The brown algae *Saccharina japonica* and *Sargassum horneri* exhibit species-specific responses to synergistic stress of ocean acidification and eutrophication

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Abstract Ocean acidification and eutrophication are two important environmental stressors. They inevitably impact marine macroalgae, and hence the coastal ecosystem of China. *Saccharina japonica*, as the main culture species in China, is suffering the harmful golden tide caused by *Sargassum horneri*. However, it remains unclear whether the detrimental effects of *S. horneri* on *S. japonica* cultivation become more severe in future acidified and eutrophic scenario. In this study, we respectively investigated the effects of pCO_2 (400 μatm and 1000 μatm) and nutrients (non-enriched and enriched seawater) on the growth, photosynthesis, respiration, chlorophyll contents, and tissue nitrogen of *S. japonica* and *S. horneri*. Results indicated that enrichment of nutrients contributed *S. horneri* to utilize HCO_3^-. The carbon acquisition pathway shifted from HCO_3^- to CO_2 in *S. japonica*, while *S. horneri* remained using HCO_3^- regulated by nutrient enrichment. *S. horneri* exhibited better photosynthetic traits than *S. japonica*, with a higher level of net photosynthetic rate and chlorophyll contents at elevated pCO_2 and enriched nutrients. Tissue nitrogen also accumulated richly in the thalli of *S. horneri* under higher pCO_2 and nutrients. Significant enhancement in growth was only detected in *S. horneri* under synergistic

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stress. Together, *S. horneri* showed competitive dominance in current study. These findings suggest that increasing risk of golden tide in acidified and eutrophic ocean can most likely result in great damage to *S. japonica* cultivation.

Keywords Eutrophication, Ocean acidification, *Saccharina japonica*, *Sargassum horneri*, Synergistic stress

1 Introduction

The concentration of atmospheric carbon dioxide (CO_2) increased approximately 130 pars per million (ppm) since the Industrial Revolution (Joos & Spahni, 2008; AOAN, 2019). Rising atmospheric CO_2 dissolve in seawater, causing pH reductions and alterations in chemical balances of dissolved inorganic carbon (DIC) (Feely et al., 2004, 2009; Doney et al., 2009). These changes in pH and DIC are ineluctable consequences of rising atmospheric CO_2, referred to as ocean acidification (OA) (Doney et al., 2009). Anthropogenic CO_2 emission is rising at the fastest rate after the Industrial Era (Joos & Spahni, 2008; AOAN, 2019), thus leading to a continuing decrease in seawater pH (Feely et al., 2004, 2009; Doney et al., 2009; Feely, Doney & Cooley, 2009). OA significantly affects the physiological processes and ecological functions of seaweeds and other marine organisms (Gazeau et al., 2007; Edmunds, 2011; Koch et al., 2013; Kroeker et al., 2013; Enochs et al., 2015; Gao et al., 2019). Previous studies showed that photosynthetic organisms including macroalgae appear to benefit from elevated CO_2 and tolerant to declined pH (Shi et al., 2012, 2019; Britton et al., 2016; Gao et al., 2019). A body of evidence indicates that OA actively stimulates the growth of kelps, such as *Saccharina latissima*, *Ulva rigida* and *Macrocystis pyrifera* which were carbon limited in nearshore environment (Swanson & Fox, 2007; Xu et al., 2019; Hurd et al., 2020; Zhang et al., 2020). On the other hand, OA simultaneously reduces the calcification of *Marginopora rossi*, *Porolithon onkodes* and other calcified algae (Reymond et al., 2013; Johnson & Carpenter, 2018).

Furthermore, human pollution, agricultural production and atmospheric deposition

have dramatically increased since 1970s, resulting in excessive nutrients input to coastal seawater (Smith et al., 2003; Felipe van der Struijk & Kroeze, 2010; Strokal et al., 2014; Brockmann et al., 2018; Murray et al., 2019). This process leads to another environmental issue known as eutrophication (Smith et al., 2003). Several studies showed that water quality slightly recovered from previous eutrophic state in the Baltic Sea, Chesapeake Bay and other coastal seas (Okino & Kato, 1987; Andersen et al., 2017; McCrackin et al., 2017; Duarte & Krause-Jensen, 2018). In contrast, severe eutrophic areas are still located at some key bays in China, including Liaodong Bay, Yangtze River Estuary and other jurisdictional seas (MEE, 2019). With exceeded nutrients supply, eutrophication can enhance the growth of phytoplankton, fast-growing filamentous and mat-forming opportunistic macroalgae (Pedersen & Borum, 1997; Wernberg et al., 2018). Degraded water quality from eutrophication is critical for the development, persistence and expansion of harmful algae blooms (HABs) (Heisler et al., 2008). Recent reports showed that microalgal blooms, *Ulva*-dominated green tides and *Sargassum*-dominated golden tides have substantially increased worldwide (Glibert et al., 2005; Smetacek & Zingone, 2013; Kudela et al., 2015; Wang et al., 2018). HAB resulted from eutrophication affects substance circulation, primary productivity, community structure and marine ecosystem service (Norkko & Bonsdorff, 1996a,b; Glibert et al., 2005; Rabouille et al., 2008; Heisler et al., 2008; Smetacek & Zingone, 2013; Anderson et al., 2015; Kudela et al., 2015; Watson et al., 2015).

Several studies have found that coral reef systems are negatively affected by OA and nutrient enrichment (Hoegh-Guldberg et al., 2007; Ge et al., 2017; Guan et al., 2020). For phytoplankton, marine diazotrophs such as *Trichodesmium* spp. increase their N_2 fixation under elevated CO_2 in nitrogen enriched cultures (Eichner, Rost & Kranz, 2014; Hutchins & Fu, 2017). However, limited investigations aimed to reveal the ecophysiological effects of OA and eutrophication on marine macrophytes. Previous studies indicated that the growth and quality of *S. japonica* were inhibited and threatened by the interactive effects of OA and eutrophication. (Chu et al., 2019,

2020). In contrast, there was an enhanced production of amino acid and fatty acid in *Ulva* species at elevated CO_2 concentration and nutrient level (Gao et al., 2018). Thus, the responses to the synergistic stress of OA and eutrophication are species-specific in macroalgae. The rise of acidity in coastal ocean was found to be greater under eutrophication (Cai et al., 2011). This severe scenario potentially aggravate the disappearance of habitat-forming seaweeds worldwide (Wernberg et al., 2018; Filbee-Dexter & Wernberg, 2018). It is thus important to understand how macroalgae will response to the future synergistic stress of OA and eutrophication.

The kelp *Saccharina japonica* is the foremost commercial harvesting alga among northwestern Pacific countries (Kim et al., 2017; Chung, Sondak & Beardall, 2017). In previous studies, the growth, photosynthesis, and nutrient uptake of *S. japonica* were significantly enhanced under elevated CO_2 concentrations (Swanson & Fox, 2007; Zhang et al., 2020). Also, excess nutrient availability significantly promoted the growth and physiological performance of *S. japonica* (Gao et al., 2017). On the other hand, the sheet-like macroalgae *Sargassum horneri* blooms frequently occur in recent years (Liu et al., 2013; Xiao, 2019), whose floating thalli have caused detrimental impacts on *S. japonica* aquaculture (Xiao, 2019). Many investigations have focused on how environmental factors affect population dynamics and distributions of *S. horneri* in East China Sea and Yellow Sea (Xiao, 2019; Xiao et al., 2020; Choi et al., 2020). However, it remains unclear whether *S. horneri* is more resilient to the synergistic stress of OA and eutrophication than *S. japonica*.

In the present study, we investigated the synergistic stress of OA and eutrophication on growth, photosynthesis, respiration, chlorophyll contents, and tissue nitrogen of sporophytes of *S. japonica* and *S. horneri* appearing in the same period. The results are expected to reveal the species-specific ecophysiological responses of *S. japonica* and *S. horneri*, and determine which alga has greater resilience and interspecific competitive dominance under synergistic stress of OA and eutrophication.

2 Materials and Methods

2.1 Algal collection and maintenance

The sporophytes of *S. japonica* (approximately 80 cm in average length, n = 20) and *S. horneri* (approximately 150 cm in average length, n = 20) were collected in Rongcheng, Shandong, China (36°07´N, 120°19´E), in December 2019. The *S. japonica* samples were from cultivated populations, with *S. horneri* twining on, or floating between their rafts. The samples were kept in cold foam boxes filled with seawater and quickly transported to the laboratory within 8 h. Healthy sporophytes were selected and rinsed several times with sterilized seawater to remove the epiphytes and detritus. More than 100 discs (1.4 cm in diameter) were punched from the meristem of *S. japonica* with a cork borer, and more than 100 segments (4-5 cm in length) were cut from the apical part of *S. horneri* branches for the subsequent experiments. The discs and segments were maintained separately in plastic tanks containing 3 L filtered seawater. The seawater was renewed daily during the maintenance. These samples were maintained at an irradiance of 90 μmol photons·m^{-2}·s^{-1} with a 12L: 12D photoperiod, and 10 □, the seawater temperature of the collection area, for 3 days to reduce the negative effects of excision.

2.2 Culture experiment

The culture experiment was conducted over a period of 6 days under combinations two pCO_2 levels (400 μatm and 1000 μatm) and two nutrient levels (non-enriched natural seawater and nutrient-enriched seawater). The nutrient-enriched level was enriched 50% PESI medium (Tatewaki, 1966), which was made by sterilized seawater from coastal Qingdao. There was a total of 4 experimental treatments and each had 3 replicates. Four individuals were cultured in each of 12 gently aerated side-arm flasks, in which each contained 500 mL non-enriched or enriched seawater at 10 □. The culture medium was renewed on the third day of the experiment.

2.3 Carbonate chemistry parameters

For the treatments under two pCO_2 levels, the samples were cultured in two CO_2 incubators (GXZ-380C-C02, Jiangnan Instruments Factory, Ningbo, China). The 400 μatm was achieved by bubbling ambient air. And the 1000 μatm was obtained through gas cylinders of the incubator. The pH value of the medium in each flask was measured by a pH meter (Orion STAR A211; Thermo Scientific). The salinity was measured by a seawater salimeter (0-100‰ , Aipli). Other indirectly measured carbonate chemistry parameters of all treatments were calculated based on the pH values, salinity, pCO_2 levels, the equilibrium constants K_1 and K_2 for carbonic acid dissociation, and K_B for boric acid, using CO2SYS software (Robbins & Kleypas, 2018).

2.4 Measurement of growth

The growth of *S. japonica* and *S. horneri* was determined by weighing fresh weight (FW) of discs or thalli. The discs and thalli were gently scrubbed with tissue paper to remove water from the surface before being weighed. The relative growth rate (RGR) was calculated as the following formula:

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

where t is the time period of culture experiment, W_0 is the initial FW, W_t is the FW after t days of culture.

2.5 Measurement of photosynthesis and respiration

The net photosynthetic rate (P_n) and the respiration rate (R_d) of the samples was measured by a manual oxygen meter (FireSting O_2 □, Pyro Science). After measuring the FW, four discs or segments of each replicates were transferred to the oxygen electrode cuvette with 330 mL culture medium from their own flasks. The medium was magnetically stirred during the measurement to ensure the even diffusion of oxygen. The irradiance and temperature conditions were set the same as the growth chambers. The samples were set to acclimate to the conditions in the cuvette for 5 min

before the measurements. The oxygen concentration in the medium was recorded per minute for 10 min. The increase of oxygen content in the medium within 5 min was defined as the P_n, and the decrease of oxygen content in darkness in the following 5 min was defined as R_d. The P_n and R_d were presented as μmol $O_2 \cdot min^{-1} \cdot g^{-1}$ FW.

2.6 Measurement of chlorophyll contents

Approximately 0.2 g (FW) of the samples from every replicate were used for the extraction of photosynthetic pigments. The discs or segments were dipped in 2 mL dimethyl sulfoxide for 5 min, and the absorbance of supernatant was determined at 665, 631, 582, and 480 nm in the ultraviolet absorbance spectrophotometer (U-2900, HITACHI, Tokyo, Japan). Then the same samples were added 3 mL acetone, setting for 2 h. Before the measurements, 1 mL methanol and 1 mL distilled water was added to the supernatant. The absorbance was obtained at 664, 631, 581, and 470 nm. The contents of chlorophyll (Chl) *a* and *c* were calculated according to the following equation:

$$\text{Chl } a(\text{mg} \cdot \text{g}^{-1}) = \frac{A_{665}}{72.8} \times V_1 + \left(\frac{A_{664}}{73.6} \times V_2\right) / (I \times W)$$

$$\text{Chl } c(\text{mg} \cdot \text{g}^{-1}) = \left[\frac{A_{631} + A_{582} - 0.297 \times A_{665}}{61.8} \times V_1 + \frac{A'_{631} + A_{581} - 0.3 \times A_{664}}{62.2} \times V_2\right] / (I \times W)$$

where A_{665}, A_{664}, A_{582} and A_{581} represent the absorbance at 665, 664, 582 and 581 nm, A_{631} is the first absorbance at 631 nm, A'_{631} is the second absorbance at 631 nm, V_1 is the volume of the first extract, V_2 is the volume of the second extract, I is the optical path length, and W is the FW of measured samples.

2.7 Measurement of tissue nitrogen

One disc or segment was randomly selected from every replicate for the measurement of tissue nitrogen (TN) contents. The samples were completely dried at 80 □, and ground into powder. About 2-3 mg powder was used to measure the TN

contents in the elemental analyzer (Vario EL □ , Elementar , Germany). The TN contents were normalized to %DW.

2.8 Data analysis

Results were expressed as mean ± standard deviation (n = 3). Prior to the analysis, the data were conformed to a normal distribution (Shapiro-Wilk test, P >0.05) and homogeneity of variance (Levene's test, P >0.05). Two-way analysis of variance (ANOVA) was conducted to assess the combined effects of pCO_2 and nutrient levels on carbonate chemistry parameters, RGR, P_n, R_d, Chl *a*, Chl *c*, and TN. Tukey honest significance difference (HSD) was conducted to determine the significance levels of factors (P <0.05). Pearson correlation coefficient (PCCs) was conducted to analyze the correlations of each experimental indicator with pCO_2 and nutrients levels (P <0.05). Data were analyzed in SPSS 22.0 software.

3 Results

3.1 Carbonate chemistry parameters of culture medium

At the same pCO_2 level, two-way ANOVA showed that nutrients had no significant effects on any parameter (Table 1). In the culture medium of *S. japonica*, elevated pCO_2 decreased the pH by 0.3 and CO_3^{2-} by 57%, but it increased the DIC by 12%, HCO_3^- by 22%, and CO_2 by 187% in both the non-enriched and enriched nutrient treatments. In the culture medium of *S. horneri*, elevated pCO_2 decreased the pH by 0.4 in both nutrient levels and CO_3^{2-} by 75% (non-enriched) and 65% (enriched), but it increased the DIC by 27% (non-enriched) and 4% (enriched), HCO_3^- by 13% (non-enriched) and 5% (enriched), and CO_2 by 191% in both nutrient treatments.

3.2 Growth

The differences in pCO_2 and nutrients yielded no significant effects on RGR of *S. japonica*, but nutrients significantly promoted the growth of *S. horneri* (Fig. 1). At both 400 μatm and 1000 μatm, the RGR of *S. japonica* decreased due to enriched

nutrient. In contrast, the RGR of *S. horneri* significantly increased in excessive nutrient availability ($F = 4.550$, $P < 0.05$). PCCs showed that RGR of *S. horneri* positively correlated with both pCO_2 and nutrients. In contrast, RGR of *S. japonica* positively correlated with pCO_2, but negatively correlated with nutrients (Table 4). Together, *S. horneri* showed more promotive growth under the synergistic stress.

3.3 Photosynthesis and respiration

As shown in Fig. 2, nutrient enrichment significantly increased the P_n of *S. japonica* at both CO_2 concentrations ($F = 5.885$, $P < 0.05$). While no significant effect was detected in *S. horneri*, P_n was lower in nutrient-enriched condition. PCCs showed that P_n in *S. japonica* had positive correlations with pCO_2 and nutrients. While *S. horneri* positively correlated with pCO_2, but negatively correlated with nutrients (Table 4). Photosynthesis of *S. horneri* was greater than that of *S. japonica* at elevated pCO_2 and nutrients.

The R_d in *S. japonica* showed a similar trend to *S. horneri* (Fig. 2). No significant effects on R_d of both algae were found in all treatments. At 400 μatm, R_d of both species was lower in excess nutrients. The correlation between R_d and nutrients of *S. japonica* was positive, but that of *S. horneri* was negative (Table 4). Respiration of *S. japonica* was also greater than that of *S. horneri* under synergistic stress.

3.4 Chlorophyll contents

The Chl *a* and *c* contents of *S. japonica* significantly increased under either elevated pCO_2 or enriched nutrient. Both chlorophyll contents reached the maximum under the synergistic stress (Fig. 3). The Chl *a* content of *S. horneri* was significantly increased at enriched nutrients, and also reached the peak in synergistic stress condition. However, the Chl *c* content of *S. horneri* increased only with pCO_2 elevated. Neither pCO_2 nor nutrients significantly affected the Chl *c* in *S. horneri*. PCCs showed positive correlations between Chl *a* with pCO_2 and nutrients in both species. However, correlation between Chl *c* and nutrients was significantly negative in *S.*

horneri (Table 4).

3.5 Tissue nitrogen

The TN contents of *S. japonica* and *S. horneri* significantly increased in nutrient-enriched condition (Fig. 4). Elevated pCO_2 had no significant effect on the TN of *S. japonica*, but significantly promoted the accumulation of TN in *S. horneri*. The correlations between nutrients and TN were significantly positive in the two species. As for the correlations between pCO_2 and TN, it was negative in *S. japonica* but positive in *S. horneri* (Table 4).

4 Discussion

There was a same increase pattern of DIC in the culture medium of *S. japonica* under two nutrient concentrations, but different case was found in the culture medium of *S. horneri* (Table 1). The effects of the synergistic stress of OA and eutrophication on algae may depend on their precise carbon acquisition pathways utilized. The HCO_3^- in the culture medium of *S. horneri* was lower in enriched nutrient than in non-enriched treatments, indicating more HCO_3^- utilization paralleled with enriched nutrients. Many macroalgae use HCO_3^- rather than dissolved CO_2 under current seawater pCO_2 concentration (Israel & Hophy, 2002; Badger, 2003; Koch et al., 2013), due to their ribulose-1,5-bisphosphate carboxylase/ oxygenase (Rubisco) is not substrate-saturated at current atmospheric CO_2 level (Reiskind, Seamon & Bowes, 1988). Marine macroalgae have species-specific responses to elevated CO_2 because of their various capacities and strategies in CO_2-concentrating mechanisms (CCMs) to utilize HCO_3^- in seawater (Wu, Zou & Gao, 2008; Raven & Hurd, 2012). Furthermore, DIC acquisition interacts with phosphorus and nitrogen availability (Giordano, Beardall & Raven, 2005), but it remains unclear how *S. horneri* regulates CCMs under excessive nutrient supply. The results indicate that enrichment of nutrients contributed *S. horneri* to the utilization of HCO_3^-. When exposed to elevated pCO_2, macroalgae may reduce the use of HCO_3^- by down-regulating their CCMs, and begin

to rely on CO_2 as the primary carbon source (Bjork et al., 1993; Axelsson, Mercado & Figueroa, 2000; Cornwall et al., 2012). This physiological process may have occurred in *S. japonica*, thus leading to the DIC of culture medium remained at the same level after increasing pCO_2 under the two nutrient conditions. In contrast, this study provides an evidence that eutrophication restrains the shift of carbon acquisition pathway in *S. horneri* to cope with higher CO_2 concentration.

In this study, promotions in RGR were detected in both *S. japonica* and *S. horneri* at elevated pCO_2 although the increases were statistically non-significant. This indicates that both *S. japonica* and *S. horneri* are capable of OA resistance with atmospheric CO_2 increased to 1000 μatm. To show which algae is competitively dominant under OA condition, we analyzed the P_n, R_d, Chl *a*, Chl *c* and TN in both species. The results showed that enhancements to P_n, R_d, and chlorophyll contents of *S. japonica* were parallel with pCO_2 elevation. These results are in line with previous investigations on *S. japonica* (Swanson & Fox, 2007; Zhang et al., 2020). The enhancement of P_n and chlorophyll contents were also found in other marine macrophytes, including *Gracilariopsis lemaneiformis*, *Pyropia yezoensis* and *Ulva prolifera* (Kang et al., 2017; Li et al., 2018; Bao et al., 2019). However, the P_n and chlorophyll contents of *S. horneri* are twice as high as those of *S. japonica*. *S. horneri* increased the utilization of HCO_3^- to maintain its photosynthesis at a higher level. Since P_n and Chl *c* of *S. horneri* also increased at 1000 μatm (Fig. 2B, Fig. 3D), photosynthesis of *S. horneri* was further improved on the basis of the original high level. These results indicate that higher photosynthetic level insured *S. horneri* potentially greater resilience to OA in comparison to *S. japonica*.

The significant enhancement in growth was observed in *S. horneri* in nutrient-enriched condition, while no promotion of growth was found in *S. japonica* (Fig.1). In this study, the concentrations of dissolved inorganic nitrate and ammonium were simultaneously increased in nutrient-enriched treatments (Tatewaki, 1966). Increase in nitrogen availability can enhance macroalgae in N uptake rates, tissue N contents, and photosynthetic rates (Valiela et al., 1997). These

enhancements accelerate the growth of macroalgae. The significant increase in Chl *a* and TN contents were detected in both species in nutrient-enriched treatments (Fig.3, Fig.4). Previous studies have also determined the same positive physiological responses in *Fucus vesiculosus*, *G. lemaneiformis*, *Hizikia fusiformis* and other macroalgae (Valiela et al., 1997; Kawamitsu & Boyer, 1999; Wu, Zou & Gao, 2008; Raven & Hurd, 2012; Ohlsson et al., 2020). The kinetics of nutrients uptake in macroalgae is affected by the physiological status and the form of nutrients (Raven & Hurd, 2012; Gao et al., 2017). It has been reported that *S. japonica* utilize ammonium first when ammonium and nitrate both exist (Wang et al., 2013), while *S. horneri* firstly takes advantage of nitrate (Yu et al., 2019). We estimated according to the measured ecophysiological traits, because the exact concentrations and formations of nitrogen in culture medium were unclear. *S. horneri* performed higher P_n, more chlorophyll and TN accumulations under nutrient-enriched condition. Thus, the eutrophic treatment in this study more significantly benefited *S. horneri*, indicating the increased risk of *Sargassum*-dominated golden tide in eutrophic condition.

The current study argued the responses of both *S. japonica* and *S. horneri* under synergistic stress of OA and eutrophication. Significant enhancement in chlorophyll and TN contents was observed in both species (Fig. 3, Fig.4). These results indicated that both *S. japonica* and *S. horneri* improved carbon and nitrogen assimilation. The exceeding nutrient availability in eutrophic scenario regulates these physiological responses in macroalgae to hence the negative effects resulting from declining pH in OA (Young & Gobler, 2016; Chu et al., 2020). However, significant increase in growth was only observed on *S. horneri* (Fig. 1). Increased carbon and nitrogen assimilation in *S. horneri* enhanced its growth more than *S. japonica*. These advantages in ecophyisiological traits may allow *S. horneri* remain dominant and cause damage to *S. japonica* cultivation in future acidified and eutrophic ocean. Furthermore, the damage resulting from golden tide to *S. japonica* cultivation is likely to be more severe in reality. *S.horneri* has vesicles in structure, which can keep

the thalli floating and increase carbon acquisition (Smetacek & Zingone, 2013; Choi et al., 2020). Floating *S. horneri* wrap the rafts, shading the cultivated *S. japonica* below (Xiao, 2019; Wu et al., 2019). Thus, we suppose that increasing *S. horneri* biomass shaded cultivated *S. japonica* in a more severe environment with lower light intensity and less carbon availability (Xiao, 2019). The *Sargassum*-dominated golden tide may cause greater damage to *S. japonica* cultivation in acidified and eutrophic ocean. In addition, we need meso-scale experiments to estimate the increasing risk of golden tide in *S. japonica* cultivation.

5 Conclusions

It is important to estimate the damage to *S. japonica* cultivation by golden tide resulting from *S. horneri* under the synergistic stress of OA and eutrophication. In this study, we determined that nutrient enrichment contributed *S. horneri* to utilize HCO_3^-. *S. horneri* exhibited better photosynthetic traits than *S. japonica*, and tissue nitrogen also accumulated more in thalli of *S. horneri* in elevated pCO_2 and nutrient-enriched treatments. Furthermore, increased carbon and nitrogen assimilation enhanced the growth of *S. horneri* in acidified and eutrophic scenario. Together, *S. horneri* may cause greater damage to *S. japonica* cultivation in acidified and eutrophic ocean.

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Figure captions

Fig. 1 Relative growth rate (RGR) of *S. japonica* (A) and *S. horneri* (B) cultured at different pCO_2 and nutrient conditions for 6 days. Data are reported as means ± SD (n =3). Different letters above the error bars indicate significant differences between

treatments (P <0.05).

Fig. 2 Net photosynthetic rate (P_n) of *S. japonica* (A) and *S. horneri* (B); Respiration rate (R_d) of *S. japonica* (C) and *S. horneri* (D) cultured at different pCO_2 and nutrient conditions for 6 days. Data are reported as means ± SD (n =3). Different letters above the error bars indicate significant differences between treatments (P <0.05).

Fig. 3 Chl *a* of *S. japonica* (A) and *S. horneri* (B); Chl *c* of *S. japonica* (C) and *S. horneri* (D) cultured at different pCO_2 and nutrient conditions for 6 days. Data are reported as means ± SD (n =3). Different letters above the error bars indicate significant differences between treatments (P <0.05).

Fig. 4 Tissue nitrogen (TN) of *S. japonica* (A) and *S. horneri* (B) cultured at different pCO_2 and nutrient conditions for 6 days. Data are reported as means ± SD (n =3). Different letters above the error bars indicate significant differences between treatments (P <0.05).

Table 1 Parameters of the seawater carbonate system at different pCO_2 and nutrient conditions.

Treatments	pH	pCO_2 (μatm)	DIC (μmol·kg^{-1})	HCO_3^- (μmol·kg^{-1})	CO_3^{2-} (μmol·kg^{-1})	CO_2 (μmol·kg^{-1})	TA (μmol·kg^{-1})
S. japonica							
L-N	8.42±0.17^b	360.29±50.85^a	4352.52±2604.58^a	3793.34±2023.28^a	541.47±583.91^b	17.42±2.11^a	4978.38±3210.98^a
L-E	8.47±0.16^b	360.29±50.85^a	4812.57±2856.49^a	4140.31±2145.30^a	654.59±713.85^b	17.67±2.50^a	5560.10±3597.04^a
H-N	8.09±0.07^a	1043.43±46.57^b	4934.55±656.25^a	4651.80±598.56^a	230.95±60.78^a	49.30±3.73^b	5163.78±722.89^a
H-E	8.13±0.05^a	1043.43±46.57^b	5371.04±637.60^a	5046.65±581.58^a	272.77±56.40^a	51.62±2.19^b	5647.01±697.17^a
S. horneri							
L-N	8.54±0.13^b	360.29±50.85^a	5324.88±831.22^a	5006.21±752.91^a	1077.56±648.92^b	17.79±2.54^a	7936.41±3033.63^a
L-E	8.57±0.12^b	360.29±50.85^a	5615.11±1104.80^a	4997.68±1705.43^a	834.21±580.90^b	17.86±2.58^a	6788.73±2879.69^a
H-N	8.13±0.07^a	1043.43±46.57^b	6742.56±2368.32^a	5647.21±1737.10^a	266.81±79.61^a	51.85±2.34^b	5592.57±916.11^a
H-E	8.14±0.09^a	1043.43±46.57^b	5849.75±2281.55^a	5268.89±1005.26^a	294.21±99.68^a	52.01±2.21^b	5910.93±1211.03^a

Notes: L-N is the low pCO_2 and non-enriched condition, L-E is the low pCO_2 and enriched condition, H-N is the high pCO_2 and non-enriched condition, and H-E is the high pCO_2 and enriched condition. DIC is dissolved inorganic carbon, and TA is total alkalinity. Data are reported as means ± SD (n =3). Different superscript letters indicate significant differences in one parameter between treatments (P <0.05).

Table 2 Analysis of variance (two-way ANOVA) examining the statistical differences of experimental parameters of *S. japonica* among pCO_2 and nutrients

	S. japonica		
Factors	df	F	P
RGR			
pCO_2	1	0.188	0.676
Nutrients	1	0.358	0.566
pCO_2×Nutrients	1	0.115	0.743
P_n			
pCO_2	1	0.364	0.563
Nutrients	1	5.885	< 0.05
pCO_2×Nutrients	1	0.001	0.972
R_d			
pCO_2	1	1.739	0.224
Nutrients	1	0.039	0.848
pCO_2×Nutrients	1	0.450	0.521
Chl *a*			
pCO_2	1	13.786	< 0.01
Nutrients	1	9.476	< 0.01
pCO_2×Nutrients	1	8.251	< 0.05
Chl *c*			
pCO_2	1	0.054	0.822
Nutrients	1	9.308	< 0.05
pCO_2×Nutrients	1	0.088	0.774
TN			
pCO_2	1	0.042	0.843
Nutrients	1	158.903	< 0.001
pCO_2×Nutrients	1	0.659	0.440

Table 3 Analysis of variance (two-way ANOVA) examining the statistical differences of experimental parameters of *S. horneri* among pCO_2 and nutrients

	S. horneri		
Factors	df	F	P
RGR			
pCO_2	1	0.569	0.475
Nutrients	1	4.550	< 0.05
pCO_2×Nutrients	1	0.749	0.415
P_n			
pCO_2	1	0.025	0.879
Nutrients	1	0.286	0.607
pCO_2×Nutrients	1	0.279	0.612
R_d			
pCO_2	1	0.015	0.904
Nutrients	1	2.607	0.145
pCO_2×Nutrients	1	0.988	0.349
Chl *a*			
pCO_2	1	1.420	0.268
Nutrients	1	13.909	< 0.01
pCO_2×Nutrients	1	0.077	0.789
Chl *c*			
pCO_2	1	0.592	0.463
Nutrients	1	0.740	0.414
pCO_2×Nutrients	1	0.060	0.812
TN			
pCO_2	1	6.053	< 0.05
Nutrients	1	27.868	< 0.01
pCO_2×Nutrients	1	2.147	0.181

Table 4 The Pearson correlation coefficient (PCCs) of various experimental indicators of *S. japonica* and *S. horneri* with pCO_2 and nutrients levels

Treatments	RGR	P_n	R_d	Chl *a*	Chl *c*	TN
S. japonica						
pCO_2	0.533**	0.241	0.883*	0.359	0.380	-0.016
Nutrients	-0.736**	0.970**	0.133*	0.933*	0.924*	0.998**
S. horneri						
pCO_2	0.021*	0.030*	0.670*	0.463*	0.953**	0.410
Nutrients	0.994**	-0.961**	-0.711**	0.777**	-0.246**	0.879*

Notes: * indicates significant correlation (P <0.05), ** indicates highly significant correlation (P <0.01).

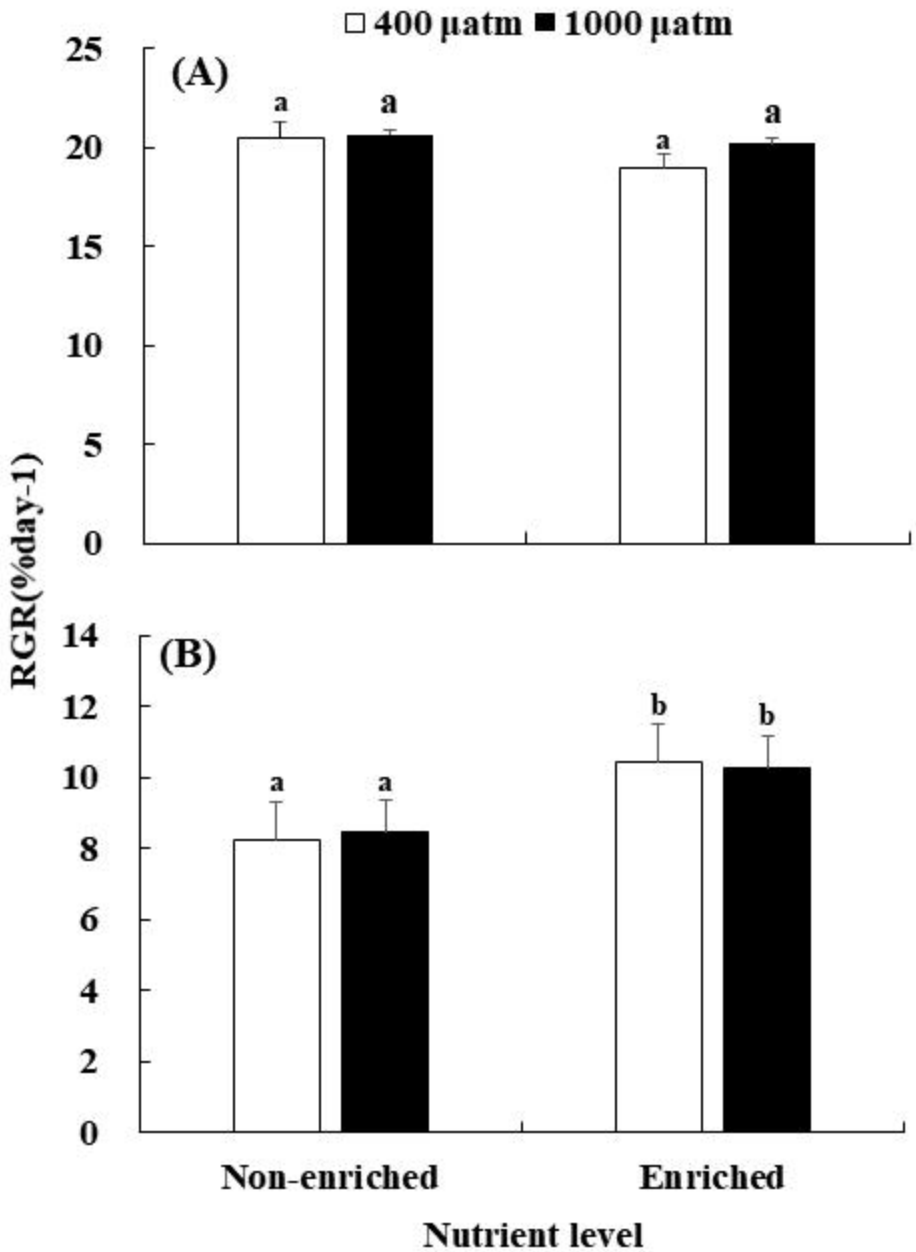

Fig. 1 Relative growth rate (RGR) of *S. japonica* (A) and *S. horneri* (B) cultured at different pCO_2 and nutrient conditions for 6 days. Data are reported as means ± SD (n =3). Different letters above the error bars indicate significant differences between treatments (P <0.05).

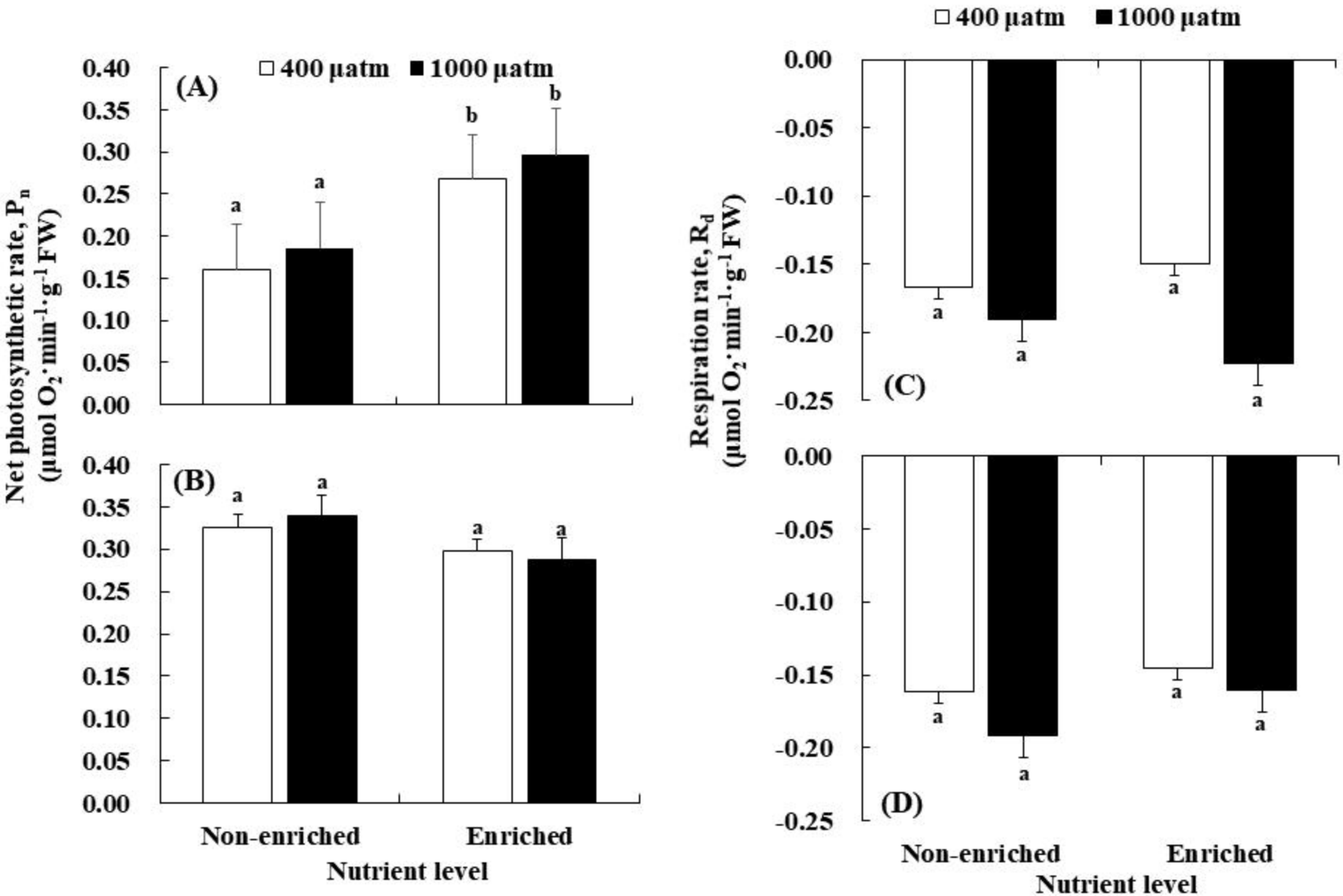

Fig. 2 Net photosynthetic rate (P_n) of *S. japonica* (A) and *S. horneri* (B); Respiration rate (R_d) of *S. japonica* (C) and *S. horneri* (D) cultured at different pCO_2 and nutrient conditions for 6 days. Data are reported as means ± SD (n =3). Different letters above the error bars indicate significant differences between treatments (P <0.05).

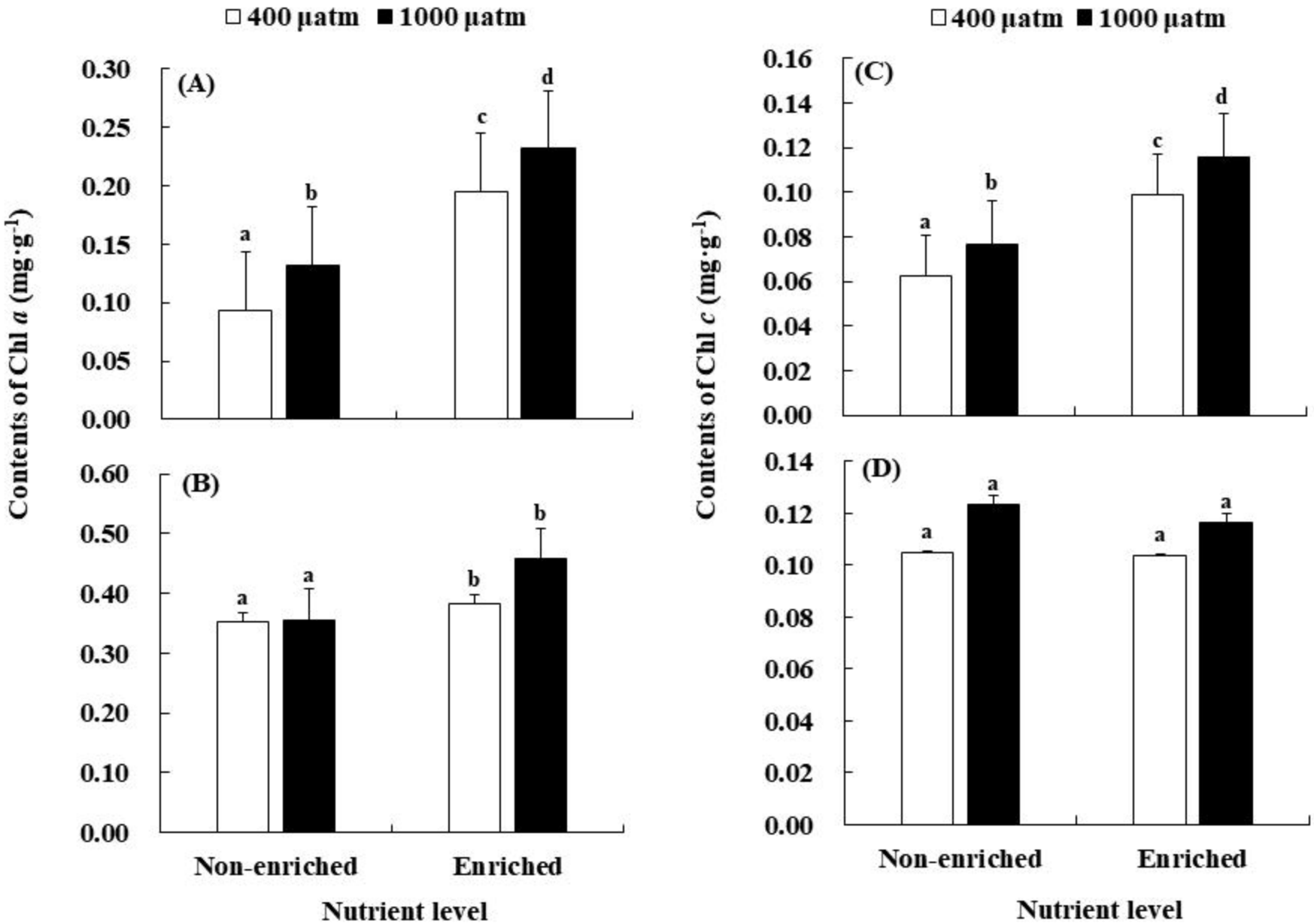

Fig. 3 Chl *a* of *S. japonica* (A) and *S. horneri* (B); Chl *c* of *S. japonica* (C) and *S. horneri* (D) cultured at different pCO_2 and nutrient conditions for 6 days. Data are reported as means ± SD (n =3). Different letters above the error bars indicate significant differences between treatments (P <0.05).

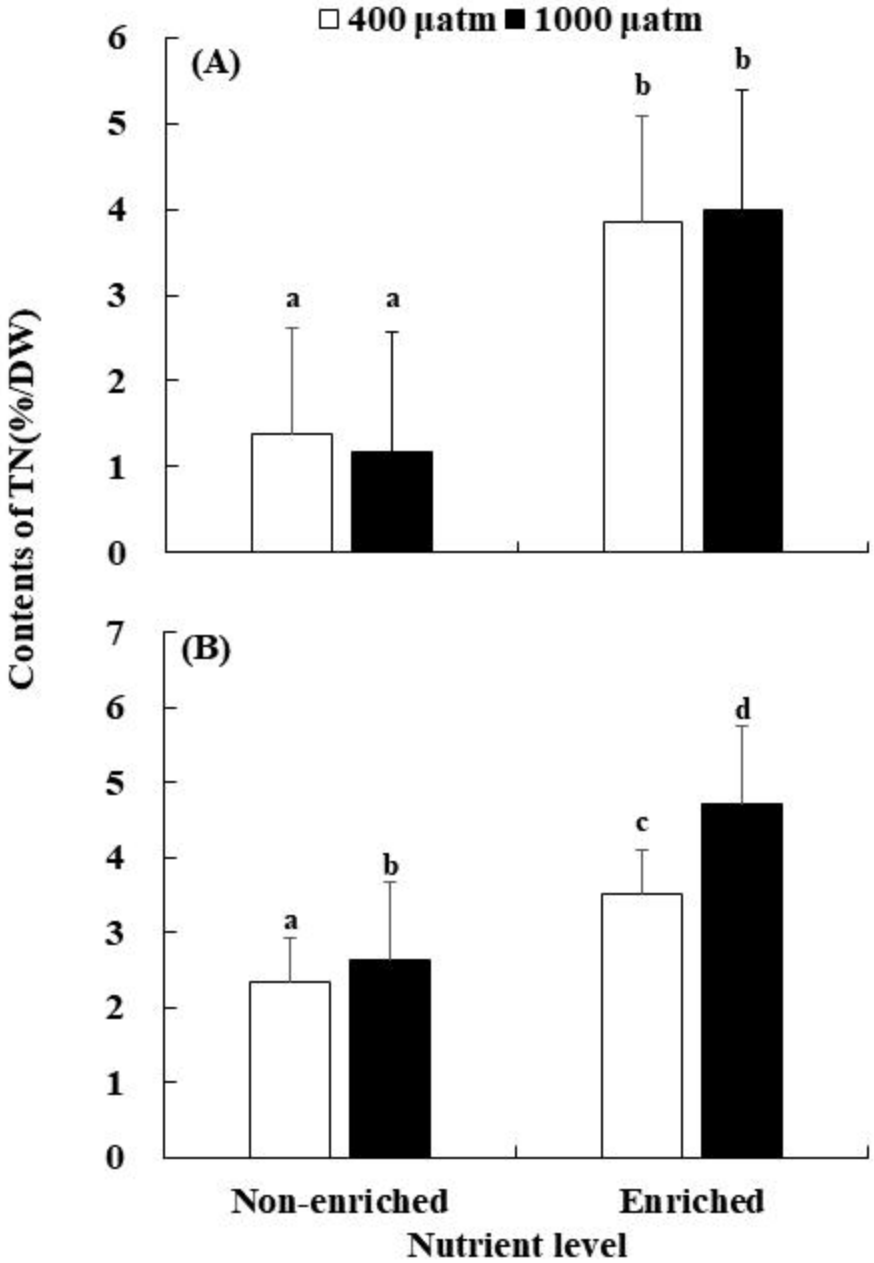

Fig. 4 Tissue nitrogen (TN) of *S. japonica* (A) and *S. horneri* (B) cultured at different pCO_2 and nutrient conditions for 6 days. Data are reported as means ± SD (n =3). Different letters above the error bars indicate significant differences between treatments (P <0.05).