched seawater (Rd_{amb375} vs. Rd_{amb700}, and $Rd_{elev375}$ vs. $Rd_{elev700}$) across all three tested temperatures (12, 20, and 28°C) and all the growth treatments. This indicated that short-term exposure to CO_2 enrichment did not affect *Rd*. Moreover, there were no statistically significant differences between *Rd* measured at growth CO_2 condition (Rd_{amb375} vs. $Rd_{elev700}$), indicating that long-term exposure to CO_2 enrichment did not alter *Rd*. However, the shift from low to high N supply in culture substantially increased ($P < 0.01$) *Rd* at 20°C and 28°C, regardless of the growth CO_2 treatment.

TABLE 1. (A) Irradiance-saturated maximum photosynthetic rate (*Pm*) and the apparent photosynthetic efficiency (α) in *Hizikia fusiformis* grown under different CO_2 and N-supply conditions. CO_2-enriched seawater was achieved from natural ambient seawater in equilibrium with 700 $\mu L \cdot L^{-1}$ CO_2 air. Values were derived from Figure 1. Unit of *Pm* is $\mu mol\ O_2 \cdot g^{-1}$ fwt $\cdot h^{-1}$, and that of α is ($\mu mol\ O_2 \cdot g^{-1}$ fwt $\cdot h^{-1}$)/ (μmol photons $\cdot m^{-2} \cdot s^{-1}$). Data are means (±SD), n = 4–6. Different superscripts indicate significant difference ($P < 0.05$) within each row. (B) The *F*- and *P*-values from analysis of variance (ANOVA) analysis assessing the single and interactive effects to *Pm* and α of growth conditions, that is, CO_2 concentrations (ambient vs. elevated) and N supplies (low vs. high), and measuring method (measured in ambient seawater vs. measured in elevated-CO_2 seawater).

(A)

	Air		+CO_2	
	−N	+N	−N	+N
Pm				
Measured in ambient seawater	9.1 ± 1.9[a]	24.0 ± 1.9[b]	11.0 ± 1.8[a]	22.3 ± 3.7[b]
Measured in CO_2-enriched seawater	13.5 ± 2.1[a]	28.3 ± 3.2[b]	16.2 ± 3.4[a]	26.9 ± 4.9[b]
α				
Measured in ambient seawater	0.09 ± 0.01[a]	0.16 ± 0.02[b]	0.10 ± 0.02[a]	0.16 ± 0.02[b]
Measured in CO_2-enriched seawater	0.11 ± 0.02[a]	0.16 ± 0.03[b]	0.12 ± 0.02[a]	0.17 ± 0.02[b]

(B)

Source	*F*	*P*
Pm		
CO_2	0.159	0.692
N	208.326	<0.001
Measuring method	26.109	<0.001
CO_2 × N	4.778	0.035
CO_2 × measuring method	0.092	0.764
N × measuring method	0.029	0.866
CO_2 × N × measuring method	0.021	0.886
α		
CO_2	2.130	0.153
N	98.782	<0.001
Measuring method	6.601	0.014
CO_2 × N	0.014	0.906
CO_2 × measuring method	0.068	0.796
N × measuring method	4.001	0.053
CO_2 × N × measuring method	0.069	0.795

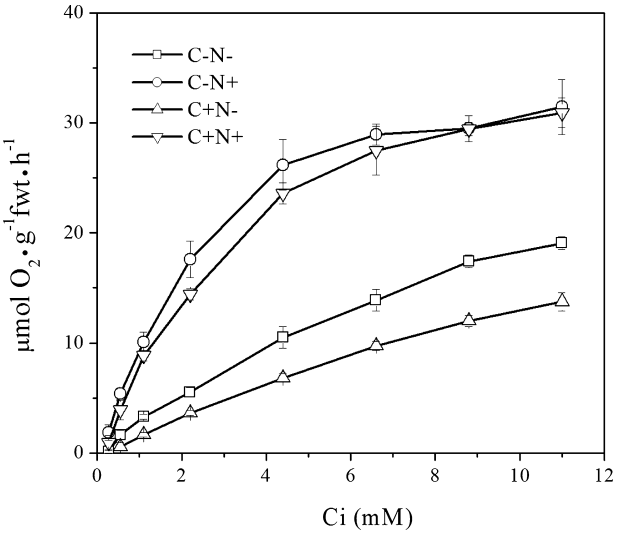

FIG. 2. Net photosynthesis versus inorganic carbon (Ci) concentration curves (*P–C* curves) of *Hizikia fusiformis* grown under different CO_2 and N-supply conditions. Photosynthesis was measured at growth temperature (20°C) and saturating irradiance (600 μmol photons $\cdot m^{-2} \cdot s^{-1}$). Symbols as in Figure 1. Values are means (±SD), n = 4.

The Q_{10} values of *Rd* were generally ~2.0, the typical value in plants, except for the algae grown at high CO_2 and high N supply, which had a Q_{10} value of 2.6 (Table 3). Short-term change of CO_2 levels in seawater did not alter Q_{10} values for all the growth treatments. Long-term exposure to CO_2 enrichment also did not result in a significant change in Q_{10} value when the N supply in culture was low. However, under the high-N growth condition, Q_{10} was significantly ($P < 0.01$) increased with the prolonged exposure to CO_2 enrichment (Table 3).

The ratio of respiration to Pg. Short-term exposure to CO_2 enrichment markedly decreased the ratios of *Rd* to *Pg* (i.e., *Pn* plus *Rd*) over the tested temperature range for the algae grown at low N, whereas it only caused a slight decrease in *Rd*:*Pg* ratios in high-N-grown algae (Fig. 4). *Rd*:*Pg* ratios over all the tested temperatures were significantly reduced ($P < 0.01$) in elevated-CO_2-grown algae than ambient-CO_2-grown algae, when the N supply was low. However, the CO_2 levels under which *H. fusiformis* were grown did not significantly alter the *Rd*:*Pg* ratios at 20°C and 28°C when the N supply was high, although a decreased ratio of *Rd*:*Pg* at 12°C

TABLE 2. (A) The apparent carboxylating efficiency (ACE) and the apparent half-saturation values [$K_{1/2}$(Ci)] in *Hizikia fusiformis* grown under different CO_2 and N-supply conditions. Values were derived from Figure 2. Unit of ACE is (μmol $O_2 \cdot g^{-1}$ fwt $\cdot h^{-1}$)/ (μM), and that of $K_{1/2}$(Ci) is μM. Data are means (±SD), $n = 4$. Different superscripts indicate significant difference ($P < 0.05$) within each row. (B) The *F*- and *P*-values from analysis of variance (ANOVA) assessing the single and interactive effects to ACE and $K_{1/2}$(Ci) of growth conditions, that is, CO_2 concentrations (ambient vs. elevated) and N supplies (low vs. high).

(A)

	Air		+CO_2	
	−N	+N	−N	+N
ACE	2.5 ± 0.1[a]	7.9 ± 1.0[b]	1.9 ± 0.2[c]	6.8 ± 0.6[b]
$K_{1/2}$(Ci)	12.0 ± 4.0[a]	4.6 ± 1.1[a]	35.8 ± 12.2[b]	8.8 ± 6.7[a]

(B)

Source	*F*	*P*
ACE		
CO_2	8.529	0.013
N	322.436	<0.001
$CO_2 \times N$	0.719	0.413
$K_{1/2}$(Ci)		
CO_2	18.461	0.001
N	27.974	<0.001
$CO_2 \times N$	9.062	0.008

was observed in elevated-CO_2-grown algae in comparison with ambient-CO_2-grown algae.

DISCUSSION

Photosynthesis. Marine macroalgae usually assimilate CO_2 via the C_3 biochemical pathway with RUBISCO as the carboxylating enzyme (Raven 1997), a process that is competitively inhibited by O_2 via photorespiration. However, many species of marine macroalgae usually display a suppressed photorespiration and exhibit a C_4-like photosynthetic gas exchange physiology due to the presence of an active CCM, which is mainly related with efficient HCO_3^- utilization (Beer 1994, Raven 1997). In the marine macroalga *H. fusiformis*, our previous works demonstrated that the exogenous Ci utilization mechanism associated with external carbonic anahydrase (CA) activity was unable to prevent photorespiration (Zou et al. 2003, Zou and Gao 2004). The present study showed that the photosynthetic rate of *H. fusiformis* was significantly enhanced in response to short-term exposure to CO_2 enrichment in seawater, and such stimulation was maintained in the long term, regardless of N supply in growth. Therefore, the results suggested that there was no down-regulation of photosynthesis in *H. fusiformis*

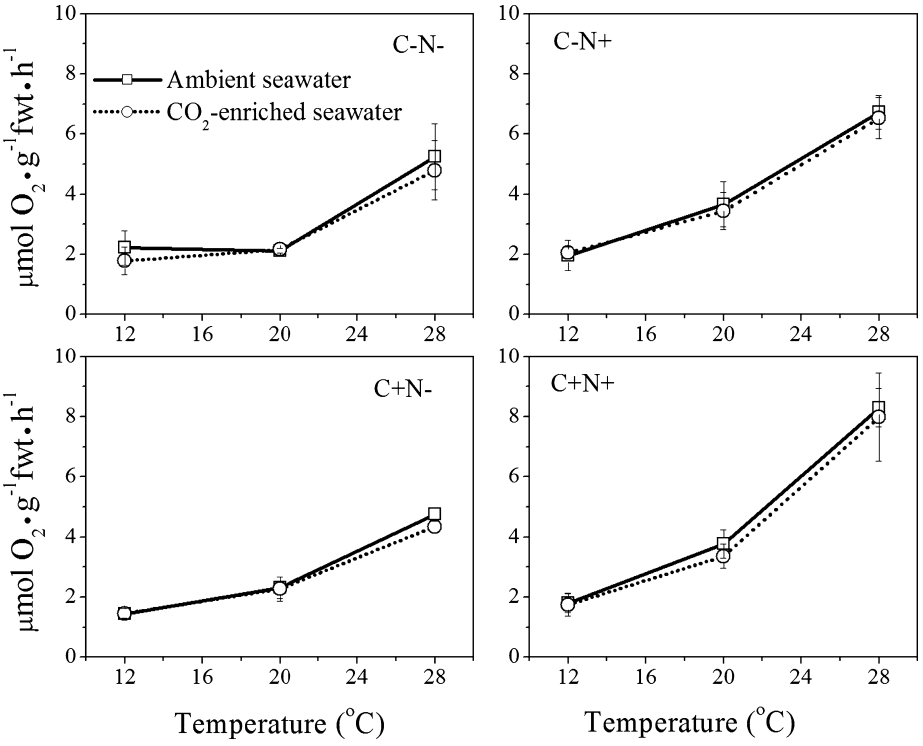

FIG. 3. Dark respiration rates versus temperature response curves for *Hizikia fusiformis* grown under different CO_2 and N-supply conditions. Curves were measured in current ambient and CO_2-enriched seawater, respectively. CO_2-enriched seawater was achieved from natural ambient seawater in equilibrium with 700 $\mu L \cdot L^{-1}$ CO_2 air. C–N−, ambient CO_2 + low N; C–N+, ambient CO_2 + high N; C+N−, elevated CO_2 + low N; C+N+, elevated CO_2 + high N. Values are means (±SD), $n = 4$–6.

TABLE 3. (A) The Q_{10} values for *Hizikia fusiformis* grown under different CO_2 and N-supply conditions. Values were measured in current ambient and CO_2-enriched seawater, respectively. CO_2-enriched seawater was achieved from natural ambient seawater in equilibrium with 700 $\mu L \cdot L^{-1}$ CO_2 air. Data are means (±SD), $n = 4–6$. Different superscripts indicate significant difference ($P < 0.05$) within each row. (B) The *F*- and *P*-values from analysis of variance assessing the single and interactive effects to Q_{10} of growth conditions, that is, CO_2 concentrations (ambient vs. elevated) and N supplies (low vs. high).

(A)

	Air		+CO_2	
	−N	+N	−N	+N
Measured in ambient seawater	1.86 ± 0.40[a]	2.14 ± 0.16[a]	2.08 ± 0.09[a]	2.59 ± 0.10[b]
Measured in CO_2-enriched seawater	1.98 ± 0.37[a]	2.06 ± 0.03[a]	2.00 ± 0.11[a]	2.61 ± 0.21[b]

(B)

Source	*F*	*P*
CO_2	19.341	<0.001
N	27.053	<0.001
Measuring method	0.003	0.959
CO_2 × N	7.175	0.012
CO_2 × measuring method	0.144	0.707
N × measuring method	0.123	0.728
CO_2 × N × measuring method	1.116	0.299

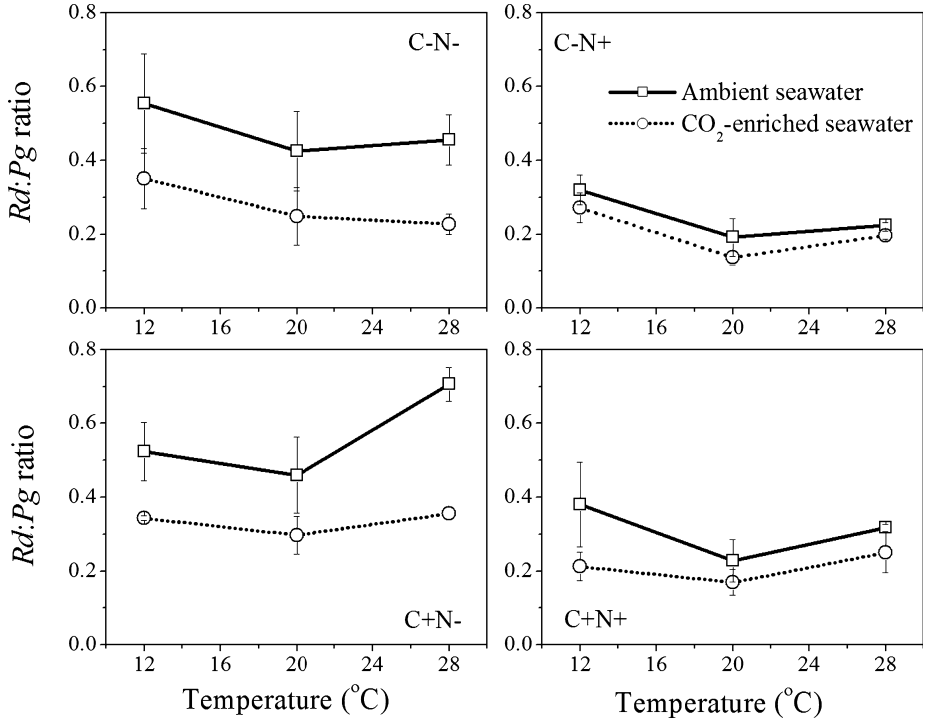

FIG. 4. The dark respiration (*Rd*) to gross photosynthesis (*Pg*) ratios at different measuring temperatures in *Hizikia fusiformis* grown under different CO_2 and N-supply conditions. The ratios were measured in current ambient and CO_2-enriched seawater, respectively. CO_2-enriched seawater was achieved from natural ambient seawater in equilibrium with 700 $\mu L \cdot L^{-1}$ CO_2 air. Symbols as in Figure 3. Values are means (±SD), $n = 4–6$.

grown at elevated CO_2. Some investigations on the terrestrial counterparts had also failed to find long-term effect of photosynthesis, either in controlled environments (Zerihum and BassiriRad 2000) or in the field (Herrick and Thomas 2001). However, photosynthetic down-regulation has been recognized as the most frequent phenomenon in the terrestrial plants (especially in C_3 plants) following prolonged exposure to elevated CO_2 (for review, see Sage 1994, Drake et al. 1997). An elevated-CO_2 growth regime usually resulted in a decrease in the contents of pigments, soluble protein, and

RUBISCO (García-Sânchez et al. 1994, Drake et al. 1997, Andría et al. 1999), producing a consequence of decreased carboxylating capacity and the maximum rate of photosynthesis. However, in elevated CO_2 concentrations, a given rate of photosynthesis could be achieved with low activities of RUBISCO, other Calvin cycle enzymes, and light reaction components because photorespiration is decreased (Woodrow 1994, Stitt and Krapp 1999). It had been shown that in *H. fusiformis*, an enrichment of CO_2 in culture could enhance the uptake and assimilation of nitrogen, which would permit decreased investment of nitrogen in the nitrogen-intensive process of photosynthesis and photorespiration, releasing nitrogen for investment in other processes and proteins (Zou 2005). This possibility could be an important physiological mechanism that empowers the thalli of *H. fusiformis* to maintain their internal N resources status, and thereby to retain the photosynthetic capacity during long-term exposure to elevated CO_2.

In marine microalgae, almost all of the species examined to date in single-species laboratory experiments or natural community-perturbation experiments show either no change or only a small increase (generally no more than 10%) in the rates of photosynthesis when grown under CO_2-enriched conditions equivalent to doubling the present atmospheric CO_2 concentration (Beardall and Raven 2004, Giordano et al. 2005). This lack of response is largely due to the presence of CCMs in algae, which bring about the carbon-saturated photosynthesis with today's CO_2 concentrations in seawater. In marine macroalgae, there are relatively more species that show carbon-limited photosynthesis, even for some species that possess the ability to utilize HCO_3^- (Mercado et al. 1999, Zou et al. 2003). It is clear that enhancement of photosynthesis under CO_2-enriched conditions is species specific, depending on their physiological characteristics, such as capacity for active HCO_3^- or CO_2 uptake and different strategies for CCM. Further experiments with more species are needed to establish whether the persistence of photosynthetic enhancement by elevated CO_2 found in *H. fusiformis* in the present study is a general phenomenon in marine macroalgae. If not so, then how the magnitude of the photosynthetic down-regulation varies with species, especially the response difference between the species that have efficient HCO_3^- utilization mechanisms or CCMs and those species that have no or only poor capacity of HCO_3^- utilization, needs to be investigated.

This study suggested that in a fluctuating environment, the ability of *H. fusiformis* to respond to CO_2 enrichment would be fundamentally dependent on the N availability under which the algae were grown. In N-deficient seawater, limitations by N might have considerable effects on the ability of *H. fusiformis* to benefit from CO_2 enrichment of seawater resulting from increased atmospheric CO_2. In contrast, under sufficient N supply, photosynthesis of *H. fusiformis* reached rather high levels, allowing a relatively small extent of enhancements owing to CO_2 enrichment. Our results that high-N-grown *H. fusiformis* had substantially higher ACE and lower $K_{1/2}$(Ci) values than the low-N-grown algae suggested that *H. fusiformis* exhibited a much higher activity and efficiency of carboxylation and higher affinity for Ci under the high-N growth condition. However, it should be pointed out that in most of the microalgae examined, the Ci affinity decreases as N increases from limiting to saturating concentrations (Giordano et al. 2005). The exception, which agrees with *Hizikia* presented in this study, is the green alga *Chlamydomonas reinhardtii* grown with ammonium as the N source (Giordano et al. 2005). On the other hand, it has been reported that, compared to N-limited growth conditions, growth at high N availability would result in increased N:C ratios, chl and protein concentrations (e.g., the RUBISCO amount), and CA activity, and thereby an increased photosynthetic capacity in marine macroalgae (Lapointe and Duke 1984, Turpin 1991, Jimenez del Rio et al. 1995, Andría et al. 1999, Gordillo et al. 2001, 2003). Therefore, compared to low-N-grown *H. fusiformis*, high-N-grown algae would run a more efficient pathway of photosynthetic carbon acquisition and assimilation. This could supply the physiological interpretations that *H. fusiformis* grown at low N supply exhibited a greater sensitivity of photosynthesis to the elevation of CO_2 concentration in seawater than high-N-grown algae. Additionally, under the N-sufficient condition, the apparent photosynthetic efficiency of *H. fusiformis* was not changed in response to elevated CO_2. This was in accordance with our previous results (Zou 2005). However, under the low-N-supply condition, *H. fusiformis* displayed an increased photosynthetic efficiency in response to the instantaneous exposure to CO_2 enrichment. This finding indicated that the photosynthetic responses in *H. fusiformis* to increasing atmospheric CO_2 were dependent on the environmental constraints (such as N), under which CO_2 enrichment was imposed.

Respiration. Since the per day CO_2 loss through *Rd* can amount to half or more of the total carbon budget of plants (Amthor 2000), the measurements of *Rd* are essential to extrapolate the gas exchange measurements to carbon budget and biomass production of plants. While there is intensive research on how plant photosynthesis may respond the CO_2 enrichment in the atmosphere, how *Rd* will respond to elevated CO_2 concentration remains uncertain (Drake et al. 1999, Gonzàlez-Meler et al. 2004, Bunce 2005). To our knowledge, very little attention has been devoted to the effects of elevated CO_2 on the *Rd* in algae, and particularly in marine macroalgae. In the present study, we found no evidence that short-term exposure to CO_2 enrichment had an effect on

respiration in the marine macroalga *H. fusiformis*, regardless of the growth conditions and the measuring temperature. Our results were consistent with the observations reported for some terrestrial plants (Roberntz and Stockfors 1998, Tjoelker et al. 1999, Amthor 2000). However, many reports on terrestrial plants showed the decreased respiration in response to short-term increase in ambient CO_2 concentration (Griffin et al. 1996, Ceulemans et al. 1997, Clinton and Vose 1999, Amthor 2000).

In addition to the short-term direct effects of CO_2 on respiration, attention has been paid to the long-term indirect effects of elevated CO_2 on respiration, which may result from the acclimation of cell biochemical components. The results in the present study showed neither long-term indirect effects nor an interaction between the direct and indirect effects of CO_2 elevation on the rates of *Rd* in *H. fusiformis*. This result might imply that respiration would be controlled by use of respiratory products (demand) and/or through the supply of respiratory substrates (nonstructural carbohydrates). Increasing atmospheric CO_2 concentration has been shown to enhance the growth of *H. fusiformis* (Zou 2005), yet it had no effect on the mass-specific respiration rates (this study). We therefore suggested that the effects of elevated CO_2 on the respiratory carbon flux are primarily at the whole-thalli level through increased biomass.

It has been shown that increased N availability would tend to increase respiratory rate in terrestrial plants by stimulating plant growth, protein (e.g., RUBISCO) accumulation, and secondary metabolism, whereas N limitation would tend to decrease it (Wullschleger et al. 1994, Amthor 2000). In the present study, we also demonstrated that in the marine macroalga *H. fusiformis*, respiration was increased in high-N-grown algae in comparison with low-N-grown algae. It was reported that high-N-grown macroalgae had higher contents of N and RUBISCO and a higher nutrient uptake rate compared with low-N-grown algae (Gordillo et al. 2001, 2003). Therefore, increased respiration in high-N-grown *H. fusiformis* might be necessary to support increased costs associated with higher maintenance demands (for example increased RUBISCO contents) and greater uptake and assimilation of extra N in seawater.

Fluctuation in temperature usually leads to an immediate alteration in the respiration rate. The extent of such alternation can be determined by the short-term temperature coefficient (Q_{10}), that is, the proportional increase in respiratory flux per 10°C rise in temperature. Our findings showed that the temperature sensitivity of respiration of *H. fusiformis* was associated with the growth condition. It was evident that Q_{10} values of respiration were significantly higher in the growth treatment with elevated CO_2 and simultaneous high N supply compared with the other three growth treatments. Our results also demonstrated that the Q_{10} values of respiration in *H. fusiformis* did not vary with the short-term change of CO_2 concentration in seawater, regardless of the condition under which the algae were grown.

The ratio of respiration to Pg. Although a number of studies have investigated the effect of increasing CO_2 concentration on photosynthesis in marine macroalgae, few have examined the effect of CO_2 change on the ratio of respiration to photosynthesis. Such information would be essential in determining if an increase of CO_2 concentration in the atmosphere or the ocean alters the algal carbon balance. Because the responses to short-term exposure to elevated CO_2 of respiration and photosynthesis were different (i.e., respiration remained unchanged with the instantaneous change of CO_2 concentration, whereas photosynthesis was sensitive to CO_2 change but was much more responsive to instantaneous elevation of CO_2 in low-N-grown *H. fusiformis* than the high-N-grown algae), the balance between respiration and *Pg* displayed different responses with the instantaneous changes of CO_2 concentration in seawater. As a result, the instantaneous CO_2 enrichment would markedly reduce the values of *Rd*:*Pg* ratios.

The effects of growth conditions on the proportion of respiration to photosynthesis have been reported in terrestrial plants, but few have been described in marine macroalgae. In wheat, Gifford (1995) showed that the *Rd*:*Pg* ratios were unchanged over a wide range of temperatures (15°C–30°C) and between ambient and elevated CO_2 concentrations at the growth temperature (20°C). However, Ziska and Bunce (1998) reported that the *Rd*:*Pg* ratios for soybean plants were not affected over the growth temperatures (20°C–35°C) but were consistently lower at the elevated relative to the ambient CO_2 concentrations. In the present study, lower *Rd*:*Pg* ratio in low-N-grown *H. fusiformis* was observed consistently lower at the elevated-CO_2 compared to at the ambient-CO_2 growth condition over the range of measurement temperatures (12°C–28°C). This suggested that, under the low-N-supply condition, future enrichment of CO_2 in seawater resulting from increasing atmospheric CO_2 may decrease the respiratory carbon lost per unit algal biomass, and thereby would enhance the capacity of carbon sequestration. However, such a phenomenon would not occur if the increasing CO_2 is coincident with the coastal eutrophication (elevated N supply). It was also worthy to note that the measuring temperature generally did not alter the balance between respiratory carbon loss and gross photosynthetic carbon gain in *H. fusiformis* thalli, except for the *H. fusiformis* grown in elevated CO_2 and simultaneous low N supply, in which the *Rd*:*Pg* ratio measured in ambient-CO_2 seawater was pronounced higher at the high measuring temperature (28°C) relative to 20°C and 12°C.

In summary, our results suggested that photosynthesis of *H. fusiformis* displayed no down-regulation

following prolonged growth in CO_2-enriched seawater. The relative enhancement of photosynthetic rates in response to elevated CO_2 was higher in low-N-grown *H. fusiformis* than in high-N-grown algae. Respiration rates of *H. fusiformis* were not sensitive to both short-term and long-term exposure to elevated CO_2, irrespective of the N-supply condition under which the algae were grown. Respiration would be more responsive to the fluctuation in temperature under future predicted atmospheric CO_2 changes and simultaneous coastal eutrophication. Additionally, under N-deficient conditions, an increase of CO_2 concentration in the atmosphere and the ocean would reduce respiratory carbon loss as a proportion of gross photosynthetic carbon gain in *H. fusiformis*.

This study was supported by the Chinese 973 Project (2009CB421207), the National Natural Science Foundation of China (41076094 and 30970450), Program for New Century Excellent Talents in University (NCET-10-0375), the Chinese 863 project (2006AA10A416), and the Fundamental Research Funds for the Central Universities, SCUT (2009ZZ0074).

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