



1 **Combined effects of elevated $p\text{CO}_2$ and temperature on biomass and carbon**

2 **fixation of phytoplankton assemblages in the northern South China Sea**

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26 Abstract

27 The individual influences of ocean warming and acidification on marine
28 organisms have been investigated intensively, but studies regarding the combined
29 effects of both global change variables on natural marine phytoplankton assemblages
30 are still scarce. Even fewer studies have addressed possible differences in the
31 responses of phytoplankton communities in pelagic and coastal zones to ocean
32 warming and acidification. We conducted shipboard microcosm experiments at both
33 off-shore (SEATS) and near-shore (D001) stations in the northern South China Sea
34 (NSCS) under three treatments, low temperature (30.5 °C at SEATS and 28.5 °C at
35 D001) and low $p\text{CO}_2$ (390.0 μatm at SEATS and 420.0 μatm at D001) (LTLC), high
36 temperature (33.5 °C at SEATS and 31.5 °C at D001) and low $p\text{CO}_2$ (390 μatm at
37 SEATS and 420 μatm at D001) (HTLC), and high temperature (33.5 °C at SEATS
38 and 31.5 °C at D001) and high $p\text{CO}_2$ (1000 μatm at SEATS and 1030 μatm at D001)
39 (HTHC). Biomass of phytoplankton at both stations were enhanced by HT. HTHC did
40 not affect phytoplankton biomass at station D001 but decreased it at station SEATS.
41 At this offshore station HT alone increased daily primary productivity (DPP, $\mu\text{g C}$ (μg
42 $\text{chl } a)^{-1} \text{d}^{-1}$) by ~64%, and by ~117% when higher $p\text{CO}_2$ was added. In contrast, HT
43 alone did not affect DPP and HTHC reduced it by ~15% at station D001. HT
44 enhanced the dark respiration rate ($\mu\text{g C}$ ($\mu\text{g chl } a)^{-1} \text{d}^{-1}$) by 64% at station SEATS, but
45 had no significant effect at station D001, and did not change the ratio of respiration to
46 photosynthesis at either station. HTHC did not affect dark respiration rate ($\mu\text{g C}$ (μg
47 $\text{chl } a)^{-1} \text{d}^{-1}$) at either station compared to LTLC. HTHC reduced the respiration to
48 photosynthesis ratio by ~41% at station SEATS but increased it ~42% at station D001.
49 Overall, our findings indicate that responses of coastal and offshore phytoplankton
50 assemblages in NSCS to ocean warming and acidification are contrasting, with the
51 pelagic phytoplankton communities being more sensitive to these two global change
52 factors.

53

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



56

57 **1 Introduction**


58 From 1979 to 2012, the mean global sea surface temperature increased at a
59 rate of ~ 0.12 °C per decade, based on in situ data records (IPCC, 2013). Particularly,
60 the warming rate in the South China Sea (~ 0.26 per decade) from 1982 to 2004 is
61 approximately 2 times faster than the global ocean mean rate (~ 0.14 per decade) from
62 1960 to 1990 (Casey and Cornillon, 2001; Fang et al., 2006). It is extremely likely
63 that more than half of the observed increase in global average surface temperature
64 from 1951 to 2010 was caused by the anthropogenic increase in greenhouse gas
65 concentrations and other anthropogenic forcings. Ocean warming is projected to
66 increase approximately by 0.6 °C (RCP 2.6) to 2.0 °C (RCP 8.5), in the upper 100 m by the
67 end of the 21st century (IPCC, 2013).

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

79 Increased atmospheric CO₂ is responsible for global warming and the ocean.
80 As a main sink for CO₂, the ocean has absorbed approximately 30% of the emitted
81 anthropogenic carbon dioxide (IPCC, 2013). Such a dissolution of CO₂ increases
82 seawater CO₂ partial pressure and bicarbonate ion levels and decreases pH and
83 carbonate ion concentrations, leading to ocean acidification (OA). By 2100, the
84 projected decline in global-mean surface pH is projected to be approximately 0.065 to



85 0.31, depending on the RCP scenario (IPCC, 2013). In terms of the South China Sea,
86 an accelerated trend of ocean acidification has been reported and the rate of pH
87 decline almost tripled between 1951 and 2000, compared to that between 1840 and
88 1950 (Liu et al., 2014).

89  CO₂ may be a potentially limiting factor for marine primary productivity
90 because of the low CO₂ level in seawater and the low affinity of the enzyme Rubisco
91 for dissolved CO₂ (Falkowski and Raven, 2013). In addition, CO₂ in seawater diffuses
92 approximately 10,000 times slower than in air, leading to its supply rate being much
93 lower than the demand of photosynthetic carbon fixation (Raven, 1993; Riebesell et
94 al., 1993). Although phytoplankton have evolved carbon-concentrating mechanisms
95 (CCMs) to cope with these problems (Giordano et al., 2005; Raven et al., 2012;
96 Reinfelder, 2011), increased CO₂ concentration may still be beneficial since energy
97 saved due to down-regulation of CCMs under elevated CO₂ can be utilized in other
98 metabolic processes (Gao et al., 2012a). Early laboratory and shipboard experiments
99 suggested that increased CO₂ indeed could enhance phytoplankton growth rates and
100 thus marine primary productivity (Riebesell et al., 1993; Hein and Sand-Jensen, 1997;
101 Schippers et al., 2004). Since then, neutral effects of increased CO₂ on growth of
102 phytoplankton assemblages have also been reported (Tortell and Morel, 2002; Tortell
103 et al., 2000). Furthermore, ocean acidification can even reduce primary productivity
104 of surface phytoplankton assemblages when exposed to incident solar radiation (Gao
105 et al., 2012b). Therefore, the effects of ocean acidification on marine primary
106 productivity remain controversial and its interactions with other environmental factors,
107 such as warming, solar UV radiation, hypoxia, etc. are incompletely understood (Gao
108 et al., 2012a; Häder and Gao, 2015; Mostofa, 2016).

109 Ocean warming and acidification, both caused by increasing atmospheric CO₂,
110 are proceeding simultaneously. The interactive or combined effects of warming and
111 OA could be completely different from that of either one stressor (Hare et al. 2007,
112 Feng et al., 2009; Gao et al., 2012a, Tatters et al. 2013). Several oceanographic cruises
113 and ship board experiments in the European sector of the Arctic Ocean, showed that



114 gross primary production increased with $p\text{CO}_2$ (145–2099 μatm) and the greatest
115 increase was observed in lower temperature regions, indicating CO_2 -enhanced
116 primary production in the European Arctic Ocean is temperature-dependent (Holding
117 et al., 2015).


118 The South China Sea (SCS) is located between the equator and 23.8°N , from
119 99.1 to 121.1°E , and is one of the largest marginal seas in the world, with a total area
120 of about $3.5 \times 10^6 \text{ km}^2$. Therefore, understanding the effects of ocean warming and
121 acidification on primary production in SCS would help us to define the role of
122 marginal seas in the global carbon cycle. However, only a very few studies on the
123 effects of ocean acidification or warming on primary productivity in the SCS have
124 been reported. Wu and Gao (2010) reported that CO_2 enrichment (700 μatm) did not
125 affect the assimilation number of phytoplankton at a near-shore site in SCS, compared
126 to the ambient CO_2 level (380 μatm). Gao et al. (2012b) demonstrated that increased
127 $p\text{CO}_2$ (800 or 1000 μatm) reduced primary productivity in off-shore stations of the
128 SCS. Therefore, we hypothesized that the effects of ocean acidification or/and
129 warming on primary productivity in SCS would be site-dependent. None of the
130 previous studies have examined co-effects of warming and increased CO_2 on primary
131 production in the SCS. In this study, to test this hypothesis we conducted shipboard
132 microcosm experiments at both near-shore and off-shore stations to determine the
133 combined effects of ocean warming and acidification on biomass, photosynthetic
134 carbon fixation, and dark respiration of phytoplankton assemblages in the SCS.

135 2 Methods

136 2.1 Experimental setup

137 The experiments were conducted at one off-shore station SEATS (17.9963°N ,
138 115.9621°E) and one near-shore station D001 (18.9740°N , 110.7166°E) in the NSCS
139 (Fig. 1). Surface seawater (0–2 m) was collected before sunrise with a 10 L
140 acid-cleaned plastic bucket, filtered (180 μm) to remove large grazers and dispensed
141 into nine microcosms. Microcosms consisted of cylindrical polymethyl methacrylate
142 tanks (32 L, 0.34 m water depth) with water-jacketed space for circulating cooled



143 water, allowing  photosynthesis active radiation (PAR, 400–700 nm), 63%
144 ultraviolet-A (UVA, 315–400 nm) and 6% ultraviolet-B (UVB, 280–315 nm)
145 transmission under incident solar radiation. Two levels of temperature (in situ, in situ
146 + 3 °C) and $p\text{CO}_2$ (ambient, ambient + 610 μatm) were used. There were three
147 triplicated treatments: low temperature and low $p\text{CO}_2$, LTLC; high temperature and
148 low $p\text{CO}_2$, HTLC; high temperature and high $p\text{CO}_2$, HTHC. The treatment of low
149 temperature and high $p\text{CO}_2$ was missing due to the lack of microcosms. Microcosm
150 temperature was controlled with circulating coolers (CTP-3000, EYELA, Japan) and
151 stable CO_2 equilibrium with the sea water (variation of $p\text{CO}_2 < 5\%$) was achieved
152 within 24 hours using a CO_2 enricher (CE-100, Wuhan Ruihua Instrument &
153 Equipment Ltd, China). The incubations were conducted for seven days for station
154 SEATS (Aug 3rd–9th 2012) and six days for station D001 (Aug 14th–19th 2012).


155 2.2 Solar radiance

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

160 2.3 Carbonate chemistry parameters

161 The seawater pH in the microcosm was recorded with a pH meter (FE20, Mettler
162 Toledo, Greifensee, Switzerland) every hour during the first day of incubation and
163 daily afterwards. The $p\text{CO}_2$ in seawater was maintained with the CO_2 enricher and
164 measured by an automated flowing $p\text{CO}_2$ measuring system (Model 8050, GO, USA).
165 Other carbonate system parameters were derived via CO2SYS (Pierrot et al., 2006),
166 using the equilibrium constants of K_1 and K_2 for carbonic acid dissociation (Roy et al.,
167 1993).

168 2.4 Chlorophyll *a* analysis

169 For the measurement of chlorophyll *a* ($\text{chl } a$),  500 mL of seawater were filtered
170 onto a Whatman GF/F glass fiber filter (25 mm). Then the filter was placed in 5 ml 93%
171 acetone at -20 °C for 24 h. $\text{Chl } a$ concentration was determined with a fluorometer
172 (Trilogy, Turner Designs, USA), following the protocol of Welschmeyer (1994).



173 2.5 Primary productivity and dark respiration

174 Seawater samples taken from each microcosm were dispensed into 50 mL quartz
175 tubes inoculated with 5 μCi (0.185 MBq) $\text{NaH}^{14}\text{CO}_3$ (ICN Radiochemicals, USA) and
176 incubated for 12 h and 24 h under natural light and day-night conditions. The
177 incubation temperature of every treatment was the same as the corresponding
178 microcosm treatment. After the incubation, the cells were filtered onto a Whatman
179 GF/F glass fiber filter (25 mm), which was immediately frozen and stored at -20°C
180 for later analysis. In the laboratory, each frozen filter was placed into a 20 mL
181 scintillation vial, exposed to HCl fumes overnight, and dried (55°C , 6 h) to expel
182 non-fixed ^{14}C (Gao et al., 2007). Then 3 mL scintillation cocktail (Perkin Elmer®)
183 was added to each vial and incorporated radioactivity was counted by a liquid
184 scintillation counting (LS 6500, Beckman Coulter, USA). The daytime primary
185 productivity (DPP) was defined as the amount of carbon fixation during 12 h
186 incubation. The dark respiration was defined as the difference in amount of carbon
187 fixation between 12 h and 24 h. Carbon fixation over 24 h was taken as daily net
188 primary productivity (NPP). Ratio of respiration to photosynthesis (R/P) was
189 expressed as that of respiratory carbon loss to daytime carbon fixation.

190 2.6 Statistical analyses

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

201 3 Results

202 The incident solar radiation during the experiment was recorded (Table 1). The



203 daytime (12 h) mean solar radiation ranged from 927 to 1592 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at
204 station SEATS while the lowest solar radiance was only 111 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$,
205 with the highest of 1583 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, at station D001. The average of
206 daytime mean solar radiation over the microcosm incubation at station SEATS was
207 15.52% higher than that at station D001.

208 The changes of the seawater carbonate system under different conditions are
209 shown in Table 2. At station SEATS, an increase of 3 °C in temperature (HTLC) did
210 not alter carbonate parameters except leading to enhanced CO_3^{2-} (Tukey HSD, $P =$
211 0.009). HTHC resulted in a significant decrease in CO_3^{2-} (Tukey HSD, $P < 0.001$) and
212 TA (Tukey HSD, $P = 0.016$) but an increase in CO_2 (Tukey HSD, $P < 0.001$)
213 compared with LTLC. The effect of temperature on carbonate parameters at station
214 D001 were similar to station SEATS, while HTHC increased HCO_3^- (Tukey HSD, $P =$
215 0.046) and did not affect TA (Tukey HSD, $P = 0.203$).

216 The in situ chl *a* level at station D001 ($0.37 \pm 0.05 \mu\text{g L}^{-1}$) was higher than that at
217 station SEATS ($0.15 \pm 0.02 \mu\text{g L}^{-1}$) (Independent samples t-test, $t = -7.483$, $df = 4$, P
218 $= 0.002$; Fig. 2a). After seven days incubation in the microcosms at station SEATS,
219 Tukey comparison ($P = 0.05$) showed that higher temperature ($0.46 \pm 0.04 \mu\text{g L}^{-1}$)
220 increased chl *a* compared with LTLC ($0.23 \pm 0.03 \mu\text{g L}^{-1}$) while HTHC (0.07 ± 0.01
221 $\mu\text{g L}^{-1}$) reduced it (Fig. 2b). The higher temperature ($0.72 \pm 0.07 \mu\text{g L}^{-1}$) also
222 increased chl *a* compared with LTLC ($0.41 \pm 0.05 \mu\text{g L}^{-1}$) at station D001 (Tukey
223 HSD, $P = 0.001$; Fig. 1b), but with no effect of HTLC ($0.41 \pm 0.04 \mu\text{g L}^{-1}$) (Tukey
224 HSD, $P = 0.988$).

225 The in situ DPP at station D001 ($49.4 \pm 4.5 \mu\text{g C L}^{-1} \text{d}^{-1}$ or $133.4 \pm 12.1 \mu\text{g C} (\mu\text{g}$
226 $\text{chl } a)^{-1} \text{d}^{-1}$) was dramatically higher than that at station SEATS ($5.1 \pm 0.5 \mu\text{g C L}^{-1} \text{d}^{-1}$
227 or $34.1 \pm 3.1 \mu\text{g C} (\mu\text{g chl } a)^{-1} \text{d}^{-1}$), whether normalized to volume of seawater
228 (Independent samples t-test, $t = -17.056$, $df = 4$, $P < 0.001$; Fig. 3a) or chl *a*
229 (Independent samples t-test, $t = -13.786$, $df = 4$, $P < 0.001$; Fig. 3b). After seven days
230 incubation in microcosms, the DPP normalized to volume of seawater under HTLC
231 ($33.2 \pm 4.8 \mu\text{g C L}^{-1} \text{d}^{-1}$) at station SEATS was significantly higher than that under
232 LTLC ($9.9 \pm 1.2 \mu\text{g C L}^{-1} \text{d}^{-1}$) and HTHC ($6.6 \pm 0.6 \mu\text{g C L}^{-1} \text{d}^{-1}$) (Tukey HSD, $P <$



233 0.001) while the difference between LTLC and HTHC was insignificant (Tukey HSD,
234 $P = 0.380$; Fig. 3c). The pattern at station D001 was similar to SEATS (Fig. 2c). When
235 DPP was normalized to chl *a*, the higher temperature increased primary productivity
236 from 43.2 ± 5.1 to $70.7 \pm 10.1 \mu\text{g C } (\mu\text{g chl } a)^{-1} \text{ d}^{-1}$ (Tukey HSD, $P = 0.014$) and
237 further to $93.9 \pm 8.1 \mu\text{g C } (\mu\text{g chl } a)^{-1} \text{ d}^{-1}$ (Tukey HSD, $P < 0.001$) when higher CO_2
238 was combined at station SEATS (Fig. 2d). In contrast, temperature did not affect DPP
239 (Tukey HSD, $P = 0.0924$) and HTHC reduced it from 150.3 ± 4.9 to $128.0 \pm 11.5 \mu\text{g}$
240 $\text{C } (\mu\text{g chl } a)^{-1} \text{ d}^{-1}$ (Tukey HSD, $P = 0.039$) at station D001 (Fig. 3d).

241 The in situ NPP at stations SEATS and D001 were $3.5 \pm 0.1 \mu\text{g C L}^{-1} \text{ d}^{-1}$ ($23.2 \pm$
242 $1.0 \mu\text{g C } (\mu\text{g chl } a)^{-1} \text{ d}^{-1}$) and $37.4 \pm 3.1 \mu\text{g C L}^{-1} \text{ d}^{-1}$ ($91.2 \pm 7.5 \mu\text{g C } (\mu\text{g chl } a)^{-1} \text{ d}^{-1}$)
243 respectively, which indicates that station D001 has higher NPP, irrespective of
244 normalizing to volume of seawater (Independent samples t-test, $t = -18.998$, $df = 4$, P
245 < 0.001 ; Fig. 4a) or chl *a* (Independent samples t-test, $t = -15.511$, $df = 4$, $P < 0.001$;
246 Fig. 4b). After a seven-day incubation in the microcosms, the higher temperature
247 increased NPP to $23.9 \pm 5.3 \mu\text{g C L}^{-1} \text{ d}^{-1}$ (Tukey HSD, $P = 0.001$) while HTHC ($5.5 \pm$
248 $0.4 \mu\text{g C L}^{-1} \text{ d}^{-1}$) did not change it (Tukey HSD, $P = 0.793$) compared with LTLC (7.2
249 $\pm 0.8 \mu\text{g C L}^{-1} \text{ d}^{-1}$). The effects of temperature and CO_2 on NPP at station D001 were
250 similar to that at station SEATS. When NPP was normalized to chl *a*, the higher
251 temperature increased NPP from 31.1 ± 3.5 to 50.9 ± 11.3 (Tukey HSD, $P = 0.044$)
252 and further to $78.3 \pm 5.9 \mu\text{g C } (\mu\text{g chl } a)^{-1} \text{ d}^{-1}$ with the addition of higher CO_2 (Tukey
253 HSD, $P < 0.001$) at station SEATS. On the other hand, neither HT (Tukey HSD, $P =$
254 0.707) nor HTHC (Tukey HSD, $P = 0.057$) affected NPP at station D001.

255 The in situ dark respiration rate at station SEATS was remarkably lower than that
256 at station D001 regardless of normalizing to volume of seawater (Independent
257 samples t-test, $t = -11.568$, $df = 4$, $P < 0.001$; Fig. 5a) or chl *a* (Independent samples
258 t-test, $t = -8.019$, $df = 4$, $P = 0.001$; Fig. 5b). The higher temperature increased dark
259 respiration rate from 2.8 ± 1.2 to $9.3 \pm 0.6 \mu\text{g C L}^{-1} \text{ d}^{-1}$ (Tukey HSD, $P < 0.001$) at
260 station SEATS while HTHC reduced it to $1.1 \pm 0.2 \mu\text{g C L}^{-1} \text{ d}^{-1}$ (Tukey HSD, $P =$
261 0.009 ; Fig. 5c). The higher temperature also promoted dark respiration rate at station
262 D001, from 16.9 ± 2.0 to $31.5 \pm 5.1 \mu\text{g C L}^{-1} \text{ d}^{-1}$ (Tukey HSD, $P = 0.007$) but HTHC



263 did not alter it (Tukey HSD, $P = 0.516$; Fig. 5c). When it was normalized to chl a ,
264 higher temperature still increased dark respiration rate from 12.0 ± 1.8 to 19.7 ± 1.4
265 $\mu\text{g C } (\mu\text{g chl } a)^{-1} \text{ d}^{-1}$ (Tukey HSD, $P = 0.006$) while the effect of temperature on
266 respiration rate at station D001 was insignificant (Tukey HSD, $P = 0.891$; Fig. 5d).
267 Compared to LTLC, HTHC did not affect respiration rate at either station (Tukey
268 HSD, $P = 0.131$ at station SEATS, $P = 0.348$ at station D001; Fig. 5d).

269 The in situ ratio of respiration to photosynthesis was $31.9 \pm 3.5\%$ at stations
270 SEATS, significantly higher than that ($24.2 \pm 1.3\%$) at station D001 (Independent
271 samples t-test, $t = 3.537$, $df = 4$, $P = 0.0024$; Fig. 6a). After seven days incubation in
272 microcosms, Tukey HSD comparison ($P = 0.05$) showed that higher temperature did
273 not affect the ratio of respiration to photosynthesis but HTHC reduced it from $27.8 \pm$
274 1.6% to $16.5 \pm 1.3\%$ at station SEATS (Fig. 6b). On the contrary, HTHC ($38.7 \pm 3.1\%$)
275 increased the ratio compared to LTLC ($27.3 \pm 2.4\%$), with insignificant effect of
276 temperature alone ($29.5 \pm 3.3\%$) at station D001 (Fig. 6b).

277 **4 Discussion**

278 **4.1 Effects of increased temperature and CO₂ on biomass**

279 The in situ chl a concentration at station D001 was higher than that at SEATS,
280 indicating more phytoplankton biomass at station D001. The chl a concentration
281 decreases with distance from the coast in the NSCS, mainly due to the change of
282 nutrients (Li et al., 2011). The higher temperature increased chl a concentration at
283 both stations, which might be attributed to increased active uptake of nutrients at the
284 elevated temperatures through enhanced enzymatic activities. Algal and
285 cyanobacterial growth commonly increases with temperature within a suitable range
286 (Goldman and Carpenter, 1974; Montagnes and Franklin, 2001; Savage et al., 2004;
287 Boyd et al. 2013) and optimum temperatures for growth of marine phytoplankton are
288 usually several degrees higher than the environmental temperatures (Thomas et al.,
289 2012).

290 On the other hand, the elevated CO₂ offset the positive effect of the higher
291 temperature on chl a at station D001, and even reduced chl a at station SEATS. High
292 CO₂ can sometimes enhance algal photosynthesis and growth, since CO₂ in seawater



293 is suboptimal for full operation of Rubisco enzymes (Wu et al., 2008 and references
294 therein). On the other hand, positive effects of elevated CO₂ can be affected by other
295 environmental factors. Gao et al. (2012b) demonstrated that rising CO₂ could enhance
296 growth of diatoms at low light intensity, but decrease it at high light intensity. It was
297 found that rising CO₂ concentration lowered the threshold for diatom growth above
298 which photosynthetic active radiation becomes excessive or stressful, owing to
299 reduced energy requirements for inorganic carbon acquisition at elevated CO₂ (Gao et
300 al., 2012b). In the present study, the mean daily solar radiation levels during
301 incubation were 1312 and 1136 μmol photons m⁻² s⁻¹ (Table 1), which were far above
302 the threshold light intensity reported for diatoms (Gao et al., 2012b). Consequently,
303 the higher CO₂ combined with the high solar radiation in summer of NSCS may have
304 imposed negative effects on phytoplankton biomass at station SEATS and D001. In
305 addition, the inhibitory effect of higher CO₂ on biomass was more significant at
306 station SEATS than D001. This can be attributed to the higher sensitivity of
307 picoplankton to high solar radiation (Li et al., 2011; Wu et al., 2015), which could be
308 delivered to the interaction of high solar radiation and high CO₂. As shown in Li et
309 al.'s (2011) study, the proportion of picoplankton in phytoplankton assemblages
310 increased with distance off the coasts. Therefore, the dominant species at station
311 SEATS are pico- and nano-phytoplankton, but micro-phytoplankton at station D001
312 (Table 3).

313 **4.2 Effects of increased levels of temperature and CO₂ on primary productivity**

314 The seawater volume-specific DPP at station D001 was higher than station
315 SEATS. This should result from both higher chl *a* concentration and chl *a*-specific
316 DPP at D001. It has been shown that smaller cells exist at SEATS than at D001 (Table
317 3). Smaller cells have been considered to have larger DPP, according to Laws's model
318 (1975). The discrepancy between our finding and Laws's model may be due to the
319 availability of nutrients. Laws's model was based on growth rates obtained from the
320 same nutrient level. Nevertheless, the nutrient level at station D001 is higher than at
321 SEATS (Table 3), leading to higher DPP. The higher temperature increased seawater
322 volume-specific DPP at both stations. This could be attributed to more biomass



323 produced at the warmer conditions, as indicated by chl *a*. High temperature enhanced
324 the chl *a*-specific DPP at station SEATS. However, no positive effects of temperature
325 were found on chl *a*-specific DPP at station D001. The differential effects of
326 temperature on chl *a*-specific DPP between the stations may be due to the
327 phytoplankton community composition, since cyanobacteria and/or
328 pico-phytoplankton have the strongest temperature response in terms of
329 photosynthetic carbon fixation compared to micro- and nano-phytoplankton
330 (Andersson et al., 1994). This finding contributes to the explanation of the dominance
331 of pico-phytoplankton in a warmer ocean (Chen et al., 2014; Montagnes and Franklin,
332 2001; Hare et al. 2007; Morán et al., 2010). HTHC reduced chl *a*-specific DPP at
333 station D001, but increased it at station SEATS. High CO₂ also reduced chl *a*-specific
334 DPP in a previous study, which could result from the interaction of high CO₂ and high
335 solar radiation during the summer in the NSCS (Gao et al., 2012b). The reason that
336 HTHC stimulated chl *a*-specific DPP at station SEATS may be due to a dramatic
337 decline in chl *a* concentration under the HTHC treatment.

338 **4.3 Effects of increased temperature and CO₂ on respiration**

339 The dark respiration rate of phytoplankton at station D001 was higher than that at
340 SEATS, regardless of normalizing to seawater or chl *a*. The respiration rate of algae or
341 cyanobacteria usually increases with cell size (López-Sandoval et al., 2014). Station
342 SEATS has more pico-phytoplankton, which would lead to a lower chl *a*-specific dark
343 respiration rate and then lower seawater volume-specific dark respiration, particularly
344 when combined with lower chl *a* level. The higher temperature increased seawater
345 volume-specific dark respiration at both stations, which could be related to increased
346 chl *a* concentration and/or enhanced respiratory carbon loss at the higher temperature.
347 The higher temperature also increased chl *a*-specific dark respiration rate at station
348 SEATS. This is consistent with Butrón et al.'s (2009) study, in which respiration rates
349 of phytoplankton along Nervión-Ibaizabal estuary showed a positive correlation with
350 temperature. Robarts and Zohary (1987) also found that respiration rate of
351 bloom-forming cyanobacteria was temperature-dependent, with optima over 25 °C.

352 HTHC reduced seawater volume-specific dark respiration at both stations, which



353 should be the consequence of the decreased chl *a* in this treatment. The higher
354 temperature increased chl *a*-specific dark respiration rate at station SEATS, but there
355 was no significant difference between HTHC and LTLC, indicating the higher CO₂
356 inhibited the chl *a*-specific dark respiration rate. Similarly, reduced respiration was
357 found in mesocosm studies (Spilling et al., 2016). In theory, higher CO₂ would inhibit
358 respiratory release of CO₂. Nevertheless, enhanced respiration rate at higher CO₂
359 conditions have been found in laboratory-grown diatoms (Wu et al., 2010),
360 coccolithophores (Jin et al., 2015), mixed phytoplankton assemblages (Jin et al.,
361 2015), and macroalgae as well (Zou et al., 2011). Such increased respiration has been
362 attributed to extra energy demand to cope with increased seawater acidity caused by
363 higher CO₂ (Gao and Campbell, 2014; Raven et al., 2014). Therefore, the effect of
364 increased CO₂ on phytoplankton respiration could be due to the combined effects of
365 CO₂ diffusive resistance and seawater acidity stress. Meanwhile, neither higher
366 temperature alone nor HTHC significantly affected chl *a*-specific dark respiration rate
367 at station D001. One possible reason could be that larger cells are less sensitive to
368 CO₂ diffusive resistance and acidic stress due to thicker diffusion boundary layers
369 around the cells. This hypothetical explanation is worthy of future testing.

370 **4.4 Effects of increased temperature and CO₂ on R/P**

371 Laws's model (1975) has suggested that large phytoplankton cells are likely to
372 consume a smaller fraction of their biomass to compete with small phytoplankton
373 cells in terms of the growth rate, considering small cells have higher gross production
374 rates. Our finding that phytoplankton in station SEATS had a higher R/P than station
375 D001 confirms Laws's model. It was theorized that autotrophic respiration is more
376 sensitive to temperature than photosynthesis and the ratio of R/P was predicted to
377 increase with temperature (Ryan, 1991; Woodwell, 1990; Woodwell et al., 1983).
378 However, the assumption that plant respiration is highly temperature dependent was
379 primarily based on short-term (a few minutes or hours) responses of plants to changes
380 of temperature (Gifford, 1994). In long-term experiments (days or months), the
381 increase in respiration with temperature tended to disappear due to acclimation
382 (Gifford, 1995; Jones, 1977; Reich et al., 2016; Slot and Kitajima, 2015; Ziska and



383 Bunce, 1998). Photosynthetic acclimation to warming is variable (Chalifour and
384 Juneau, 2011; Hancke and Glud, 2004; Schlüter et al., 2014; Staehr and Birkeland,
385 2006). However, a general acclimation response to long-term increased temperature is
386 a rise in optimal temperature of photosynthesis (Gunderson et al., 2010; Kattge and
387 Knorr, 2007; Staehr and Birkeland, 2006). Such shifts in the temperature response of
388 photosynthesis and respiration via physiological acclimation can dampen the increase
389 in R/P at high temperatures, or climate warming would not increase R/P (Reich et al.,
390 2016). In other words, phytoplankton would down-regulate the high sensitivity of
391 respiration to temperature, and maintain a relatively consistent net primary production
392 and hence food web structure in a warming ocean. Although the ratio of R/P did not
393 vary with increased temperature at both stations in our work either, both
394 photosynthesis and respiration were enhanced by the higher temperature. The constant
395 ratio was due to the similar amplitude of increase in photosynthesis and respiration.
396 The incubation period of 7–8 days might not be enough for phytoplankton to
397 acclimate to the increased temperature completely, and therefore the stimulated effects
398 of high temperature on photosynthesis and respiration were still notable. Opposite
399 effects of HTHC on R/P were detected at station SEATS and D001, negative at
400 SEATS and positive at D001. This can be attributed to differential responses of
401 photosynthesis at both stations to HTHC, considering the responses of respiration
402 were similar.

403 **5 Conclusions**

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



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- 627
- 628



629 Table 1. The daytime (12 h) mean solar radiation (PAR, $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) during
 630 incubation at stations SEATS and 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		

631 ^aThe dates for measurements of photosynthetic carbon fixation in situ. ^bThe dates for
 632 measurements of photosynthetic carbon fixation experiencing temperature and $p\text{CO}_2$
 633 treatments. ^cMean represents the average of daytime mean solar radiation over seven
 634 or six days microcosm incubation.

635



636 Table 2. Parameters of the seawater carbonate system at different incubation
 637 conditions. Measurements and estimation of the parameters were described in
 638 Methods. Data are the means \pm SD ($n = 3$). LTLC, low temperature and low $p\text{CO}_2$;
 639 HTLC, high temperature and low $p\text{CO}_2$; HTHC, high temperature and high $p\text{CO}_2$.
 640 DIC = dissolved inorganic carbon, TA = total alkalinity. Different superscript letters
 641 indicate significant differences between treatments within one station.

642

	SEATS			D001		
	LTLC	HTLC	HTHC	LTLC	HTLC	HTHC
Temperature (°C)	30.5 \pm 1.0	33.5 \pm 1.0	33.5 \pm 1.0	28.5 \pm 1.0	31.5 \pm 1.0	31.5 \pm 1.0
pH _T	8.07 \pm 0.01	8.05 \pm 0.01	7.68 \pm 0.01	8.02 \pm 0.01	8.01 \pm 0.01	7.68 \pm 0.01
$p\text{CO}_2$ (μatm)	390.0 \pm 19.5	390.0 \pm 19.5	1000.0 \pm 70.0	420.0 \pm 25.2	420.0 \pm 25.2	1030.0 \pm 60.0
DIC ($\mu\text{mol kg}^{-1}$)	2056.4 \pm 49.2 ^a	1986.6 \pm 47.1 ^a	1999.9 \pm 91.4 ^a	1969 \pm 67.7 ^a	1896.5 \pm 64.8 ^a	2039.1 \pm 69.5 ^a
HCO ₃ ⁻ ($\mu\text{mol kg}^{-1}$)	1758.4 \pm 47.5 ^{ab}	1681.2 \pm 45.4 ^a	1838.4 \pm 86.5 ^b	1719.2 \pm 63.7 ^a	1640.9 \pm 60.7 ^a	1882.3 \pm 66.4 ^b
CO ₃ ²⁻ ($\mu\text{mol kg}^{-1}$)	288.2 \pm 1.2 ^b	296.2 \pm 1.2 ^c	138.1 \pm 3.3 ^a	238.8 \pm 3.4 ^b	245.3 \pm 3.5 ^b	131.6 \pm 1.7 ^a
CO ₂ ($\mu\text{mol kg}^{-1}$)	9.8 \pm 0.5 ^a	9.1 \pm 0.5 ^a	23.5 \pm 1.7 ^b	11.0 \pm 0.7 ^a	10.3 \pm 0.7 ^a	25.2 \pm 1.5 ^b
TA ($\mu\text{mol kg}^{-1}$)	2443.5 \pm 47.9 ^a	2387.7 \pm 45.8 ^a	2170.7 \pm 92.0 ^b	2294.2 \pm 68.6 ^a	2260.0 \pm 33.9 ^a	2199.4 \pm 68.5 ^a

643



644 Table 3. Physical, chemical, and biological parameters at stations SEATS and D001.

645 SST: seawater surface temperature; N: $\text{NO}_3^- + \text{NO}_2^-$ ($\mu\text{mol L}^{-1}$); P: PO_4^{3-} ($\mu\text{mol L}^{-1}$).

646 Data of nutrients and phytoplankton composition are derived from literatures.

647

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

648 ^aDu et al. (2013); ^bWu et al. (2003); ^cLi et al. (2011); ^dLi et al. (2014); ^eZhang et al. (2014).

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658 **Figure captions**

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

660 **Figure 2.** Chl *a* concentration in situ (a) and after temperature and *p*CO₂ treatments in
661 microcosms (b). The microcosm incubations lasted seven days at station SEATS and
662 six days at station D001. The error bars indicate the standard deviations (*n* = 3). The
663 different letters above the error bars represent significant (*P* < 0.05) differences
664 between stations in panel (a) and between treatments in panel (b).

665 **Figure 3.** Daytime primary productivity (DPP) in situ (a, b) and after temperature and
666 *p*CO₂ treatments in microcosms (c, d). The microcosm incubations lasted seven days
667 at station SEATS and six days at station D001. The error bars indicate the standard
668 deviations (*n* = 3). The different letters above the error bars represent significant (*P* <
669 0.05) differences between stations in panels (a, b) and between treatments in panels (c,
670 d).

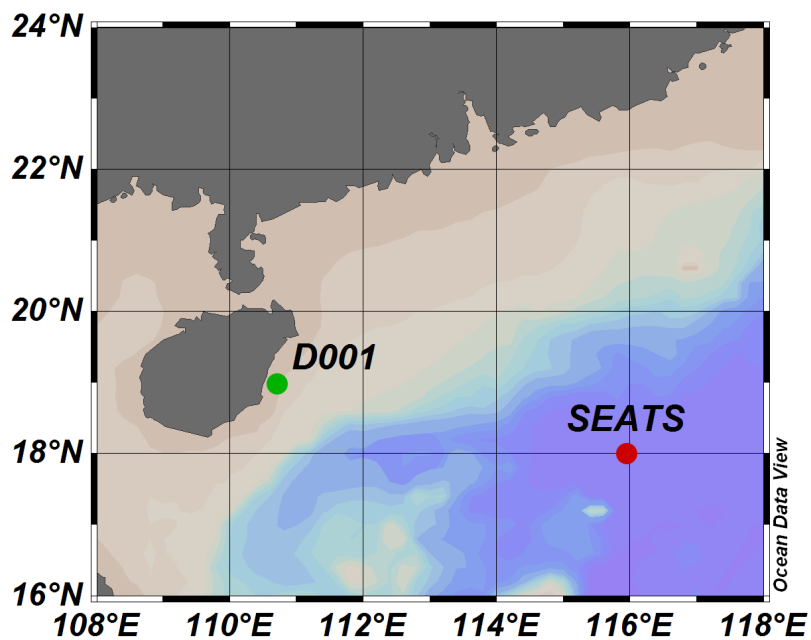
671 **Figure 4.** Net primary productivity (NPP) in situ (a, b) and after temperature and
672 *p*CO₂ treatments in microcosms (c, d). The microcosm incubations lasted seven days
673 at station SEATS and six days at station D001. The error bars indicate the standard
674 deviations (*n* = 3). The different letters above the error bars represent significant (*P* <
675 0.05) differences between stations in panels (a, b) and between treatments in panels (c,
676 d).

677 **Figure 5.** Dark respiration in situ (a, b) and after temperature and *p*CO₂ treatments in
678 microcosms (c, d). The microcosm incubations lasted seven days at station SEATS and
679 six days at station D001. The error bars indicate the standard deviations (*n* = 3). The
680 different letters above the error bars represent significant (*P* < 0.05) differences
681 between stations in panels (a, b) and between treatments in panels (c, d).

682 **Figure 6.** The ratio of respiration to photosynthesis in situ (a, b) and after temperature
683 and *p*CO₂ treatments (c, d) in microcosms. The microcosm incubations lasted seven
684 days at station SEATS and six days at station D001. The error bars indicate the
685 standard deviations (*n* = 3). The different letters above the error bars represent
686 significant (*P* < 0.05) differences between stations in panels (a, b) and between
687 treatments in panels (c, d).



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Figure 1

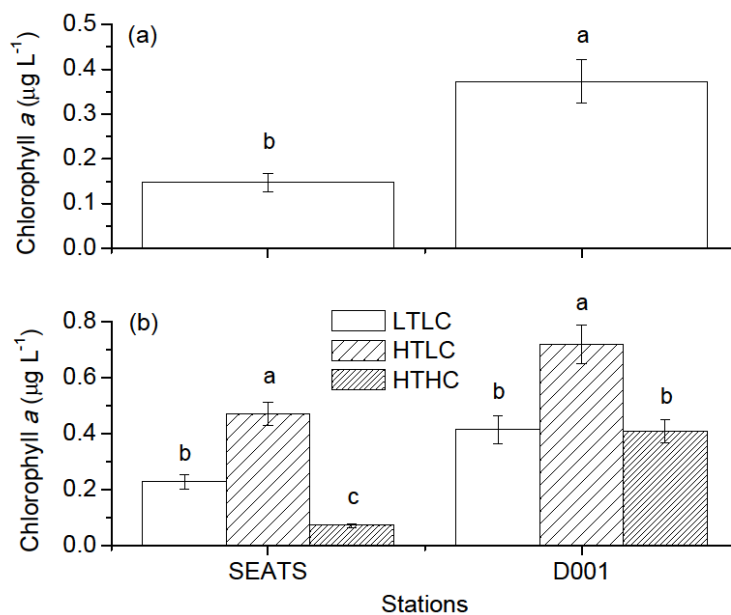


Figure 2

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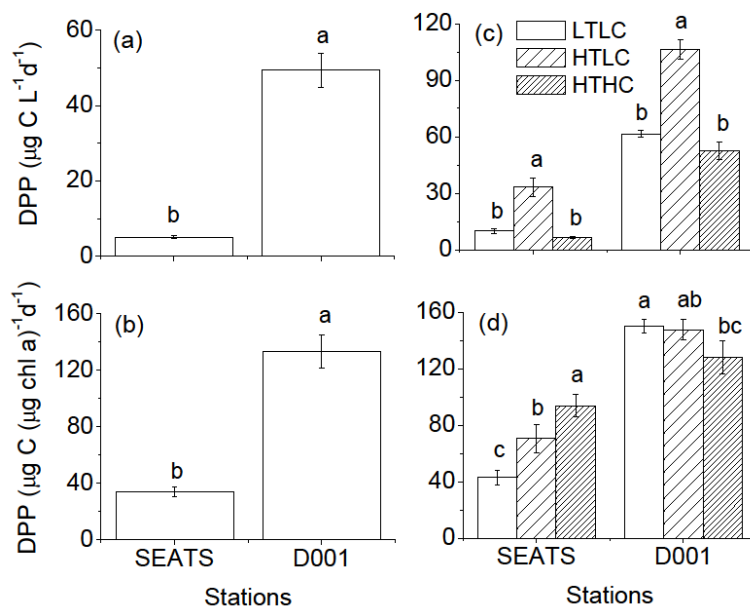


Figure 3

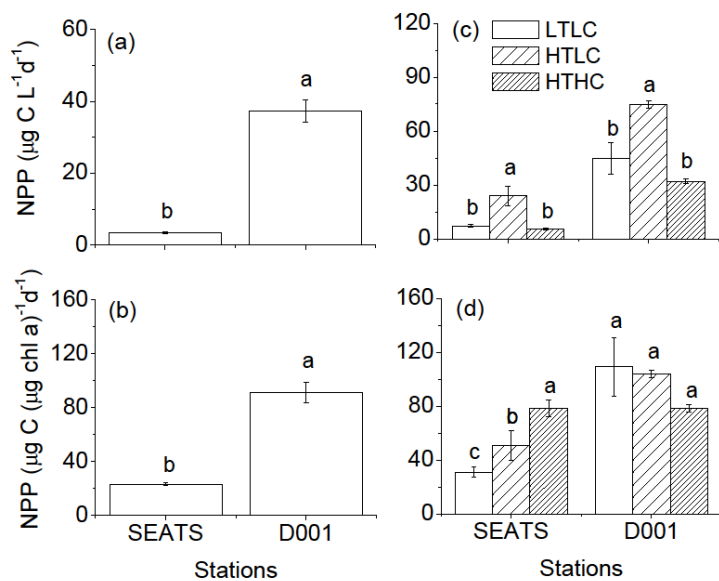


Figure 4

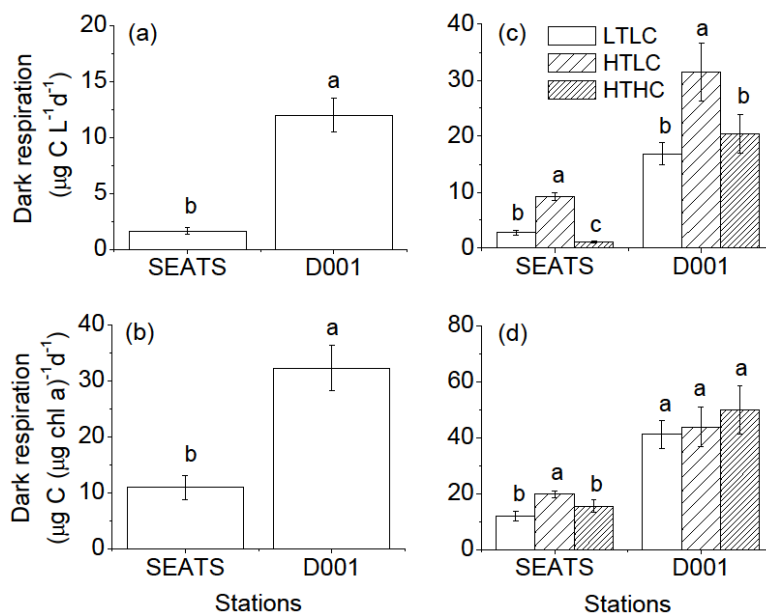


Figure 5

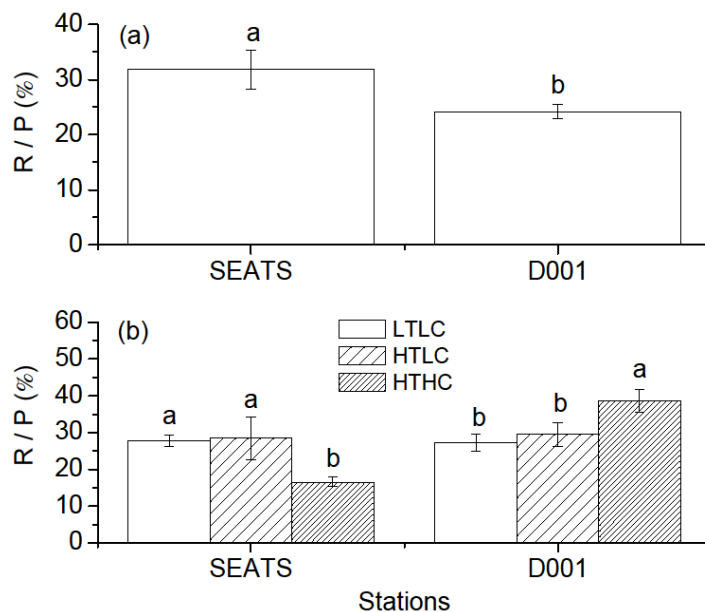


Figure 6