

# Effects of elevated CO<sub>2</sub> and phosphorus supply on growth, photosynthesis and nutrient uptake in the marine macroalga *Gracilaria lemaneiformis* (Rhodophyta)

Zhiguang Xu<sup>1,\*</sup>, Dinghui Zou<sup>2</sup> and Kunshan Gao<sup>3</sup>

<sup>1</sup> Marine Biology Institute, Shantou University, Shantou, Guangdong, 515063, China, e-mail: g\_zgxu@stu.edu.cn

<sup>2</sup> The Key Laboratory of Pollution Control and Ecosystem Restoration in Industry Clusters, Ministry of Education of China, College of Environmental Science and Engineering, South China University of Technology, Guangzhou, 510006, China

<sup>3</sup> State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, 361005, China

\* Corresponding author

## Abstract

The red alga *Gracilaria lemaneiformis* was cultured under different CO<sub>2</sub> and phosphorus conditions for 16 days, and its growth, photosynthesis and uptake of nitrate and phosphate were examined in order to establish the longer-term impacts of elevated CO<sub>2</sub> and phosphorus supplies on this economically important seaweed. Enrichment with either CO<sub>2</sub> or phosphorus in culture markedly increased the growth of *G. lemaneiformis* compared to the control. Light-saturated photosynthetic rate was enhanced significantly by phosphorus enrichment, but hardly affected by the elevation of CO<sub>2</sub> when *G. lemaneiformis* was grown under low phosphorus conditions. High phosphorus stimulated photosynthetic inorganic carbon utilization and nitrogen uptake. Under low phosphorus conditions, the thalli grown at the high level of CO<sub>2</sub> had a lower carbon utilization capacity and a higher nitrogen uptake rate compared to those grown under ambient CO<sub>2</sub>. Reversed results were found when the algae were grown under high phosphorus conditions. Hence, available phosphorus may regulate inorganic carbon utilization of *G. lemaneiformis* grown at different CO<sub>2</sub> levels, and growth reflected a balance between carbon and nutrient metabolism.

**Keywords:** CO<sub>2</sub>; *Gracilaria lemaneiformis*; growth; phosphorus; photosynthesis.

## Introduction

Atmospheric CO<sub>2</sub> concentration has been rising since the Industrial Revolution and is predicted to double by the end of this century (IPCC 2001). Marine macroalgae play an

important role in the carbon cycle of coastal ecosystems and their responses to elevated atmospheric CO<sub>2</sub> are of considerable interest. High CO<sub>2</sub> levels generally enhance photosynthesis in many species due to the diffusion of more CO<sub>2</sub> from the external bulk medium to the active site of Rubisco, although most of them possess CO<sub>2</sub> concentrating mechanisms (CCMs) and mainly utilize HCO<sub>3</sub><sup>-</sup> (Giordano et al. 2005). However, the effects of longer-term (days to weeks) CO<sub>2</sub> enrichment are variable (Wu et al. 2008). This variation may depend on the availability of nutrients and the ways in which they are utilized by the alga (Rivers and Peckol 1995, Andria et al. 1999) because higher growth rates with elevated CO<sub>2</sub> will lead to an increased demand for nutrients. Therefore, studies on longer-term effects of the interaction between CO<sub>2</sub> and nutrients are very important.

Phosphorus is an essential nutrient which macroalgae require in relatively large quantities and continuous supply for their survival and growth (Lobban et al. 1985). Therefore, changes in available phosphorus in the environment will significantly affect the growth and physiology of seaweeds. It has been reported that increased dissolved inorganic phosphorus (Pi) supply (e.g., due to eutrophication) causes increased algal growth and hence increases algal abundance (Diaz-Pulido and McCook 2005). The uptake rate of phosphorus is greatly enhanced by high external Pi level, and photosynthesis and the content of pigments exhibit an increment under high Pi conditions (García-Sánchez et al. 1996). In addition, due to its important metabolic functions (Irihimovitch and Yehudai-Resheff 2008), supply of available phosphorus may affect responses of macroalgae to high CO<sub>2</sub> levels. Indeed, it has been reported that phosphorus limitation down-regulates CCMs activity (Beardall et al. 2005), and high dissolved inorganic carbon (Ci) concentration stimulates the modification of acclimation to phosphate deficiency (Kozłowska-Szerenos et al. 2004) in freshwater microalgae. Conversely, Spijkerman et al. (2007) have shown that Ci concentration has no clear influence on Pi limitation of *Chlamydomonas acidophila* Negoro in a very acidic lake (pH 2.7).

*Gracilaria lemaneiformis* (Bory) Weber-van Bosse is an economically important red seaweed that is cultivated on a large scale in China due to the quantity and quality of agar in its cell walls. This alga was also suggested an excellent species for alleviating coastal eutrophication in China (Fei 2004). Much attention has been paid to the biofiltration capacity of the species (Yang et al. 2005, 2006, Zhou et al. 2006). The characteristics of photosynthetic inorganic car-

bon utilization have been investigated in our previous works (Zou et al. 2004). We showed that *G. lemaneiformis* mainly used HCO<sub>3</sub><sup>-</sup> as its carbon source via external carbonic anhydrase (CA). In addition, effects on growth and photosynthesis of environmental factors, such as solar ultraviolet radiation (Gao and Xu 2008) and nitrate enrichment (Zheng and Gao 2009), have been studied. However, no data have been published on how the species responds to elevated CO<sub>2</sub> concentration when grown at different Pi levels. The aim of the present study was to investigate physiological responses to elevated CO<sub>2</sub> of *G. lemaneiformis* grown under low and high Pi concentrations.

## Materials and methods

### Plant material

Thalli of *Gracilaria lemaneiformis* were collected at 0.5 m depth from a farm located in Shen'ao Bay, Nanao Island (23.3° N, 116.6° E), Shantou, China during April 2006. The algae collected were transported to the laboratory in an insulated cooler (5°C) within 2 h. Samples were maintained for 2 days in a glass tank with natural seawater at constant temperature (23°C) and an irradiance of 100 μmol photons m<sup>-2</sup> s<sup>-1</sup> provided by fluorescent tubes on a 12:12 h photoperiod. Healthy individuals were selected for use in subsequent experiments.

### Experimental design

About 4 g fresh weight (FW) of *Gracilaria lemaneiformis* thalli were introduced to flasks containing 3 l of natural seawater and cultured in CO<sub>2</sub> chambers (Conviron, EF7, Winnipeg, Canada). Algae were treated with two different levels of CO<sub>2</sub> and Pi for 16 days (i.e., 4 treatments): C<sub>L</sub>P<sub>L</sub>, low CO<sub>2</sub> and low Pi; C<sub>H</sub>P<sub>L</sub>, high CO<sub>2</sub> and low Pi; C<sub>L</sub>P<sub>H</sub>, low CO<sub>2</sub> and high Pi; and C<sub>H</sub>P<sub>H</sub>, high CO<sub>2</sub> and high Pi. Here, low (C<sub>L</sub>) and high CO<sub>2</sub> (C<sub>H</sub>) represent CO<sub>2</sub> concentrations of 370 (actual atmosphere) and 720 μl l<sup>-1</sup>, respectively; and low (P<sub>L</sub>) and high Pi (P<sub>H</sub>) were Pi concentrations of 0.5 (natural seawater) and 30 μM, respectively. High CO<sub>2</sub> level was obtained by adjusting the CO<sub>2</sub> concentration in the CO<sub>2</sub> chamber, and high Pi concentration was obtained by dissolving NaH<sub>2</sub>PO<sub>4</sub> in natural seawater.

Light and temperature conditions in the culture were those mentioned above, and nitrate concentration was simultaneously enriched to 500 μM with NaNO<sub>3</sub> for each of the treatments. The cultivation media were renewed every other day. The pH values in different treatments were measured every 3 h before renewing media, and the values were almost stable after the first 3 h at about 8.25 in C<sub>L</sub>P<sub>L</sub>, 8.42 in C<sub>L</sub>P<sub>H</sub> and 7.85 in both C<sub>H</sub>P<sub>L</sub> and C<sub>H</sub>P<sub>H</sub> treatments. After 16 days of incubation, the thalli were harvested and physiological indicators were determined.

### Growth rate

Changes in biomass (FW) were measured at the end of incubation, and the mean relative growth rate (RGR) was estimated according to the logarithmic model:

$$\text{RGR} = \ln(W_t/W_0) \times t^{-1} \times 100,$$

where  $W_0$  refer to the initial FW and  $W_t$  the FW after  $t$  days.

### Photosynthetic oxygen evolution

Photosynthetic rates at different photon irradiances ( $P$  vs.  $I$  curves) were determined as oxygen evolution with a Clark-type oxygen electrode (YSI Model 5300, Yellow Springs, Ohio, USA) at 23°C. Before measurements, algae were cut into small segments and then incubated for at least 2 h to minimize the effect of cutting damage on photosynthesis (Z. Xu, unpublished data). About 0.15 g FW segments were transferred into the oxygen electrode chamber containing 8 ml of autoclaved natural seawater. Light was provided by a halogen lamp and varied within the range from 0 to 800 μmol photons m<sup>-2</sup> s<sup>-1</sup>. The different levels of irradiance were obtained by altering the distance between the light source and the oxygen electrode chamber, and were measured with a PAR quantum sensor (SKP200, ELE International, Leighton Buzzard, UK). Dark respiration rate was measured after covering the chamber with an opaque cloth.

The values of kinetic parameters of the  $P$  vs.  $I$  curves were calculated. The apparent photosynthetic efficiency ( $\alpha$ ) was estimated as the irradiance-limited slope of the  $P$  vs.  $I$  curve. The irradiance compensation point ( $I_c$ ) and irradiance saturation point ( $I_k$ ) were calculated as  $R_d/\alpha$  and  $P_m/\alpha$ , respectively (Henley 1993). Here,  $R_d$  was the dark respiration rate and  $P_m$  was the irradiance-saturated maximum photosynthetic rate, which was calculated as the mean in the asymptote region of the  $P$  vs.  $I$  curve.

Photosynthetic rates in different dissolved inorganic carbon (Ci) concentrations ( $P$  vs.  $C_i$  curves) were measured at the saturating irradiance of 600 μmol photons m<sup>-2</sup> s<sup>-1</sup>. The different Ci concentrations (from 0 to 6.6 mM) were obtained by dissolving NaHCO<sub>3</sub> in buffered Ci-free seawater. Here, buffered Ci-free seawater was prepared by adding 1.0 M HCl to lower the pH to about 3.0, and then aerating with pure N<sub>2</sub> gas for 2 h to drive out CO<sub>2</sub> dissolved in seawater, finally adding Tris buffer and adjusting the pH to 8.1 with HCl and NaOH.

The parameters of the  $P$  vs.  $C_i$  curves were calculated with Michaelis-Menten saturation kinetics (Von Caemmerer and Farquhar 1981):

$$V = V_{max} \times [S] / (K_{0.5} + [S])$$

The maximum rate of Ci utilization ( $V_{max}$ ) and the half saturation constant ( $K_{0.5}$ ) for Ci were determined by fitting the photosynthetic rates ( $V$ ) at various Ci concentrations ( $[S]$ ).

### Uptake rates of nutrients

The uptake rates of nutrients were determined by the disappearance of nitrate and/or phosphate from the culture media over a given time interval and expressed by the following equation:

$$\text{nutrient uptake rate} = (N_o - N_t) \times V \times W_o^{-1} \times t^{-1},$$

where  $N_o$  is the initial concentration of nutrient,  $N_t$  the concentration after  $t$  hours,  $V$  the volume of the culture medium and  $W_o$  the initial FW of the alga (Harrison 1988). The light and temperature conditions were those mentioned above.

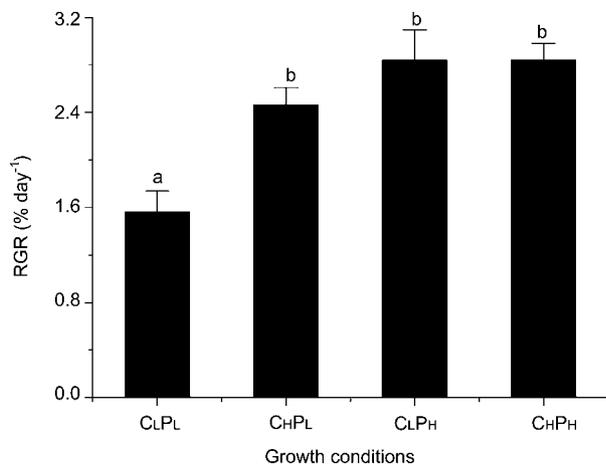
### Statistical analysis

All data were expressed as means ± SD ( $n \geq 3$ ). One-way ANOVA and Tukey's test were used to analyze differences among treatments and the significance level was set at 0.05.

## Results

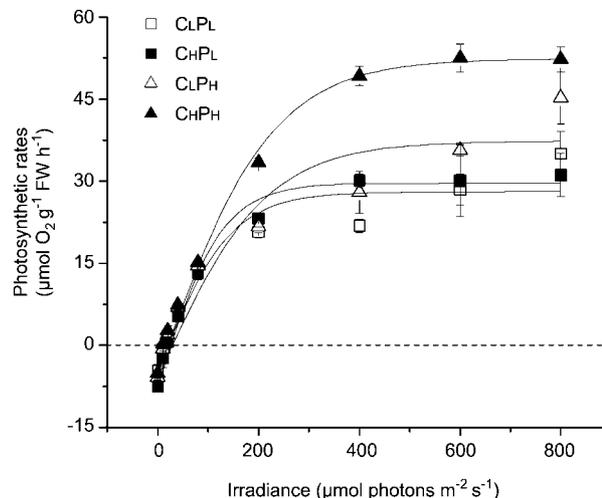
### Growth

Higher mean RGRs of *Gracilaria lemaneiformis* were found at high CO<sub>2</sub> or Pi levels compared to C<sub>L</sub>P<sub>L</sub> treatment (Tukey's test,  $p < 0.05$ ). However, there was no significant difference among the C<sub>H</sub>P<sub>L</sub>, C<sub>L</sub>P<sub>H</sub> and C<sub>H</sub>P<sub>H</sub> treatments (Tukey's test,  $p > 0.05$ , Figure 1). These results showed that either CO<sub>2</sub> or Pi enrichment in culture may accelerate the growth of *G. lemaneiformis*, but they had no synergistic effects.



**Figure 1** *Gracilaria lemaneiformis*: relative growth rate (RGR) at different CO<sub>2</sub> and phosphorus levels determined over 2 days at the end of 16 days incubation.

C<sub>L</sub>P<sub>L</sub>, low CO<sub>2</sub> and low Pi; C<sub>H</sub>P<sub>L</sub>, high CO<sub>2</sub> and low Pi; C<sub>L</sub>P<sub>H</sub>, low CO<sub>2</sub> and high Pi; and C<sub>H</sub>P<sub>H</sub>, high CO<sub>2</sub> and high Pi. Significant (Tukey's test,  $p < 0.05$ ) differences among the treatments are indicated by different lowercase letters. Values are means + SD for triplicate samples.



**Figure 2** *Gracilaria lemaneiformis*: photosynthetic oxygen evolution rates vs. photon irradiance ( $P$  vs.  $I$ ) curves of plants grown at different CO<sub>2</sub> and phosphorus levels.

The curves were measured in natural seawater (i.e., pH 8.1, Ci 2.2 mM). C<sub>L</sub>P<sub>L</sub>, low CO<sub>2</sub> and low Pi; C<sub>H</sub>P<sub>L</sub>, high CO<sub>2</sub> and low Pi; C<sub>L</sub>P<sub>H</sub>, low CO<sub>2</sub> and high Pi; and C<sub>H</sub>P<sub>H</sub>, high CO<sub>2</sub> and high Pi. Values are means ± SD for triplicate samples.

### Photosynthesis

The photosynthetic rate vs. irradiance ( $P$  vs.  $I$ ) curves of *Gracilaria lemaneiformis* grown at different CO<sub>2</sub> and Pi levels are presented in Figure 2. Both elevated CO<sub>2</sub> and Pi concentration in the culture medium affected photosynthetic rate in response to photon irradiance. Enrichment of CO<sub>2</sub> or Pi in the culture medium dramatically enhanced  $P_m$ ,  $R_d$  and  $I_c$  (Tukey's test,  $p < 0.05$ ), but  $\alpha$  did not change markedly with the culture conditions (Tukey's test,  $p > 0.05$ ).  $I_k$  was enhanced by high Pi level (Tukey's test,  $p < 0.05$ ) but not affected by elevated CO<sub>2</sub> (Tukey's test,  $p > 0.05$ , Table 1).

Characteristics of the photosynthetic responses to exogenous inorganic carbon ( $P$  vs.  $C_i$  curves) were significantly affected by both CO<sub>2</sub> and Pi levels in the culture medium (Figure 3, Table 2). At low Pi level, CO<sub>2</sub> enrichment decreased the maximum rate of Ci utilization ( $V_{max}$ ) and the  $V_{max}/K_{0.5}$  ratio (Tukey's test,  $p < 0.05$ ) by 34% and 37%, respectively; however, it had no significant effect on the half saturation constant,  $K_{0.5}$  for either total Ci or dissolved CO<sub>2</sub> (Tukey's test,  $p > 0.05$ ). Algae grown at high Pi concentration had a greater  $V_{max}$  and a higher  $V_{max}/K_{0.5}$  ratio, but a lower value of  $K_{0.5}$  (Tukey's test,  $p < 0.05$ ) compared to the algae grown at low Pi level, which suggests an elevated affinity for exogenous inorganic carbon in high Pi-grown algae.

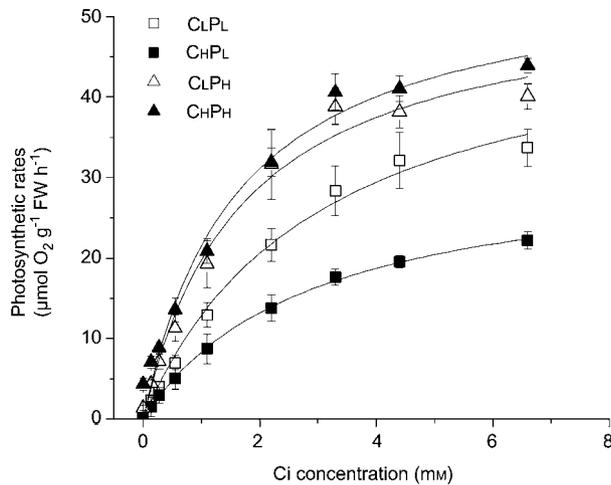
### Nitrate and phosphate uptake

Nitrate and phosphate uptake rates of *Gracilaria lemaneiformis* grown under different conditions are shown in Figure 4. Algae grown at a high Pi level had a faster uptake rate of phosphate than those at low Pi level. However, enrichment of CO<sub>2</sub> in cultures had no significant effect on phosphate

**Table 1** *Gracilaria lemaneiformis*: parameters for photosynthetic responses to photon irradiance (*P* vs. *I* curves) of plants grown at different CO<sub>2</sub> and phosphorus levels.

	C <sub>L</sub> P <sub>L</sub>	C <sub>H</sub> P <sub>L</sub>	C <sub>L</sub> P <sub>H</sub>	C <sub>H</sub> P <sub>H</sub>
<i>P<sub>m</sub></i>	32.68±2.65 <sup>a</sup>	37.30±4.11 <sup>ab</sup>	43.82±3.88 <sup>b</sup>	57.51±2.78 <sup>c</sup>
<i>α</i>	0.23±0.03 <sup>a</sup>	0.29±0.03 <sup>b</sup>	0.25±0.05 <sup>ab</sup>	0.26±0.01 <sup>ab</sup>
<i>R<sub>d</sub></i>	-4.64±1.20 <sup>a</sup>	-7.52±1.10 <sup>b</sup>	-7.52±1.10 <sup>b</sup>	-5.11±0.60 <sup>a</sup>
<i>I<sub>k</sub></i>	144.18±7.40 <sup>a</sup>	128.40±16.73 <sup>a</sup>	180.26±41.06 <sup>ab</sup>	224.61±7.77 <sup>b</sup>
<i>I<sub>c</sub></i>	20.19±3.10 <sup>a</sup>	25.65±1.76 <sup>b</sup>	30.42±4.91 <sup>b</sup>	19.92±1.52 <sup>a</sup>

*P<sub>m</sub>*, maximal net photosynthetic rate (μmol O<sub>2</sub> g<sup>-1</sup> FW h<sup>-1</sup>); *α*, apparent photosynthetic efficiency (μmol O<sub>2</sub> g<sup>-1</sup> FW h<sup>-1</sup>)/(μmol photons m<sup>-2</sup> s<sup>-2</sup>); *R<sub>d</sub>*, dark respiration rate (μmol O<sub>2</sub> g<sup>-1</sup> FW h<sup>-1</sup>); *I<sub>k</sub>*, irradiance saturation point (μmol photons m<sup>-2</sup> s<sup>-2</sup>) and *I<sub>c</sub>*, irradiance compensation point (μmol photons m<sup>-2</sup> s<sup>-2</sup>). <sup>a,b,c</sup> Within each row of the data, different superscript letters or letter combinations indicate significant differences at p=0.05 (Tukey's test). Values are means±SD (n=3).



**Figure 3** *Gracilaria lemaneiformis*: photosynthetic oxygen evolution rates vs. exogenous inorganic carbon concentration (*P* vs. *C<sub>i</sub>*) curves in plants grown at different CO<sub>2</sub> and phosphorus levels. The curves were measured at an irradiance of 600 μmol photons m<sup>-2</sup> s<sup>-1</sup> and pH 8.1. The increasing inorganic carbon levels were obtained by dissolving NaHCO<sub>3</sub> into carbon-free seawater. C<sub>L</sub>P<sub>L</sub>, low CO<sub>2</sub> and low Pi; C<sub>H</sub>P<sub>L</sub>, high CO<sub>2</sub> and low Pi; C<sub>L</sub>P<sub>H</sub>, low CO<sub>2</sub> and high Pi; and C<sub>H</sub>P<sub>H</sub>, high CO<sub>2</sub> and high Pi. Values are means±SD for triplicate samples.

uptake rate (Tukey's test, p>0.05, Figure 4B). Both CO<sub>2</sub> and Pi-enriched cultures increased nitrate uptake markedly (Figure 4A). The uptake rates of nitrate were increased by 40% and 178% with CO<sub>2</sub> and Pi enrichment, respectively. How-

ever, in high Pi-grown algae, CO<sub>2</sub> enrichment inhibited nitrate uptake by 28% compared to normal CO<sub>2</sub> in culture.

In order to investigate the capability for absorbing phosphate in plants grown under different conditions, algae from different treatments were transferred into culture medium with equal Pi concentrations of 5 or 50 μM; the media used for measurements were not CO<sub>2</sub>-enriched. Phosphate uptake rates were measured and the results are presented in Figure 5. For both initial Pi concentrations (5 and 50 μM), high Pi-grown algae had a lower uptake rate of phosphate (Tukey's test, p<0.05) than low Pi-grown algae. Similar effects on phosphate uptake capability were found in the algae grown at high CO<sub>2</sub> level. Additionally, phosphate uptake rates of low Pi-grown algae increased by about 10 and 50 times, respectively, when they were transferred into medium with Pi concentrations of 5 and 50 μM. However, high Pi-grown algae did not change markedly (contrast Figure 5 with Figure 4B). These results indicate that the phosphate uptake capacities of *Gracilaria lemaneiformis* were dependent on the Pi levels under which the algae were grown.

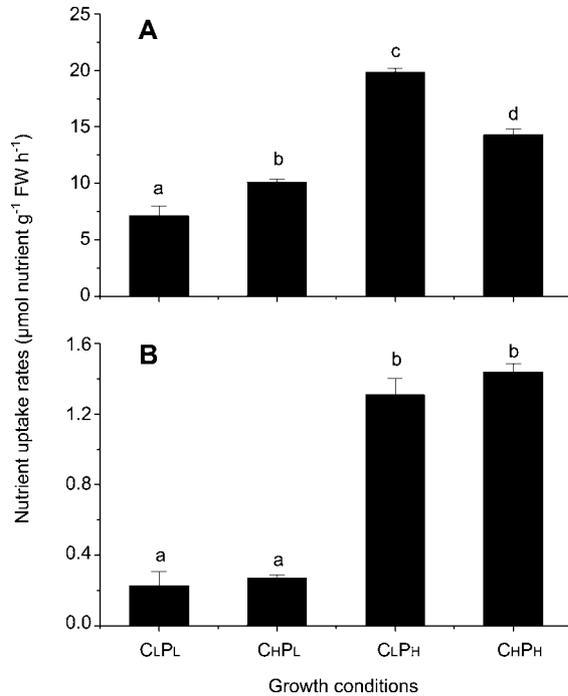
## Discussion

The present study shows that the light-saturated photosynthetic rate, photosynthetic efficiency, irradiance saturation and compensation point in *Gracilaria lemaneiformis* in natural seawater with normal carbon concentration were not markedly affected by the CO<sub>2</sub> levels in the culture medium at low Pi level. Similarity of photosynthetic parameters

**Table 2** *Gracilaria lemaneiformis*: parameters for the photosynthetic responses to exogenous inorganic carbon (*P* vs. *C<sub>i</sub>*) curves) of plants grown at different CO<sub>2</sub> and phosphorus levels.

	C <sub>L</sub> P <sub>L</sub>	C <sub>H</sub> P <sub>L</sub>	C <sub>L</sub> P <sub>H</sub>	C <sub>H</sub> P <sub>H</sub>
<i>V<sub>max</sub></i>	49.30±1.20 <sup>a</sup>	32.64±2.99 <sup>b</sup>	54.33±4.18 <sup>ac</sup>	56.36±2.56 <sup>c</sup>
<i>K<sub>0.5</sub>(TC)</i>	2.80±0.56 <sup>a</sup>	3.03±0.27 <sup>a</sup>	1.81±0.55 <sup>b</sup>	1.63±0.30 <sup>b</sup>
<i>K<sub>0.5</sub>(CO<sub>2</sub>)</i>	15.37±3.01 <sup>a</sup>	16.67±1.48 <sup>a</sup>	9.93±3.00 <sup>b</sup>	9.00±1.65 <sup>b</sup>
<i>V<sub>max</sub>/K<sub>0.5</sub>(TC)</i>	18.13±3.78 <sup>a</sup>	11.50±3.21 <sup>b</sup>	31.81±8.51 <sup>c</sup>	35.04±4.65 <sup>c</sup>

*V<sub>max</sub>* (μmol O<sub>2</sub> g<sup>-1</sup> FW h<sup>-1</sup>, the maximum rate of Ci utilization; *K<sub>0.5</sub>(TC)* (mM) and *K<sub>0.5</sub>(CO<sub>2</sub>)* (μM), the concentration of total Ci and dissolved CO<sub>2</sub> supporting half of *V<sub>max</sub>*. <sup>a,b,c</sup> Within each row of the data, different superscript letters or letter combinations indicate significant differences at p=0.05 (Tukey's test). Values are means±SD (n=3).



**Figure 4** *Gracilaria lemaneiformis*: nitrate (A) and phosphate (B) uptake rates at different CO<sub>2</sub> and phosphorus levels. C<sub>L</sub>P<sub>L</sub>, low CO<sub>2</sub> and low Pi; C<sub>H</sub>P<sub>L</sub>, high CO<sub>2</sub> and low Pi; C<sub>L</sub>P<sub>H</sub>, low CO<sub>2</sub> and high Pi; and C<sub>H</sub>P<sub>H</sub>, high CO<sub>2</sub> and high Pi. Significant (Tukey's test, p<0.05) differences among the treatments are indicated by different lowercase letters. Values are means+SD for triplicate samples.

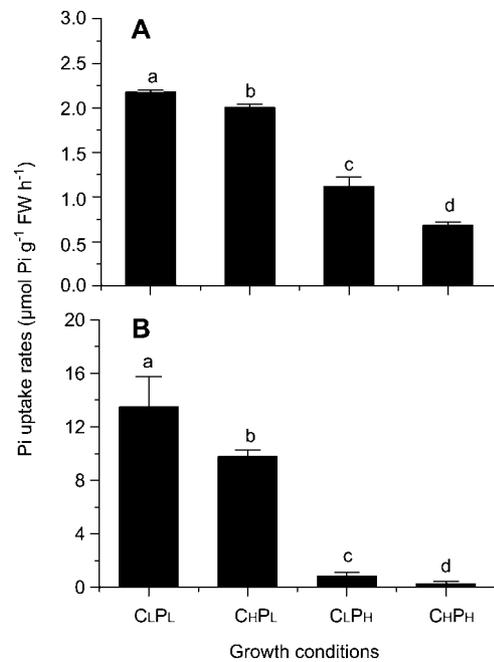
across CO<sub>2</sub> concentrations might be associated with the similarity in Chl-*a* contents, which were unchanged at different CO<sub>2</sub> levels (data not shown). However, capability, efficiency and affinity for Ci were all depressed by high CO<sub>2</sub> level at low Pi concentration. In our previous study, we found that *G. lemaneiformis* has CO<sub>2</sub> concentrating mechanisms (CCMs) and primarily utilizes exogenous HCO<sub>3</sub><sup>-</sup> using external CA, which catalyzes the HCO<sub>3</sub><sup>-</sup> to CO<sub>2</sub> conversion (Zou et al. 2004). Thus, one possible reason for the decrease in capability for Ci utilization might be that the activity and content of external CA were inhibited by CO<sub>2</sub> enrichment (Mercado et al. 1997).

At high Pi level, elevated CO<sub>2</sub> enhanced the photosynthetic capability of *Gracilaria lemaneiformis* (Tables 1 and 2). High levels of phosphorus are required for various chloroplast functions, including generation of ATP and phosphorylation of photosynthetic proteins and enzymes (Zer and Ohad 2003). Sufficient phosphorus supply gives the energy requirement for active transport of Ci, as well as the energy requirement for synthesis of proteins involved in Ci utilization, and hence affects the capacity of algae to drive CCMs, which also costs energy. Beardall et al. (2005) have reported that CCM activity of a microalga is down-regulated by phosphorus limitation. In addition, Pi enrichment results in enhancement of the capacity for regenerating ribulose-1,5-bisphosphate (RuBP) (Rao and Terry 1989), or the increment

of both activity and amount of Rubisco, and augmentation of RuBP pool size (Lauer et al. 1989). Therefore, enrichment of Pi may change the photosynthetic response of *G. lemaneiformis* to high CO<sub>2</sub> by regulating the activity of related enzymes.

The present study demonstrated that nitrate uptake rates were increased by high CO<sub>2</sub>. This indicated that elevated CO<sub>2</sub> enhanced nitrogen assimilation, which would presumably be required to support the higher growth rate of *Gracilaria lemaneiformis* grown at high CO<sub>2</sub>. This enhancement of nitrate uptake rate may be due to the increased activity of nitrate reductase by high levels of CO<sub>2</sub>, as shown by Gordillo et al. (2001) and Zou (2005). However, the enhancement of nitrate uptake was not accompanied by an increased uptake rate of phosphorus, which may depend mainly on exogenous Pi concentration (Huertas et al. 2000).

The growth of seaweeds is a complicated physiological and biochemical process related to photosynthetic carbon fixation and metabolism of nitrogen and phosphorus. Elevated levels of CO<sub>2</sub> in seawater increase the growth rate of many seaweed species despite the variety of ways in which carbon is utilized in these algae. Some species, such as *Porphyra yezoensis* Ueda (Gao et al. 1991) and *Hizikia fusiforme* (Harv.) Okamura (Zou 2005) are capable of using HCO<sub>3</sub><sup>-</sup>, but are limited by the current ambient carbon concentration in seawater. Enrichment of CO<sub>2</sub> relieves this limitation and



**Figure 5** *Gracilaria lemaneiformis*: phosphate uptake rate of plants transferred into culture medium with low Pi (5 µM, A) and high Pi (50 µM, B) after culture at different CO<sub>2</sub> and phosphorus levels for 16 days.

The medium for measurement was not CO<sub>2</sub>-enriched. C<sub>L</sub>P<sub>L</sub>, low CO<sub>2</sub> and low Pi; C<sub>H</sub>P<sub>L</sub>, high CO<sub>2</sub> and low Pi; C<sub>L</sub>P<sub>H</sub>, low CO<sub>2</sub> and high Pi; and C<sub>H</sub>P<sub>H</sub>, high CO<sub>2</sub> and high Pi. Significant (Tukey's test, p<0.05) differences among treatments are indicated by different lowercase letters. Values are means+SD for triplicate samples.

enhances growth. Some species cannot utilize exogenous HCO<sub>3</sub><sup>-</sup>, but their growth is stimulated by aerating with high CO<sub>2</sub>, which can be attributed to accelerated photosynthetic carbon fixation by increasing carbon availability and/or depressed photorespiration by elevating the ratio of CO<sub>2</sub>/O<sub>2</sub> in the culture medium (Kübler et al. 1999). In this study, *Gracilaria lemaneiformis*, which efficiently uses HCO<sub>3</sub><sup>-</sup> and whose photosynthesis is saturated at current inorganic carbon concentration of natural seawater (Zou et al. 2004), showed an increment of growth rate in the CO<sub>2</sub>-enriched treatment (Figure 1). This enhancement of growth could be due to the increased nitrogen uptake rates at elevated CO<sub>2</sub> levels. High CO<sub>2</sub> may enhance the activity of nitrate reductase (NR) (Mercado et al. 1999, Gordillo et al. 2001, Zou 2005) and stimulate the accumulation of nitrogen, which could contribute to growth.

Our results showed that high levels of Pi also stimulated the growth rate of *Gracilaria lemaneiformis*. And we demonstrated that photosynthesis and nitrogen uptake in *G. lemaneiformis* improved markedly at high Pi levels. Therefore, improvement of these biological processes may lead to the increment of growth rate. Similar effects have been found in other macroalgae and microalgae (García-Sánchez et al. 1996, Geider et al. 1998).

We also found that simultaneous enrichment of CO<sub>2</sub> and Pi did not result in a growth rate higher than that of algae enriched with CO<sub>2</sub> or Pi alone (Figure 1). When we transferred the algae cultured at high Pi into lower Pi concentration, the uptake rate of Pi declined. This might be due to the synthesis of sufficient stored polyphosphates (Vance et al. 2003). Therefore, growth rate of algae cultured at high Pi level was mainly dependent on photosynthetic carbon utilization and nitrogen metabolism. Both nitrate assimilation and carbon fixation cost much energy and many carbon skeletons (Foyer et al. 1994, Ferrario-Méry et al. 1997), so they must compete for assimilatory energy (reduced ferredoxin, NADPH and ATP) and carbon skeletons needed to form amino acids or synthesize carbohydrates (Turpin 1991, Ferrario-Méry et al. 1997). In the present study, high CO<sub>2</sub> enhanced photosynthetic Ci utilization, but there was a decreased uptake of nitrate at high Pi level, which led to similar growth rates between simultaneous and sole enrichment by CO<sub>2</sub> or Pi.

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