Combined Influences of Light and Nitrogen Enrichment on the Physiological Performance of a Golden Tide Alga (Sargassum horneri)

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Abstract: Sargassum golden tides (GT) are common in numerous coastal areas all over the world, and it adversely affects local marine life. Eutrophication is critical for Sargassum GT development. However, its physiological and ecological mechanism remains unclear. To investigate the responses of drifting Sargassum horneri, the species causing GT in the western Pacific, to light and enriched nitrogen, we set three light conditions (Low-light (LL), 10 µmol photons m−2 s−1; Middle-light (ML), 60 µmol photons m−2 s−1; and High-light (HL), 300 µmol photons m−2 s−1) and three nitrogen conditions (Natural seawater, the final concentration of N was 31.8 µmol L−1; Enrichment of NO3−, the final concentration of N was 200 µmol L−1; and Enrichment of NH4+, the final concentration of N was 200 µmol L−1), and grew the thalli under varying conditions for 10 days before determining the growth and utilization of carbon and nitrogen. Based on the accumulated data, the elevated light level led to a higher growth rate of alga. In the LL culture, the higher capacity for carbon utilization, which was reflected by the higher maximum photosynthetic carbon fixation rate (Vmax), resulted in the elevated growth rates of thalli in the nitrogen-enriched media as compared with the natural seawater. Furthermore, a higher growth rate was found in the enrichment of NH4+ despite a low affinity for inorganic carbon indicated by a higher value of the half-saturation constant (Ks). In the ML treatment, an insignificant difference in growth rate was found in three nitrogen cultures, except for a slight increase in the enrichment of NH4+ than the enrichment of NO3−. In the HL treatment, compared with natural seawater culture, enrichment of NO3− or NH4+ accelerated the growth of alga, with no significant difference between the two nitrogen sources. Such enhancement in growth was related to the more photosynthetic carbon fixation, indicated by the higher value of Vmax and soluble carbohydrates content of alga cultured with NO3− and NH4+ enrichments. Additionally, the uptake and assimilation products of nitrogen, such as pigments and soluble proteins, remained unaffected by nitrogen source enrichment of NO3− or NH4+ at all three light levels. In conclusion, enrichment of NO3− and NH4+ exhibited different influences on the growth of S. horneri at different light levels, which was mainly associated with the capacity and efficiency of photosynthetic carbon utilization. At the HL level, both the enrichment of NO3− and NH4+ dramatically accelerate the growth of alga by stimulating the photosynthetic carbon fixation. Accordingly, we speculated that drifting S. horneri, exposed to HL level on the surface of the sea, were likely to develop rapidly to form GT in eutrophic oceanic areas with upwelled and river plume NO3− or NH4+ nutrients.

Keywords: Sargassum horneri; golden tide; nitrogen source; photosynthetic carbon fixation; nitrogen uptake
1. Introduction

Recently, there is massive accumulations of drifting Sargassum, termed ‘golden tides’ (GT), on the sea surface in certain regions like the Gulf of Mexico in the Atlantic Ocean [1–5], East China Sea and Yellow Sea [6,7]. Two species, S. natans and S. fluitans, are the most abundant in the Atlantic [1,4], whereas S. horneri is the only species found in the East China and Yellow Seas [6,7]. With the blooming of an invasive genus, massive amounts of drifting Sargassum can alter the original sea area ecosystem [8–10], negatively impacting offshore tourism and maritime transport [11,12], and worsening seawater quality once it sinks and decays [13]. If it breaks out in aquaculture regions, GT can produce major losses to the local mariculture industry [6].

In previous studies, GT prevalence was associated with alterations in local environmental conditions, namely, nutrients, temperature, light, and so on [1,14,15]. Using satellite remote sensing monitoring, multiple studies verified GT development in eutrophic oceanic areas with upwelled [4,16] and river [14,17] plume nutrients. Therefore, many scholars speculate that eutrophication is critical to Sargassum GT formation [4,8,14,15,17].

On the other hand, when drifting on the sea surface from submarine attachment, GT algae suffer from higher light levels due to the lack of attenuation of light by seawater [18–20]. Generally, most macroalgae exhibit enhanced photosynthetic and developmental rates in high light (HL) conditions [21,22]. However, excessive light may suppress macroalgal photosynthesis, which is known as photoinduction [23,24]. In addition, the availability of nitrogen and phosphorus usually alleviates the photoinduction [25–27], and even promotes photosynthetic carbon fixation and growth rate in some seaweed species under light stress [26]. Those Sargassum in a drifting state, compared with the attached individuals, receiving enhanced light energy, may rapidly develop to form a GT in the presence of sufficient nutrients. Further, the top and bottom edges of the drifting Sargassum layer may experience different light conditions, depending on the thickness of the drifting layer. However, there is limited published information regarding the impact of light conditions on photosynthetic carbon fixation and growth of drifting Sargassum, especially on nutrient enrichment.

GTs of S. horneri erupting frequently in the East China Sea and the Yellow Sea, similarly, may also be associated with over-enrichment by nutrients in seawater [6,7]. In fact, coastal eutrophication in the China Seas is now very serious [28]. The dissolved inorganic nitrogen, including both ammonia and nitrate, often exhibits high concentrations, especially in mariculture areas [29,30]. It has been reported that several species of microalgae cultured under different nitrogen sources showed different growth rates and photophysiological responses under environmental stress, due to different utilization pathways for different nitrogen sources [31–33]. However, the physiological responses of S. horneri under different nitrogen sources to environmental stress remain unknown. This study attempts to investigate the impacts of light conditions on the development and related carbon and nitrogen utilization of S. horneri cultured under different levels of nitrogen enrichment with ammonia or nitrate, which would provide necessary data for revealing the environmental physiological mechanism of S. horneri GT formation in eutrophic seawater.

2. Materials and Methods

2.1. Materials

Sargassum horneri samples were collected from a drifting population entangled in the kelp culture raft in the Lidao Bay, Rongcheng City, Shandong Province, China (37°15′ N, 122°35′ E) on 25 July 2020. Thalli were about 50 cm long and in the growing period with no receptacle. The collected samples were transported to the research center in a 4 °C cooler over a 2-h journey. Healthy individuals were selected and cleaned with autoclaved seawater. About 5 cm long segments were arbitrarily sliced from several branches and subsequently grown in autoclaved natural seawater (salinity, 30 psu; NO$_3^-$, 30.5 µmol L$^{-1}$; NH$_4^+$, 1.3 µmol L$^{-1}$; dissolved inorganic phosphorus (DIP), 1.5 µmol L$^{-1}$) in the growth chamber for 48 h for the main experiment. The temperature was adjusted to 18 °C and light
intensity 60 μmol photons m$^{-2}$ s$^{-1}$ with a light and dark period of 12:12 h. The culture media received continuous air.

2.2. Experiment Design

Following a 48-h pre-incubation, 5–6 algae segments (fresh weight (FW), ~6.0 g) were grown in a conical flask with 3 L of autoclaved natural seawater and 1% modified Provasoli enriched seawater (PES) medium [34] under varying nitrogen and light status for 10 days. The PES medium was prepared with distilled water without nitrogen, it was added separately for different nitrogen source supplies when required. Three nitrogen treatments were set in the experiment as: natural seawater, enrichment with NO$_3^-$ and enrichment with NH$_4^+$. For natural seawater, the final nitrogen concentration in cultured media was 31.8 μmol L$^{-1}$, including NO$_3^-$ (30.5 μmol L$^{-1}$) and NH$_4^+$ (1.3 μmol L$^{-1}$). For the enrichment with NO$_3^-$ and with NH$_4^+$, the final nitrogen concentration was 200 μmol L$^{-1}$ and obtained by adding NaNO$_3$ and NH$_4$Cl, respectively. The media were continuously aerified with air and refreshed every two days. Moreover, the culture density of 2.0 g FW L$^{-1}$ was employed in these experiments. The excess culture was removed while renewing the media.

The growth containers were placed in a growth chamber (MGC-250P, Yiheng Technical Co., Ltd., Shanghai, China) at 18 °C, with a 12 h:12 h light and dark period. Light intensities reaching the algae were adjusted to three conditions (Low-light (LL), 10 μmol photons m$^{-2}$ s$^{-1}$; Middle-light (ML), 60 μmol photons m$^{-2}$ s$^{-1}$ and HL, 300 μmol photons m$^{-2}$ s$^{-1}$) by modulating the light source attached to the algae containers. A portable optical quantum meter (QRT1, Hansatech, Norfolk, UK) was used to measure light intensities. Each sample was grown under varying nitrogen and light conditions for 10 days with 3 replicates, and then the growth rate and utilization of carbon and nitrogen of algae were determined.

2.3. Measurement of Growth

Algae development was measured under varying nitrogen and light conditions during the last two days of culture, and the daily growth rate (DGR) was computed as follows [35]:

$$\text{DGR} = \frac{\ln N_t - \ln N_o}{t} \times 100,$$

where $N_t$ represented day t (day 10) FW, $N_o$ represented the initial day 8 FW, and t represented the duration (2 days).

2.4. Assessment of Photosynthetic Carbon Fixation

The photosynthesis-based algae carbon fixation rates were expressed as the photosynthetic oxygen evolution rates [36], and they were assessed at varying dissolved inorganic carbon (DIC) levels via a Clark-type oxygen electrode (Chlorolab-3, Hansatech, Norfolk, UK) at the end of the experiment. Thalli were cut into ~1.0 cm segments, before measuring the segments were maintained for at least 2 h to reduce photosynthetic damage brought by the cut. Approximately 0.1 g FW sample was placed in an oxygen electrode cuvette containing 8 mL media with various DIC levels. During measurement, the media were stirred, the temperature was adjusted to 18 °C, and the irradiance was set as 600 μmol photons m$^{-2}$ s$^{-1}$ (saturated but not causing photoinhibition on photosynthesis in this alga (our pre-experimental result)) to avoid the influence of light restriction on the measurement result of photosynthetic oxygen evolution rates. The variable DIC concentrations (0–13.2 mmol L$^{-1}$) were acquired by introducing variable quantities of NaHCO$_3$ to the Tris-buffered DIC-free seawater, which was freshly prepared by removing DIC from the autoclaved natural seawater through pH reduction to about 3.0, with 1.0 mol L$^{-1}$ HCl addition, prior to sparging for 2 h with pure N$_2$ gas. Lastly, Tris buffer (25 mmol L$^{-1}$) was introduced, prior to pH adjustment to 8.1 with 1 mol L$^{-1}$ NaOH and 1 mol L$^{-1}$ HCl. The value of pH was determined by calibrated pH meter (INES-3CW, Shanghai Lida Instrument Factory).

The curve of photosynthetic carbon fixation rate vs. DIC was obtained from the equation [37]:

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

whereby, $V$ was the photosynthesis-based carbon fixation rate under different DIC ([S]) concentrations. The largest photosynthetic
carbon fixation rate $V_{\text{max}}$ and the half-saturation constant $K_{0.5}$ were calculated from the equation.

2.5. Identification of Photosynthetic Pigments

FW thalli (0.1 g) from the treated and control groups were crushed in 90% acetone solution, which was then increased to 10 mL to induce extraction at 4 °C without light for 12 h. Once centrifuged (4000 r/min, 15 min), supernatant absorption at 400–700 nm was assessed with a spectrophotometer (DU 530, Beckman Coulter, Fullerton, CA, USA). The chlorophyll (Chl a, Chl c) and carotenoid (Car) contents (mg g$^{-1}$ FW) were computed according to Jeffrey and Humphrey [38] and Parsons et al [39], respectively.

\[
\text{Content of Chl a} = (11.47 \times A_{664} - 0.40 \times A_{630}) \times \frac{V}{M}
\]

\[
\text{Content of Chl c} = (24.36 \times A_{630} - 3.73 \times A_{664}) \times \frac{V}{M}
\]

\[
\text{Content of Car} = (7.6 \times (A_{480} - 1.49 \times A_{510})) \times \frac{V}{M}
\]

whereby, $A$ was the supernatant absorbance at varying wavelengths, $V$ was the solution volume at constant volume (10 mL), and $M$ was the fresh algal weight (g).

2.6. Estimate of Nitrogen Uptake Rate (NUR)

The thalli NUR was predicted by using the decreased rate of nitrogen nutrients in the culture seawater over the interval of 2 d, with a refreshed medium. The concentrations both of NO$_3^-$ – N and NH$_4^+$ – N were determined according to Lobban et al. [40], and the NUR was computed as follows: NUR = ($N_0 - N_1$) $\times$ V/M/t. whereby, $N_0$ and $N_1$ were the medium nitrogen nutrient levels at the beginning and end of the culture, respectively. $V$ was the culture medium volume (10 mL), $M$ was the original fresh thallus weight (0.1 g), and $t$ was the culture duration (2 days).

2.7. Evaluation of Soluble Carbohydrates and SOLUBLE Protein

Once culturing was over, 0.1 g FW thalli from different treatments were crushed in distilled water, and the solutions were finally increased to 5 mL. To extract soluble carbohydrates, the solutions were placed in an 80 °C water bath for 30 min. After centrifugation for 10 min at 5000 $\times$ g, the absorbance value at 620 nm of the supernatant was measured, and the content of soluble carbohydrates was assessed using the phenol-sulfuric acid technique [41].

To extract soluble protein, about 0.5 g FW thalli from all treated groups were crushed using a mortar and pestle and added with 5 mL of phosphate buffer (0.1 mol L$^{-1}$, pH 6.8). Following centrifugation at 5000 $\times$ g for 10 min, soluble protein in the supernatant was evaluated with the Bradford [42] assay using bovine serum albumin as the reference.

2.8. Statistical Analyses

Data are presented as means ± standard deviation and assessed with SPSS v.21 (Licence No.: QA3AW8U62Z4ZWTPV44VX65P59OLE547WHIQVZYWLARL9JEYQEGD-UBLH8Z3ZCJAL3FLXMS98V95TSY7FOEXUPR). The accumulated data from all treatments followed a normal distribution (Shapiro–Wilk, $P > 0.05$), and the variances were deemed as equal (Levene’s test, $P > 0.05$). Two-way analysis of variance (ANOVA) was performed to evaluate the impacts of light and N on DGR, $V_{\text{max}}$, $K_{0.5}$, pigments, soluble carbohydrates, soluble protein, and NUR. Tukey’s honest significance difference (HSD) was carried out for a post hoc examination. All examinations had a confidence interval of 95%.

3. Result

3.1. Growth Rate

The daily growth rates (DGR) of *S. horneri* grown under varying light and nitrogen conditions are provided in Figure 1. Light interacted with nitrogen on *S. horneri* DGR (ANOVA, $P < 0.01$), and all factors produced a major effect (ANOVA, $P < 0.001$ for light;
ANOVA, \( P < 0.001 \) for nitrogen). Regardless of the nitrogen supply, the DGR of thalli was enhanced by increased light levels (Tukey’s HSD, \( P < 0.05 \)). Relative to LL exposure, ML and HL improved DGR by 2.26 times and 5.24 times in the enrichment of \( \text{NO}_3^- \), 1.12 times and 2.28 times in the enrichment of \( \text{NH}_4^+ \), and 10.14 times and 15.61 in the natural seawater, respectively. Moreover, enrichment of N augmented the algal developmental rate at LL and HL conditions (Tukey’s HSD, \( P < 0.05 \)). Enrichments of \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) increased DGR by 2.09 times and 4.64 times at the LL level, and 16.0% and 11.5% at the HL level, respectively. While at the ML level, enrichments of both \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) showed no influence on the algal developmental rate (Tukey’s HSD, \( P > 0.05 \)).

![Figure 1](image1.jpg)

**Figure 1.** Daily growth rate (DGR) of *Sargassum horneri* grown at different light and N conditions for 10 days. Data are reported as means ± SD (n = 3). Different letters above the error bars indicate significant differences between treatments (\( P < 0.05 \)).

3.2. Photosynthetic Carbon Fixation

Photosynthetic rates of *S. horneri* increased with supplies of DIC (P-C curves) under all light and nitrogen conditions (Figure 2). Both carbon-saturating maximum photosynthetic rate (\( V_{\text{max}} \)) and half saturation constant (\( K_{0.5} \)) acquired from P-C curves showed different manifestations at varying light and nitrogen conditions (Figure 3). Light interacted with nitrogen on algal \( V_{\text{max}} \) (ANOVA, \( P < 0.001 \)), and all factors produced a major influence (ANOVA, \( P < 0.001 \) for light; ANOVA, \( P < 0.001 \) for nitrogen). A post hoc Tukey’s HSD assessment (\( P < 0.05 \)) revealed that compared with natural seawater culture, enrichments of \( \text{NO}_3^- \) and \( \text{NH}_4^+ \), respectively, increased the \( V_{\text{max}} \) by 74.5% and 67.8% at the LL level, with insignificant differences between enrichments of \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) (\( P > 0.05 \)). At the ML level, enrichment of \( \text{NO}_3^- \) enhanced the \( V_{\text{max}} \) by 20.9%, while enrichment of \( \text{NH}_4^+ \) had no effect on it (\( P > 0.05 \)). At the HL level, enrichments of \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) increased the \( V_{\text{max}} \) by 31.8% and 79.1%, respectively. The highest value of \( V_{\text{max}} \) was found in \( \text{NH}_4^+ \)-enriched culture. Light levels had different effects on \( V_{\text{max}} \) in different nitrogen source cultures (Figure 3A). In the natural seawater culture, ML and HL treatments enhanced the \( V_{\text{max}} \) with insignificant differences between themselves (\( P > 0.05 \)). In the \( \text{NO}_3^- \)-enriched culture, the \( V_{\text{max}} \) remained unaffected by light levels (\( P > 0.05 \)). In the enrichment culture with \( \text{NH}_4^+ \), ML treatment decreased the \( V_{\text{max}} \) by 28.4%, while HL increased it by 44.5%, compared with the LL treatment.
insignificant difference between enrichments of NO$_3^-$ and NH$_4^+$ (P > 0.05). At the ML level, enrichment of NO$_3^-$ enhanced the $V_{\text{max}}$ by 20.9%, while enrichment of NH$_4^+$ had no effect (P > 0.05). At the HL level, enrichments of NO$_3^-$ and NH$_4^+$ increased the $V_{\text{max}}$ by 31.8% and 79.1%, respectively. The highest value of $V_{\text{max}}$ was found in NH$_4^+$-enriched culture.

Light levels had different effects on $V_{\text{max}}$ in different nitrogen source cultures (Figure 3A). In the natural seawater culture, ML and HL treatments enhanced the $V_{\text{max}}$ with insignificant differences between themselves (P > 0.05). In the NO$_3^-$-enriched culture, the $V_{\text{max}}$ remained unaffected by light levels (P > 0.05). In the enrichment culture with NH$_4^+$, ML treatment decreased the $V_{\text{max}}$ by 28.4%, while HL increased it by 44.5%, compared with the LL treatment.

Figure 2. Photosynthesis versus DIC curves of *S. horneri* after being cultured under different light and N conditions for 10 days (A, Low-light; B, Middle-light; C, High-light). Data are reported as means ± SD (n = 3). DIC is dissolved inorganic carbon.
Figure 3. The carbon-saturating maximum photosynthetic rate ($V_{\text{max}}$, A) and half saturation constant ($K_{0.5}$, B) for *S. horneri* cultured under different light and N conditions for 10 days. Data are reported as means ± SD (n = 3). Different letters above the error bars indicate significant differences between treatments ($P < 0.05$).

Light and nitrogen interacted on the $K_{0.5}$ of *S. horneri* (ANOVA, $P < 0.001$), and all factors produced a major impact (ANOVA, $P < 0.001$ for light; ANOVA, $P < 0.001$ for nitrogen), as shown in Figure 3B. A post hoc Tukey's HSD evaluation ($P < 0.05$) revealed that at the low and HL levels, the highest value of $K_{0.5}$ was found in the culture enriched with NH$_4^+$. Compared with the natural seawater, enrichment of NH$_4^+$ enhanced $K_{0.5}$ by 1.10 and 1.85 times at low and HL levels, respectively. However, at the ML level, $K_{0.5}$ decreased by 31.4% in the NH$_4^+$-enriched culture, compared with the natural seawater. In contrast, enrichment of NO$_3^-$ remarkably lowered $K_{0.5}$ of alga at the LL level ($P < 0.05$), and showed no obvious difference from the natural seawater at the ML and HL levels ($P > 0.05$). Light levels had different effects on $K_{0.5}$ in different nitrogen source cultures (Figure 3B). In the natural seawater, both middle and HL depressed $K_{0.5}$ significantly ($P < 0.05$), with no discernible difference between them ($P > 0.05$), compared with LL. However, in the NO$_3^-$-enriched culture, the $K_{0.5}$ remained unaffected by light levels ($P > 0.05$), and in the NH$_4^+$-enriched culture, the lowest value of $K_{0.5}$ was found at ML, while the highest at LL.
3.3. Photosynthetic Pigments

Figure 4 showed photosynthetic pigment contents of *S. horneri* grown under varying light and nitrogen conditions. Light and nitrogen showed no interaction in terms of the Chl a, Chl c and Car contents (ANOVA, $P > 0.5$ for these three pigments), and all factors showed no major impact (ANOVA, $P > 0.5$), except that a slight decrease in Chl a content in the enrichment of NO$_3^-$ was induced by ML, compared with LL (Tukey's HSD evaluation, $P < 0.05$).

![Figure 4](image)

**Figure 4.** Pigment content (A, Chl a; B, Chl c; C, Car) of *S. horneri* after being grown at different light and N conditions for 10 days. Data are reported as means ± SD (n = 3). Different letters above the error bars indicate significant differences between treatments ($P < 0.05$).

3.4. Nitrogen Uptake

To evaluate the significance of light and nitrogen sources on nitrogen assimilation in *S. horneri*, the nitrate and ammonia uptake rates under varying light and nitrogen conditions were examined (Figure 5). Light and nitrogen demonstrated no interaction with nitrate, ammonia and total nitrogen uptakes (ANOVA, $P > 0.5$), and light has no impact on them (ANOVA, $P > 0.5$). However, nitrogen supply had main effects on uptake rates of NO$_3^-$, NH$_4^+$ and total N, respectively (ANOVA, $P < 0.001$). Compared with natural seawater, enrichment of NO$_3^-$ or NH$_4^+$ remarkably enhanced the uptake rate of NO$_3^-$ or NH$_4^+$, respectively (Tukey’s HSD evaluation, $P < 0.05$), resulting in an enhanced uptake rate of total N, with insignificant difference between them (Tukey’s HSD evaluation, $P > 0.05$).
rate of total N, with insignificant difference between them (Tukey’s HSD evaluation, $P > 0.05$).

Figure 5. N uptake rate of *S. horneri* after being grown at different light and N conditions for 10 days (A, NO$_3$−-N; B, NH$_4$+−N; C, Total N). Data are reported as means ± SD (n = 3). Different letters above the error bars indicate significant differences between treatments ($P < 0.05$).

3.5. Soluble Protein and Soluble Carbohydrates

The soluble protein (Figure 6A) and carbohydrates (Figure 6B) were predicted to elucidate the impacts of light and nitrogen on carbon and nitrogen assimilation products in *S. horneri*. The content of soluble protein was not affected by light and/or nitrogen (ANOVA, $P > 0.5$). Nevertheless, light and nitrogen showed an association with soluble carbohydrates content in *S. horneri* (ANOVA, $P < 0.001$), and all factors produced a major impact (ANOVA, $P = 0.001$ for light; ANOVA, $P < 0.001$ for nitrogen). A post hoc Tukey’s HSD evaluation ($P < 0.05$) revealed that LL increased soluble carbohydrates content of alga in the NH$_4$+-enriched culture, while HL increased it in the NO$_3$−-enriched culture. Moreover, HL significantly decreased it in the culture with natural seawater. Thus, the positive effect on the soluble carbohydrates content was caused by the enrichment of NH$_4$+ at LL, while by the enrichment of NO$_3$− at HL. Additionally, at ML, nitrogen treatment had an insignificant impact on soluble carbohydrates content in *S. horneri* (Tukey’s HSD evaluation, $P > 0.05$).
while NO

Gracilaria lemaneiformis

The promotion of light on development rate was likely due to the improvement of photosynthetic pigment content, and improving related enzymatic activity [21–24]. In this study, the enrichments of two nitrogen sources showed different promoting effects on the growth of this alga, suggesting significant interaction between light and nitrogen source, which was closely related to the photosynthetic carbon fixation of algae under different conditions.

Light is the basis of photosynthesis and the major environmental agent affecting algal development. Therefore, increased light can promote photosynthesis and algae growth by accelerating light energy absorption, promoting electron transfer, increasing pigment content, and improving related enzymatic activity [21–24]. In this study, the content of photosynthetic pigments, as well as the uptake rate of nitrogen and the metabolism of nitrogen metabolites (soluble protein) in S. horneri suggested significant interaction between light and nitrogen source, which was closely related to the photosynthetic carbon fixation of algae under different conditions.

NH₄⁺

The growth of algae depends not only on photosynthesis, but also on the level of N metabolism, thus it is the result of the joint action of C and N metabolism [43]. The increased N generally promotes the growth rate of algae [44]. However, the growth of algae supplied with different forms of N sources (such as NO₃⁻–N and NH₄⁺–N) may be different, due to different ways for algae to absorb different forms of N [45]. It has been reported that NH₄⁺–N is used by algae through passive diffusion without energy consumption, while NO₃⁻–N is through active absorption with energy consumption [46]. Therefore, the absorption of different N sources and photosynthesis of algae are interdependent and compete for energy, resulting in different physiological effects of N sources on algae at different light levels. It has been reported that at the LL intensity of 17 μmol photons m⁻² s⁻¹, a brown tide alga Aureococcus anophagefferens cultured with NO₃⁻–N presents the lowest growth rate in all N sources culture [31]. At a light level of 60 μmol photons m⁻² s⁻¹, N sources, including NO₃⁻–N and NH₄⁺–N, had no marked impact on the growths of two marine diatoms Phaeodactylum tricornutum and Chaetoceros muelleri [32]. Conversely, at an HL level of 250 μmol photons m⁻² s⁻¹, microalgae Chlorella sp. Cul-

Figure 6. The contents of soluble protein (A) and carbohydrates (B) of S. horneri after being grown at different light and N conditions for 10 days. Data are reported as means ± SD (n = 3). Different letters above the error bars indicate significant differences between treatments (P < 0.05).

4. Discussion

Herein, both increased light level and enriched nitrogen (including NO₃⁻–N and NH₄⁺–N) enhanced Sargassum horneri development. However, at different light levels, the enrichments of two nitrogen sources showed different promoting effects on the growth of this alga, suggesting significant interaction between light and nitrogen source, which was closely related to the photosynthetic carbon fixation of algae under different conditions.

Photosynthesis transforms light energy into chemical energy, which is the most important physiological activity of algae and directly determines the growth and other processes of algae. Light is the basis of photosynthesis and the major environmental agent affecting algal development. Therefore, increased light can promote photosynthesis and algae growth by accelerating light energy absorption, promoting electron transfer, increasing pigment content, and improving related enzymatic activity [21–24]. In this study, the content of photosynthetic pigments, as well as the uptake rate of nitrogen and the content of nitrogen metabolites (soluble protein) in S. horneri was not affected by the level of light. The promotion of light on development rate was likely due to the improvement of photosynthetic carbon fixation capacity and/or affinity for inorganic carbon substrates, which had been also confirmed by the results of the effect of UVR on the growth of Rhodophyta Gracilaria lemaneiformis [26].

The growth of algae depends not only on photosynthesis, but also on the level of N metabolism, thus it is the result of the joint action of C and N metabolism [43]. The increased N generally promotes the growth rate of algae [44]. However, the growth of algae supplied with different forms of N sources (such as NO₃⁻–N and NH₄⁺–N) may be different, due to different ways for algae to absorb different forms of N [45]. It has been reported that NH₄⁺–N is used by algae through passive diffusion without energy consumption, while NO₃⁻–N is through active absorption with energy consumption [46]. Therefore, the absorption of different N sources and photosynthesis of algae are interdependent and compete for energy, resulting in different physiological effects of N sources on algae at different light levels. It has been reported that at the LL intensity of 17 μmol photons m⁻² s⁻¹, a brown tide alga Aureococcus anophagefferens cultured with NO₃⁻–N presents the lowest growth rate in all N sources culture [31]. At a light level of 60 μmol photons m⁻² s⁻¹, N sources, including NO₃⁻–N and NH₄⁺–N, had no marked impact on the growths of two marine diatoms Phaeodactylum tricornutum and Chaetoceros muelleri [32]. Conversely, at an HL level of 250 μmol photons m⁻² s⁻¹, microalgae Chlorella sp. Cul-
tured with NO$_3^-$–N showed a higher growth rate compared with those cultured with NH$_4^+$–N [33]. Additionally, in an invasive marine macroalga Chaetomorpha valida, a higher growth rate and photosynthetic activity were found in thalli cultured with NO$_3^-$–N than NH$_4^+$–N [47]. In this study, under LL (10 µmol photons m$^{-2}$ s$^{-1}$) and ML (60 µmol photons m$^{-2}$ s$^{-1}$), despite no advantage in photosynthesis, enrichment of NH$_4^+$ manifested more obvious promotion on the growth of S. horneri than the enrichment of NO$_3^-$ [33]. Such a result might be due to the fact that the absorption of NH$_4^+$–N consumed less energy and N metabolism was more favorable, alga could contribute saved energy to its growth, in case of insufficient energy supply at lower light levels [31]. Under HL (300 µmol photons m$^{-2}$ s$^{-1}$), due to adequate energy supply from vigorous photosynthesis, energy-consuming NO$_3^-$–N metabolism showed a significant increment of the growth in S. horneri similar to NH$_4^+$–N, even the higher content of soluble carbohydrates. It seems that under sufficient light conditions, S. horneri tends to use NO$_3^-$–N as a nitrogen source for growth, although NH$_4^+$–N had better absorption convenience. It is scientifically recognized that NO$_3^-$–N can be reduced to NH$_4^+$–N for N metabolism inside a cell only through the active processes of nitrate reductase and nitrite reductase, after being absorbed into cells by algae [46]. These processes can be actively regulated by the cells themselves to benefit survival and growth. However, NH$_4^+$–N can penetrate the cell membrane and passively enter the algal cells. Excessive NH$_4^+$–N may accumulate in the cells, and even cause toxic damage to algae under certain conditions [48]. Fortunately, such toxic damage of NH$_4^+$–N has not been found in S. horneri cultured at NH$_4^+$–N concentration of 200 µmol L$^{-1}$ in the present study.

5. Conclusions

This investigation was the first to demonstrate the synergistic effects of light and N source on a golden tide alga, S. horneri’s, growth and physiological traits. Based on our results, enrichment of NO$_3^-$ and NH$_4^+$ showed different effects on the growth of S. horneri under different light intensities, which was mainly associated with algal photosynthetic carbon fixation. Especially, at the HL level, the enrichment of both NO$_3^-$ and NH$_4^+$ dramatically promoted the growth of S. horneri, which suggested that drifting S. horneri, exposed to HL on the surface of the sea, was easy to grow rapidly and form GT, when encountering eutrophic seawater with a higher concentration of nitrate or ammonia. Therefore, our results may provide important references for the prediction of the booming of Sargassum golden tides.

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