

Author's Response to Anonymous Referee 2

Anonymous Referee #2

General comment:

The authors address the combined effect of ocean acidification, nutrient availability and presence of UV irradiation on the marine diatom *Phaeodactylum tricornutum*. Previous studies have considered the single effect of the tested factors on phytoplankton. This work goes a step further by analysing (using a set of appropriate and well described methods) the effects of the interactions between factors. According to the results presented, the expected suite of environmental changes might have important implications for primary production and biogeochemical cycling. Thus, the manuscript gives important insight on an actual subject, calling attention to the importance of species response to multiple stressors. *Phaeodactylum tricornutum* isn't a sensitive and typical diatom. However, existing information on this species provides a good basis to a study such as this with so many variables. Although, this manuscript reveals important data such as that ocean acidification and UVB showed a stronger effect under low nutrient concentrations, it would benefit from synthesizing and clarifying the most significant conclusions in the Abstract. Moreover, the manuscript would benefit from additional references in the introduction and discussion sections to support statements concerning the various effects of ocean acidification on natural communities and / or other diatoms.

Response: We are glad to know the work is recognized as a good contribution to multiple stressors studies in relation to global climate change. We have added some further references and discussion in the introduction and discussion according to the suggestions (page 5, line 94-96; page 19-20, line 459-473). We have also added a statement to the Abstract that sets out the main conclusions in a clearer way (page 2, line 38-40).

Specific points:

The title reflects the content of the paper. The Abstract of the manuscript could be more clear on the main results and their repercussions. Finally, figures have a considerable amount of information, becoming difficult to quickly understand.

Response: We have added a statement to the Abstract that sets out the main conclusions in a clearer way (page 2, line 38-40). We also have added one-line titles (page 37, line 847-848, 856, 865; page 38, 882-883, 890-891) of the main features of each figure to the legend, which we feel will be help to improve understanding by the readers.

Technical points:

Introduction

- **P. 5, lines 81 to 85:** Introduce CO₂ range or the concentration of the referred enhanced CO₂ for comparison.

Response: The CO₂ concentrations of the studies referred to were added.

- **P. 7, lines 111 to 121:** The connection between the sentences should be more fluid.

Response: We re-worded the sentences according to the suggestions.

Material and Methods

- **P. 8, line 143 to 144:** Provide information on nitrate range during the 24h of incubations between dilutions. This will be useful to show nitrate limitation throughout the experiment.

Response: The referee makes a good point. According to our pre-experiment the initial nitrate concentration of 10 $\mu\text{mol L}^{-1}$ could be totally consumed (0-10 $\mu\text{mol L}^{-1}$); and the initial nitrate concentration of 110 $\mu\text{mol L}^{-1}$ treatment, the nitrate ranged from ca. 85-110 $\mu\text{mol L}^{-1}$ during the culture. We have added the descriptions in page 7-8, line 169-177.

- **P. 9, line 165:** Subtitle "2.3 Radiation treatments" should be more ambiguous in order to include all treatments referred in the text (CO₂ and nitrate).

Response: We have changed the subtitle "Radiation treatments" to "Radiation treatments under the solar simulator".

- **P. 9, lines 171 to 175:** Facilitate understanding of the nomenclature given to the treatments by inverting their order of appearance in the text.

Response: Corrected.

- **P. 10, line 181:** Specify "middle of the photic...".

Response: The light intensity of PAR level under solar simulator was ca. 190.11 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ which is close to 25-42% of incident surface solar PAR levels in the SCS (22-36 m depth in South China Sea, SEATS station), based on the vertical profiles of PAR at the SEATS station (Gao et al., 2012). We added the description in page 9, line 213-215 as suggested.

- **P. 12, lines 238 to 240:** Order of the parameters of subtitle 2.6 could follow their corresponding order in the subtitle.

Response: We re-ordered the subtitle to "Cells counts and chlorophyll a measurements" of 2.6 as suggested.

Discussion

- **P. 18, line 370 to 373:** Explain reasoning and potential causes for this statement.

Response: As the reviewer pointed out, the statement that "OA appeared to counteract UVB-induced damage under NO₃⁻ replete conditions, but when combined with decreased availability of nitrate, it increased the diatom's sensitivity to UV radiation." could be explained as follows:

Many studies have shown that the sensitivity of cells to high levels of PAR and UV under OA condition could be stimulated and then induce higher inhibition of

photosynthesis or growth rate (Sobrino et al., 2008; Xu and Gao et al., 2012; Gao et al., 2012). However, this phenomenon is not always found in all species especially when the intensity of PAR or UV is not that high. For example, a recent study reported that the unicellular chlorophyte (*Dunaliella tertiolecta*) acclimated to high CO₂ under nutrient replete conditions could alleviate the stress induced by high PAR and UV (García-Gómez et al., 2014). This could be due to the energy saving as a result of down-regulation of CCM activity. However, in the present study, we did not find that the synergistic effects of OA and UVR induced a higher inhibition at the light intensity of PAR+ UVA+UVB (44.11 + 14.19 + 0.75 Wm⁻²) used, than found under LC. This may be due to the light intensity of PAR or UVR not being high enough to exceed the energy dissipating capacity of the cells. Furthermore, under high N the nutrient supply would be sufficient to support the repair processes of UV or high PAR induced damage.

We have made a description as the reviewer suggested on page 20, line 467-473.

- **P. 20, line 398:** UVR would be easier to read as presented in other parts of the text, specifically "UV radiation".

Response: Corrected.

Figure captions

- **P. 36, Figure 1 (Line 734):** Replace "in *P.tricornutum*" by "of *P. tricornutum*"

Response: Corrected.

- **P. 36, Figure 1 (Line 735):** a of Chl a should be in italic and one space after Chl a should be removed

Response: Corrected.

- **P. 36, Figure 1 (Line 738):** Standard errors are commonly referred as SE not SD.

Response: We apologise for this error in stating SE instead of SD. We should have used 'SD' throughout as all data are expressed as means +/- standard deviation. This correction has now been made.

- **P. 36, Figure 1 (Line 739):** Provide further information concerning the letters that indicate significant differences.

Response: To make a clear indication of significance, we changed the description from "Different letters indicated significant differences among different treatments at $P < 0.05$ level." into "Treatments with the same lowercase superscript letters, means the difference is not significant. In contrast, treatments with different lowercase superscript letters indicate the difference is significant ($P < 0.05$ level)." in page 34 line 783-785.

- **P. 36, Figure 2 (Line 747):** Explain meaning of dashed line.

Response: The dashed line indicates the time point at which the culture was moved from the solar simulator (P, PA and PAB) to the culture light level for recovery. We have

added a simple description of the dashed line in Page 37, line 861.

1 **Nitrate limitation and ocean acidification interact with UV-B to reduce**
2 **photosynthetic performance in the diatom *Phaeodactylum tricornutum***

3
4 **Running Title:** Combined effects of NO₃⁻, OA and UV

5
6 Wei Li^{1,2}, Kunshan Gao^{1*}, John Beardall³

7
8 ¹ State Key Laboratory of Marine Environmental Science, Xiamen University
9 (Xiang-An campus), Xiamen, Fujian, 361102 China

10 ²College of Life and Environmental Sciences, Huangshan University, 245041,
11 Huangshan, China

12 ³School of Biological Sciences, Monash University, Clayton, VIC 3800, Australia

13
14 *Author for correspondence: ksgao@xmu.edu.cn (Kunshan Gao)

23 **Abstract**

24 It has been proposed that ocean acidification (OA) will interact with other
25 environmental factors to influence the overall impact of global change on biological
26 systems. Accordingly we investigated the influence of nitrogen limitation and OA on
27 the physiology of diatoms by growing the diatom *Phaeodactylum tricornutum* Bohlin
28 under elevated (1000 μatm , HC) or ambient (390 μatm , LC) levels of CO_2 with
29 replete (110 $\mu\text{mol L}^{-1}$, HN) or reduced (10 $\mu\text{mol L}^{-1}$, LN) levels of NO_3^- and
30 subjecting the cells to solar radiation with or without UV irradiance to determine their
31 susceptibility to UV radiation ([UVR](#), 280-400 nm). Our results indicate that OA and
32 UVB induced significantly higher inhibition of both the photosynthetic rate and
33 quantum yield under LN than under HN conditions. UVA or/and UVB increased the
34 cells' non-photochemical quenching (NPQ) regardless of the CO_2 levels. Under LN
35 and OA conditions, activity of superoxide dismutase and catalase activities were
36 enhanced, along with the highest sensitivity to UVB and the lowest ratio of repair to
37 damage of PSII. HC-grown cells showed a faster recovery rate of yield under HN but
38 not under LN conditions. [We conclude therefore that nutrient limitation makes cells](#)
39 [more prone to the deleterious effects of UV radiation and that HC conditions \(ocean](#)
40 [acidification\) exacerbate this effect.](#) The finding that nitrate limitation and ocean
41 acidification interact with UV-B to reduce photosynthetic performance of the diatom *P.*
42 *tricornutum* implies that ocean primary production and the marine biological C pump
43 will be affected by OA under multiple stressors.

删除的内容: the

44

46 **Key words:** CO₂, diatom, multiple stressors, nutrients, ocean acidification,
47 photosynthesis, UV radiation

48 **Abbreviations:** DIC, dissolved inorganic carbon; NPQ, non-photochemical
49 quenching; SOD, superoxide dismutase; CAT, catalase; Inh_{UVR}, inhibition due to UVR;
50 *r*, repair rate; *k*, damage rate; CCMs, CO₂ concentrating mechanisms.

51

52 **1 Introduction**

53 Increasing atmospheric levels of CO₂ and the associated dissolution of CO₂ into
54 the oceans has resulted in ocean acidification (OA), with increased levels of pCO₂,
55 HCO₃⁻ and H⁺ and decreased CO₃²⁻ concentration. The acidity of surface oceans has
56 increased by 30% (lowered pH by 0.1 unit) since the Industrial Revolution and is
57 expected to increase by 100-150% (0.3-0.4 pH units) by the year 2100 (Orr et al.,
58 2005). At the same time, increased sea surface temperatures are predicted to cause a
59 shoaling of the surface mixed layer, which in turn will lead to enhanced exposure to
60 sunlight (both as photosynthetically active radiation (PAR) and as UVR). This
61 enhanced stratification will also decrease upward transport of nutrients from deeper,
62 nutrient rich layers, leading to more frequent/marked nutrient limitation (Cerreño et
63 al., 2008). Global change is thus likely to cause changes in a multiplicity of factors
64 that influence phytoplankton growth and it is thus critical to examine OA in the
65 context of interactive effects with these other environmental drivers (Boyd, 2011).

66 Increased availability of CO₂ in seawater appears in some cases to bring a low
67 level of benefit to growth and photosynthesis of natural phytoplankton populations

删除的内容: radiation

69 (Riebesell and Tortell, 2011) and references therein), though in most cases laboratory
70 experiments have shown little effect of OA alone (Doney et al., 2009). However, the
71 effects can differ according to changes in solar radiation and/or other physical or
72 chemical factors (Gao et al., 2012a). Increased acidity of seawater may lead to
73 physiological stress (Pörtner and Farrell, 2008) and affect phytoplankton nutrient
74 uptake (Beman et al., 2011; Shi et al., 2012). Therefore, OA could most likely result
75 in differential effects on different photosynthetic organisms or under different
76 environmental conditions (Gao, 2011).

77 Diatoms account for about 20% of the total global primary production
78 and about 40% of that in the oceans (Granum et al., 2005). Early reports
79 suggested that growth of diatom species could be limited by the availability of CO₂
80 (Riebesell et al., 1993). However, the growth rate of diatom-dominated natural
81 phytoplankton populations was not affected by CO₂ enrichment to 800 µatm (Tortell,
82 2000), and not all diatom species were sensitive to seawater pCO₂ rise under
83 nutrient-replete conditions in a mesocosm study (Kim et al., 2006). In laboratory
84 experiments, growth of *Skeletonema costatum* was not stimulated by elevated CO₂
85 (800 µatm) (Chen and Gao, 2011). *Phaeodactylum tricorutum* grown under
86 nitrate-limited conditions also showed no enhancement of growth under high CO₂
87 (1000 µatm) (Li et al., 2012a). Nevertheless, in other work, the diatoms
88 *Phaeodactylum tricorutum* (1000 µatm) (Wu et al., 2010) and *Attheya* sp. (670 µatm)
89 (King et al., 2011) showed enhanced growth rate in nutrient replete conditions under
90 elevated CO₂ levels. These variable findings reflect physiologically differential

91 responses among different species or under different experimental or environmental
92 conditions. Changes in light intensity can lead to enhanced, unaffected or inhibited
93 growth rates under OA conditions, even for the same diatom species (Gao et al.,
94 2012b). Recently, microcosm studies have shown that the species abundance and
95 physiological responses (eg. Chl *a*, DNA damage, ROS, photosynthetic efficiency)
96 could be regulated by nutrients and light availability under high CO₂ conditions
97 (Neale et al., 2014; Sobrino et al., 2014). Therefore, the effects of OA should be
98 considered in the context of the influence of multiple factors, such as temperature,
99 nutrient status, light and UV_R (Boyd, 2011; IPCC, 2011; Gao et al., 2012a).

删除的内容: a series

删除的内容: y

删除的内容: showed

带格式的: 字体: 倾斜

带格式的: 下标

删除的内容: radiation

100 Solar UVB radiation (280-315 nm), which is increasing due to interactions of
101 global change and ozone depletion (Häder et al., 2011), is known to damage DNA
102 (Buma et al., 2003; Gao et al., 2008), lower photosynthetic rates (Helbling et al.,
103 2003), perturb the uptake of nutrients (Hessen et al., 2008) and alter morphological
104 development (Wu et al., 2005) of phytoplankton. In contrast, under moderate levels of
105 solar radiation, solar UVA radiation (315-400 nm) is known to stimulate
106 photosynthesis (Gao et al., 2007), signaling (Cashmore, 1998) and photo-repair of
107 UVB-induced damage (Buma et al., 2003) in phytoplankton. Previously, it was shown
108 that UV-induced inhibition of dinoflagellates was lower under nutrient replete
109 conditions but higher under nutrient limitation, due to less efficient repair resulting
110 from lowered nutrient availability (Litchman et al., 2002). Similar enhancement of
111 UVB impacts under nutrient (N, P) limitation were shown for a green microalga,
112 *Dunaliella tertiolecta* (Shelly et al., 2002; Heraud et al., 2005). Recently, OA was

117 found to enhance UVB-induced damage to a red tide alga, *Phaeocystis globosa*,
118 leading to a greater decrease in growth rate and photochemical yield under 1000 μatm
119 CO_2 (Chen and Gao, 2011).

120 Marine phytoplankton often experience nutrient limitation in offshore waters;
121 with progressive ocean warming, such limitation will be intensified due to decreased
122 depth of the surface mixed layer (enhanced stratification) (Cerreño et al., 2008).

123 Combined effects of nutrient levels and CO_2 have been reported in many studies. For

124 example, photosynthetic carbon fixation of the coccolithophorid *Emiliana huxleyi*

125 was enhanced under high light and low nitrogen conditions when the seawater CO_2

126 concentration was raised to 2000 μatm (Leonardos and Geider, 2005). However,

127 increased seawater CO_2 concentration also showed antagonistic effects with iron in

128 modulating (down- or up-regulating) primary production of marine phytoplankton in

129 the Gulf of Alaska (a nutrient replete but low chlorophyll area) (Hopkinson et al.,

130 2010). In some toxin producing species, for example the dinoflagellate *Karlodinium*

131 veneficum, toxicity was enhanced under high CO_2 and low phosphate conditions (Fu

132 et al., 2010). However, to the best of our knowledge, there is little information

133 concerning the combined effects of OA and NO_3^- limitation on diatoms and their

134 susceptibility to damage from solar UV_R (280-400 nm).

135 Nutrient availability can influence phytoplankton responses to UV and to

136 CO_2 -induced seawater acidification. Theoretically, increased seawater acidity can

137 perturb intracellular acid-base balance and thus lead to differential interactions

138 between nutrients and solar UV_R. In this study, we hypothesize that reduced

带格式的：下标

删除的内容：P

删除的内容：t

删除的内容：on

删除的内容：In the

删除的内容：take

删除的内容：as an example

删除的内容：the,

删除的内容：radiation

删除的内容：UVR,

删除的内容：radiation

149 availability of NO_3^- under OA would affect the photosynthetic performance under
150 solar radiation with or without UVR. We used the diatom *Phaeodactylum tricorutum*,
151 to test this hypothesis.

删除的内容: ,

删除的内容: whose genome has been completely sequenced (<http://genome.jgi-psf.org/Phatr2/Phatr2.home.html>),

带格式的: 突出显示

153 2 Materials and methods

154 2.1 Growth conditions

155 The diatom *Phaeodactylum tricorutum* Bohlin (strain CCMA 106), isolated
156 from the South China Sea (SCS) and maintained in the Center for Collections of
157 Marine Bacteria and Phytoplankton (CCMBP) of the State Key Laboratory of Marine
158 Environmental Sciences (Xiamen University), was grown mono-specifically in
159 artificial seawater enriched with Aquil medium (Morel et al., 1979). Cells were
160 cultured in 500 mL vessels containing 250 mL medium under two levels of NO_3^- (110
161 $\mu\text{mol L}^{-1}$, HN; 10 $\mu\text{mol L}^{-1}$, LN) and aerated with ambient (outdoor) air (LC, 390
162 μatm) or elevated (1000 μatm , HC) CO_2 levels within a plant CO_2 chamber
163 (HP1000G-D, Ruihua instrument & equipment Co. Ltd, China). Gas flow rate was
164 300 ml min^{-1} , and the CO_2 concentrations varied by less than 3% of the target value.
165 The low NO_3^- level of 10 $\mu\text{mol L}^{-1}$ was based on its concentration range (ca. 0-20
166 $\mu\text{mol L}^{-1}$) in the oligotrophic SCS, from where the diatom strain was isolated.
167 Dilutions were made every 24 h, so that the seawater carbonate system was
168 maintained stable under each CO_2 level within the cell density range of 6×10^4 to $3 \times$
169 10^5 cells ml^{-1} (exponential growth phase). According to the pre-experiment, the initial
170 nitrate concentration of 10 $\mu\text{mol L}^{-1}$ could be totally consumed (0-10 $\mu\text{mol L}^{-1}$); and the

176 | initial nitrate concentration of 110 $\mu\text{mol L}^{-1}$ treatment, the nitrate ranged from ca. 85-110
177 | $\mu\text{mol L}^{-1}$ during the culture. The cells were grown at 70 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (cool
178 | white fluorescent tubes) under a 12L: 12D photoperiod for at least 10 generations
179 | before being used for the solar radiation treatments described below. Three
180 | independent cultures were grown at each condition.

181

182 | **2.2 Determination of seawater carbonate system parameters**

183 | The pH in the cultures was determined daily during the light period with a pH
184 | potentiometric titrator (DL15, Mettler-Toledo, Schwerzenbach, Switzerland), which
185 | was calibrated with NBS (National Bureau of Standards) buffer solutions (Hanna).
186 | DIC (dissolved inorganic carbon) was estimated with an automatic system (AS-C3,
187 | Apollo Scitech) linked to an infrared gas detector (Li-Cor 7000, Li-Cor). DIC, pH,
188 | nutrient concentrations (phosphate, 10 $\mu\text{mol L}^{-1}$; silicate, 100 $\mu\text{mol L}^{-1}$), salinity (35)
189 | and temperature (20°C) were used to calculate the parameters of the seawater
190 | carbonate system (HCO_3^- , CO_3^{2-} , CO_2 and TA) using the CO_2 system analyzing
191 | software CO_2SYS (Lewis and Wallace, 1998) as described previously (Li et al.,
192 | 2012a). The carbonic acid dissociation constants (K_1 and K_2) used were those of Roy
193 | et al. (1993), and that for boric acid (K_B) was from Dickson (1990).

194

195 | **2.3 Radiation treatments under the solar simulator**

196 | To determine the effects of growth conditions on the sensitivity of carbon fixation
197 | and chlorophyll fluorescence to short-term exposure to UV~~R~~, *P. tricornutum* cells,

删除的内容: the

删除的内容: radiation

200 grown under LC-LN (low CO₂ + low nitrate), HC-LN (high CO₂ + low nitrate),
201 LC-HN (low CO₂ + high nitrate) and HC-HN (high CO₂ + high nitrate) conditions,
202 were exposed for 1 h to different radiation treatments with or without UV_R, as
203 follows: 1) P treatment, tubes wrapped with Ultraphan film 395 (UV Opak, DigeFra),
204 being exposed to PAR alone; 2) PA treatment, tubes wrapped with Folex 320
205 (Montagefolie, Folex, Dreieich, Germany), receiving wavelengths above 320 nm
206 (PAR+UVA); 3) PAB treatment, tubes wrapped with Ultraphan Film 295 (DigeFra,
207 Munich, Germany), so that the cells received wavelengths above 295 nm
208 (PAR+UVA+UVB). The transmission spectra of the cut-off filters are available
209 elsewhere (Zheng and Gao, 2009). Samples were placed at a distance of 1.2 m from a
210 solar simulator (Sol 1200W, Dr. Hönle, Martinsried, Germany), so that the actual PAR
211 light intensities to which the cells were exposed within the tubes (calculated taking
212 into account the transmission properties of the quartz tubes and the filters) was 44.11
213 Wm⁻² (ca. 190.11 μmol photons m⁻² s⁻¹) which is close to the daytime mean photon
214 flux in the middle of the photic zone (22-36 m depth in South China Sea, SEATS
215 station). The corresponding UVA and UVB irradiances were 14.19 Wm⁻² (ca. 41.99
216 μmol photons m⁻² s⁻¹) and 0.75 Wm⁻² (ca. 1.89 μmol photons m⁻² s⁻¹). Irradiances
217 were measured with a broad-band filter radiometer (ELDONET, Real Time Computer,
218 Möhrendorf, Germany). After the radiation treatments, the cells were replaced under
219 their growth light level (70 μmol photons m⁻² s⁻¹) to examine the recovery of
220 photosynthetic performance. During the incubations, the tubes were maintained in a
221 water bath at 20 °C using a circulating cooler (Eyela, CAP-3000, Tokyorikakikai Co.

删除的内容: radiation

删除的内容: 1) PAB treatment, tubes wrapped with Ultraphan Film 295 (DigeFra, Munich, Germany), so that the cells received wavelengths above 295 nm (PAR+UVA+UVB,)

删除的内容: 3) P treatment, tubes wrapped with Ultraphan film 395 (UV Opak, DigeFra), being exposed to PAR alone

删除的内容: (

删除的内容:)

234 Ltd., Tokyo, Japan).

235

236 **2.4 Measurement of carbon fixation**

237 The ^{14}C method was applied to measurements of marine photosynthetic carbon
238 fixation (Nielsen, 1952), and has been detailed with modified protocols in many
239 publications (Holm-Hansen and Helbling, 1995; Gao et al., 2007). Cells were
240 harvested in the middle of the light phase, diluted with freshly made medium
241 equilibrated with the designated concentrations of CO_2 to a cell concentration of $2\text{-}3 \times$
242 10^4 cells ml^{-1} and transferred to 35 ml quartz tubes. Each tube was injected with 100
243 μl -5 μCi (0.185 MBq) $\text{NaH}^{14}\text{CO}_3$ solution (ICN Radiochemicals). Triplicate
244 incubations were carried out for each treatment as mentioned above and, additionally,
245 3 tubes were wrapped in aluminum foil and incubated as a dark control. The cells
246 were collected on Whatman GF/F glass filters either immediately after 1 h exposure
247 to the solar simulator or after a period of recovery under their growth light for another
248 hr. The filters were put into 20 ml scintillation vials, fumed with HCl for 12 h and
249 then dried for 6 h at 45 $^\circ\text{C}$ to expel the non-fixed inorganic carbon as CO_2 .
250 Scintillation cocktail (3 mL of Tri-Carb 2800TR, Perkin Elmer®) was added to the
251 vials, and radioactivity in the vials counted with a liquid scintillation counter (LS
252 6500, Beckman Coulter, USA). Carbon fixation rates were calculated from these
253 counts and are presented on a per cell basis or per chl *a*.

254

255 **2.5 Measurement of Chlorophyll fluorescence**

256 For chlorophyll fluorescence measurements, cell collection and radiation
257 treatments were carried out as described above. The effective quantum yield (yield)
258 was measured every 20 min either during the solar simulator exposure or during
259 recovery under the growth light level.

260 The effective quantum yield (yield) and non-photochemical quenching (NPQ)
261 parameters were calculated according to Genty et al. (1990) as $\text{yield} = (F'_m - F_t) / F'_m$
262 and $\text{NPQ} = (F_m - F'_m) / F'_m$, respectively, where F_m is the maximum fluorescence yield
263 after 15 min dark adaptation, F'_m is the light-adapted maximal chlorophyll
264 fluorescence yield measured during the exposures, and F_t is the steady fluorescence
265 level during the exposures. The actinic light was set at the growth light level, and the
266 saturating pulse ($5000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) lasted for 0.8 s.

267 Repair (r) and damage (k) rates during the 60 min exposure period in the presence
268 of UV were calculated using the Kok model (Heraud and Beardall, 2000): $P/P_{\text{initial}} =$
269 $r/(k+r) + [k/(k+r)]e^{-(k+r)t}$, where P_{initial} and P were the yield values at beginning and at
270 exposure time t .

271 During the recovery period, the exponential rate constant for recovery (R) was
272 calculated from the following equation: $y = y_o + b[1 - \exp(-R \times t)]$, where y represents
273 the yield value at time t , y_o is the starting value before recovery and b is a constant.

274 The relative inhibitions of carbon fixation or yield caused by UVA or UVB were
275 calculated as follows:

276
$$\text{Inh}_{\text{UVR}} = (P_{\text{PAR}} - P_{\text{PAB}}) / P_{\text{PAR}} \times 100\%;$$

277
$$\text{Inh}_{\text{UVA}} = (P_{\text{PAR}} - P_{\text{PA}}) / P_{\text{PAR}} \times 100\%;$$

278 $\text{Inh}_{\text{UVB}} = \text{Inh}_{\text{UVR}} - \text{Inh}_{\text{UVA}}$;

279 where P_{PAR} , P_{PA} and P_{PAB} represent carbon fixation or yield values under PAR,
280 PAR + UVA, PAR + UVA + UVB treatments, respectively.

281

282 **2.6 Cells counts and chlorophyll *a* measurements**

283 The cells were counted using a Z2™ Coulter Counter (Beckman, USA). Where
284 needed, we used the values for chlorophyll *a* (chl *a*) contents of the cells grown under
285 the same CO₂ and nitrate levels reported previously (Li et al., 2012a).

286

287 **2.7 Total protein content, superoxide dismutase (SOD) and catalase (CAT)**

288 **measurements**

289 To determine the total protein content and activities of SOD and CAT, cells were
290 collected, in the middle of the light phase, onto a polycarbonate membrane (0.22 μm,
291 Whatman) under vacuum at a pressure of less than 0.1 Pa and washed into a 1 ml
292 centrifuge tube with phosphate buffer (pH 7.6). The enzyme extractions were carried
293 out in 0.6 ml phosphate buffer (pH 7.6) that contained 50 mM KH₂PO₄, 1 mM
294 Ethylene Diamine Tetraacetic Acid (EDTA), 0.1% Triton X-100 and 1% (w/v)
295 polyvinyl pyrrolidone. The cells were broken by sonication in an ice-water bath
296 (4 °C), and the homogenized extract was centrifuged at 12000 g (4 °C) for 10 min
297 before the activities of SOD and CAT were tested with SOD and CAT Assay Kits
298 (Nanjing Jiancheng Biological Engineering Company, China). One unit of SOD was
299 defined as the amount causing a 50% inhibition of nitroblue tetrazolium (NBT)

删除的内容: C

删除的内容: Chlorophyll *a*
measurements and cells counts

303 reduction (Wang and Wang, 2010). One unit of CAT activity was defined as the
304 amount required to decompose 1 $\mu\text{mol H}_2\text{O}_2$ per second. The SOD and CAT activities
305 were expressed as U mg^{-1} protein and per 10^6 cells (Fig. S1). The total protein content
306 was determined according to Bradford (1976) using bovine serum albumin as the
307 standard.

308

309 **2.8 Statistical analyses and calculations**

310 One-way analysis of variance (ANOVA) was used, followed by a multiple
311 comparison using a Tukey-test to establish differences among the treatments.

312 Interactive effects among CO_2 , NO_3^- and UV~~R~~ on carbon fixation and yield were
313 determined using a two- or three-way ANOVA to establish significant differences
314 among the variables.

315

316 **3 Results**

317 **3.1 Carbon fixation**

318 Carbon fixation was significantly inhibited by UV~~R~~ in both HN and LN-grown
319 cells either based on per cell or chl *a* (Fig.1). Under the HN conditions, the carbon
320 fixation rates of LC and HC cultures, compared to that of PAR alone treatment, were
321 inhibited by 29.4% ($P = 0.0002$) and 36.7% ($P < 0.0001$) in the presence of UVA (PA
322 treatment: PAR+UVA), and by 47.7% ($P < 0.0001$) and 46.1% ($P = 0.0029$) with both
323 UVA and UVB (PAB, PAR+UVA+B) (Fig. 1 A, C). However, the carbon fixation per
324 cell in the LC grown cells was 10.0% ($P = 0.0058$) higher in those exposed to PA, and

删除的内容: radiation

删除的内容: radiation

327 that based on chl *a* was higher under the PAR alone or PA treatments, by about 8.4%
328 ($P = 0.0253$) and 17.9% ($P = 0.005$) compared to that of the HC-grown cells. For PAB
329 treatments, there were no significant differences between the HC and LC-grown cells
330 (Fig. 1 A, C).

331 Under LN conditions, carbon fixation rates of LC and HC grown cells were
332 decreased by 14.7 % ($P = 0.0039$) and 1.1% ($P = 0.8658$) in the presence of UVA (PA)
333 and by 23.3% ($P = 0.0019$) and 27.3% ($P = 0.0123$) with UVA and UVB (PAB)
334 treatments, respectively (Fig. 1 B, D), compared with that of PAR alone treatment.
335 That is, both UVA and UVB resulted in significant impacts in the LN-grown cells
336 under LC, but only UVB brought about significant reduction of the rate under HC. In
337 the PA treatment, the HC-LN cells fixed carbon at a rate 21.7% ($P = 0.0071$) higher
338 than in the LC-LN cells (Fig. 1 B), however, there were no significant differences
339 between HC and LC cells in the **PAR** and the PAB treatments under N-limitation.
340 Under the LN level, the carbon fixation rate per chl *a* was about 30.8% ($P = 0.01$),
341 51.6% ($P = 0.0013$) and 24.0% ($P = 0.03$) higher in HC than in LC-grown cells (Fig.
342 1 D).

343

344 **3.2 Photochemical quantum yield**

345 When exposed to different irradiation treatments, photochemical quantum yields
346 (‘yield’) in the cells grown under either HC or LN conditions showed similar patterns
347 with those grown at LC and HN conditions (Fig. 2), decreasing rapidly during the
348 initial 20 min and leveling off after 40 to 60 min. Under HN conditions, the yield in

349 the HC-grown cells decreased to a similar level among the treatments (P , $P = 0.1568$;
350 PA, $P = 0.0879$; PAB, $P = 0.1341$) as that in the LC-treatments (Fig. 2A, B). Under
351 the LN condition, the yield decreased to much lower levels compared to those under
352 HN treatments (Fig. 2C, D). Cells exposed to all treatments showed recovery of the
353 yield, under their growth light ($70 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), to approximately their initial
354 levels in about 80 min (Fig 3).

355

356 **3.3 UVA and UVB induced inhibition of photosynthetic performance**

357 While UVA induced significantly higher ($P = 0.0114$) inhibition of photosynthetic
358 carbon fixation in the HC-HN but lower ($P = 0.0038$) in the HC-LN grown cells (Fig.
359 3A, B), it did not cause significant changes in the yield between the HC- and LC-
360 grown cells (HN, $P = 0.1375$; LN, $P = 0.0500$) (Fig. 3C). While the contribution of
361 UVB did not induce significant inhibition of either carbon fixation ($P = 0.2308$) or
362 yield ($P = 0.5319$) in the HN-grown cells, under both the HC and LC conditions (Fig.
363 3A, C), it caused significantly higher inhibition of the photosynthetic rate (by 203.3%,
364 $P = 0.0006$) and the yield (by 76.8%, $P = 0.0451$) in the HC- than the LC- grown cells
365 under NO_3^- limited conditions (Fig. 3B, D). Interactive effects among CO_2 , NO_3^- and
366 radiation treatments on yield were significant (Table 1).

367

368 **3.4 Repair, damage rates and constant for recovery rate**

369 The HC-grown cells had higher rates of damage, k , than the LC-grown cells
370 under nitrogen limitation but not under N replete conditions (HN, $P = 0.2109$; LN, P

371 = 0.0092). No effect was observed for repair rates r (HN, $P = 0.1655$; LN, $P =$
372 0.5276). The repair:damage (r/k) ratios in the HC-grown cells showed a 21.0% (but
373 statistically insignificant) increase under HN ($P = 0.3450$) but decreased significantly
374 by 31.1% under LN ($P = 0.0320$) conditions, compared to the LC-grown cells,
375 respectively (Table 2). Under the low PAR, the exponential rate constant for recovery
376 (R) showed dependency on previous light treatments with lowered rate in the cells
377 exposed to UV~~R~~, while HC stimulated the rate under the HN but not LN condition
378 (Table 3). Obviously, the cells exposed to the radiation treatments with UVB took
379 longer ($P < 0.05$) to recover their photochemical yield, and pre-exposure to UVA had
380 little ($P > 0.05$) effect on the recovery; HC-HN-grown cells had faster ($P < 0.05$)
381 photochemical recovery (Table 4).

删除的内容: radiation

382

383 **3.5 Non-photochemical quenching (NPQ)**

384 Non-photochemical quenching (NPQ) showed an opposite pattern of change to
385 yield during both the exposure and recovery periods (Fig. 4). Under HN conditions,
386 HC treatments triggered the highest NPQ within 20 min (Fig. 4A), while NPQ
387 reached its maximal values at 40 min under the ambient (LC) CO₂ level (Fig. 4B).
388 Similar trends were found in both the LN and HN grown cells regardless of the
389 radiation treatments (Fig. 4). Both UVA and UVB caused additional ($P < 0.05$) rises in
390 NPQ in HN-grown cells regardless of the CO₂ levels (Fig. 4A, B). However, neither
391 UVA nor UVB induced significant ($P > 0.05$) change in NPQ in LN-grown cells,
392 regardless of the CO₂ levels (Fig. 4C, D). Lower NPQ values were found in

394 HN-grown cells compared with LN, under either PAR alone or PAR+UVA treatments.
395 Addition of UVB, however, resulted in an approximately 17.0% higher, but
396 statistically insignificant (LC, $P = 0.1150$; HC, $P = 0.1660$), increase of NPQ in HN
397 compared to LN-grown cells. Transfer to the growth light level without UV, to allow
398 recovery, led to a rapid decline of NPQ with time. For the cells that were pre-exposed
399 to the PAR+UVA+B treatment, relaxation of NPQ during the recovery period showed
400 no difference ($P > 0.05$) between HC- and LC-grown cells except that NPQ in the
401 HC-HN grown cells declined faster ($P = 0.0242$) than in LC-HN cells. Two-way
402 ANOVA showed that both nitrogen levels and radiation treatments individually, and
403 also interactively, affected the NPQ (Table 1).

404

405 **3.6 Protein content, SOD and CAT activities**

406 Protein contents were enhanced in HN cultures under both LC (3.21 ± 0.98 pg
407 cell^{-1}) and HC (3.38 ± 1.35 pg cell^{-1}) conditions, compared with LN grown cells (LC,
408 2.58 ± 0.46 pg cell^{-1} ; HC, 2.28 ± 0.68 pg cell^{-1}), though statistically there were no
409 significant differences among the treatments ($P = 0.4296$) (Fig. 5A). There was no
410 significant difference in protein content between LC and HC treatments at a given
411 NO_3^- concentration. However, NO_3^- -limitation enhanced SOD (LC, by 62.5%, $P =$
412 0.0004 ; HC, by 72.5%, $P = 0.0007$) and CAT (LC, by 67.5%, $P = 0.0759$; HC, by
413 67.1%, $P = 0.0747$) activities in both LC and HC-grown cells, when based on protein
414 content (Fig. 5B, C), though such enhancement was insignificant ($P > 0.1$) when
415 normalized to per cell (Fig. S1).

416

417 **4 Discussion**

418 This study shows that nitrate limitation interacts with OA to affect the overall
419 impacts of solar UV~~R~~ on the diatom *P. tricornutum*. OA and UVB caused
420 significantly higher inhibition of the photosynthetic rate and the quantum yield under
421 LN than under HN conditions. Interactive effects of reduced nitrate availability and
422 OA increased protein-based activity of superoxide dismutase (SOD) and catalase
423 (CAT) but decreased the rate of repair of PSII from UV-induced damage. OA
424 appeared to counteract UVB-induced damage under NO₃⁻ replete conditions, but
425 when combined with decreased availability of nitrate, it increased the diatom's
426 sensitivity to UV~~R~~.

删除的内容: radiation

删除的内容: radiation

427 Most diatoms have evolved CO₂ concentrating mechanisms (CCMs) as a
428 response to low availability of CO₂ in the present-day oceans (Raven et al., 2011).
429 Increasing pCO₂ may, to some extent, benefit marine phytoplankton due to increased
430 availability of CO₂ (Burkhardt et al., 2001; Rost et al., 2003). CCMs are known to be
431 down-regulated under a CO₂ level doubling that of the current ambient concentration,
432 saving about 20% of the energy cost for active inorganic carbon acquisition in some
433 diatoms (including *P. tricornutum*) (Hopkinson et al., 2011). Such a down-regulation
434 of CCMs was equally obvious in *P. tricornutum* grown under nitrate-limited or replete
435 conditions (Wu et al., 2010; Li et al., 2012a). However, this down-regulated CCM and
436 its effects may be mediated by many other factors. A recent study found that different
437 acclimation times (short term, 15-16 generations and longer term, 33-57 generations)

440 to increased CO₂ and nitrate limitation may have different effects on the DIC and DIN
441 uptake rate in diatom *Thalassiosira pseudonana*, with short-term acclimated cells
442 showing a linear correlation with changes in fCO₂ although this was not the case in
443 long-term acclimated cells (Hennon et al., 2014). On the other hand, the
444 down-regulation of CCM operation was recently shown to decrease the growth of 3
445 diatoms (*Phaeodactylum tricornutum*, *Thalassiosira pseudonana* and *Skeletonema*
446 *costatum*) under high levels of sunlight but to enhance it under low light (Gao et al.,
447 2012b). The growth rate of *P. tricornutum* under high CO₂ (1000 µatm) decreased at
448 light levels higher than 180 µmol m⁻² s⁻¹ to be lower than that of the low CO₂-grown
449 cells (Gao et al., 2012b). In the present study, under the near-saturation light level (ca.
450 190 µmol photons m⁻² s⁻¹ of PAR), photosynthetic carbon fixation rate per chl *a* under
451 the nitrate limited condition was higher in the HC-grown cells. Obviously, the nutrient
452 limitation influenced the effects of OA.

453 UVR is known to damage photosynthetic pigments and proteins (for example D1
454 and Rubisco proteins) (Zacher et al., 2007) and therefore would reduce the
455 photosynthetic capacity of algae (Häler et al., 2011). UVA induced significantly
456 higher inhibition of carbon fixation in HC-HN than in LC-HN grown cells, reflecting
457 a synergistic effect of UVA and OA; however, for the same cells, UVB induced no
458 greater inhibition of the photosynthetic carbon fixation in HC compared to LC cells,
459 which is in contrast to the findings reported in another study (Li et al., 2012b). Many
460 studies have shown that the sensitivity of cells to high levels of PAR and UV under
461 OA condition could be stimulated and then induce higher inhibition rate of

462 photosynthesis (Sobrino et al., 2008; Gao et al., 2012b; Xu and Gao, 2012). However,
463 this phenomenon is not always found in all species especially when the intensity of
464 PAR or UV is not that high. For example, a recent study reported that the unicellular
465 chlorophyte (*Dunaliella tertiolecta*) acclimated with high CO₂ under nutrient replete
466 conditions could alleviate the stress induced by high PAR and UV (García-Gómez et
467 al., 2014). This could be due to the energy saving as a result of down-regulation of CCM
468 activity. However, in the present study, we did not find that the synergistic effects of OA
469 and UVR induced a higher inhibition at the light intensity of PAR+ UVA+UVB (44.11 +
470 14.19 + 0.75 Wm⁻²) used, than found under LC. This may be due to the light intensity of
471 PAR or UVR not being high enough to exceed the energy dissipating capacity of the cells.
472 Furthermore, under high N the nutrient supply would be sufficient to support the repair
473 processes of UV or high PAR induced damage. In the LN-grown cells, UVB induced
474 greater inhibition of both carbon fixation and yield, probably due to a decreased
475 repair/damage ratio (Table 2) and decreased levels of both chl *a* and other light
476 harvesting pigments (Li et al., 2012a), since the (re)synthesis of both proteins and
477 UV-screening compounds depends on nitrogen availability (Beardall et al., 2009;
478 Beardall et al., 2014). Such an inhibition by UVB in LN-grown cells was more
479 pronounced under OA conditions (Fig. 3B, D), though UVB appeared to counteract
480 the OA effect under the HN condition. When the cells are exposed to lower external
481 pH, they would need additional energy to cope with the acid-base perturbation
482 (Kanazawa and Kramer, 2002). By impairing photosynthesis, nitrogen limitation
483 could decrease the supply of energy, especially in the presence of UVB (Döhler,

删除的内容: hold

带格式的: 字体: 倾斜

带格式的: 下标

带格式的: 非突出显示

删除的内容: higher

删除的内容: reduced

删除的内容: .

删除的内容: N

489 1998). Though SOD and CAT normalized per cell showed no change in all treatments
490 (Fig. S1), the fact that nitrogen limitation led to decreased protein contents per cell
491 and with higher activity of SOD and CAT (based on protein content) implies that
492 these enzymes are preferentially retained in the face of decreasing protein per cell and
493 thus reflects an enhanced defense strategy (Fig. 5), so that reactive oxygen species
494 (ROS) that were formed under N-limitation could be scavenged. The differential
495 impacts of UVB on HN and LN-grown cells under the OA treatment could be due to
496 differences in the repair and damage rates (Table 2) and differential stimulation of
497 periplasmic proteins (Wu and Gao, 2009), which are important transporters of ions
498 and play important roles in maintaining intracellular acid-base stability. On the other
499 hand, NO_3^- scarcity usually leads to an impaired PSII reaction center activity due to
500 decreased synthesis of key proteins, therefore, leading to decreased quantum yields of
501 PSII (Geider et al., 1993). In this study, *P. tricornutum* showed much lower yield (Fig.
502 2C, D), as well as NPQ, in the nitrogen limited cells (Fig. 4C, D), indicating smaller
503 functional PSII reaction centers and a lower heat dissipating capability, when
504 combined with the OA treatment, consistent with these cells having the highest
505 damage and the lowest repair (Table 2). In the HN-grown cells, better recovery of
506 both photosynthetic carbon fixation (data not shown) and photochemical performance
507 (Table 3, 4) under the OA condition could be attributed to faster repair rate of PSII
508 and related metabolic up-regulations.

509 The results from the present work suggest that nutrient limitation can alter the
510 effects of OA or UV_R and their interactions. In the oligotrophic oceans, such as the

删除的内容: radiation

512 surface mixed layers of the South China Sea (SCS), where averaged total inorganic
513 nitrogen concentrations range from 0-20 μmol , UVB and OA can act synergistically
514 to bring about a higher inhibition of photosynthetic carbon fixation. Higher
515 UVB-induced inhibition of photosynthesis was found in pelagic low-nutrient waters
516 than in coastal waters in the SCS (Li et al., 2011). With enhanced stratification and
517 reduced thickness of the upper mixed layer due to ocean warming, fewer nutrients
518 will be transported from deeper layers to the photic zones, and interactions of
519 enhanced nutrient limitation, OA and increased solar exposures will become the main
520 drivers influencing marine primary production (Gao et al., 2012a). For the diatoms,
521 such as *P. tricornutum*, OA and other ocean changes may result in transitions in their
522 vertical and horizontal distributions and changes in phytoplankton community
523 structure.

524

525 **Author contribution**

526 K.G. and W.L. conceived and designed the experiments, W.L. performed the
527 experiments. W.L., K.G. and J.B. analyzed the data and wrote the paper.

528

529 **Acknowledgements**

530 This study was supported by National Natural Science Foundation (41120164007,
531 41430967), by Joint project of NSFC and Shandong province (Grant No. U1406403),
532 Strategic Priority Research Program of CAS (Grant No. XDA11020302), Program for
533 Chang-jiang Scholars and Innovative Research Team (IRT_13R51), SOA

534 (GASI-03-01-02-04) and China-Japan collaboration project from MOST
535 (S2012GR0290). JB's work on climate change effects on algae has been funded by
536 the Australian Research Council and his visit to Xiamen was supported by '111'
537 project from Ministry of Education. We thank Yahe Li (Xiamen University, China) for
538 her kind assistance during the experiments.

539

540 **References**

- 541 Beardall, J., Sobrino, C., and Stojkovic, S.: Interactions between the impacts of
542 ultraviolet radiation, elevated CO₂, and nutrient limitation on marine primary
543 producers, Photochemical & Photobiological Sciences, 8, 1257-1265, 2009.
- 544 Beardall, J., Stojkovic, S., and Gao, K.: Interactive effects of nutrient supply and other
545 environmental factors on the sensitivity of marine primary producers to
546 ultraviolet radiation: implications for the impacts of global change, *Aquat. Biol.*,
547 doi: 10.3354/ab00582, 2014. 2014.
- 548 Beman, J. M., Chow, C.-E., King, A. L., Feng, Y., Fuhrman, J. A., Andersson, A.,
549 Bates, N. R., Popp, B. N., and Hutchins, D. A.: Global declines in oceanic
550 nitrification rates as a consequence of ocean acidification, *Proc. Natl. Acad. Sci.*
551 *U. S. A.*, 108, 208-213, 2011.
- 552 Boyd, P. W.: Beyond ocean acidification, *Nat. Geosci.*, 4, 273-274, 2011.
- 553 Bradford, M. M.: A rapid and sensitive method for the quantitation of microgram
554 quantities of protein utilizing the principle of protein-dye binding, *Anal.*
555 *Biochem.*, 72, 248-254, 1976.
- 556 Buma, A. G. J., Boelen, P., and Jeffrey, W. H.: UVR-induced DNA damage in aquatic

带格式的：缩进：左侧：0 厘米，
悬挂缩进：2 字符，首行缩进：-2
字符，行距：2 倍行距

域代码已更改

带格式的：字体：小四

带格式的: 字体: 小四

- 557 organisms. In: UV effects in aquatic organisms and ecosystems, Helbling, E. W.
558 and Zagarese, H. E. (Eds.), The Royal Society of Chemistry, Cambridge, UK,
559 [291-327](#), 2003.
- 560 Burkhardt, S., Amoroso, G., Riebesell, U., and Sültemeyer, D.: CO₂ and HCO₃⁻ uptake
561 in marine diatoms acclimated to different CO₂ concentrations, *Limnol. Oceanogr.*,
562 46, 1378-1391, 2001.
- 563 Cashmore, A. R.: The cryptochrome family of blue/UV-A photoreceptors, *J. Plankton*
564 *Res.*, 111, 267-270, 1998.
- 565 Cermeño, P., Dutkiewicz, S., Harris, R. P., Follows, M., Schofield, O., and Falkowski,
566 P. G.: The role of nutricline depth in regulating the ocean carbon cycle, *Proc.*
567 *Natl. Acad. Sci. U. S. A.*, 105, 20344-20349, 2008.
- 568 Chen, S. and Gao, K.: Solar ultraviolet radiation and CO₂-induced ocean acidification
569 interacts to influence the photosynthetic performance of the red tide alga
570 *Phaeocystis globosa* (Prymnesiophyceae), *Hydrobiologia*, 675, 105-117, 2011.
- 571 Dähler, G.: Effect of ultraviolet radiation on pigmentation and nitrogen metabolism of
572 Antarctic phytoplankton and ice algae, *J. Plant Physiol.*, 153, 603-609, 1998.
- 573 Dickson, A. G.: Standard potential of the reaction: AgCl(s) + 1/2 H₂(g) = Ag(s) +
574 HCl(aq), and the standard acidity constant of the ion HSO₄⁻ in synthetic seawater
575 from 273.15 to 318.15 K, *J. Chem. Thermodyn.*, 22, 113-127, 1990.
- 576 Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A.: Ocean acidification: The
577 other CO₂ problem, *Annu. Rev. Mar. Sci.*, 1, 169-192, 2009.
- 578 Fu, F., Place, A. R., Garcia, N. S., and Hutchins, D. A.: CO₂ and phosphate

579 availability control the toxicity of the harmful bloom dinoflagellate *Karlodinium*
580 *veneficum*, *Aquat. Microb. Ecol.*, 59, 55-65, 2010.

581 Gao, K.: Positive and negative effects of ocean acidification: Physiological responses
582 of algae, *Journal of Xiamen University (Natural Science)*, 50, 411-417, 2011.

583 Gao, K., Helbling, E. W., Häder, D. P., and Hutchins, D. A.: Responses of marine
584 primary producers to interactions between ocean acidification, solar radiation,
585 and warming, *Mar. Ecol. Prog. Ser.*, 470, 167-189, 2012a.

586 Gao, K., Li, P., Watanabe, T., and Helbling, E. W.: Combined effects of ultraviolet
587 radiation and temperature on morphology, photosynthesis, and DNA of
588 *Arthrospira (Spirulina) platensis* (Cynophyta), *J. Phycol.*, 44, 777-786, 2008.

589 Gao, K., Wu, Y., Li, G., Wu, H., Villafañe, V. E., and Helbling, E. W.: Solar UV
590 radiation drives CO₂ fixation in marine phytoplankton: a double-edged sword,
591 *Plant Physiol.*, 144, 54-59, 2007.

592 Gao, K., Xu, J., Gao, G., Li, Y., Hutchins, D. A., Huang, B., Wang, L., Zheng, Y., Jin,
593 P., Cai, X., Häder, D. P., Li, W., Xu, K., Liu, N., and Riebesell, U.: Rising CO₂
594 and increased light exposure synergistically reduce marine primary productivity,
595 *Nat. Clim. Change.*, 2, 519-523, 2012b.

596 Garc á-Gómez, C., Gordillo, F. J., Palma, A., Lorenzo, M. R., and Segovia, M.:
597 Elevated CO₂ alleviates high PAR and UV stress in the unicellular chlorophyte
598 *Dunaliella tertiolecta*, *Photochemical & Photobiological Sciences*, 13,
599 1347-1358, 2014.

600 Geider, R. J., Roche, J., Greene, R. M., and Olaizola, M.: Response of the

601 photosynthetic apparatus of *Phaeodactylum tricornutum* (Bacillariophyceae) to
602 nitrate, phosphate, or iron starvation, *J. Phycol.*, 29, 755-766, 1993.

603 Genty, B., Harbinson, J., and Baker, N. R.: Relative quantum efficiencies of the
604 two-photosystems of leaves in photorespiratory and non-photorespiratory
605 conditions, *Plant Physiol. Bioch.*, 28, 1-10, 1990.

606 Granum, E., Raven, J. A., and Leegood, R. C.: How do marine diatoms fix 10 billion
607 tonnes of inorganic carbon per year?, *Can. J. Bot.*, 83, 898-908, 2005.

608 Häder, D.-P., Helbling, E. W., Williamson, C. E., and Worrest, R. C.: Effects of UV
609 radiation on aquatic ecosystems and interactions with climate change,
610 *Photochemical Photobiological Sciences*, 10, 242-260, 2011.

611 Helbling, E. W., Gao, K., Gonçalves, R. J., Wu, H., and Villafañe, V. E.: Utilization of
612 solar UV radiation by coastal phytoplankton assemblages off SE China when
613 exposed to fast mixing, *Mar. Ecol. Prog. Ser.*, 259, 59-66, 2003.

614 Hennon, G. M. M., Quay, P., Morales, R. L., Swanson, L. M., and Virginia Armbrust,
615 E.: Acclimation conditions modify physiological response of the diatom
616 *Thalassiosira pseudonana* to elevated CO₂ concentrations in a nitrate-limited
617 chemostat, *J. Phycol.*, 50, 243-253, 2014.

618 Heraud, P. and Beardall, J.: Changes in chlorophyll fluorescence during exposure of
619 *Dunaliella tertiolecta* to UV radiation indicate a dynamic interaction between
620 damage and repair processes, *Photosynth. Res.*, 63, 123-134, 2000.

621 Heraud, P., Roberts, S., Shelly, K., and Beardall, J.: Interactions between UV-B
622 exposure and phosphorus nutrition. II. Effects on rates of damage and repair, *J.*

623 Phycol., 41, 1212-1218, 2005.

624 Hessen, D. O., Leu, E., Færøvig, P. J., and Falk Petersen, S.: Light and spectral
625 properties as determinants of C: N: P-ratios in phytoplankton, Deep-Sea Res.
626 Part II, 55, 2169-2175, 2008.

627 Holm-Hansen, O. and Helbling, E. W.: Técnicas para la medición de la productividad
628 primaria en el fitoplancton. In: Manual de métodos ficológicos, Alveal, K.,
629 Ferrario, M. E., Oliveira, E. C., and Sar, E. (Eds.), Universidad de Concepción,
630 Concepción, Chile, [329-350](#), 1995.

631 Hopkinson, B. M., Dupont, C. L., Allen, A. E., and Morel, F. M. M.: Efficiency of the
632 CO₂-concentrating mechanism of diatoms, Proc. Natl. Acad. Sci., 108,
633 3830-3837, 2011.

634 Hopkinson, B. M., Xu, Y., Shi, D., McGinn, P. J., and Morel, F. M. M.: The effect of
635 CO₂ on the photosynthetic physiology of phytoplankton in the Gulf of Alaska,
636 Limnol. Oceanogr., 55, 2011-2024, 2010.

637 IPCC: Workshop Report of the Intergovernmental Panel on Climate Change
638 Workshop on Impacts of Ocean Acidification on Marine Biology and
639 Ecosystems. Field, C. B., Barros, V., Stocker, T. F., Qin, D., Mach, K. J., Plattner,
640 G.-K., Mastrandrea, M. D., Tignor, M., and Ebi, K. L. (Eds.), IPCC Working
641 Group II Technical Support Unit, Carnegie Institution, Stanford, California,
642 United States of America, 2011.

643 Kanazawa, A. and Kramer, D. M.: In vivo modulation of nonphotochemical exciton
644 quenching (NPQ) by regulation of the chloroplast ATP synthase, Proc. Natl.

带格式的: 字体: 小四

645 Acad. Sci., 99, 12789-12794, 2002.

646 Kim, J. M., Lee, K., Shin, K., Kang, J. H., Lee, H. W., Kim, M., Jang, P. G., and Jang,
647 M. C.: The effect of seawater CO₂ concentration on growth of a natural
648 phytoplankton assemblage in a controlled mesocosm experiment, *Limnol.*
649 *Oceanogr.*, 51, 1629-1636, 2006.

650 King, A. L., Sañudo-Wilhelmy, S. A., Leblanc, K., Hutchins, D. A., and Fu, F.: CO₂
651 and vitamin B₁₂ interactions determine bioactive trace metal requirements of a
652 subarctic Pacific diatom, *The ISME journal*, 5, 1388-1396, 2011.

653 Leonardos, N. and Geider, R. J.: Elevated atmospheric carbon dioxide increases
654 organic carbon fixation by *Emiliana Huxleyi* (Haptophyta), under
655 nutrient-limited high-light conditions, *J. Phycol.*, 41, 1196-1203, 2005.

656 Lewis, E. and Wallace, D. W. R.: Program developed for CO₂ system calculations. In:
657 ORNL/CDIAC-105, Carbon Dioxide Information Analysis Center, Oak Ridge
658 National Laboratory, US Department of Energy, Oak Ridge, Tennessee, 1998.

659 Li, G., Gao, K., and Gao, G.: Differential impacts of solar UV radiation on
660 photosynthetic carbon fixation from the coastal to offshore surface waters in the
661 South China Sea, *Photochem. Photobiol.*, 87, 329-334, 2011.

662 Li, W., Gao, K., and Beardall, J.: Interactive effects of ocean acidification and
663 nitrogen-limitation on the diatom *Phaeodactylum tricornutum*, *PLoS One*, 7,
664 e51590, 2012a.

665 Li, Y., Gao, K., Villafañe, V., and Helbling, E.: Ocean acidification mediates
666 photosynthetic response to UV radiation and temperature increase in the diatom

667 *Phaeodactylum tricornutum*, Biogeosciences, 9, 3931-3942, 2012b.

668 Litchman, E., Neale, P. J., and Banaszak, A. T.: Increased sensitivity to ultraviolet
669 radiation in nitrogen-limited dinoflagellates: Photoprotection and repair, Limnol.
670 Oceanogr., 47, 86-94, 2002.

671 Morel, F. M. M., Rueter, J. G., Anderson, D. M., and Guillard, R. R. L.: Aquil: A
672 chemically defined phytoplankton culture medium for trace metal studies, J.
673 Phycol., 15, 135-141, 1979.

674 Neale, P., Sobrino, C., Segovia, M., Mercado, J., Leon, P., Cortés, M., Tuite, P., Picazo,
675 A., Salles, S., and Cabrerizo, M.: Effect of CO₂, nutrients and light on coastal
676 plankton. I. Abiotic conditions and biological responses, Aquat. Biol., 22, 25-41,
677 2014.

678 Nielsen, E. S.: The use of radioactive carbon (C¹⁴) for measuring organic production
679 in the sea, Journal du Conseil, 18, 117-140, 1952.

680 Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A.,
681 Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R. M., Lindsay, K.,
682 Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R. G., Plattner,
683 G. K., Rodgers, K. B., Sabine, C. L., Sarmiento, J. L., Schlitzer, R., Slater, R. D.,
684 Totterdell, I. J., Weirig, M. F., Yamanaka, Y., and Yool, A.: Anthropogenic ocean
685 acidification over the twenty-first century and its impact on calcifying organisms,
686 Nature, 437, 681-686, 2005.

687 Pörtner, H. O. and Farrell, A. P.: Physiology and climate change, Science, 322,
688 690-692, 2008.

689 Raven, J. A., Giordano, M., Beardall, J., and Maberly, S. C.: Algal and aquatic plant
690 carbon concentrating mechanisms in relation to environmental change,
691 Photosynth. Res., 109, 281-296, 2011.

692 Riebesell, U. and Tortell, P. D.: Effects of ocean acidification on pelagic organisms
693 and ecosystems. In: Ocean acidification, Gattuso, J.-P. and Hansson, L. (Eds.),
694 Oxford University Press, New York, 99-121, 2011.

695 Riebesell, U., Wolf-Gladrow, D. A., and Smetacek, V.: Carbon dioxide limitation of
696 marine phytoplankton growth rates, Nature, 361, 249-251 1993.

697 Rost, B., Riebesell, U., Burkhardt, S., and Sültemeyer, D.: Carbon acquisition of
698 bloom-forming marine phytoplankton, Limnol. Oceanogr., 48, 55-67, 2003.

699 Roy, R. N., Roy, L. N., Vogel, K. M., Porter-Moore, C., Pearson, T., Good, C. E.,
700 Millero, F. J., and Campbell, D. M.: The dissociation constants of carbonic acid
701 in seawater at salinities 5 to 45 and temperatures 0 to 45 °C, Mar. Chem., 44,
702 249-267, 1993.

703 Shelly, K., Heraud, P., and Beardall, J.: Nitrogen limitation in *Dunaliella tertiolecta*
704 Butcher (Chlorophyceae) leads to increased susceptibility to damage by
705 ultraviolet-B radiation but also increased repair capacity, J. Phycol., 38, 713-720,
706 2002.

707 Shi, D., Kranz, S. A., Kim, J. M., and Morel, F. M. M.: Ocean acidification slows
708 nitrogen fixation and growth in the dominant diazotroph *Trichodesmium* under
709 low-iron conditions, Proc. Natl. Acad. Sci., 109, E3094-E3100, 2012.

710 Sobrino, C., Segovia, M., Neale, P. J., Mercado, J. M., García-Gómez, C., Kulk, G.,

带格式的：字体：小四

711 Lorenzo, M. R., Camarena, T., van de Poll, W. H., Spilling, K., and Ruan, Z.:
712 Effect of CO₂, nutrients and light on coastal plankton. IV. Physiological
713 responses, *Aquat. Biol.*, 22, 77-93, 2014.

714 Sobrino, C., Ward, M. L., and Neale, P. J.: Acclimation to elevated carbon dioxide and
715 ultraviolet radiation in the diatom *Thalassiosira pseudonana*: Effects on growth,
716 photosynthesis, and spectral sensitivity of photoinhibition, *Limnol. Oceanogr.*,
717 53, 494-505, 2008.

718 Tortell, P. D.: Evolutionary and ecological perspectives on carbon acquisition in
719 phytoplankton, *Limnol. Oceanogr.*, 45, 744-750, 2000.

720 Wang, M. and Wang, G.: Oxidative damage effects in the copepod *Tigriopus*
721 *japonicus* Mori experimentally exposed to nickel, *Ecotoxicology*, 19, 273-284,
722 2010.

723 Wu, H. and Gao, K.: Responses of a marine red tide alga *Skeletonema costatum*
724 (Bacillariophyceae) to long-term UV radiation exposures, *J. Photoch. Photobio.*
725 B, 94, 82-86, 2009.

726 Wu, H., Gao, K., Villafañe, V. E., Watanabe, T., and Helbling, E. W.: Effects of solar
727 UV radiation on morphology and photosynthesis of filamentous cyanobacterium
728 *Arthrospira platensis*, *Appl. Environ. Microb.*, 71, 5004-5013, 2005.

729 Wu, Y., Gao, K., and Riebesell, U.: CO₂-induced seawater acidification affects
730 physiological performance of the marine diatom *Phaeodactylum tricorutum*,
731 *Biogeosciences*, 7, 2915-2923, 2010.

732 Xu, K. and Gao, K.: Reduced calcification decreases photoprotective capability in the

733 coccolithophorid *Emiliana huxleyi*, *Plant Cell Physiol.*, 53, 1267-1274, 2012.

734 Zacher, K., Hanelt, D., Wiencke, C., and Wulff, A.: Grazing and UV radiation effects
735 on an Antarctic intertidal microalgal assemblage: a long-term field study, *Polar*
736 *Biol.*, 30, 1203-1212, 2007.

737 Zheng, Y. and Gao, K.: Impacts of solar UV radiation on the photosynthesis, growth,
738 and UV-absorbing compounds in *Gracilaria Lemaneiformis* (Rhodophyta) grown
739 at different nitrate concentrations, *J. Phycol.*, 45, 314-323, 2009.

740

741

742

743

744

745

746

747

748

749

750

751

752

753

754

755
756
757
758
759
760

761
762
763
764
765
766
767
768
769

Table 1. Interactive effects among NO₃⁻ concentrations, CO₂ levels and radiation treatments. Two or three way ANOVA analysis of individual and interactive effects among NO₃⁻ concentrations, CO₂ levels and radiation treatments. Stars indicate significance at *P* < 0.05. Where “Ni” indicates nitrate, “OA” CO₂/pH, “Rad-Treat” radiation treatments, “Inh-C” inhibition of carbon fixation and “Inh- yield” inhibition of yield.

Parameter	Ni &		Ni &		OA &	Ni, OA &
	Ni	OA	Rad-Treat	OA	Rad-Treat	Rad-Treat
Carbon fixation	*	*	*	*	*	*
Inh-C	*		*		*	*
yield	*		*	*	*	
Inh- yield	*		*	*	*	
NPQ	*		*		*	

刪除的內容: -
-
-
-
-
-
-

779 **Table 2.** The PSII damage (k) and repair (r) rate constants (min^{-1}) in *Phaeoductylum*
 780 *tricornutum* cells grown in LC-HN, LC-LN, HC-HN and HC-LN during the 60 min
 781 exposures to PAR+ UVA+UVB ($44.11 + 14.19 + 0.75 \text{ Wm}^{-2}$). Parameters of repair
 782 and damage rates were calculated based on Fig. 2 according to Heraud and Beardall
 783 (2000). SD was for triplicate cultures. Treatments with the same lowercase superscript
 784 letters, means the difference is not significant. In contrast, treatments with different
 785 lowercase superscript letters indicate the difference is significant ($P < 0.05$ level).

	R^2 for fit	Repair rate(r)	Damage rate(k)	r/k
LC-HN	>0.99	0.044 ± 0.007^a	0.068 ± 0.007^a	0.666 ± 0.216^{ab}
HC-HN	>0.99	0.064 ± 0.019^{ab}	0.079 ± 0.010^{ab}	0.806 ± 0.145^{ab}
LC-LN	>0.99	0.054 ± 0.012^{ab}	0.062 ± 0.008^a	0.854 ± 0.138^a
HC-LN	>0.99	0.059 ± 0.005^b	0.095 ± 0.010^b	0.588 ± 0.073^b

删除的内容: it

删除的内容: ,

删除的内容: instead

删除的内容: Different letters indicated significant differences among different treatments at $P < 0.05$ level.

786

787

788

789

790

791

792

793

794

795

803 **Table 3.** The exponential rate constant for recovery (R, min^{-1}) under growth light after
 804 80 min exposure to solar radiation with or without UV. Different letters of
 805 superscripts indicate significant differences between the CO_2 and NO_3^- treatments at P
 806 < 0.05 .

删除的内容: .

删除的内容: level

	LC-HN	LC-LN	HC-HN	HC-LN
P	0.038 ± 0.006^{ab}	0.029 ± 0.011^b	0.043 ± 0.009^a	0.038 ± 0.002^{ab}
PA	0.028 ± 0.002^a	0.023 ± 0.007^a	0.037 ± 0.002^b	0.027 ± 0.008^{ab}
PAB	0.019 ± 0.002^a	0.024 ± 0.001^b	0.029 ± 0.003^c	0.021 ± 0.003^d

807
808
809
810
811
812
813
814
815
816
817
818
819
820

823 **Table 4.** The recovery time to half maximal yield values under growth light after 80
 824 min exposure to solar radiation with or without UV. Different letters of superscripts
 825 indicate significant differences between the radiation treatments at $P < 0.05$.

删除的内容: .

删除的内容: level

	LC-HN	LC-LN	HC-HN	HC-LN
	(min)	(min)	(min)	(min)
P	16.78±2.94 ^a	20.81±5.93 ^a	15.41±2.57 ^{ab}	16.79±0.64 ^a
PA	20.38±1.28 ^a	23.36±4.47 ^a	16.83±0.67 ^a	21.66±4.52 ^{ab}
PAB	25.82±1.51 ^b	22.73±1.25 ^a	20.05±1.78 ^b	24.64±1.57 ^b

826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843

846 **Figure captions**

847 **Figure 1.** Photosynthetic carbon fixation rates of *P. tricornutum* under different
848 treatments. Photosynthetic carbon fixation rates of *P. tricornutum* cells represented as
849 rates (A, B) per cell and (C, D) per chl *a*, grown at ambient (390 μatm , LC) or elevated
850 CO_2 (1000 μatm , HC) under NO_3^- replete (110 $\mu\text{mol L}^{-1}$, HN) (A, C) or limited
851 condition (10 $\mu\text{mol L}^{-1}$, LN) (B, D) when exposed to PAR (P), PAR+UVA (PA) and
852 PAR+UVA+UVB (PAB) for 60 min, respectively. Vertical bars indicate $\pm\text{SD}$, the
853 means and standard deviation were based on 3 replicates. The different lowercase
854 letters indicate significant differences between different treatments at $P < 0.05$ level.

855

856 **Figure 2.** The effective quantum yield of *P. tricornutum* under different treatments.

857 Changes of effective quantum yield in *P. tricornutum* cells at ambient (390 μatm , LC)
858 or elevated CO_2 (1000 μatm , HC) under (A, B) NO_3^- replete (110 $\mu\text{mol L}^{-1}$, HN) or (C,
859 D) limited (10 $\mu\text{mol L}^{-1}$, LN) when exposed to PAR (P), PAR+UVA (PA) and
860 PAR+UVA+UVB (PAB) for 60 min and another 80 min under the growth light level
861 (the time of the switch to growth light levels is indicated by the dashed line),
862 respectively. The irradiance intensities under solar simulator or growth light were the
863 same as mentioned above. Vertical bars are means $\pm\text{SD}$, $n=3$.

864

865 **Figure 3.** UV induced inhibition of carbon fixation and PSII activity. UVA and UVB

866 induced inhibition of (A, B) photosynthetic carbon fixation and (C, D) PSII of *P.*

867 *tricornutum* cells grown at ambient (390 μatm , LC) or elevated CO_2 (1000 μatm , HC)

带格式的：字体：非加粗，检查拼写和语法

带格式的：字体：非加粗，倾斜，检查拼写和语法

带格式的：字体：非加粗，非倾斜，检查拼写和语法

带格式的：字体：非加粗，检查拼写和语法

删除的内容：in

带格式的：字体：倾斜，检查拼写和语法

删除的内容：

带格式的：检查拼写和语法

删除的内容：errors

带格式的：检查拼写和语法，突出显示

删除的内容：d

带格式的：字体：非加粗，检查拼写和语法

删除的内容：

删除的内容：point

删除的内容：with

删除的内容：

带格式的：字体：非加粗，检查拼写和语法

876 | under (A, C) NO_3^- replete ($110 \mu\text{mol L}^{-1}$, HN) or (B, D) NO_3^- limited condition (10
877 $\mu\text{mol L}^{-1}$, LN) when exposed to PAR (P), PAR+UVA (PA) and PAR+UVA+UVB
878 (PAB) for 60 min, respectively. The irradiance intensity under solar simulator was the
879 same as mentioned above. Vertical bars are means $\pm\text{SD}$, $n=3$, the different letters
880 indicate significant differences between different treatments at $P < 0.05$ level.

删除的内容: l

删除的内容: d

881
882 | **Figure 4. Non-photochemical quenching (NPQ) of *P. tricornutum* under different**
883 **treatments.** NPQ of *P. tricornutum* grown at ambient ($390 \mu\text{atm}$, LC) or elevated CO_2
884 ($1000 \mu\text{atm}$, HC) under (A, B) NO_3^- replete ($110 \mu\text{mol L}^{-1}$, HN) or (C, D) limited
885 condition ($10 \mu\text{mol L}^{-1}$, LN) when exposed to PAR (P), PAR+UVA (PA) and
886 PAR+UVA+UVB (PAB) for 60 min and another 80 min under the growth light level,
887 respectively. The irradiance intensities under solar simulator or growth light were the
888 same as mentioned above. Vertical bars means $\pm\text{SD}$, $n=3$.

带格式的: 检查拼写和语法

带格式的: 字体: 倾斜, 检查拼写和语法

带格式的: 检查拼写和语法

删除的内容: Non-photochemical quenching (

删除的内容:)

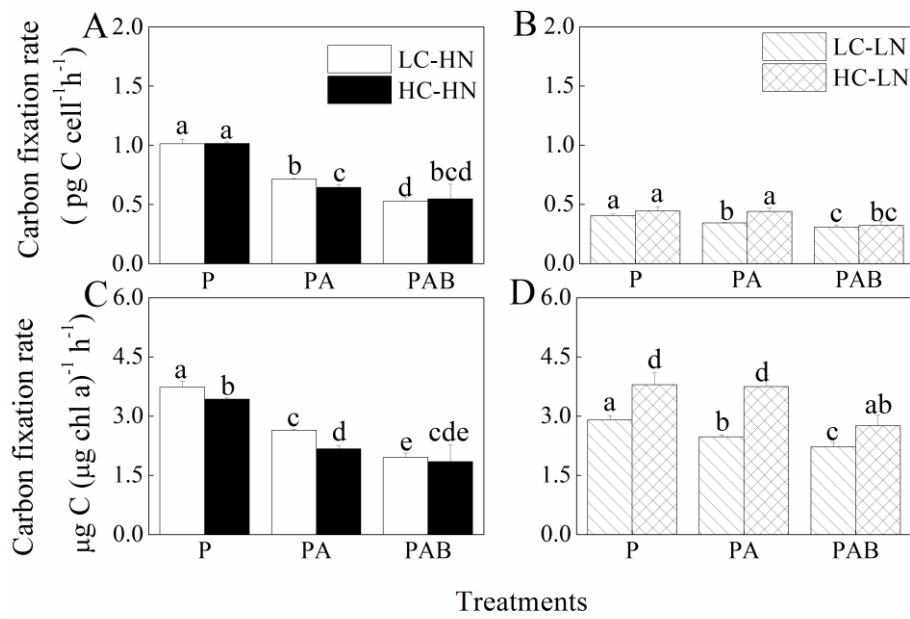
889
890 | **Figure 5. Protein contents, SOD and CAT activities of *P. tricornutum* under different**
891 **treatments.** (A) Protein contents, (B) SOD and (C) CAT activities (represented as per
892 milligram protein) of *P. tricornutum* grown at ambient ($390 \mu\text{atm}$, LC) or elevated
893 CO_2 ($1000 \mu\text{atm}$, HC) under NO_3^- replete ($110 \mu\text{mol L}^{-1}$, HN) or limited ($10 \mu\text{mol L}^{-1}$,
894 LN). The different letters above each column indicate significant differences between
895 different treatments at $P < 0.05$ level. Vertical bars means $\pm\text{SD}$, except the CAT value
896 in HC-LN for which there were only 2 replicates, other treatments used at least 3
897 replicates ($n=3-7$).

带格式的: 字体: 非加粗, 检查拼写和语法

带格式的: 字体: 非加粗, 检查拼写和语法

带格式的: 字体: 非加粗, 倾斜, 检查拼写和语法

带格式的: 字体: 非加粗, 检查拼写和语法



删除的内容: .
.
.

903
904
905
906
907
908
909
910
911
912
913
914
915
916

Fig. 1

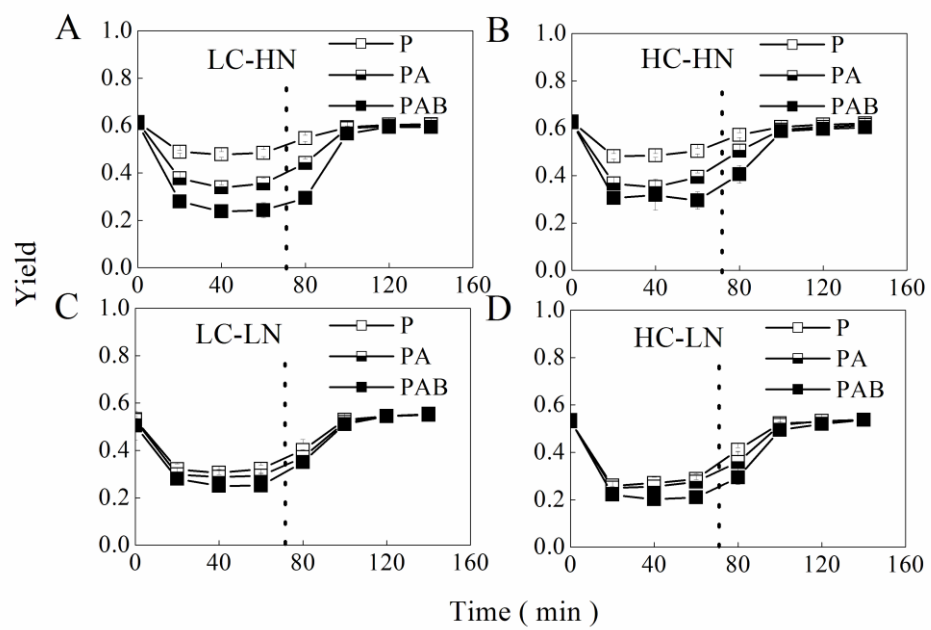
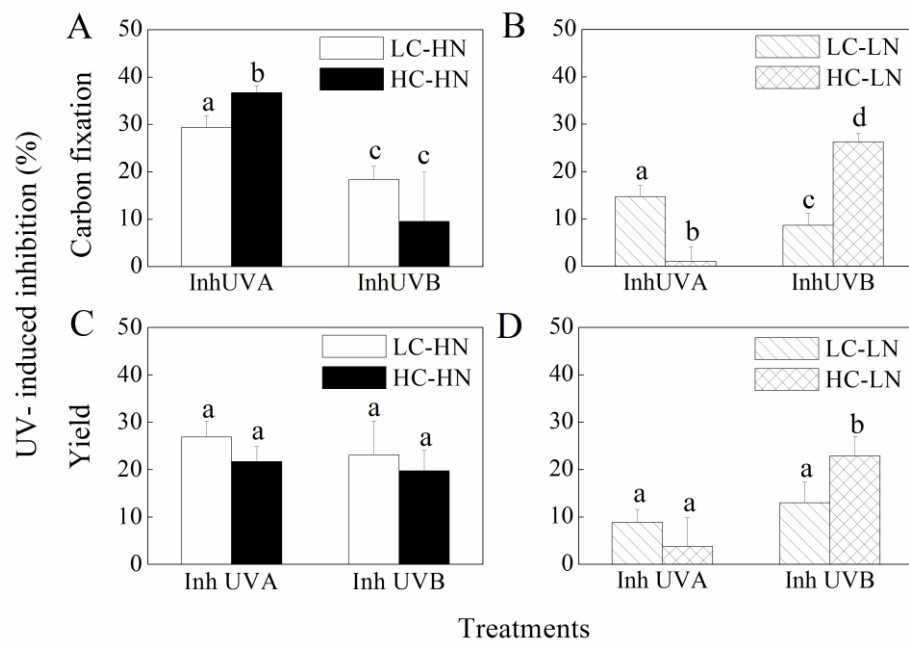


Fig. 2

921
 922
 923
 924
 925
 926
 927
 928
 929
 930
 931
 932
 933
 934



935

936

937

938

939

940

941

942

943

944

945

946

947

948

Fig. 3

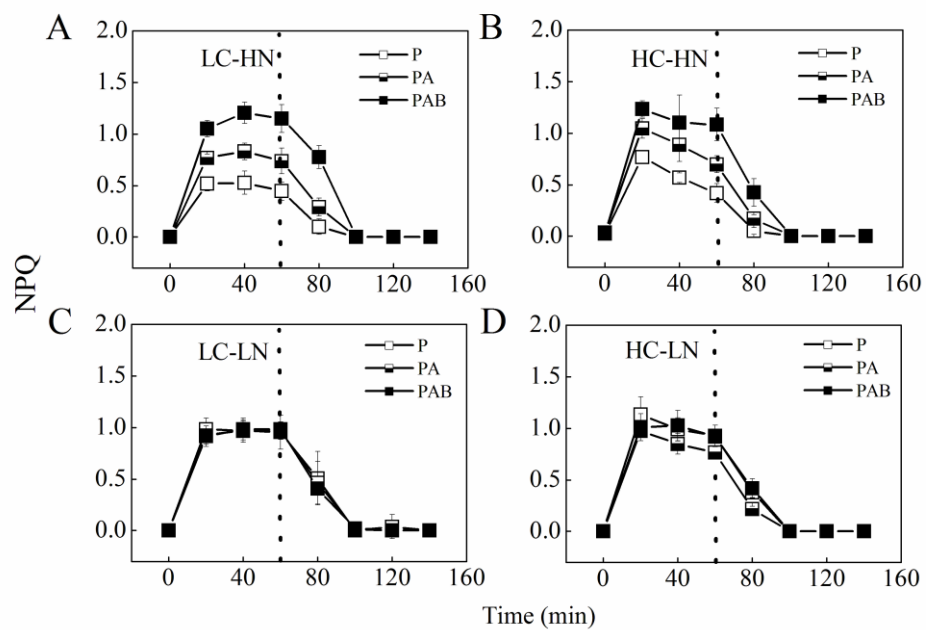


Fig. 4

949
 950
 951
 952
 953
 954
 955
 956
 957
 958
 959
 960
 961
 962

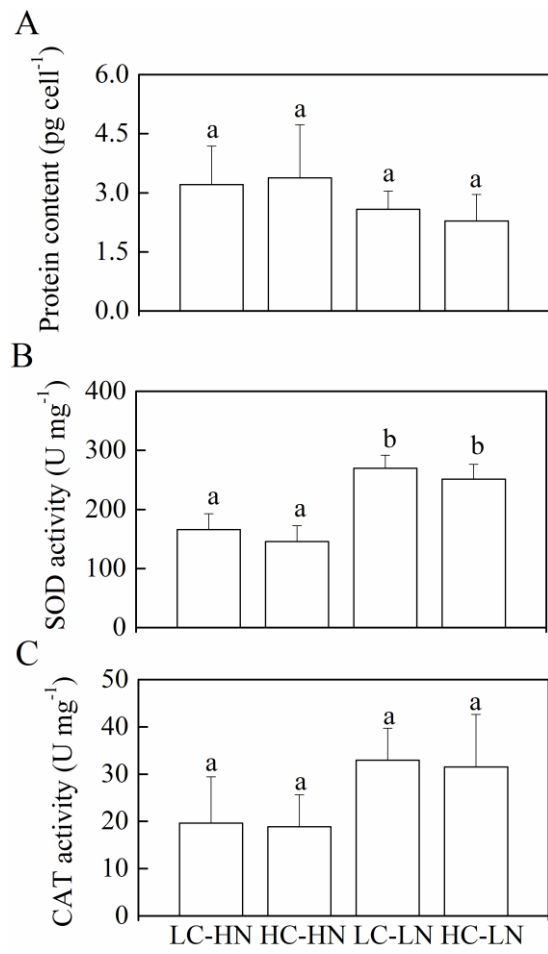


Fig. 5

963

964