Combined effects of elevated pCO_2 and temperature on biomass and carbon fixation of phytoplankton assemblages in the northern South China Sea

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Abstract

The individual influences of ocean warming and acidification on marine organisms have been investigated intensively, but studies regarding the combined effects of both global change variables on natural marine phytoplankton assemblages are still scarce. Even fewer studies have addressed possible differences in the responses of phytoplankton communities in pelagic and coastal zones to ocean warming and acidification. We conducted shipboard microcosm experiments at both off-shore ~~(SEATS)~~ and near-shore ~~(D001)~~ stations in the northern South China Sea (NSCS) under three treatments, low temperature ~~(30.5 °C at SEATS and 28.5 °C at D001)~~ and low pCO_2 (~~390.0 µatm at SEATS and 420.0 µatm at D001) (~~LTLC, ambient temperature and ambient pCO_2), high temperature ~~(33.5 °C at SEATS and 31.5 °C at D001)~~ and low pCO_2 (~~390 µatm at SEATS and 420 µatm at D001) (~~HTLC, ambient temperature + 3 °C and ambient pCO_2), and high temperature ~~(33.5 °C at SEATS and 31.5 °C at D001)~~ and high pCO_2 (~~1000 µatm at SEATS and 1030 µatm at D001) (~~HTHC, ambient temperature + 3 °C and ambient pCO_2 + 610 µatm). Biomass of phytoplankton at both stations were enhanced by HT. HTHC did not affect phytoplankton biomass at near-shore station ~~D001~~ but decreased it at the off-shore station ~~SEATS~~. At this off-shore station HT alone increased daily primary productivity (DPP, µg C (µg chl *a*)$^{-1}$ d^{-1}) by ~64%, and by ~117% when higher pCO_2 was added. In contrast, HT alone did not affect DPP and HTHC reduced it by ~15% at the near-shore station ~~D001~~. HT enhanced the dark respiration rate (µg C (µg chl *a*)$^{-1}$ d^{-1}) by 64% at the near-shore station ~~SEATS~~, but had no significant effect at the near-shore station ~~D001~~, and did not change the ratio of respiration to photosynthesis at either station. HTHC did not affect dark respiration rate (µg C (µg chl *a*)$^{-1}$ d^{-1}) at either station compared to LTLC. HTHC reduced the respiration to photosynthesis ratio by ~41% at the off-shore station ~~SEATS~~ but increased it ~42% at the near-shore station ~~D001~~. Overall, our findings indicate that responses of coastal and offshore phytoplankton assemblages in NSCS to ocean warming and acidification are contrasting, with the pelagic phytoplankton communities being more easily affected by these two global change factors in terms of carbon fixation and respiration

compared to the coastal phytoplankton communities ~~sensitive to these two global change factors~~.

Key words: ocean acidification, ocean warming, photosynthesis, primary productivity, respiration, South China Sea

1 Introduction

From 1979 to 2012, the mean global sea surface temperature (SST) increased at a rate of ~0.12 ℃ per decade~~, based on in-situ data records~~ (IPCC, 2013). Particularly, the warming rate in the surface of South China Sea (~0.26 ℃ per decade) from 1982 to 2004 (Fang et al., 2006) appears to be about ~~is approximately~~ 2 times faster ~~than the global ocean mean rate (~0.14 per decade) from 1960 to 1990 (Casey and Cornillon, 2001; Fang et al., 2006)~~. It is extremely likely that more than half of the observed increase in global average surface temperature from 1951 to 2010 was caused by the anthropogenic increase in greenhouse gas concentrations and other anthropogenic forcings. Ocean warming is projected to rise approximately by 0.6 ℃ (Representative Concentration Pathway (RCP) 2.6) to 2.0 ℃ (RCP 8.5), in the upper 100 m of the water column by the end of the 21st century (IPCC, 2013).

Ocean warming is known to affect primary productivity both directly and indirectly. In situ surface chl *a* declined exponentially with rise of SST (13–23 ℃) in the northeast Atlantic Ocean from latitudes 29 to 63 °N in spring and summer, which was attributed to enhanced stratification and consequent reduced upward transport of nutrients into the upper mixed layer (Poll et al., 2013). The seawater volume-specific primary produtivity also decreased with temperature rise due to lower phytoplankton biomass (Poll et al., 2013). Mesocosm experiments have also demonstrated that ocean warming (an increase of 6 ℃) can decrease phytoplankton biomass by ~80% in the Baltic Sea (~~Lewandowska et al., 2012;~~ Sommer et al., 2015). On the other hand, ocean warming did not affect volume-specific primary produtivity in the Baltic Sea (Lewandowska et al., 2012).

Increased atmospheric CO_2 is responsible for global warming, and the oceans. As a main sink for CO_2, the ocean has have absorbed approximately 30% of the emitted anthropogenic carbon dioxide (IPCC, 2013). Such a dissolution of CO_2 increases seawater CO_2 partial pressure and bicarbonate ion levels and decreases pH and carbonate ion concentrations, leading to ocean acidification (Orr et al., 2005OA). By 2100, the projected decline in global-mean surface pH is projected to be approximately 0.065 (RCP 2.6) to 0.31 , (RCP 8.5) depending on the RCP scenario (IPCC, 2013). In terms of the South China Sea, an accelerated trend of ocean acidification has been reported and the rate of pH decline almost tripled between 1951 and 2000, compared to that between 1840 and 1950 (Liu et al., 2014).

Dissolved CO_2 may be a potentially limiting factor for marine primary productivity because of the low CO_2 level in seawater and the low affinity of the enzyme Rubisco for dissolved CO_2 (Falkowski and Raven, 2013). In addition, CO_2 in seawater diffuses approximately 10,000 times slower than in air, leading to its supply rate being much lower than the demand of photosynthetic carbon fixation (Raven, 1993; Riebesell et al., 1993). Although phytoplankton have evolved carbon-concentrating mechanisms (CCMs) to cope with these problems challenges (Giordano et al., 2005; Raven et al., 2012; Reinfelder, 2011), increased CO_2 concentration may still be beneficial since energy saved due to down-regulation of CCMs under elevated CO_2 can be utilized in other metabolic processes (Gao Wu et al., 2012a2010). Early laboratory and shipboard experiments suggested that increased CO_2 indeed could enhance phytoplankton growth rates and thus marine primary productivity (Riebesell et al., 1993; Hein and Sand-Jensen, 1997; Schippers et al., 2004). Since then, neutral effects of increased CO_2 on growth of phytoplankton assemblages have also been reported (Tortell and Morel, 2002; Tortell et al., 2000Gao et al., 2012 and references therein). Furthermore, ocean acidification can even reduce primary productivity of surface phytoplankton assemblages when exposed to incident solar radiation (Gao et al., 2012b). Therefore, the effects of ocean acidification on marine primary productivity remain controversial and its interactions with other

environmental factors, such as warming, solar UV radiation, hypoxia, etc. are incompletely understood (Gao et al., 2012a; Häder and Gao, 2015; Mostofa, 2016).

Ocean warming and acidification, both caused by increasing atmospheric CO_2, are proceeding simultaneously. The interactive or combined effects of warming and OA could be completely different from that of either one stressor (Hare et al. 2007, Feng et al., 2009; Gao et al., 2012a, Tatters et al. 2013). Several oceanographic cruises and ship board experiments in the European sector of the Arctic Ocean, showed that gross primary production increased with pCO_2 (145–2099 µatm) and the greatest increase was observed in lower temperature regions, indicating CO_2-enhanced primary production in the European Arctic Ocean is temperature-dependent (Holding et al., 2015).

The South China Sea (SCS) is located between the equator and 23.8 °N, from 99.1 to 121.1 °E, and is one of the largest marginal seas in the world, with a total area of about 3.5×10^6 km^2. Therefore, understanding the effects of ocean warming and acidification on primary production in SCS would help us to define the role of marginal seas in the global carbon cycle. However, only a very few studies on the effects of ocean acidification or warming on primary productivity in the SCS have been reported. Wu and Gao (2010) reported that CO_2 enrichment (700 µatm) did not affect the photosynthetic carbon fixation rate ~~assimilation number~~ of phytoplankton at a near-shore site in SCS, compared to the ambient CO_2 level (380 µatm). Gao et al. (2012b) demonstrated that increased pCO_2 (800 or 1000 µatm) reduced primary productivity in off-shore stations of the SCS. Therefore, we hypothesized that the effects of ocean acidification or/and warming on primary productivity in SCS would be site-dependent. None of the previous studies have examined co-effects of warming and increased CO_2 on primary production in the SCS. In this study, to test this hypothesis we conducted shipboard microcosm experiments at both near-shore and off-shore stations to determine the combined effects of ocean warming and acidification on biomass, photosynthetic carbon fixation, and dark respiration of phytoplankton assemblages in the SCS.

2 Methods

2.1 Experimental setup

The experiments were conducted at one off-shore station SEATS (17.9963° N, 115.9621° E) and one near-shore station D001 (18.9740° N, 110.7166° E) in the NSCS (Fig. 1). Surface seawater (0–2 m) was collected before sunrise with a 10 L acid-cleaned plastic bucket, filtered (180 μm) to remove large grazers and dispensed into nine microcosms. Microcosms consisted of cylindrical polymethyl methacrylate tanks (32 L, 0.34 m water depth) with water-jacketed space for circulating cooled water~~,~~. In the microcosms, phytoplankton assemblages could be exposed to 81-91% and 64-91% of full sunlight at the off-shore and near-shore stations respectively, due to the shielding of the cover, water-jacket and the depth of the water.~~, allowing 91% photosynthesis active radiation (PAR, 400–700 nm), 63% ultraviolet-A (UVA, 315–400 nm) and 6% ultraviolet-B (UVB, 280–315 nm) transmission under incident solar radiation.~~ Since we aimed to investigate the impacts on surface phytoplankton assemblages, and provide comparable data with the previous study in the SCS (Gao et al., 2012b), we decided not to shield the microcosms. ~~–~~Two levels of temperature (in situ, in situ + 3 °C) and pCO_2 (ambient, ambient + 610 μatm) were used. There were three triplicated treatments: low temperature and low pCO_2, LTLC; high temperature and low pCO_2, HTLC; high temperature and high pCO_2, HTHC. The treatment of low temperature and high pCO_2 was missing due to the lack of microcosms. Microcosm temperature was controlled and monitored ~~with~~ via circulating coolers with a variation of ± 1.0°C (CTP-3000, EYELA, Japan) and stable CO_2 equilibrium with the sea water (variation of pCO_2 < 5%) was achieved within 24 hours using a CO_2 enricher (CE-100, Wuhan Ruihua Instrument & Equipment Ltd, China). The incubations were conducted for seven days for the off-shore station ~~SEATS~~ (Aug 3rd–9th 2012) and six days for the near-shore station ~~D001~~ (Aug 14th–19th 2012).

2.2 Solar radiance

The incident solar radiation was continuously monitored using an Eldonet broadband filter radiometer (Eldonet XP, Real Time Computer, Germany) that was fixed at the top of the ship. It measured every second and recorded the means over

each minute.

2.3 Carbonate chemistry parameters

The seawater pH in the microcosm was recorded with a pH meter (FE20, Mettler Toledo, Greifensee, Switzerland) every hour during the first day of incubation and daily afterwards. The pCO_2 in seawater was maintained with the CO_2 enricher and measured by an automated flowing pCO_2 measuring system (Model 8050, GO, USA). Other carbonate system parameters were derived via CO2SYS (Pierrot et al., 2006), using the equilibrium constants of K_1 and K_2 for carbonic acid dissociation (Roy et al., 1993).

2.4 Chlorophyll *a* analysis

For the measurement of chlorophyll *a* (chl *a*), 500 mL of seawater were filtered onto a Whatman GF/F glass fiber filter (25 mm).Then the filter was placed in 5 ml 93% acetone at -20 ℃ for 24 h. Chl *a* concentration was determined with a fluorometer (Trilogy, Turner Designs, USA), following the protocol of Welschmeyer (1994). The concentrations of chl *a* in situ and in microcosms were measured at the beginning and end of the experiment, respectively.

2.5 Primary productivity and dark respiration

Seawater samples taken from each microcosm at the end of the experiment were dispensed into 50 mL quartz tubes, inoculated with 5 μCi (0.185 MBq) $NaH^{14}CO_3$ (ICN Radiochemicals, USA) and then incubated for 12 h (from 6:00 a.m. to 6:00 p.m.) and 24 h (from 6:00 a.m. to 6:00 a.m. next day) under natural light and day-night conditions. The incubation temperature of every treatment was the same as the corresponding microcosm treatment. After the incubation, the cells were filtered onto a Whatman GF/F glass fiber filter (25 mm), which was immediately frozen and stored at -20^{o}C for later analysis. In the laboratory, each frozen filter was placed into a 20 mL scintillation vial, exposed to HCl fumes overnight, and dried (55 ^{o}C, 6 h) to expel non-fixed ^{14}C ~~(Gao et al., 2007)~~. Then 3 mL scintillation cocktail (Perkin Elmer®) was added to each vial and incorporated radioactivity was counted by a liquid scintillation counting (LS 6500, Beckman Coulter, USA). The daytime primary productivity (DPP) was defined as the amount of carbon fixation during 12 h

incubation. The dark respiration was defined as the difference in amount of carbon fixation between 12 h and 24 h. Carbon fixation over 24 h was taken as daily net primary productivity (NPP). Ratio of respiration to photosynthesis (R/P) was expressed as that of respiratory carbon loss to daytime carbon fixation. The primary productivity and dark respiration in situ were measured at the beginning of the experiment.

2.6 Statistical analyses

Results were expressed as means of replicates ± standard deviation. Data were analyzed using the software SPSS v.21. The data from each treatment conformed to a normal distribution (Shapiro-Wilk, $P > 0.05$) except the DPP (P = 0.034) and dark respiration (P = 0.034) under HTLC at the near-shore station, and the variances could be considered equal (Levene's test, $P > 0.05$). One-way ANOVAs were conducted to assess the significant differences in carbonate chemistry parameters, chl *a*, DPP, NPP, dark respiration, ratio of dark respiration to photosynthesis between three treatments. Tukey HSD was conducted for post hoc investigation. Independent samples t-tests were conducted to compare in situ chl *a*, DPP, NPP, dark respiration, and ratio of dark respiration to photosynthesis between both stations. The threshold value for determining statistical significance was $P < 0.05$.

3 Results

The incident solar radiation during the experiment was recorded (Table 1). The daytime (12 h) mean solar radiation ranged from 927 to 1592 μmol photons m^{-2} s^{-1} at the off-shore station ~~SEATS~~ while the lowest solar radiance was only 111 μmol photons m^{-2} s^{-1}, with the highest of 1583 μmol photons m^{-2} s^{-1}, at the near-shore station ~~D001~~. The average of daytime mean solar radiation over the microcosm incubation at the off-shore station ~~SEATS~~ was 15.52% higher than that at the near-shore station ~~D001~~.

The changes of the seawater carbonate system under different conditions are shown in Table 2. At the off-shore station ~~SEATS~~, an increase of 3 ^{o}C in temperature (HTLC) did not alter carbonate parameters except leading to enhanced CO_3^{2-} (Tukey HSD, $P = 0.009$). HTHC resulted in a significant decrease in CO_3^{2-} (Tukey HSD, $P <$

0.001) and TA (Tukey HSD, P = 0.016) but an increase in CO_2 (Tukey HSD, P < 0.001) compared with LTLC. The effect of temperature on carbonate parameters at the near-shore station ~~D001~~ were similar to the off-shore station ~~SEATS~~, while HTHC increased HCO_3^- (Tukey HSD, P = 0.046) and did not affect TA (Tukey HSD, P = 0.203).

The in situ chl *a* levels at the near-shore station and the off-shore station were ~~D001 (~~0.37 ± 0.05 μg L^{-1}~~)~~ and ~~was higher than that at station SEATS (~~0.15 ± 0.02 μg L^{-1}, respectively.~~) (Independent samples t-test, t = –7.483, df = 4, P = 0.002; Fig. 2a).~~ After seven days incubation in the microcosms at the off-shore station ~~SEATS,~~ Tukey comparison (P = 0.05) showed that higher temperature (0.46 ± 0.04 μg L^{-1}) increased chl *a* compared with LTLC (0.23 ± 0.03 μg L^{-1}) while HTHC (0.07 ± 0.01 μg L^{-1}) reduced it (Fig. 2b). The higher temperature (0.72 ± 0.07 μg L^{-1}) also increased chl *a* compared with LTLC (0.41 ± 0.05 μg L^{-1}) at the near-shore station ~~D001~~ (Tukey HSD, P = 0.001; Fig. 1b), but with no effect of HTLC (0.41 ± 0.04 μg L^{-1}) (Tukey HSD, P = 0.988).

The in situ DPP at the near-shore station ~~D001~~ (49.4 ± 4.5 μg C L^{-1} d^{-1} or 133.4 ± 12.1 μg C (μg chl *a*)$^{-1}$ d^{-1}) was dramatically higher than that at the off-shore station ~~SEATS~~ (5.1 ± 0.5 μg C L^{-1} d^{-1} or 34.1 ± 3.1 μg C (μg chl *a*)$^{-1}$ d^{-1}), whether normalized to volume of seawater (Independent samples t-test, t = –17.056, df = 4, P < 0.001; Fig. 3a) or chl *a* (Independent samples t-test, t = –13.786, df = 4, P < 0.001; Fig. 3b). After seven days incubation in microcosms, the DPP normalized to volume of seawater under HTLC (33.2 ± 4.8 μg C L^{-1} d^{-1}) at the off-shore station ~~SEATS~~ was significantly higher than that under LTLC (9.9 ± 1.2 μg C L^{-1} d^{-1}) and HTHC (6.6 ± 0.6 μg C L^{-1} d^{-1}) (Tukey HSD, P < 0.001) while the difference between LTLC and HTHC was insignificant (Tukey HSD, P = 0.380; Fig. 3c). The pattern at the near-shore station ~~D001~~ was similar to the off-shore station~~SEATS~~ (Fig. 2c). When DPP was normalized to chl *a*, the higher temperature increased primary productivity from 43.2 ± 5.1 to 70.7 ± 10.1 μg C (μg chl *a*)$^{-1}$ d^{-1} (Tukey HSD, P = 0.014) and further to 93.9 ± 8.1 μg C (μg chl *a*)$^{-1}$ d^{-1} (Tukey HSD, P < 0.001) when higher CO_2 was combined at the off-shore station ~~SEATS~~ (Fig. 2d). In contrast, temperature did not affect DPP (Tukey HSD, P =

0.0924) and HTHC reduced it from 150.3 ± 4.9 to 128.0 ± 11.5 μg C (μg chl *a*)$^{-1}$ d^{-1} (Tukey HSD, P = 0.039) at the near-shore station ~~D001~~ (Fig. 3d).

The in situ NPP at the off-shore and the near-shore ~~stations SEATS and D001~~ were 3.5 ± 0.1 μg C L^{-1} d^{-1} (23.2 ± 1.0 μg C (μg chl *a*)$^{-1}$ d^{-1}) and 37.4 ± 3.1 μg C L^{-1} d^{-1} (91.2 ± 7.5 μg C (μg chl *a*)$^{-1}$ d^{-1}) respectively, which indicates that the near-shore station ~~D001~~ has higher NPP, irrespective of normalizing to volume of seawater (Independent samples t-test, t = –18.998, df = 4, P < 0.001; Fig. 4a) or chl *a* (Independent samples t-test, t = –15.511, df = 4, P < 0.001; Fig. 4b). After a seven-day incubation in the microcosms, the higher temperature increased NPP to 23.9 ± 5.3 μg C L^{-1} d^{-1} (Tukey HSD, P = 0.001) while HTHC (5.5 ± 0.4 μg C L^{-1} d^{-1}) did not change it (Tukey HSD, P = 0.793) compared with LTLC (7.2 ± 0.8 μg C L^{-1} d^{-1}). The effects of temperature and CO_2 on NPP at the near-shore station ~~D001~~ were similar to that at the off-shore station ~~SEATS~~. When NPP was normalized to chl *a*, the higher temperature increased NPP from 31.1 ± 3.5 to 50.9 ± 11.3 (Tukey HSD, P = 0.044) and further to 78.3 ± 5.9 μg C (μg chl *a*)$^{-1}$ d^{-1} with the addition of higher CO_2 (Tukey HSD, P < 0.001) at the off-shore station ~~SEATS~~. On the other hand, neither HT (Tukey HSD, P = 0.707) nor HTHC (Tukey HSD, P = 0.057) affected NPP at the near-shore station ~~D001~~.

The in situ dark respiration rate at the off-shore station ~~SEATS~~ was remarkably lower than that at the near-shore station ~~D001~~ regardless of normalizing to volume of seawater (Independent samples t-test, t = –11.568, df = 4, P < 0.001; Fig. 5a) or chl *a* (Independent samples t-test, t = –8.019, df = 4, P = 0.001; Fig. 5b). The higher temperature increased dark respiration rate from 2.8 ± 1.2 to 9.3 ± 0.6 μg C L^{-1} d^{-1} (Tukey HSD, P < 0.001) at the off-shore station ~~SEATS~~ while HTHC reduced it to 1.1 ± 0.2 μg C L^{-1} d^{-1} (Tukey HSD, P = 0.009; Fig. 5c). The higher temperature also promoted dark respiration rate at the near-shore station ~~D001~~, from 16.9 ± 2.0 to 31.5 ± 5.1 μg C L^{-1} d^{-1} (Tukey HSD, P = 0.007) but HTHC did not alter it (Tukey HSD, P = 0.516; Fig. 5c). When it was normalized to chl *a*, higher temperature still increased dark respiration rate from 12.0 ± 1.8 to 19.7 ± 1.4 μg C (μg chl *a*)$^{-1}$ d^{-1} (Tukey HSD, P = 0.006) while the effect of temperature on respiration rate at the near-shore station

~~D001~~ was insignificant (Tukey HSD, P = 0.891; Fig. 5d). Compared to LTLC, HTHC did not affect respiration rate at either station (Tukey HSD, P = 0.131 at the off-shore station ~~SEATS~~, P = 0.348 at near-shore station ~~D001~~; Fig. 5d).

The in situ ratio of respiration to photosynthesis was 31.9 ± 3.5% at the off-shore station ~~SEATS,~~ significantly higher than that (24.2 ± 1.3%) at the near-shore station ~~D001~~ (Independent samples t-test, t = 3.537, df = 4, P = 0.0024; Fig. 6a). After seven days incubation in microcosms, Tukey HSD comparison (P = 0.05) showed that higher temperature did not affect the ratio of respiration to photosynthesis but HTHC reduced it from 27.8 ± 1.6% to 16.5 ± 1.3% at the off-shore station ~~SEATS~~ (Fig. 6b). On the contrary, HTHC (38.7 ± 3.1%) increased the ratio compared to LTLC (27.3 ± 2.4%), with insignificant effect of temperature alone (29.5 ± 3.3%) at the near-shore station ~~D001~~ (Fig. 6b).

4 Discussion

4.1 Effects of increased temperature and CO_2 on biomass

~~The in situ chl *a* concentration at station D001 was higher than that at SEATS, indicating more phytoplankton biomass at station D001. The chl *a* concentration decreases with distance from the coast in the NSCS, mainly due to the change of nutrients (Li et al., 2011).~~ The higher temperature increased chl *a* concentration at both stations, which might be attributed to increased active uptake of nutrients at the elevated temperatures through enhanced enzymatic activities. Algal and cyanobacterial growth commonly increases with temperature within a suitable range and then decreases after the optimal temperature point/range (Goldman and Carpenter, 1974; Montagnes and Franklin, 2001; Savage et al., 2004; Boyd et al. 2013) and optimum temperatures for growth of marine phytoplankton are usually several degrees higher than the environmental temperatures (Thomas et al., 2012), which could explained the increase chl a level of phytoplankton grown at the higher temperature ~~.~~ in the present study.

On the other hand, the elevated CO_2 offset the positive effect of the higher temperature on chl *a* at the near-shore station ~~D001~~, and even reduced chl *a* at the off-shore station ~~SEATS~~. High CO_2 can sometimes enhance algal photosynthesis and

growth, since CO_2 in seawater is suboptimal for full operation of Rubisco enzymes (~~Wu~~ Giordano et al., ~~2008~~ 2005 and references therein). On the other hand, positive effects of elevated CO_2 can be affected by other environmental factors. Gao et al. (2012b) demonstrated that rising CO_2 could enhance growth of diatoms at low light intensity, but decrease it at high light intensity. It was found that rising CO_2 concentration lowered the threshold for diatom growth above which photosynthetic active radiation becomes excessive or stressful, owing to reduced energy requirements for inorganic carbon acquisition at elevated CO_2 (Gao et al., 2012b). In the present study, the mean daily solar radiation levels during incubation were 1312 and 1136 μmol photons m^{-2} s^{-1} (Table 1), corresponding to phytoplankton in the microcosms exposed to 1068-1194 μmol photons m^{-2} s^{-1} at the off-shore station and 729-1034 μmol photons m^{-2} s^{-1} at the near-shore station, which were far above the threshold light intensity reported for diatoms (Gao et al., 2012b). Consequently, the higher CO_2 combined with the high solar radiation in summer of NSCS may have imposed negative effects on phytoplankton biomass at both the off-shore and the near-shore stations ~~SEATS and D001~~. In addition, the inhibitory effect of higher CO_2 on biomass was more significant at the off-shore station ~~SEATS~~ than the near-shore station~~D001~~. This can be attributed to the higher sensitivity of picoplankton to high solar radiation (Li et al., 2011; Wu et al., 2015), which could be delivered to the interaction of high solar radiation and high CO_2. As shown in Li et al.'s (2011) study, the proportion of picoplankton in phytoplankton assemblages increased with distance off the coasts. Therefore, the dominant species at the off-shore station ~~SEATS~~ are pico- and nano-phytoplankton, but micro-phytoplankton at the near-shore station ~~D001~~ (Table 3).

4.2 Effects of increased levels of temperature and CO_2 on primary productivity

The seawater volume-specific DPP at the near-shore station ~~D001~~ was higher than the off-shore station ~~SEATS~~. This should result from both higher chl *a* concentration and chl *a*-specific DPP at the near-shore station~~D001~~. It has been shown that more smaller cells exist at the off-shore station~~SEATS~~ than at the near-shore station~~D001~~ (Table 3). Smaller cells have been considered to have larger DPP,

according to Laws's model (1975). The discrepancy between our finding and Laws's model may be due to the availability of nutrients. Laws's model was based on growth rates obtained from the same nutrient level. Nevertheless, the nutrient level at the near-shore station ~~D001~~ is higher than at the off-shore station ~~SEATS~~ (Table 3), leading to higher DPP. The higher temperature increased seawater volume-specific DPP at both stations. This could be attributed to more biomass produced at the warmer conditions, as indicated by chl *a*. High temperature enhanced the chl *a*-specific DPP at the off-shore station ~~SEATS~~. However, no positive effects of temperature were found on chl *a*-specific DPP at the near-shore station ~~D001~~. The differential effects of temperature on chl *a*-specific DPP between the stations may be due to the phytoplankton community composition, since cyanobacteria and/or pico-phytoplankton have the strongest temperature response in terms of photosynthetic carbon fixation compared to micro- and nano-phytoplankton (Andersson et al., 1994). This finding contributes to the explanation of the dominance of pico-phytoplankton in a warmer ocean (Chen et al., 2014; Montagnes and Franklin, 2001; Hare et al. 2007; Morán et al., 2010). HTHC reduced chl *a*-specific DPP at the near-shore station ~~D001~~, but increased it at the off-shore station ~~SEATS.~~ High CO_2 also reduced chl *a*-specific DPP in a previous study, which could result from the interaction of high CO_2 and high solar radiation during the summer in the NSCS (Gao et al., 2012b). The reason that HTHC stimulated chl *a*-specific DPP at the off-shore station ~~SEATS~~ may be due to a dramatic decline in chl *a* concentration under the HTHC treatment.

4.3 Effects of increased temperature and CO_2 on respiration

The dark respiration rate of phytoplankton at the near-shore station ~~D001~~ was higher than that at the off-shore station~~SEATS~~, regardless of normalizing to seawater or chl *a*. The respiration rate of algae or cyanobacteria usually increases with cell size (López-Sandoval et al., 2014). The off-shore s~~S~~tation ~~SEATS~~ has more pico-phytoplankton, which would lead to a lower chl *a*-specific dark respiration rate and then lower seawater volume-specific dark respiration, particularly when combined with lower chl *a* level. The higher temperature increased seawater

volume-specific dark respiration at both stations, which could be related to increased chl *a* concentration and/or enhanced respiratory carbon loss at the higher temperature. The higher temperature also increased chl *a*-specific dark respiration rate at the off-shore station ~~SEATS~~. This is consistent with Butrón et al.'s (2009) study, in which respiration rates of phytoplankton along Nervión–Ibaizabal estuary showed a positive correlation with temperature. Robarts and Zohary (1987) also found that respiration rate of bloom-forming cyanobacteria was temperature-dependent, with optima over 25 ℃.

HTHC reduced seawater volume-specific dark respiration at both stations, which should be the consequence of the decreased chl *a* in this treatment. The higher temperature increased chl *a*-specific dark respiration rate at the off-shore station ~~SEATS~~, but there was no significant difference between HTHC and LTLC, indicating the higher CO_2 inhibited the chl *a*-specific dark respiration rate. Similarly, reduced respiration was found in mesocosm studies (Spilling et al., 2016). In theory, higher CO_2 would inhibit respiratory release of CO_2. Nevertheless, enhanced respiration rate at higher CO_2 conditions have been found in laboratory-grown diatoms (Wu et al., 2010), coccolithophores (Jin et al., 2015), mixed phytoplankton assemblages (Jin et al., 2015), and macroalgae as well (Zou et al., 2011). Such increased respiration has been attributed to extra energy demand to cope with increased seawater acidity caused by higher CO_2 (Gao and Campbell, 2014; Raven et al., 2014). Therefore, the effect of increased CO_2 on phytoplankton respiration could be due to the combined effects of CO_2 diffusive resistance and seawater acidity stress. Meanwhile, neither higher temperature alone nor HTHC significantly affected chl *a*-specific dark respiration rate at the near-shore station ~~D001~~. One possible reason could be that larger cells are less sensitive to CO_2 diffusive resistance and acidic stress due to thicker diffusion boundary layers around the cells. This hypothetical explanation is worthy of future testing.

4.4 Effects of increased temperature and CO_2 on R/P

Laws's model (1975) has suggested that large phytoplankton cells are likely to consume a smaller fraction of their biomass to compete with small phytoplankton

cells in terms of the growth rate, considering small cells have higher gross production rates. Our finding that phytoplankton ~~in~~ at the off-shore station ~~SEATS~~ had a higher R/P than the near-shore station ~~D001~~ confirms Laws's model. It was theorized that autotrophic respiration is more sensitive to temperature than photosynthesis and the ratio of R/P was predicted to increase with temperature (Ryan, 1991; Woodwell, 1990; Woodwell et al., 1983). However, the assumption that plant respiration is highly temperature dependent was primarily based on short-term (a few minutes or hours) responses of plants to changes of temperature (Gifford, 1994). In long-term experiments (days or months), the increase in respiration with temperature tended to disappear due to acclimation (Gifford, 1995; Jones, 1977; Reich et al., 2016; Slot and Kitajima, 2015; Ziska and Bunce, 1998). Photosynthetic acclimation to warming is variable (Chalifour and Juneau, 2011; Hancke and Glud, 2004; Schlüter et al., 2014; Staehr and Birkeland, 2006). However, a general acclimation response to long-term increased temperature is a rise in optimal temperature of photosynthesis (Gunderson et al., 2010; Kattge and Knorr, 2007; Staehr and Birkeland, 2006). Such shifts in the temperature response of photosynthesis and respiration via physiological acclimation can dampen the increase in R/P at high temperatures, or climate warming would not increase R/P (Reich et al., 2016). In other words, phytoplankton would down-regulate the high sensitivity of respiration to temperature, and maintain a relatively consistent net primary production and hence food web structure in a warming ocean. Although the ratio of R/P did not vary with increased temperature at ~~both~~ either station~~s~~ in our work either, both photosynthesis and respiration were enhanced by the higher temperature. The constant ratio was due to the similar amplitude of increase in photosynthesis and respiration. The incubation period of 7–8 days might not be enough for phytoplankton to acclimate to the increased temperature completely, and therefore the stimulated effects of high temperature on photosynthesis and respiration were still notable. Opposite effects of HTHC on R/P were detected at the two station~~s SEATS and D001~~, negative at the off-shore station~~SEATS~~ and positive at the near-shore station~~D001~~. This can be attributed to differential responses of photosynthesis at both stations to HTHC, considering the responses of respiration

were similar.

5 Conclusions

~~This study demonstrates that ocean warming expected to occur by the end of the century would simulate the DPP and also dark respiration of phytoplankton assemblages in NSCS, but this positive effect can be damped or offset when ocean acidification is combined. The responses of phytoplankton assemblages locating different areas to ocean warming and acidification could be contrasting due to various phytoplankton compositions and physical and chemical environments. It seems that phytoplankton assemblages in pelagic areas are more sensitive to ocean warming and acidification. More exhaustive investigations are needed to obtain an accurate view of primary production under future ocean environment.~~

This study demonstrates that a short-term rise of SST appeared to simulate the DPP and dark respiration of phytoplankton assemblages in the NSCS. However, this positive effect ~~could be~~was dampened or offset when warming and ocean acidification treatments were combined. The regional responses of phytoplankton assemblages at the two stations to ocean warming and acidification may differ due to differences in physical and chemical environment as well as phytoplankton community structure. The combined treatment of warming and acidification reduced biomass and dark respiration rate at the off-shore, but did not affect them at the near-shore station. Ecologically and geographically, our data implies differential responses of primary production to ocean climate change. This short-term experiment suggests the need to determine whether similar effects may occur over the longer timescales of future anthropogenic change.

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686 87-97, 2011.

687 Table 1. The daytime (12 h) mean solar radiation (PAR, μmol photons m^{-2} s^{-1}) during
688 incubation at off-shore station~~s~~ SEATS and near-shore station D001.

SEATS		D001	
Date	Solar radiation	Date	Solar radiation
03/08/2012[a]	1454	14/08/2012[a]	1512
04/08/2012	1304	15/08/2012	1480
05/08/2012	1146	16/08/2012	400
06/08/2012	1113	17/08/2012	111
07/08/2012	927	18/08/2012	1520
08/08/2012	1592	19/08/2012	1583
09/08/2012	1582	20/08/2012[b]	1346
10/08/2012[b]	1381	Mean[c]	1136
Mean[c]	1312		

689 [a]The dates for measurements of photosynthetic carbon fixation in situ. [b]The dates for
690 measurements of photosynthetic carbon fixation experiencing temperature and pCO_2
691 treatments. [c]Mean represents the average of daytime mean solar radiation over seven
692 or six days microcosm incubation.
693

694 Table 2. Parameters of the seawater carbonate system at different incubation
695 conditions. Measurements and estimation of the parameters were described in
696 Methods. Data are the means ± SD (n = 3). LTLC, low temperature and low pCO_2;
697 HTLC, high temperature and low pCO_2; HTHC, high temperature and high pCO_2.
698 DIC = dissolved inorganic carbon, TA = total alkalinity. Different superscript letters
699 indicate significant differences between treatments within one station.
700

	SEATS			D001		
	LTLC	HTLC	HTHC	LTLC	HTLC	HTHC
Temperature (^oC)	30.5 ±1.0	33.5 ±1.0	33.5 ±1.0	28.5 ±1.0	31.5 ±1.0	31.5 ±1.0
pH_T	8.07 ±0.01	8.05 ±0.01	7.68 ±0.01	8.02 ±0.01	8.01 ±0.01	7.68 ±0.01
pCO_2 (μatm)	390.0 ±19.5	390.0 ±19.5	1000.0 ±70.0	420.0 ±25.2	420.0 ±25.2	1030.0 ±60.0
DIC (μmol kg^{-1})	2056.4 ±49.2^a	1986.6 ±47.1^a	1999.9 ±91.4^a	1969 ±67.7^a	1896.5 ±64.8^a	2039.1 ±69.5^a
HCO_3^- (μmol kg^{-1})	1758.4 ±47.5^{ab}	1681.2 ±45.4^a	1838.4 ±86.5^b	1719.2 ±63.7^a	1640.9 ±60.7^a	1882.3 ±66.4^b
CO_3^{2-} (μmol kg^{-1})	288.2 ±1.2^b	296.2 ±1.2^c	138.1 ±3.3^a	238.8 ±3.4^b	245.3 ±3.5^b	131.6 ±1.7^a
CO_2 (μmol kg^{-1})	9.8 ±0.5^a	9.1 ±0.5^a	23.5 ±1.7^b	11.0 ±0.7^a	10.3 ±0.7^a	25.2 ±1.5^b
TA (μmol kg^{-1})	2443.5 ±47.9^a	2387.7 ±45.8^a	2170.7 ±92.0^b	2294.2 ±68.6^a	2260.0 ±33.9^a	2199.4 ±68.5^a

701

Table 3. Physical, chemical, and biological parameters at off-shore station~~s~~ SEATS and near-shore station D001. SST: seawater surface temperature; N: NO_3^-+NO_2 (μmol L^{-1}); P: PO_4^{3-} (μmol L^{-1}). Data of nutrients and phytoplankton composition are derived from literatures.

Station	SST	Salinity	pH_T	N	P	Dominant phytoplankton
SEATS	28.7	32.9	8.07	<0.1[a]	<0.01[b]	Pico- and nano-phytoplankton[c]
D001	26.8	33.5	8.03	>1[d]	>0.1[d]	Micro-phytoplankton[e]

[a]Du et al. (2013); [b]Wu et al. (2003); [c]Li et al. (2011); [d]Li et al. (2014); [e]Zhang et al. (2014).

Figure captions

Figure 1. Experimental stations in the northern South China Sea.

Figure 2. Chl *a* concentration in situ (a) and after temperature and pCO_2 treatments in microcosms (b). The microcosm incubations lasted seven days at off-shore station SEATS and six days at near-shore station D001.The error bars indicate the standard deviations (n = 3). The different letters above the error bars represent significant ($P < 0.05$) differences between stations in panel (a) and between treatments in panel (b).

Figure 3. Daytime primary productivity (DPP) in situ (a, b) and after temperature and pCO_2 treatments in microcosms (c, d). The microcosm incubations lasted seven days at off-shore station SEATS and six days at near-shore station D001. The error bars indicate the standard deviations (n = 3). The different letters above the error bars represent significant ($P < 0.05$) differences between stations in panels (a, b) and between treatments in panels (c, d).

Figure 4. Net primary productivity (NPP) in situ (a, b) and after temperature and pCO_2 treatments in microcosms (c, d). The microcosm incubations lasted seven days at off-shore station SEATS and six days at near-shore station D001. The error bars indicate the standard deviations (n = 3). The different letters above the error bars represent significant ($P < 0.05$) differences between stations in panels (a, b) and between treatments in panels (c, d).

Figure 5. Dark respiration in situ (a, b) and after temperature and pCO_2 treatments in microsoms (c, d). The microcosm incubations lasted seven days at off-shore station SEATS and six days at near-shore station D001. The error bars indicate the standard deviations (n = 3). The different letters above the error bars represent significant ($P < 0.05$) differences between stations in panels (a, b) and between treatments in panels (c, d).

Figure 6. The ratio of respiration to photosynthesis in situ (a, b) and after temperature and pCO_2 treatments (c, d) in microcosms. The microcosm incubations lasted seven days at off-shore station SEATS and six days at near-shore station D001. The error bars indicate the standard deviations (n = 3). The different letters above the error bars represent significant ($P < 0.05$) differences between stations in panels (a, b) and

between treatments in panels (c, d).

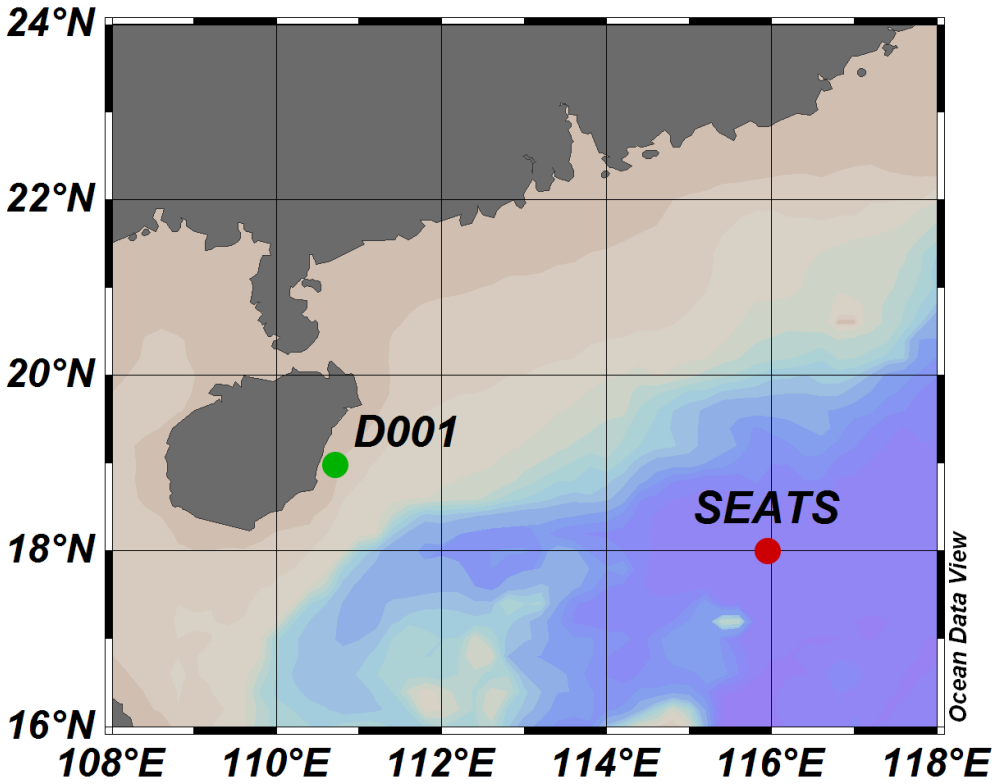
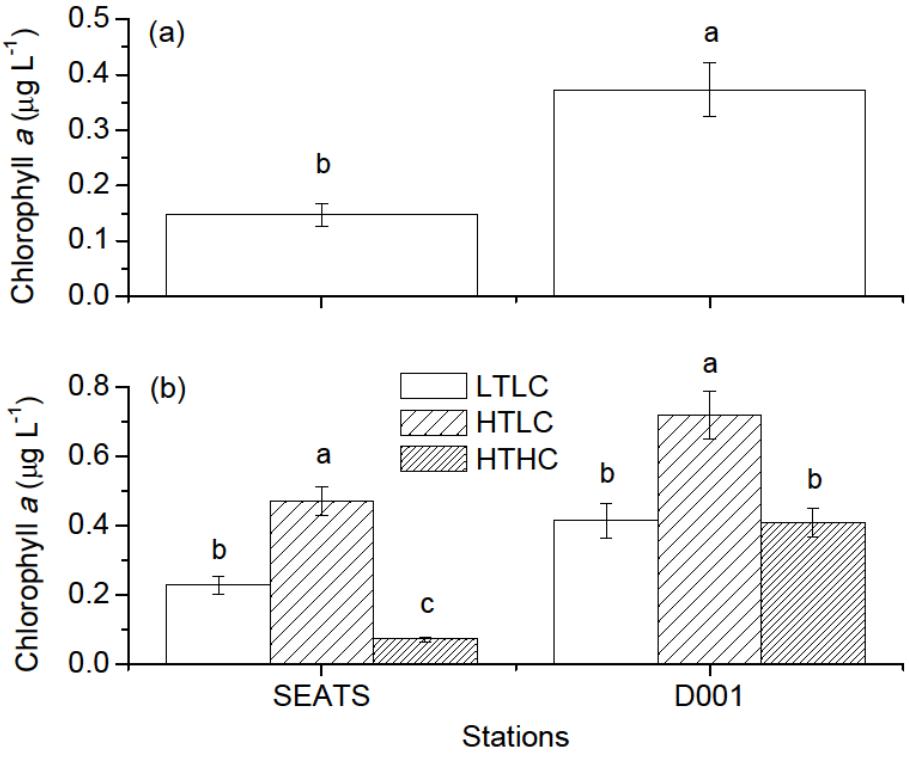


Figure 1



754

755 Figure 2

756

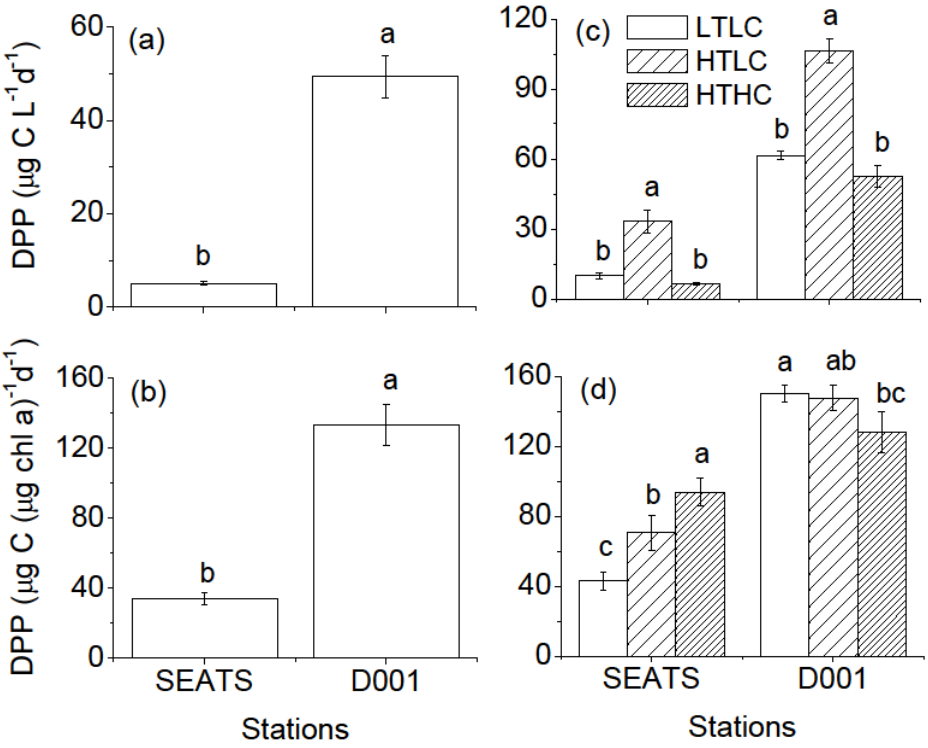

(a)
(b)
(c)
(d)
DPP (μg C L-1d-1)
DPP (μg C (μg chl a)-1d-1)
LTLC
HTLC
HTHC
SEATS
D001
Stations

Figure 3

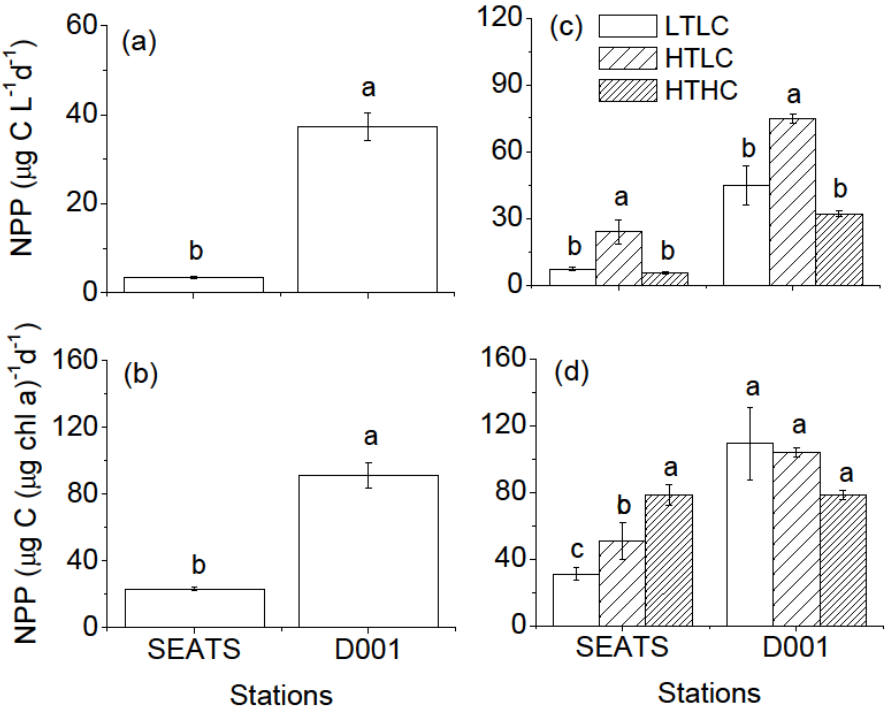

(a)
(b)
(c)
(d)
NPP (μg C L-1d-1)
NPP (μg C (μg chl a)-1d-1)
LTLC
HTLC
HTHC
SEATS
D001
Stations

Figure 4

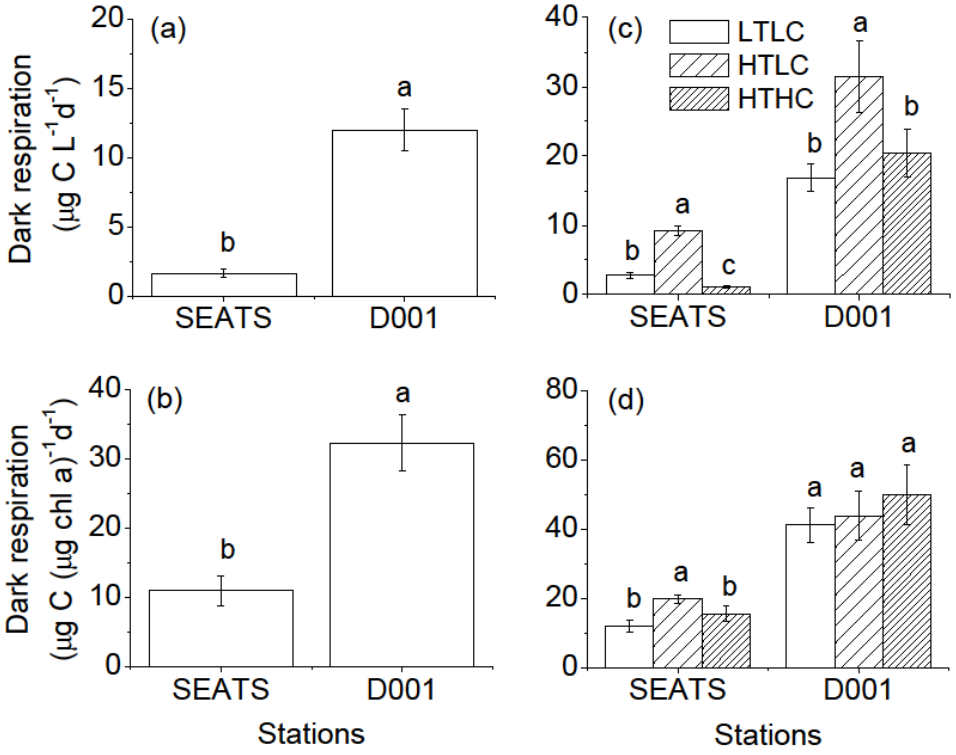

(a)
(b)
(c)
(d)
Dark respiration (μg C L-1d-1)
Dark respiration (μg C (μg chl a)-1d-1)
LTLC
HTLC
HTHC
SEATS
D001
Stations

Figure 5

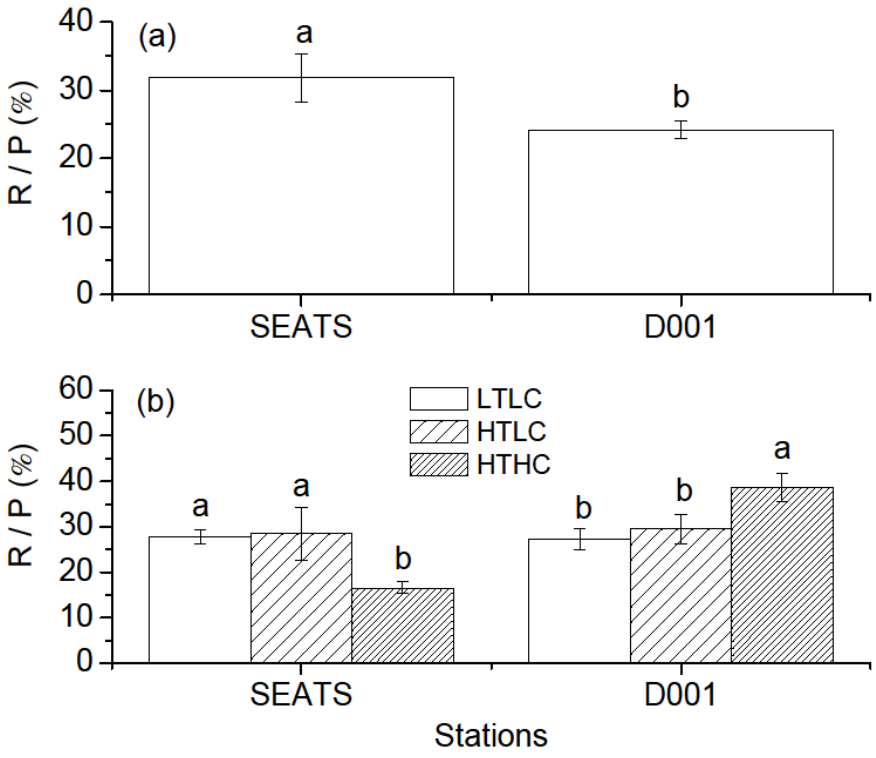

(a)
a
b
R / P (%)
40
30
20
10
0
SEATS
D001
(b)
LTLC
HTLC
HTHC
a
a
b
b
b
a
R / P (%)
60
50
40
30
20
10
0
SEATS
D001
Stations

Figure 6