

Interactive comment on “Combined effects of elevated $p\text{CO}_2$ and temperature on biomass and carbon fixation of phytoplankton assemblages in the northern South China Sea” by G. Gao et al.

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Anonymous Referee #2 Received and published: 18 November 2016 The MS “Combined effects of elevated $p\text{CO}_2$ and temperature on biomass and carbon fixation of phytoplankton assemblages in the northern South China Sea” by Gao et al. describes one of the first studies trying to understand changes in productivity at two stations (on shore and off shore) the South China Sea to a combination of both enhanced temperature as well as CO_2 .

The hypothesis to be answered is an important one and we do need high quality datasets to understand potential changes in productivity in a future ocean. Unfortunately I find this manuscript to be written very confusingly with some significant issues

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on experimental design, data evaluation and interpretation. Please find my detailed comments below.

Response: We have carefully examined the comments raised by the reviewer, and believe our manuscript has been largely improved now.

Abstract: Please keep “near shore” and “off-shore” as descriptions of the experimental sites. It becomes very confusing reading SEATS and D001 over and over again.

Response: Corrected.

The flow of the text is constantly interrupted by parentheses – please change.

Response: Corrected. Please see lines 32-40.

What does the last sentence mean? “...being more sensitive to these two global change factors”. What does more sensitive mean? In comparison to what? Please clarify.

Response: More sensitive means being more easily affected by these two global change factors in comparison to the coastal phytoplankton communities. It has been clarified to “with the pelagic phytoplankton communities being more easily affected by these two global change factors in terms of carbon fixation and respiration compared to the coastal phytoplankton communities” at lines 54-56.

Introduction: Line 58 to 67: The authors state different changes in SST increase over time (global SST, South China Sea, global mean rate. At least one of these temperatures is redundant. Also define if you mean South China Sea SST or average temp.

Response: The global mean rate has been removed and the warming rate for South China Sea is now what is meant by SST. Therefore, that sentence has been changed to “From 1979 to 2012, the mean global sea surface temperature increased at a rate of $\sim 0.12^\circ\text{C}$ per decade (IPCC, 2013). Particularly, the warming rate in the surface of South China Sea (~ 0.26 per decade) from 1982 to 2004 (Fang et al., 2006) appears

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to be about 2 times faster” at lines 63-68.

Line 73: correct the typo in phytoplankton. (in general please only submit a manuscript after carefully revising it – obvious typos as well as incomplete sentences -see below) should have been revised by at least one co-author!)

Response: We truly apologize for that. We have double checked the text and corrected phytoplankton to phytoplankton at line 316.

Line 79: this is not a sentence. – also this non-sentence needs a reference.

Response: It has been corrected to “Increased atmospheric CO₂ is responsible for global warming, and the oceans have absorbed approximately 30% of the emitted anthropogenic carbon dioxide (IPCC, 2013)” at lines 85-87.

Line 82/83: Add a reference.

Response: The following reference has been added at line 89.

Orr J C, Fabry V J, Aumont O, et al. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, 2005, 437: 681-686.

Line 85: Define RCP scenario

Response: It has been clarified to “By 2100, the projected decline in global-mean surface pH is approximately 0.065 (RCP 2.6) to 0.31 (RCP 8.5) (IPCC, 2013).” at lines 90-92.

Line 95: change the wording “problem”

Response: It has been changed to “challenges” at line 101.

Line 98: Gao et al 2012a was certainly not the first suggesting energy and metabolite allocation from the down-regulation of the CCM. Despite -to my knowledge the cited study (being a great study) did not investigate any CCM parameters.

Response: That reference has been changed to Wu et al. (2010), which shows in-

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creased K_m (DIC concentration required for half-maximal photosynthetic rate) by different techniques (PAM and C14), down-regulated CCM, and enhanced growth in *Phaeodactylum tricornutum* grown at the higher pCO₂ condition.

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

Line 101: The cited papers represent only a minor fraction of papers with a “neutral” CO₂ response – maybe use a review paper as citation instead adding “and references therein”.

Response: It has been changed to “(Gao et al., 2012 and references therein)” at line 109.

Gao K, Helbling E W, Häder D P, Hutchins D A. Responses of marine primary producers to interactions between ocean acidification, solar radiation, and warming. *Marine Ecology Progress Series*, 2012, 470: 167-189.

Line 125: what do the authors mean with “assimilation number”?

Response: It is the carbon fixed per chl a per time, now it has been changed to “photosynthetic carbon fixation rate” at line 132.

Methods: Some general remarks: Why did the authors not shield the incubations from the very high light intensity? Intensities of >1000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ as they correctly stated are pretty high and most (all) other studies used much lower light intensities. And phytoplankton change their vertical position over the day!

Response: The reviewer raised an important point. In the microcosms, practically, due to the shielding of the cover, water-jacket and the depth of the water, phytoplankton assemblages could be exposed to 81-91% and 64-91% of full sunlight at the off-shore and near-shore stations respectively. Since we aimed to investigate the impacts on surface phytoplankton assemblages, and provide comparable data with the previous study

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in the SCS (Gao et al., 2012), we decided not to shield the microcosms. These explanations have been added to lines 151-158. As reported previously (Gao et al. 2012), diatoms' response to OA depends on light levels. We have discussed this context at lines 324-338.

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

Did the authors monitor the temperature in the incubation continuously? The tank can easily heat up several degrees over the day if not monitored carefully. Please add this information to the MS.

Response: It has been clarified to "Microcosm temperature was controlled and monitored via circulating coolers (CTP-3000, EYELA, Japan) with a variation of $\pm 1.0^{\circ}\text{C}$ " at lines 162-164.

For future reference, it is best practice to measure at least DIC or TA additional to pH for these kinds of experiments. I know several reviewers who would not accept this MS just based on the "sloppy" characterization of the carbonate chemistry.

Response: We would like to thank the reviewer for this advice. It would be helpful to double check the chemistry stability. In this work, we measured pH and pCO₂ in the microcosms and other carbonate system parameters were derived via CO₂SYS (Pierrot et al., 2006), using the equilibrium constants of K₁ and K₂ for carbonic acid dissociation (Roy et al., 1993).

Pierrot D, Lewis E, and Wallace D W R. MS Excel program developed for CO₂ system calculations, ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee, 2006. 2006. Roy RN, Roy LN, Vogel KM, Porter-Moore C, Pearson T, Good CE, et al. The dis-

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sociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45°C. *Marine Chemistry*, 1993, 44: 249-267.

Detailed comments: It is unclear when the DDT was actually incubated. When was the sample taken and when was the ¹⁴C added. Was the experiment run every day or was it run once at the end of the incubation. Please revise the method section of the experimental setup and the corresponding measurements in order to understand the timeline of the measurements during the incubation.

Response: Detailed information has been added to the Methods section. It reads "The concentrations of chl a in situ and in microcosms were measured at the beginning and end of the experiment, respectively" at line 186-188; "Seawater samples taken from each microcosm at the end of the experiment were dispensed into 50 mL quartz tubes, inoculated with 5 μCi (0.185 MBq) NaH¹⁴CO₃ (ICN Radiochemicals, USA) and then incubated for 12 h (from 6:00 a.m. to 6:00 p.m.) and 24 h (from 6:00 a.m. to 6:00 a.m. next day) under natural light and day-night conditions" at lines 190-194; "The primary productivity and dark respiration in situ were measured at the beginning of the experiment." at lines 206-208.

Urgently needed additional information: Nutrient concentrations (specific values and not > or < (see Table 3)) prior to the acclimation start as well as nutrient concentrations at the end of the incubation.

Response: We realize that nutrient concentration is an important parameter. Unfortunately, we did not measure it during this ship-board experiment. Instead, we used reported data of the nutrients in the same regions for the reference (Table 3). We will measure nutrient concentrations in our future work by all means.

Regarding the statistical analysis: I assume that the authors had a maximum of three replicates. The authors state that the data "were conformed to a normal distribution". This seems to me pretty much impossible. How can n=3 be considered a normal distribution? Did the authors verify the outcome of the Shapiro-Wilk test? Please

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clarify!

Response: Yes, we had triplicate microcosms for each treatment. We realize that triplicates are the minimum for statistical analysis, but we did not have more replicates due to the breakdown of some microcosms during transport. We double checked the outcome of the Shapiro-Wilk tests and found all data sets were conformed to a normal distribution except the daily primary productivity ($P = 0.034$) and dark respiration ($P = 0.034$) under HTLC at the near-shore station. Consequently, we have changed the text to “The data from each treatment conformed to a normal distribution (Shapiro-Wilk, $P > 0.05$) except the DPP ($P = 0.034$) and dark respiration ($P = 0.034$) under HTLC at the near-shore station” at lines 211-213. We are happy to supply the outcome of statistical analysis if necessary.

Results: Line 208-215: Most of the results listed here are basic carbonate chemistry responses – please shorten this section.

Response: This paragraph describes changes of carbonate chemistry under three treatments at two stations using only eight lines. To shorten this section will affect clarification of the results and therefore we hope the current text could be sustained.

The authors compare the initial chl a concentration of both stations. As the authors know – the chlorophyll concentration is changing daily – even hourly, can be different 500 meter next to the sampling spot and obviously will vary with season. This whole section including the discussion does not mean anything if you don't look at long-term changes and differences. Please revise!

Response: We agree with the reviewer on the dynamics of chl a concentration, which could be affected by both physical and chemical forcings. The two stations, one is pelagic and highly oligotrophic, and the other station is near the coastal up-welling. Therefore, naturally, mean chl a concentration would be very different, despite the inevitable variability the reviewer points out. We have revised this part, trying to focus on responses of different regions or water bodies to changed pH, pCO₂ and temperature,

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at lines 238-247 and 307-319.

Line 255 -276: I feel that without the information on nutrient concentration in the different acclimations any data obtained are oblivious. Please revise if nutrient data are available.

Response: Yes, we totally agree with the reviewer that the conditions of nutrients are important. Since we compared the regional (different stations) response to pH and temp changes, and we had control for each station, it is, we believe, informative in terms of the phytoplankton assemblages' response to climate change in different areas. We did not measure the nutrients in the seawater but we know the general ranges of nutrients for both stations based on the references, as mentioned in the above response. We will measure nutrient concentrations in our future work by all means.

Discussion: Line 284-288: The authors state that phytoplankton growth commonly increases with temperature. This statement is simply wrong as phytoplankton which growth at its optimal temperature will be heat stressed at even higher temperature. Please revise. Also – the authors did not test a full temperature growth response curve for the phytoplankton in the North China Sea.

Response: We have realized that the wordings are confusing. We have revised, and now it reads “Algal and cyanobacterial growth commonly increases with temperature within a suitable range and then decreases after the optimal temperature point/range (Goldman and Carpenter, 1974; Montagnes and Franklin, 2001; Savage et al., 2004; Boyd et al. 2013) and optimum temperatures for growth of marine phytoplankton are usually several degrees higher than the environmental temperatures (Thomas et al., 2012), which could explained the increase chl a level of phytoplankton grown at the higher temperature in the present study.” at lines 312-319. We admit that more information would have been obtained if a full temperature growth response curve had been conducted. We did not do it because our aim was only to examine the effect of projected temperature and pCO₂ by the end of this century on primary productivity in

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SCS.

Line 293: Please change the citation (Wu et al. 2008) to a more original work. I also feel that the citation culture in this manuscript should be improved.

Response: This citation has been replaced with Giordano et al. (2005). We have tried to improve the citation culture by citing more original and more related literature. For instance, the citation Gao et al 2012a at line 104 was replaced with Wu et al., 2010, the citation Wu et al., 2008 at line 199 was replaced with Giordano et al. (2005), and the citation (Gao et al., 2007) at line 324 was removed.

Giordano M, Beardall J, Raven J A. CO₂ concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. *Annual Review of Plant Biology*, 2005, 56: 99-131. Wu Y, Gao K, Riebesell U. CO₂-induced seawater acidification affects physiological performance of the marine diatom *Phaeodactylum tricornutum*. *Biogeosciences*, 2010, 7: 2915–2923.

In the conclusion the authors state that the study demonstrates that ocean warming would stimulate DPP and that the effect can be dampened by OA. This would be an important result – but I feel that the data quantity and quality does not support this very general conclusion. The authors also disqualified their results with their own discussion in line 378-402 when they talk about the shortcomings of short time vs. long time acclimations. This manuscript is a short time incubation and should be discussed as including the potential shortcomings.

Response: We have to admit that ship-board tests are hard to extend longer than the ship-cruising period. And we take the advice from the reviewer and revised the conclusion section. It reads now: “This study demonstrates that a short-term rise of SST appeared to simulate the DPP and dark respiration of phytoplankton assemblages in the NSCS. However, this positive effect was dampened or offset when warming and ocean acidification treatments were combined. The regional responses of phytoplankton assemblages at the two stations to ocean warming and

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acidification may differ due to differences in physical and chemical environment as well as phytoplankton community structure. The combined treatment of warming and acidification reduced biomass and dark respiration rate at the off-shore, but did not affect them at the near-shore station. Ecologically and geographically, our data implies differential responses of primary production to ocean climate change. This short-term experiment suggests the need to determine whether similar effects may occur over the longer timescales of future anthropogenic change.” at lines 454-465.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/bg-2016-403/bg-2016-403-AC2-supplement.pdf>

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