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Physiological response of a golden tide alga (*Sargassum muticum*) to the interaction of ocean acidification and phosphorus enrichment

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Abstract. The development of golden tides is potentially influenced by global change factors, such as ocean acidification and eutrophication, but related studies are very scarce. In this study, we cultured a golden tide alga, *Sargasssum muticum*, at two levels of pCO_2 (400 and 1000 µatm) and phosphate (0.5 and 40 µM) to investigate the interactive effects of elevated pCO_2 and phosphate on the physiological properties of the thalli. Higher pCO_2 and phosphate (P) levels alone increased the relative growth rate by 41 and 48 %, the net photosynthetic rate by 46 and 55 %, and the soluble carbohydrates by 33 and 62 %, respectively, while the combination of these two levels did not promote growth or soluble carbohydrates further. The higher levels of pCO_2 and P alone also enhanced the nitrate uptake rate by 68 and 36 %, the nitrate reductase activity (NRA) by 89 and 39 %, and the soluble protein by 19 and 15 %, respectively. The nitrate uptake rate and soluble protein was further enhanced, although the nitrate reductase activity was reduced when the higher levels of pCO_2 and P worked together. The higher pCO_2 and higher P levels alone did not affect the dark respiration rate of the thalli, but together they increased it by 32 % compared to the condition of lower pCO_2 and lower P. The neutral effect of the higher levels of pCO_2 and higher P on growth and soluble carbohydrates, combined with the promoting effect on soluble protein and dark respiration, suggests that more energy was drawn from carbon assimilation to nitrogen assimilation under conditions of higher pCO_2 and higher P; this is most likely to act against the higher pCO_2 that caused acid–base perturbation via synthesizing H^+ transport-related protein. Our results indicate that ocean acidification and eutrophication may not boost golden tide events synergistically, although each one has a promoting effect.

1 Introduction

Sargassum C. Agardh (1820) is the most species-rich genus in the Phaeophyta and has a global distribution (Mattio and Payri, 2011). The species of this genus constitutes an important part of the marine flora and is considered a valuable and unique habitat for a number of highly adapted marine animal species (Laffoley et al., 2011). Some species of *Sargassum* are economically important and are used in animal fodder, agricultural manure, and alginate production (Ashok-Kumar et al., 2012; Fenoradosoa et al., 2010; González-López et al., 2012). On the other hand, *Sargassum* is an aggressive genus, and it can rapidly spread and invade new areas (Sfriso and Facca, 2013). The invasion of *Sargassum* would accordingly compete with indigenous species for nutrients and light, leading to the alteration of the macroalgal community structure (Rueness, 1989; Stæhr et al., 2000). For instance, the increased abundance of *S. muticum* in Limfjorden (Denmark) between 1990 and 1997 led to decreased cover of several indigenous species belonging to the genera *Codium*, *Fucus*, and *Laminaria*, and thus reduced the species richness and diversity of the macroalgal community (Stæhr et al., 2000). Recently, species of *Sargassum* have inundated the coasts along the Gulf of Mexico, West Africa, the Caribbean, and Brazil in unprecedented biomass, which are termed golden tides (Schell et al., 2015; Smetacek and Zingone, 2013). Apart

from the negative effect on aesthetics and tourism, the occurrence of golden tides could kill the fish within the algal mass, mainly due to hypoxia or anoxia in the waters caused by decomposition of *Sargassum* thalli (Cruzrivera et al., 2015). In addition, the dense *Sargassum* accumulation could clog fishing nets and impede the passage of boats, leading to food shortages for local people who depend on artisanal fisheries (Smetacek and Zingone, 2013). The occurrence of golden tides has been linked to higher nutrient levels in seawater (Lapointe, 1995; Smetacek and Zingone, 2013). The distribution pattern and biomass of *Sargassum* spp. are environment-dependent (temperature, light, nutrients, etc.) (Ang, 2006; Sfriso and Facca, 2013).

Due to the burning of fossil fuels and changes in land use, the atmospheric concentrations of carbon dioxide increased to the level of 401.72 ppm in July 2016 (http://www.esrl.noaa.gov/gmd/ccgg/trends/global.html), which is an unprecedented high over the last 800 000 years (IPCC, 2013). When CO_2 dissolves in seawater, it forms carbonic acid, and as more CO_2 is taken up by the ocean's surface, the pH decreases, moving towards a less alkaline and therefore more acidic state; this is termed ocean acidification. The mean surface ocean pH has already decreased by 0.1 units since the beginning of the industrial era, corresponding to a 26 % increase in hydrogen ion concentration (IPCC, 2013). By 2100, concentrations of CO_2 (aq) and HCO_3^- are predicted to increase by 192 and 14 %, respectively, and CO_3^{2-} is predicted to decrease by 56 % with a concomitant decline in pH to 7.65 (Raven et al., 2005). Increased CO_2 could exert positive, neutral, or negative effects on the physiological properties of macroalgae (Ji et al., 2016; Wu et al., 2008). In terms of *Sargassum* species, increased CO_2 (800 ppm) enhanced the photosynthetic rate (based on CO_2 uptake) in *S. muticum* (Longphuirt et al., 2014). On the other hand, the same level of increased CO_2 (750 ppm) did not affect growth, Rubisco's maximal activity, affinity for CO_2, or quantity in *S. vulgare* (Alvaro and Mazal, 2002). Furthermore, increased CO_2 (750 ppm) significantly decreased the net photosynthetic rate and light saturation point of *S. henslowianum* (Chen and Zou, 2014).

Apart from ocean acidification, eutrophication is another environmental challenge. Eutrophication can occur naturally in lakes through the transfer of nutrients from the sediment to the water via living or decomposing macrophytes, resuspension, diffusion, and bioturbation (Carpenter, 1981). However, anthropogenic activities have accelerated the rate and extent of eutrophication (Carpenter et al., 1998). The inevitable urbanization of a growing human population, the increased use of coastal areas, and rising fertilizer use for agricultural intensification has led to accelerated nutrient inputs from land water to coastal waters (Smith et al., 1999). These changes in nutrient availability result in eutrophication, an increasing threat for coastal ecosystems (Bricker et al., 2008). One consequence of eutrophication is that it can lead to algal bloom, such as green tides and golden tides (Smetacek and Zingone, 2013). There are intensive studies regarding the effect of nutrients on the physiological properties of *Sargassum* species (Hwang et al., 2004; Incera et al., 2009; Lapointe, 1995; Liu and Tan, 2014; Nakahara, 1990). Enrichment of nutrients can usually enhance the growth and photosynthetic parameters of *Sargassum*. For instance, the growth rate of *S. baccularia* almost doubled when nutrients increased from 3 µM ammonium plus 0.3 µM phosphate to 5 µM ammonium plus 0.5 µM phosphate (Schaffelke and Klumpp, 1998), and the photosynthetic rates of *S. fluitans* and *S. natans* were also 2-fold higher with 0.2 mM PO_4^{3-} enrichment compared to the control (Lapointe, 1986). Furthermore, some studies have demonstrated that macroalgae experience more phosphorus limit than nitrogen limit (Lapointe, 1986; Lapointe et al., 1987, 1992; Littler et al., 1991). For instance, nitrogen enrichment did not affect the growth rates of *S. fluitans* or *S. natans*, while phosphorus enrichment increased them from 0.03–0.04 (control) to 0.05–0.08 doublings d^{-1} (Lapointe, 1986).

Neither ocean acidification nor eutrophication is proceeding in isolation; rather, they occur simultaneously, particularly in coastal areas. The interactive effects of the two factors may be completely different or of greater magnitude compared to the effects of any single stressor. To the best of our knowledge, no studies have been reported regarding the interactive effects of ocean acidification and eutrophication on *Sargassum*. In this study, we chose the species *S. muticum* to investigate its responses to the interaction of ocean acidification and eutrophication. *S. muticum* is an invasive macroalga that commonly inhabits rocky shores (Karlsson and Loo, 1999). It originates from Japan and was imported to the Northern Pacific coast of the United States in the early 20th century (Scagel, 1956). It was also introduced to Europe along with the Japanese oyster in the late 1960s (Jones and Farnham, 1973). Its distribution is now worldwide due to its introduction and subsequent rapid expansion (Cheang et al., 2010). Our study supplies insight into how ocean acidification and eutrophication affect the physiological properties of *S. muticum* and thus the development of golden tides.

2 Materials and methods

2.1 Sample collection and experimental design

S. muticum was collected from lower intertidal rocks on the coast of Lidao, Rongcheng, China (37°15′ N, 122°35′ E). The samples were transported to the laboratory in an insulated polystyrene cooler (4–6 °C) within 3 h. Healthy thalli were selected and rinsed with sterile seawater to remove sediments, epiphytes, and small grazers. The thalli were maintained in an intelligent illumination incubator (MGC-250P, Yiheng Technical Co. Ltd., Shanghai, China) for 24 h before the experiment. The temperature in the incubator was set at 20 °C with a 12 h–12 h (light–dark) photoperiod of

150 µmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR). After the maintenance, a two-way factorial experiment was set up to investigate the interactive effects of pCO_2 and phosphate on *S. muticum*. The thalli were placed in 3 L flasks with 2 L of sterile seawater (one thallus per flask) and cultured at fully crossed two pCO_2 (400 µatm, lower pCO_2, LC; 1000 µatm, higher pCO_2, HC) and two phosphate (0.5 µM, lower phosphate, LP; 40 µM, higher phosphate, HP) levels with continuous aeration for 13 days. Phosphorus was selected as a nutrient variable, because some findings have displayed that phosphorus, rather than nitrogen, is the primary limiting nutrient for macroalgae (Lapointe, 1986; Lapointe et al., 1987, 1992; Littler et al., 1991). The conditions of natural seawster are 400 µatm pCO_2 and 0.5 µM phosphate. The 400 µatm pCO_2 was achieved by bubbling ambient air and 1000 µatm pCO_2 was obtained through a CO_2 plant chamber (HP1000 G-D, Wuhan Ruihua Instrument & Equipment Ltd, China) with a CO_2 variation of less than 5 %. The higher P level (40 µM) was achieved by adding NaH_2PO_4 to natural seawater, and the nitrate concentration was set at 200 µM for all treatments to avoid N limit. The media were refreshed every day.

2.2 Carbonate chemistry parameters

The seawater pH was recorded with a pH meter (pH 700, Eutech Instruments, Singapore), and total alkalinity (TA) was measured by titrations. The salinity of the seawater was 29. Other carbonate system parameters, which were not directly measured, were calculated via CO2SYS (Pierrot et al., 2006) using the equilibrium constants of K_1 and K_2 for carbonic acid dissociation (Roy et al., 1993).

2.3 Measurement of growth

The growth of *S. muticum* was determined by weighing fresh thalli. The thalli of *S. muticum* were blotted gently with tissue paper to remove water on the surface from the thalli before weighing them. The relative growth rate (RGR) was estimated as follows: $\mathrm{RGR} = (\ln W_t - \ln W_0)/t \times 100$, where W_0 is the initial fresh weight (FW) and W_t is the weight after t days of culture.

2.4 Determination of photosynthesis and respiration

The net photosynthetic rate of the thalli was measured by a Clark-type oxygen electrode (Chlorolab-3, Hansatech, Norfolk, UK) at the end of the experiment. Approximately 0.1 g of fresh-weight algae harvested from the culture flask was transferred to the oxygen electrode cuvette with 8 mL of sterilized media, and the media were stirred during measurement. The irradiance and temperature conditions were set the same as in the growth incubators. The increase of oxygen content in seawater within 5 min was defined as the net photosynthetic rate, and the decrease of oxygen content in seawater in darkness within 10 min was defined as the respiration rate. The net photosynthetic rate (NPR) and respiration rate were presented as µmol O_2 g^{-1} FW h^{-1}.

Photosynthetic rates at different dissolved inorganic carbon (DIC) levels were measured under saturating irradiance of 600 µmol photons m^{-2} s^{-1} at the end of the experiment. The various DIC concentrations (0–13.2 mM) were obtained by adding different amounts of $NaHCO_3$ to the Tris-buffered DIC-free seawater. DIC was removed from the natural seawater by reducing pH to approximately 4.0 with the addition of 1.0 M HCl and then sparging for 2 h with pure N_2 gas (99.999 %). Finally, Tris buffer (25 mM) was added and the pH was adjusted to 8.1 with freshly prepared 1 M NaOH and 1 M HCl. The parameters, which are the maximum photosynthetic rate (V_{max}) and the half saturation constant ($K_{0.5}$, i.e., the DIC concentration required to give half of inorganic carbon (Ci)-saturated maximum rate of photosynthetic O_2 evolution), were calculated from the Michaelis–Menten kinetics equation (Caemmerer and Farquhar, 1981)

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

where $[S]$ is the DIC concentration.

2.5 Assessment of photosynthetic pigments

At the end of the experiment, approximately 100 mg of fresh-weight thalli from each culture condition were ground thoroughly in 2 mL 80 % acetone and placed in darkness for 12 h. Then the homogenate was centrifuged for 10 min at 5000 *g* and the supernatant was used to determine Chl *a* content spectrophotometrically according to the equation of Lichtenthaler (1987).

2.6 Measurement of nitrate uptake rate

The nitrate uptake rate (NUR) of the thalli was estimated from the decrease of NO_3^- concentration in the culture medium over a given time interval (12 h) during the light period using the following equation: $\mathrm{NUR} = (N_0 - N_t) \times V/W/12$, where N_0 is the initial concentration of NO_3^-, N_t is the concentration after 12 h, V is the volume of the culture medium, and W is the fresh weight of the thalli in culture. NO_3^- concentration in the seawater was measured according to Strickland and Parsons (1972).

2.7 Estimate of nitrate reductase activity

The nitrate reductase activity of the thalli was assayed according to modified in situ method of Corzo and Niell (1991). The measurement was conducted during the local noon period (13:00 UTC + 8 h (Chinese Standard Time)), because the activity of nitrate reductase usually displays circadian periodicity; a maximum during the light period and a minimum in darkness (Deng et al., 1991; Velasco and Whitaker, 1989). Approximately 0.3 g (FW) of thalli from each culture condition was incubated for 1 h at 20 °C in darkness in the reaction

solution (10 mL), which contained 0.1 M phosphate buffer, 0.1 % propanol (v/v), 50 mM KNO_3, 0.01 mM glucose, and 0.5 mM EDTA with a pH of 8.0. The mixture was flushed with pure N_2 gas (99.999 %) for 2 min to obtain an anaerobic state before the incubation. The concentration of nitrite produced was determined colorimetrically at 540 nm (Zou, 2005). The NRA was expressed as $\mu mol\ NO_2^-\ g^{-1}\ FW\ h^{-1}$.

2.8 Analysis of biochemical composition

At the end of the experiment, about 0.2 g of FW thalli from each culture condition were ground in a mortar with distilled water, and soluble carbohydrates were extracted in a water bath of 80 °C for 30 min. After being centrifuged for 10 min at 5000 g, the supernatant was volumed to 25 mL with distilled water, and soluble carbohydrate content was determined by the phenol-sulfuric acid method (Kochert, 1978).

Approximately 0.2 g of FW thalli from each culture condition were ground in a mortar with extraction buffer (0.1 mol L^{-1} phosphate buffer, pH 6.8) and then centrifuged for 10 min at 5000 g. Soluble protein was estimated from the supernatant using the Bradford (1976) assay with bovine serum albumin as a standard.

2.9 Data analysis

Results were expressed as means of replicates ± standard deviation. Data were analyzed using the software SPSS v.21. The data under every treatment conformed to a normal distribution (Shapiro–Wilk, $P > 0.05$), and the variances can be considered equal (Levene's test, $P > 0.05$). Two-way analysis of variance (ANOVA) was conducted to assess the effects of pCO_2 and P on carbonate parameters, relative growth rate, net photosynthesis rate, V_{max}, $K_{0.5}$, Chl a, nitrate uptake rate, nitrate reductase activity, soluble carbohydrates, soluble protein, and dark respiration rate. Tukey's honest significance difference (HSD) was conducted for a post hoc investigation. A confidence interval of 95 % was set for all tests.

3 Results

The effects of ocean acidification and P enrichment on seawater carbonate parameters were detected (Table 1). Two-way ANOVA analysis ($P = 0.05$) showed that pCO_2 had a main effect on all parameters except TA, while P did not affect any parameter. A post hoc Tukey's HSD comparison ($P = 0.05$) showed that elevated pCO_2 decreased pH by 0.31 at both LP and HP and CO_3^{2-} by 45 % (LP) and 45 % (HP), but it increased DIC by 10 % (LP) and 9 % (HP), HCO_3^- by 14 % (LP) and 14 % (HP), and CO_2 by 139 % (LP) and 134 % (HP).

The growth of *S. muticum* cultured at different pCO_2 and P conditions was recorded (Fig. 1). pCO_2 and P had an interactive effect on the relative growth rate of *S. muticum* (ANOVA, $F = 5.776$, $df = 1, 8$, $P = 0.043$), and each factor had a main effect (ANOVA, $F = 19.145$, $df = 1, 8$, $P = 0.002$ for pCO_2; ANOVA, $F = 30.592$, $df = 1, 8$, $P = 0.001$ for P). A post hoc Tukey's HSD comparison ($P = 0.05$) showed that the higher levels of pCO_2 and higher P alone increased the relative growth rate by 41 and 48 %, respectively, compared to the relative growth rate (3.1 ± 0.4 %) at lower pCO_2 and lower P. The combination of the higher pCO_2 and higher P levels did not enhance the relative growth rate as much as the sum of the higher pCO_2 alone plus the higher P alone, with an increase of 59.66 %. Although the higher P level increased the relative growth rate at lower pCO_2, it did not affect the relative growth rate at higher pCO_2.

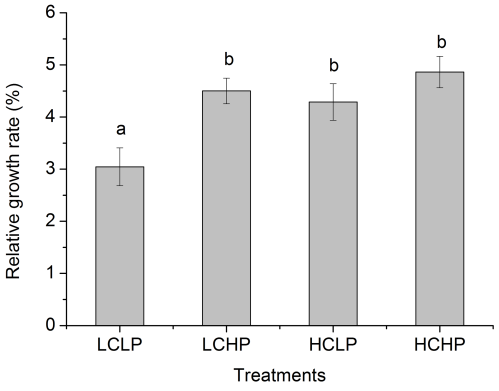

Figure 1. Relative growth rate (RGR) of *S. muticum* grown at different pCO_2 and P conditions for 13 days. Data are reported as means ±SD ($n = 3$). LCLP is the low pCO_2 and low P condition, LCHP is the low pCO_2 and high P condition, HCLP is the high pCO_2 and low P condition, and HCHP is the high pCO_2 and high P condition. Different letters above the error bars indicate significant differences between treatments ($P < 0.05$).

In terms of the net photosynthetic rate (Fig. 2), both pCO_2 (ANOVA, $F = 26.556$, $df = 1, 8$, $P = 0.001$) and P had main effects (ANOVA, $F = 38.963$, $df = 1, 8$, $P < 0.001$). A post hoc Tukey's HSD comparison ($P = 0.05$) showed that the higher pCO_2 level increased the net photosynthetic rates by 46 and 24 % at lower P and higher P, respectively. The higher P level increased the net photosynthetic rates by 55 and 31 % at lower pCO_2 and higher pCO_2, respectively. The difference in the net photosynthetic rate between LCHP and HCLP was statistically insignificant.

The carbon-saturating maximum photosynthetic rate (V_{max}) and the half saturation constant ($K_{0.5}$) obtained from the photosynthesis versus DIC curves (Fig. 3) are shown in Table 2. The pCO_2 and P had an interactive effect on the V_{max} of *S. muticum* (ANOVA, $F = 10.095$, $df = 1, 8$, $P = 0.013$), and each factor had a main effect (ANOVA, $F = 31.402$, $df = 1, 8$, $P = 0.001$ for pCO_2; ANOVA, $F = 105.116$, $df = 1, 8$, $P < 0.001$ for P). A post hoc Tukey's HSD comparison ($P = 0.05$) showed that the higher pCO_2 level increased the V_{max} by 42 % at lower P, while the increase at higher P was statistically insignificant. The higher

Table 1. Parameters of the seawater carbonate system at different CO_2 and phosphate conditions. Measurements and estimation of the parameters are described in the "Materials and methods" section. Data are reported as means ±SD ($n = 3$). LCLP is the low pCO_2 and low P condition, LCHP is the low pCO_2 and high P condition, HCLP is the high pCO_2 and low P condition, and HCHP is the high pCO_2 and P condition. DIC is dissolved inorganic carbon, and TA is total alkalinity.

Treatment	pH	pCO_2 (µatm)	HCO_3^- (µmol kg^{-1})	CO_3^{2-} (µmol kg^{-1})	CO_2 (µmol kg^{-1})	DIC (µmol kg^{-1})	TA (µmol kg^{-1})
LCLP	8.07 ± 0.02^b	426.9 ± 31.1^a	2000.2 ± 51.7^a	200.9 ± 5.8^b	14.2 ± 1.0^a	2215.3 ± 49.7^a	2475.2 ± 44.2
LCHP	8.07 ± 0.02^b	423.9 ± 21.1^a	1987.6 ± 10.9^a	199.8 ± 11.4^b	14.1 ± 0.7^a	2201.5 ± 19.3^a	2504.7 ± 33.8
HCLP	7.76 ± 0.02^a	1017.2 ± 83.2^b	2282.5 ± 27.6^b	110.0 ± 10.0^a	34.0 ± 2.9^b	2426.5 ± 32.5^b	2541.5 ± 44.2
HCHP	7.76 ± 0.02^a	992.2 ± 44.9^b	2261.8 ± 35.9^b	110.5 ± 5.9^a	33.1 ± 1.5^b	2405.4 ± 39.4^b	2563.6 ± 44.2

[a, b] Different superscript letters indicate significant differences in one parameter between treatments ($P < 0.05$).

Table 2. The carbon-saturating maximum photosynthetic rate (V_{max}, µmol O_2 g^{-1} FW h^{-1}) and half saturation constant ($K_{0.5}$, mM) for *S. muticum* cultured under different pCO_2 and P conditions for 13 days.

	LCLP	LCHP	HCLP	HCHP
V_{max}	57.00 ± 2.88^a	93.99 ± 0.98^c	81.18 ± 5.94^b	100.67 ± 6.81^c
$K_{0.5}$	0.21 ± 0.02^a	0.14 ± 0.05^a	0.42 ± 0.08^b	0.19 ± 0.05^a

[a, b, c] Different superscript letters indicate significant differences in one parameter between treatments ($P < 0.05$).

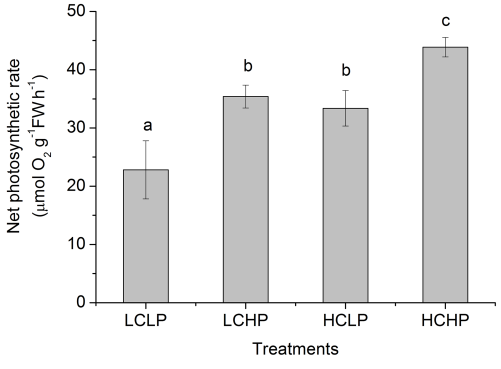

Figure 2. Net photosynthetic rate (NPR) of *S. muticum* after being grown at different pCO_2 and P conditions for 13 days. Data are reported as means ±SD ($n = 3$). LCLP is the low pCO_2 and low P condition, LCHP is the low pCO_2 and high P condition, HCLP is the high pCO_2 and low P condition, and HCHP is the high pCO_2 and high P condition. Different letters above the error bars indicate significant differences between treatments ($P < 0.05$).

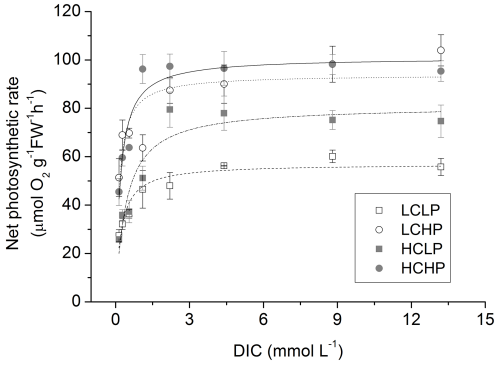

Figure 3. The photosynthesis versus DIC curves of *S. muticum* after being cultured under pCO_2 and P conditions for 13 days. Data are reported as means ±SD ($n = 3$). LCLP is the low pCO_2 and low P condition, LCHP is the low pCO_2 and high P condition, HCLP is the high pCO_2 and low P condition, and HCHP is the high pCO_2 and high P condition. DIC is dissolved inorganic carbon.

P level increased the V_{max} at the conditions of both lower pCO_2 (65 %) and higher pCO_2 (24 %) with a larger promoting effect at lower pCO_2.

pCO_2 and P interacted on the $K_{0.5}$ of *S. muticum* (ANOVA, $F = 5.928$, d$f = 1, 8$, $P = 0.041$), and each factor had a main effect (ANOVA, $F = 14.713$, d$f = 1, 8$, $P = 0.005$ for pCO_2; ANOVA, $F = 20.857$, d$f = 1, 8$, $P = 0.002$ for P). A post hoc Tukey's HSD comparison ($P = 0.05$) showed that the higher pCO_2 level increased the $K_{0.5}$ by 98 % at lower P but did not affect it at higher P. In contrast, the higher P level decreased the $K_{0.5}$ by 55 % at higher pCO_2 and the negative effect of the higher P level at lower pCO_2 was insignificant.

The amounts of the photosynthetic pigment Chl *a* under various treatments were also estimated (Fig. 4). pCO_2 and P had an interactive effect on the Chl *a* content (ANOVA, $F = 8.184$, d$f = 1, 8$, $P = 0.021$), and P had a main effect (ANOVA, $F = 22.828$, d$f = 1, 8$, $P = 0.001$), while pCO_2 did not affect it (ANOVA, $F = 0.676$, d$f = 1, 8$, $P = 0.435$). A post hoc Tukey's HSD comparison

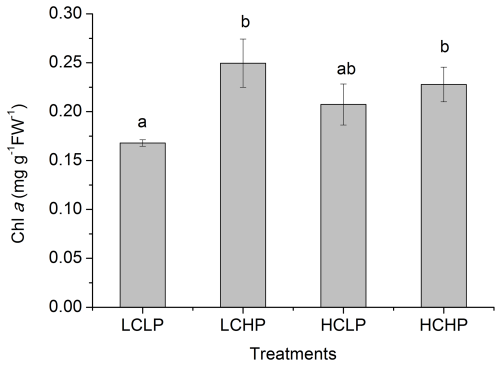

Figure 4. Chl *a* content of *S. muticum* after being grown at different pCO_2 and P conditions for 13 days. Data are reported as means ±SD ($n = 3$). LCLP is the low pCO_2 and low P condition, LCHP is the low pCO_2 and high P condition, HCLP is the high pCO_2 and low P condition, and HCHP is the high pCO_2 and high P condition. Different letters above the error bars indicate significant differences between treatments ($P < 0.05$).

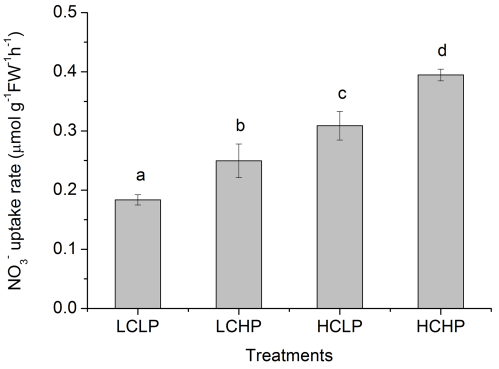

Figure 5. Nitrate uptake rate of *S. muticum* after being grown at different pCO_2 and P conditions for 13 days. Data are reported as means ±SD ($n = 3$). LCLP is the low pCO_2 and low P condition, LCHP is the low pCO_2 and high P condition, HCLP is the high pCO_2 and low P condition, and HCHP is the high pCO_2 and high P condition. Different letters above the error bars indicate significant differences between treatments ($P < 0.05$).

($P = 0.05$) showed that the higher P level increased the Chl *a* content from 0.17 ± 0.00 to 0.25 ± 0.02 mg g^{-1} FW at lower pCO_2, whereas the difference in the Chl *a* content between HCLP (0.21 ± 0.02 mg g^{-1} FW) and HCHP (0.23 ± 0.02 mg g^{-1} FW) was not statistically significant.

To assess the effects of ocean acidification and P enrichment on the nitrogen assimilation in *S. muticum*, the nitrate uptake rate under various pCO_2 and P treatments was investigated (Fig. 5). Both pCO_2 (ANOVA, $F = 139.916$, $df = 1, 8$, $P < 0.001$) and P (ANOVA, $F = 43.923$, $df = 1, 8$, $P < 0.001$) had main effects on the nitrate uptake rate of *S. muticum*. The nitrate uptake rates at lower pCO_2 were 0.18 ± 0.01 (LP) and 0.25 ± 0.03 μmol NO_3^- g^{-1} FW h^{-1} (HP), respectively. A post hoc Tukey's HSD comparison ($P = 0.05$) showed that the higher pCO_2 level increased the nitrate uptake rate to 0.31 ± 0.02 μmol NO_3^- g^{-1} FW h^{-1} at lower P and to 0.39 ± 0.01 μmol NO_3^- g^{-1} FW h^{-1} at higher P, compared to the rates at lower pCO_2. The higher P level also increased the nitrate uptake rate by 36 % at lower pCO_2 and by 28 % at higher pCO_2, compared to the rates at lower P.

Apart from nitrate uptake, the NRA of *S. muticum* under various pCO_2 and P treatments was also detected (Fig. 6). pCO_2 and P interacted on the NRA of *S. muticum* (ANOVA, $F = 28.435$, $df = 1, 8$, $P = 0.001$), and pCO_2 had a main effect (ANOVA, $F = 59.038$, $df = 1, 8$, $P < 0.001$). The NRAs at lower pCO_2 were 0.10 ± 0.01 (LP) and 0.14 ± 0.02 μmol NO_2^- g^{-1} FW h^{-1} (HP), respectively. The higher pCO_2 level increased it to 0.19 ± 0.00 μmol NO_2^- g^{-1} FW h^{-1} at lower P and to 0.15 ± 0.02 μmol NO_2^- g^{-1} FW h^{-1} at higher P. The higher P level increased the NRA by 39 % at lower pCO_2; however, it decreased the NRA by 18 % at higher pCO_2.

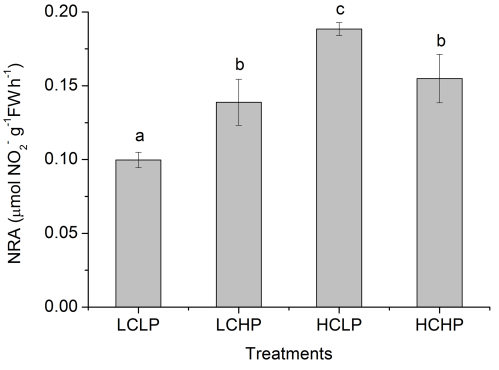

Figure 6. Nitrate reductase activity (NRA) of *S. muticum* after being grown at different pCO_2 and P conditions for 13 days. Data are reported as means means ±SD ($n = 3$). LCLP is the low pCO_2 and low P condition, LCHP is the low pCO_2 and high P condition, HCLP is the high pCO_2 and low P condition, and HCHP is the high pCO_2 and high P condition. Different letters above the error bars indicate significant differences between treatments ($P < 0.05$).

The soluble carbohydrates (Fig. 7a) and protein (Fig. 7b) were estimated to understand the effects of ocean acidification and P enrichment on the products of carbon and nitrogen assimilation in *S. muticum*. pCO_2 and P had an interactive effect on the soluble carbohydrates (ANOVA, $F = 18.294$, $df = 1, 8$, $P = 0.003$), and P had a main effect (ANOVA, $F = 23.129$, $df = 1, 8$, $P = 0.001$). The higher P level increased the soluble carbohydrates from 25.40 ± 1.66 to 41.10 ± 1.74 mg g^{-1} FW at lower pCO_2 but did not alter them at higher pCO_2. The higher pCO_2 level increased the soluble carbohydrates to 33.72 ± 3.31 mg g^{-1} FW at lower P, while the decrease of soluble carbohydrates caused by the higher pCO_2 level was not statistically significant at higher P.

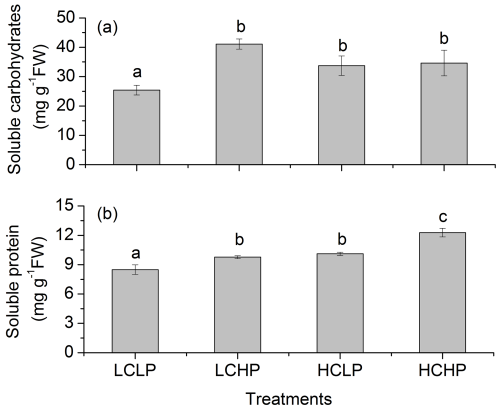

Figure 7. The contents of soluble carbohydrates **(a)** and protein **(b)** of *S. muticum* after being grown at different pCO_2 and P conditions for 13 days. Data are reported as means ±SD ($n = 3$). LCLP is the low pCO_2 and low P condition, LCHP is the low pCO_2 and high P condition, HCLP is the high pCO_2 and low P condition, and HCHP is the high pCO_2 and high P condition. Different letters above the error bars indicate significant differences between treatments ($P < 0.05$).

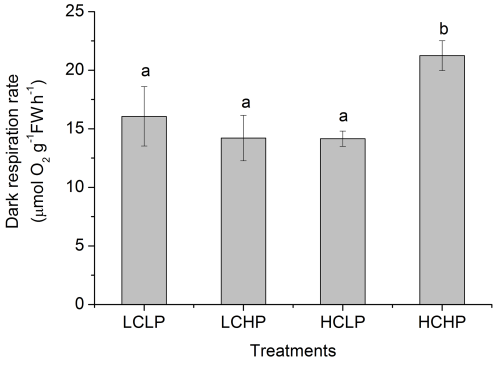

Figure 8. Dark respiration rate of *S. muticum* after being grown at different pCO_2 and P conditions for 13 days. Data are reported as means ±SD ($n = 3$). LCLP is the low pCO_2 and low P condition, LCHP is the low pCO_2 and high P condition, HCLP is the high pCO_2 and low P condition, and HCHP is the high pCO_2 and high P condition. Different letters above the error bars indicate significant differences between treatments ($P < 0.05$).

Both pCO_2 (ANOVA, $F = 106.663$, $df = 1, 8$, $P < 0.001$) and P (ANOVA, $F = 75.003$, $df = 1, 8$, $P < 0.001$) had main effects on the soluble protein of *S. muticum*, and an interactive effect of the two factors was not detected (ANOVA, $F = 4.961$, $df = 1, 8$, $P = 0.057$). The soluble protein contents at lower pCO_2 were 8.49 ± 0.49 (LP) and 9.77 ± 0.14 mg g^{-1} FW (HP), respectively. The higher pCO_2 level increased it to 10.11 ± 0.16 mg g^{-1} FW at lower P and to 12.28 ± 0.44 mg g^{-1} FW at higher P. The higher P level also increased the soluble protein contents by 15 % at lower pCO_2 and by 21 % at higher pCO_2.

Finally, the effects of ocean acidification and P enrichment on the dark respiration rate of *S. muticum* were investigated (Fig. 8). pCO_2 and P had an interactive effect on the dark respiration rate (ANOVA, $F = 19.584$, $df = 1, 8$, $P = 0.002$), and each factor had a main effect (ANOVA, $F = 6.428$, $df = 1, 8$, $P = 0.035$ for pCO_2; ANOVA, $F = 6.754$, $df = 1, 8$, $P = 0.032$ for P). The higher pCO_2 level increased the dark respiration rate from 14.21 ± 1.94 to 21.24 ± 1.28 μmol O_2 g^{-1} FW h^{-1} at higher P but did not affect it at lower P. Likewise, the higher P level increased the respiration rate from 14.15 ± 0.65 to 21.24 ± 1.28 μmol O_2 g^{-1} FW h^{-1} at higher pCO_2 but did not change it at lower pCO_2.

4 Discussion

4.1 Effects of pCO_2 and P on carbon assimilation

The higher pCO_2 level increased the net photosynthetic rate in *S. muticum* at lower P in the present study. Although the dissolved inorganic carbon in seawater is around 2 mM, the dominant form is HCO_3^- with CO_2 typically accounting for less than 1 % (Dickson, 2010). In addition, CO_2 in seawater diffuses ~8000 times more slowly than in air (Gao and Campbell, 2014). Furthermore, marine macroalgae have high $K_{0.5}$ values (40–70 μM CO_2) for Rubisco, the carbon assimilating enzyme (Ji et al., 2016). The evidence above indicates that the CO_2 in seawater should be carbon limited for marine macroalgae. The promoting effect of elevated CO_2 on photosynthesis was also reported in other macroalgae species, such as the green algae *Ulva linza* (Gao et al., 1999), the red algae *Pyropia haitanensis* (Zou and Gao, 2002), and the brown algae *Petalonia binghamiae* (Zou and Gao, 2010). The higher pCO_2 level increased $K_{0.5}$ of *S. muticum* at lower P in the present study, which indicates that a plant grown under conditions of higher pCO_2 reduces its photosynthetic affinity for DIC. This phenomenon is commonly found in both microalgae and macroalgae (Gao and Campbell, 2014; Ji et al., 2016; Wu et al., 2008) and is considered a sign of downregulated CCMs at high CO_2 conditions (Gao and Campbell, 2014). However, this decrease of photosynthetic affinity for DIC did not lead to reduced photosynthesis in *S. muticum* compared to that at the lower pCO_2 in the present study, mainly because of increased CO_2 availability for Rubisco and depressed photorespiration at the elevated ratio of CO_2 to O_2, which has been confirmed in the red seaweed *Lomentaria articulata* (Kübler et al., 1999).

The higher P level also increased the net photosynthetic rate of *S. muticum* in the present study, which can be partially explained by the decreased $K_{0.5}$ at higher P. The decreased $K_{0.5}$ is an indication of increased photosynthetic carbon-use capability. Phosphorus is a key macronutrient component for organisms, and high levels of P availability are not only essential for chloroplast DNA and RNA syn-

thesis (Vered and Shlomit, 2008), but are also required for various chloroplast functions referring to the phosphorylation of photosynthetic proteins, the synthesis of phospholipids, and the generation of adenosine triphosphate (ATP; Zer and Ohad, 2003). Therefore, high P levels could speed up the transport of Ci from media to the site of Rubisco by supplying necessary energy. In addition, P enrichment can increase both the activity and the amount of Rubisco (Lauer et al., 1989). Phosphorus, with low concentrations in seawater, is generally considered to be limiting for marine primary producers (Elser et al., 2007; Howarth, 1988; Müller and Mitrovic, 2015). Therefore, adding extra phosphorus to natural seawater can stimulate the photosynthesis of algae. For instance, the midday (12:00) photosynthetic rates increased from 1.3 to 2.3 mg C g^{-1} DW h^{-1} for *S. natans* and from 0.9 to 2.1 mg C g^{-1} DW h^{-1} for *S. fluitans* when 0.2 mM P was added (Lapointe, 1986). In the present study, the addition of 40 µmol P also resulted in a nearly 2-fold increase of the net photosynthetic rate and the V_{max}, which suggests the importance of P in the photosynthesis of this alga. In addition, the higher P level promoted the synthesis of Chl *a* at the condition of lower pCO_2, which may also contribute to the increased net photosynthetic rate in *S. muticum* at higher P. Although P is not a component constituting Chl *a*, a higher P supply may stimulate the content of Chl *a* synthesis-related enzymes and thus the production of Chl *a*. The positive effect of P on Chl *a* was also reported in *S. thunbergii* (Nakahara, 1990). On the other hand, the higher P level did not increase the Chl *a* content at higher pCO_2 in the present study. A possible reason is that there is more ATP available at higher pCO_2 due to the downregulation of CCMs, and thus there is no need to synthesize more Chl *a* to capture more light for cells, as excessive energy can harm the photosynthesis and growth of algae (Gao et al., 2012; Xu and Gao, 2012).

4.2 Effect of pCO_2 and P on nitrogen assimilation

The higher pCO_2 level noticeably enhanced the nitrate uptake rate in *S. muticum* regardless of P concentration in the present study. This could be attributed to the increased NRA at the condition of higher pCO_2. The enhanced NRA at the conditions of high CO_2 was also reported in *U. rigida* (Gordillo et al., 2001), *Hizikia fusiforme* (Zou, 2005), *P. haitanensis* (Liu and Zou, 2015), and *Corallina officinalis* (Hofmann et al., 2013), as well as in the higher plants *Plantago major* (Fonseca et al., 1997) and tomatoes (Yelle et al., 1987). Taken together, these findings indicate that the response of NRA in plants to elevated CO_2 may be homogeneous.

The higher P level also enhanced the nitrate uptake in *S. muticum* regardless of the pCO_2 level, which could be partially due to the increased NRA at higher P. This is very evident at lower pCO_2. However, the higher P level decreased the NRA at higher pCO_2, which did not lead to reduced nitrate uptake. This indicates that there should be other mechanisms to account for the promoting effect of the higher P level on the nitrate uptake. One possible mechanism is the higher P level increasing the availability of ATP required for the active uptake of nitrate across the plasma membrane. The phenomenon of ATP concentration increasing with P level has been found in higher plants (Olivera et al., 2004; Rychter et al., 2006). Apart from *S. muticum*, the positive effect of a higher P level on nitrate uptake was also reported in the red macroalgae *Gracilaria lemaneiformis* (Xu et al., 2010) and the higher plant *Phaseolus vulgaris* (Gniazdowska and Rychter, 2000). The increased nitrate uptake, NRA, and soluble protein at higher P in the present study suggest that high P availability promoted nitrogen assimilation in *S. muticum*. It is worth noting that the nitrate uptake rates were commonly higher than the corresponding reduction rates of NO_3^- to nitrite NO_2^- by nitrate reductase in the present study, which might be due to the intercellular nitrate storage (Collos, 1982; Lartigue and Sherman, 2005) and the underestimation of RNA measured by the in situ assay (Lartigue and Sherman, 2002). The higher P level increased the nitrate uptake rate and soluble protein at both lower pCO_2 and higher pCO_2, but it only increased the NRA in *S. muticum* at lower pCO_2 in the present study. Surprisingly, it decreased the NRA at higher pCO_2. There may be more than one reason related to interaction of pCO_2 and P. High pCO_2, on the one hand, could enhance photosynthetic carbon fixation and thus growth by supplying sufficient CO_2. On the other hand, it also results in the decrease of pH and the increase of seawater acidity, which can disturb the acid–base balance on the cell surface of algae (Flynn et al., 2012). Algae may accordingly allocate additional energy to act against the acid–base perturbation in some way. This hypothesis is supported by increased respiration at higher pCO_2 and higher P in the present study. The increased soluble protein and decreased NRA at higher pCO_2 and higher P suggest that some H^+ transport-related protein, such as plasma membrane H^+-ATPase, might be synthesized to counteract the acid–base perturbation caused by increased pCO_2 and H^+. The additional production of an H^+ transport-related protein, like plasma membrane H^+-ATPase, could competitively decrease the synthesis of nitrate reductase. This hypothesis needs further experimental evidence to confirm, even though it could explain the results in the present study.

4.3 Connection between carbon and nitrogen assimilation

The increased net photosynthetic rate at higher pCO_2 and higher P did not result in higher soluble carbohydrates compared to higher pCO_2 and lower P. The additional ATP produced by photosynthetic electron transport higher pCO_2 and higher P may be drawn to nitrogen assimilation as more soluble protein was synthesized at higher pCO_2 and higher P. The additional energy allocation to protein synthesis, possibly an H^+ transport-related protein, to maintain the balance of acid–base hindered the increase of growth, which may be the rea-

son that the higher P increased the net photosynthetic rate but not the growth rate at higher pCO_2. Although synthesized protein can also contribute to the increase of thalli weight, it is not as energy-effective as carbohydrates (Norici et al., 2011; Raven, 1982). It seems that *S. muticum* tends to maintain a steady state in vivo, even if it can sacrifice growth to some extent, considering that the regulation of the intracellular acid–base balance is crucial for organismal homoeostasis (Flynn et al., 2012; Smith and Raven, 1979). The increased respiration at HC was also demonstrated in *G. lemaneiformis* (Xu et al., 2010) and *U. prolifera* (Xu and Gao, 2012). The respiration at higher pCO_2 and lower P did not increase compared to at lower pCO_2 and lower P in the present study, suggesting that action against acid–base perturbation did not commence. The acid–base perturbation at higher pCO_2 and lower P may lead to the decreased photosynthetic rate compared to that at lower pCO_2 and lower P.

5 Conclusion

Our study, for the first time, demonstrates the combined effects of elevated pCO_2 and P enrichment on the physiological traits of a golden alga, *S. muticum.* It suggests that the current ocean environment is both CO_2 and P limited for the photosynthesis and growth of *S. muticum*. Therefore, future ocean acidification and eutrophication may promote the growth of *S. muticum* and thus the occurrence of golden tide events. *S. muticum* tends to maintain homoeostasis by taking advantage of phosphate enrichment at the cost of growth. Accordingly, the combination of ocean acidification and eutrophication may not boost golden tides further compared to ocean acidification or eutrophication alone.

6 Data availability

The data to this paper can be found in the Supplement.

The Supplement related to this article is available online at doi:10.5194/bg-14-671-2017-supplement.

Competing interests. The authors declare that they have no conflict of interest.

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References

Alvaro, I. and Mazal, H.: Growth, photosynthetic properties and Rubisco activities and amounts of marine macroalgae grown under current and elevated seawater CO_2 concentrations, Glob. Change Biol., 30, 831–840, 2002.

Ang, P. O.: Phenology of *Sargassum* spp. in Tung Ping Chau Marine Park, Hong Kong SAR, China, J. Appl. Phycol., 18, 403–410, 2006.

Ashok-Kumar, N., Vanlalzarzova, B., Sridhar, S., and Baluswami, M.: Effect of liquid seaweed fertilizer of *Sargassum wightii* Grev. on the growth and biochemical content of green gram (*Vigna radiata* (L.) R. Wilczek), Recent Res. Sci. Technol., 4, 40–45, 2012.

Bradford, M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem., 72, 248–254, 1976.

Bricker, S. B., Longstaff, B., Dennison, W., Jones, A., Boicourt, K., Wicks, C., and Woerner, J.: Effects of nutrient enrichment in the nation's estuaries: a decade of change, Harmful Algae, 8, 21–32, 2008.

Caemmerer, S. V. and Farquhar, G. D.: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves, Planta, 153, 376–387, 1981.

Carpenter, S. R.: Submersed vegetation: an internal factor in lake ecosystem succession, Am. Nat., 118, 372–383, 1981.

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., and Smith, V. H.: Nonpoint pollution of surface waters with phosphorus and nitrogen, Ecol. Appl., 8, 559–568, 1998.

Cheang, C. C., Chu, K. H., Fujita, D., Yoshida, G., Hiraoka, M., Critchley, A., Choi, H. G., Duan, D., Serisawa, Y., and Ang, P. O.: Low genetic variability of *Sargassum muticum* (Phaeophyceae) revealed by a global analysis of native and introduced populations, J. Phycol., 46, 1063–1074, 2010.

Chen, B. and Zou, D.: Growth and photosynthetic activity of *Sargassum henslowianum* (Fucales, Phaeophyta) seedlings in responses to different light intensities, temperatures and CO_2 levels under laboratory conditions, Mar. Biol. Res., 10, 1019–1026, 2014.

Collos, Y.: Transient situations in nitrate assimilation by marine diatoms. III. Short-term uncoupling of nitrate uptake and reduction, J. Exp. Mar. Bio. Ecol., 62, 285–295, 1982.

Corzo, A. and Niell, F. X.: Determination of nitrate reductase activity in *Ulva rigida* C. Agardh by the in situ method, J. Exp. Mar. Bio. Ecol., 146, 181–191, 1991.

Cruzrivera, E., Floresdíaz, M., and Hawkins, A.: A fish kill coincident with dense *Sargassum accumulation* in a tropical bay, Bull. Mar. Sci., 91, 455–456, 2015.

Deng, M. D., Moureaux, T., Cherel, I., Boutin, J. P., and Caboche, M.: Effects of nitrogen metabolites on the regulation and cir-

cadian expression of tobacco nitrate reductase, Plant Physiol. Biochem., 29, 239–247, 1991.

Dickson, A. G.: The carbon dioxide system in seawater: Equilibrium chemistry and measurements, in: Guide to best practices for ocean acidification research and data reporting, edited by: Riebesell, U., Fabry, V. J., Hansson, L., and Gattuso, J. P., Publications Office of the European Union, Luxembourg, 2010.

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., and Smith, J. E.: Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems, Ecol. Lett., 10, 1135–1142, 2007.

Fenoradosoa, T. A., Ali, G., Delattre, C., Laroche, C., Petit, E., Wadouachi, A., and Michaud, P.: Extraction and characterization of an alginate from the brown seaweed *Sargassum turbinarioides* Grunow, J. Appl. Phycol., 22, 131–137, 2010.

Flynn, K. J., Blackford, J. C., Baird, M. E., Raven, J. A., Clark, D. R., Beardall, J., Brownlee, C., Fabian, H., and Wheeler, G. L.: Changes in pH at the exterior surface of plankton with ocean acidification, Nat. Clim. Change, 2, 510–513, 2012.

Fonseca, F., Bowsher, C. G., and Stulen, I.: Impact of elevated atmospheric CO_2 on nitrate reductase transcription and activity in leaves and roots of *Plantago major*, Physiol. Plantarum, 100, 940–948, 1997.

Gao, K. and Campbell, D. A.: Photophysiological responses of marine diatoms to elevated CO_2 and decreased pH: a review, Funct. Plant Biol., 41, 449–459, 2014.

Gao, K., Yan, J., and Aruga, Y.: Relationship of CO_2 concentrations to photosynthesis of intertidal macroalgae during emersion, Hydrobiologia, 398/399, 355–359, 1999.

Gao, K., Xu, J., Gao, G., Li, Y., Hutchins, D. A., Huang, B., Wang, L., Zheng, Y., Jin, P., and Cai, X.: Rising CO_2 and increased light exposure synergistically reduce marine primary productivity, Nat. Clim. Change, 2, 519–523, 2012.

Gniazdowska, A. and Rychter, A. M.: Nitrate uptake by bean (*Phaseolus vulgaris* L.) roots under phosphate deficiency, Plant Soil, 226, 79–85, 2000.

González-López, N., Moure, A., and Domínguez, H.: Hydrothermal fractionation of *Sargassum muticum* biomass, J. Appl. Phycol., 24, 1569–1578, 2012.

Gordillo, F. J. L., Niell, F. X., and Figueroa, F. L.: Non-photosynthetic enhancement of growth by high CO_2 level in the nitrophilic seaweed *Ulva rigida* C. Agardh (Chlorophyta), Planta, 213, 64–70, 2001.

Hofmann, L., Straub, S., and Bischof, K.: Elevated CO_2 levels affect the activity of nitrate reductase and carbonic anhydrase in the calcifying rhodophyte *Corallina officinalis*, J. Exp. Bot., 64, 899–908, 2013.

Howarth, R. W.: Nutrient limitation of net primary production in marine ecosystems, Annu. Rev. Ecol. Syst., 19, 89–110, 1988.

Hwang, R. L., Tsai, C. C., and Lee, T. M.: Assessment of temperature and nutrient limitation on seasonal dynamics among species of sargassum from a coral reef in southern taiwan, J. Phycol., 40, 463–473, 2004.

Incera, M., Olabarria, C., Troncoso, J. S., and López, J.: Response of the invader *Sargassum muticum* to variability in nutrient supply, Mar. Ecol.-Prog. Ser., 377, 91–101, 2009.

IPCC: Climate change 2013: The physical science basis, in: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, edited by: Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P. M., Cambridge Univ Press, New York, 2013.

Ji, Y., Xu, Z., Zou, D., and Gao, K.: Ecophysiological responses of marine macroalgae to climate change factors, J. Appl. Phycol., 28, 2953–2967, 2016.

Jones, G. and Farnham, W.: Japweed: new threat to British coasts, New Sci., 60, 394–395, 1973.

Kübler, J. E., Johnston, A. M., and Raven, J. A.: The effects of reduced and elevated CO_2 and O_2 on the seaweed *Lomentaria articulata*, Plant Cell Environ., 22, 1303–1310, 1999.

Karlsson, J. and Loo, L. O.: On the distribution and continuous expansion of the Japanese seaweed – *Sargassum muticum* – in Sweden, Bot. Mar., 42, 285–294, 1999.

Kochert, G.: Carbohydrate determination by the phenol-sulfuric acid method, in: Handbook of Phycological Methods: Physiological and Biochemical Methods, edited by: Hellebust, J. A. and Graigie, J. S., Cambridge University Press, Cambridge, 1978.

Laffoley, D. A., Roe, H. S. J., Angel, M. V., Ardron, J., Bates, N. R., Boyd, I. L., Brooke, S., Buck, K. N., Carlson, C. A., and Causey, B.: The protection and management of the Sargasso Sea: The golden floating rainforest of the Atlantic Ocean, Sargasso Sea Alliance, Washington, DC, USA, 2011.

Lapointe, B. E.: Phosphorus-limited photosynthesis and growth of *Sargassum natans* and *Sargassum fluitans* (Phaeophyceae) in the western North Atlantic, Deep-Sea Res. Pt. I, 33, 391–399, 1986.

Lapointe, B. E.: A comparison of nutrient – productivity in *Sargassum natans* from neritic vs. oceanic waters of the western North Atlantic Ocean, Limnol. Oceanogr., 40, 625–633, 1995.

Lapointe, B. E., Littler, M. M., and Littler, D. S.: A comparison of nutrient-limited productivity in macroalgae from a Caribbean barrier reef and from a mangrove ecosystem, Aquat. Bot., 28, 243–255, 1987.

Lapointe, B. E., Littler, M. M., and Littler, D. S.: Nutrient availability to marine macroalgae in siliciclastic versus carbonate-rich coastal waters, Estuar. Coast., 15, 75–82, 1992.

Lartigue, J. and Sherman, T. D.: Field assays for measuring nitrate reductase activity in *Enteromorpha* sp. (Chlorophyceae), *Ulva* sp. (Chlorophyceae), and *Gelidium* sp. (Rhodophyceae), J. Phycol., 38, 971–982, 2002.

Lartigue, J. and Sherman, T. D.: Response of *Enteromorpha* sp. (Chlorophyceae) to a nitrate pulse: nitrate uptake, inorganic nitrogen storage and nitrate reductase activity, Mar. Ecol.-Prog. Ser., 292, 147–157, 2005.

Lauer, M. J., Pallardy, S. G., Blevins, D. G., and Randall, D. D.: Whole leaf carbon exchange characteristics of phosphate deficient soybeans (*Glycine max* L.), Plant Physiol., 91, 848–854, 1989.

Lichtenthaler, H. K.: Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes, Methods Enzymol., 148, 350–382, 1987.

Littler, M. M., Littler, D. S., and Titlyanov, E. A.: Comparisons of N- and P-limited productivity between high granitic islands versus low carbonate atolls in the Seychelles Archipelago: a test of the relative-dominance paradigm, Coral Reefs, 10, 199–209, 1991.

Liu, C. and Zou, D.: Effects of elevated CO_2 on the photosynthesis and nitrate reductase activity of *Pyropia haitanensis* (Ban-

giales,Rhodophyta) grown at different nutrient levels, Chin. J. Oceanol. Limnol., 33, 419–429, 2015.

Liu, Y. and Tan, H.: Changes of growth and nutrient-relating enzymatic activities of *Sargassum thunbergii* when exposed to different nutrient conditions, Aquat. Sci. Technol., 2, 1–13, 2014.

Longphuirt, S. N., Eschmann, C., Russell, C., and Stengel, D. B.: Seasonal and species specific response of five brown macroalgae to high atmospheric CO_2, Mar. Ecol.-Prog. Ser., 493, 91–102, 2014.

Mattio, L. and Payri, C. E.: 190 years of *Sargassum* taxonomy, facing the advent of DNA phylogenies, Bot. Rev., 77, 31–70, 2011.

Müller, S. and Mitrovic, S. M.: Phytoplankton co-limitation by nitrogen and phosphorus in a shallow reservoir: progressing from the phosphorus limitation paradigm, Hydrobiologia, 744, 255–269, 2015.

Nakahara, K. G. H.: Effects of nutrients on the photosynthesis of *Sargassum thunbergii*, Bot. Mar., 33, 375–384, 1990.

Norici, A., Bazzoni, A. M., Pugnetti, A., Raven, J. A., and Giordano, M.: Impact of irradiance on the C allocation in the coastal marine diatom *Skeletonema marinoi* Sarno and Zingone, Plant Cell Environ., 34, 1666–1677, 2011.

Olivera, M., Tejera, N., Iribarne, C., Ocaña, A., and Lluch, C.: Growth, nitrogen fixation and ammonium assimilation in common bean (*Phaseolus vulgaris*): effect of phosphorus, Physiol. Plant., 121, 498–505, 2004.

Pierrot, D., Lewis, E., and Wallace, D. W. R.: MS Excel program developed for CO_2 system calculations, ORNL/CDIAC-105a, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee, 2006.

Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P., Riebesell, U., Shepherd, J., Turley, C., and Watson, A.: Ocean acidification due to increasing atmospheric carbon dioxide, The Royal Society, London, 2005.

Raven, J. A.: The energetics of freshwater algae; energy requirements for biosynthesis and volume regulation, New Phytol., 92, 1–20, 1982.

Roy, R. N., Roy, L. N., Vogel, K. M., Porter-Moore, C., Pearson, T., Good, C. E., Millero, F. J., and Campbell, D. M.: The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45 °C, Mar. Chem., 44, 249–267, 1993.

Rueness, J.: *Sargassum muticum* and other introduced Japanese macroalgae: Biological pollution of European coasts, Mar. Pollut. Bull., 20, 173–176, 1989.

Rychter, A. M., Chauveau, M., Bomsel, J. L., and Lance, C.: The effect of phosphate deficiency on mitochondrial activity and adenylate levels in bean roots, Physiol. Plant., 84, 80–86, 2006.

Scagel, R. F.: Introduction of a Japanese alga, *Sargassum muticum*, into the northeast Pacific, Fisheries Research Papers, 1, 49–58, 1956.

Schaffelke, B. and Klumpp, D. W.: Nutrient-limited growth of the coral reef macroalga *Sargassum baccularia* and experimental growth enhancement by nutrient addition in continuous flow culture, Mar. Ecol.-Prog. Ser., 164, 199–211, 1998.

Schell, J. M., Goodwin, D. S., and Siuda, A. N. S.: Recent sargassum inundation events in the Caribbean, Oceanography, 28, 8–10, 2015.

Sfriso, A. and Facca, C.: Annual growth and environmental relationships of the invasive species *Sargassum muticum* and *Undaria pinnatifida* in the lagoon of Venice, Estuar. Coast. Shelf S., 129, 162–172, 2013.

Smetacek, V. and Zingone, A.: Green and golden seaweed tides on the rise, Nature, 504, 84–88, 2013.

Smith, F. A. and Raven, J. A.: Intracellular pH and its regulation, Annu. Rev. Plant Phys., 30, 289–311, 1979.

Smith, V. H., Tilman, G. D., and Nekola, J. C.: Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems, Environ. Pollut., 100, 179–196, 1999.

Stæhr, P. A., Pedersen, M. F., Thomsen, M. S., Wernberg, T., and KrauseJensen, D.: Invasion of *Sargassum muticum* in Limfjorden (Denmark) and its possible impact on the indigenous macroalgal community, Mar. Ecol.-Prog. Ser., 207, 79–88, 2000.

Strickland, J. D. H. and Parsons, T. R.: A practical handbook of seawater analysis, 2nd Edn., Fisheries Research Board of Canada, Ottawa, 1972.

Velasco, P. J. and Whitaker, J. R.: Synthesis and degradation of nitrate reductase during the cell cycle of *Chlorella sorokiniana*, Plant Physiol., 89, 220–224, 1989.

Vered, I. and Shlomit, Y. R.: Phosphate and sulfur limitation responses in the chloroplast of *Chlamydomonas reinhardtii*, FEMS Microbiol. Lett., 283, 1–8, 2008.

Wu, H. Y., Zou, D. H., and Gao, K. S.: Impacts of increased atmospheric CO_2 concentration on photosynthesis and growth of micro- and macro-algae, Sci. China Ser. C, 51, 1144–1150, 2008.

Xu, J. and Gao, K.: Future CO_2-induced ocean acidification mediates the physiological performance of a green tide alga, Plant Physiol., 160, 1762–1769, 2012.

Xu, Z., Zou, D. H., and Gao, K.: Effects of elevated CO_2 and phosphorus supply on growth, photosynthesis and nutrient uptake in the marine macroalga *Gracilaria lemaneiformis* (Rhodophyta), Bot. Mar., 53, 123–129, 2010.

Yelle, S., Gosselin, A., and Trudel, M. J.: Effect of atmospheric CO_2 concentration and root-zone temperature on growth, mineral nutrition, and nitrate reductase activity of greenhouse tomato, J. Am. Soc. Hort. Sci., 112, 1036–1040, 1987.

Zer, H. and Ohad, I.: Light, redox state, thylakoid-protein phosphorylation and signaling gene expression, Trends Biochem. Sci., 28, 467–470, 2003.

Zou, D.: Effects of elevated atmospheric CO_2 on growth, photosynthesis and nitrogen metabolism in the economic brown seaweed, *Hizikia fusiforme* (Sargassaceae, Phaeophyta), Aquaculture, 250, 726–735, 2005.

Zou, D. and Gao, K.: Effects of desiccation and CO_2 concentrations on emersed photosynthesis in *Porphyra haitanensis* (Bangiales, Rhodophyta), a species farmed in China, Eur. J. Phycol., 37, 587–592, 2002.

Zou, D. and Gao, K.: Acquisition of inorganic carbon by *Endarachne binghamiae* (Scytosiphonales, Phaeophyceae), Eur. J. Phycol., 45, 117–126, 2010.