Biogeosciences
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Discussions
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Interactive comment on "Inter- and intra-specific responses of coccolithophores to CO_2-induced ocean acidification" *by* D. S. Wang et al.

Anonymous Referee #1

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The authors investigate the effect of the change in carbonate chemistry on elemental composition (particulate organic and inorganic C, particulate N) and photosynthetic performance of two coccolithopholids Emiliania huxleyi and Gephyrocapsa oceanica. Interestingly, they used naked and calcified strains of both species. Experimental approach and data may be interesting to publish in a scientific journal, but many problems can be seen which should be addressed before publication. One major drawback is that they performed experiments improperly in terms of the sense of ocean acidification study. Presumably, phytoplankton biomass in a culture bottle was too high to regulate carbonate chemistry properly; accordingly, pH changed 0.3 to 0.5 during the first several days. It is also improper method in ocean acidification study that cells used in the experiments were not acclimated in the experimental conditions. In addition, measurement and/or calculation of carbonate chemistry is no good which I checked the values

listed in a Table 1. The authors should check thoroughly concerning carbonate chemistry measurement and calculations. Please read "Guide to best practice for ocean acidification research and data reporting", published from EPOCA, with the greatest attention. In my opinion, the authors are better to change the logic of this manuscript from ocean acidification study to the effect of the rapid change in carbonate chemistry on the ecophysiology of coccolithophores after the proper revision concerning carbonate chemistry. Finally, this paper is poorly written in terms of English language, which often disrupt the proper understanding of this paper. No good English renders ineffective communication of the science. Overall, this manuscript is hard to accept in Biogeosciences in the present status.

Thank you.

General comments

1. Manipulation or measurement of carbonate chemistry seems wrong. Did the authors calibrate pH electrode precisely for seawater analysis and use reference material of TA? Did CO2 concentration of air used in the experiment measure directly? TA values (2587-2788 umol kg-1) of seawater which salinity 32 seem very high and incomprehensible variations among treatments. According to the Table S1, pCO2 values calculated by CO2SYS using TA DIC or TA pH do not fit with the values listed in Table S1. It is also questionable that as shown in Figure S2, pH values changed dramatically during the course of experiments, but standard deviation of measured and calculated values listed in Table S1 are relatively small. Calcification also alter TA during experiments. Please clarify what the Table S1 shows. In addition, the authors conducted 4 experiments but showed only one table. What $\pm$ indicates in Table S1? When the authors collect samples? Please provide all data on the time course change in TA.

2. Bioassay studies concerning ocean acidification should be conducted under low biomass to minimize the change in carbonate chemistry (Guide to best practices for ocean acidification research and data reporting; http://www.epoca-

project.eu/index.php/guide-to-best-practices-for-ocean-acidification-research-and-data-reporting.html). At this point of view, this study was not well done as seen in Figure S2. Further, this study did not acclimate algal strains under experimental conditions before experiment. Such improper practices should be rule out from the ocean acidification study. The rate of change in carbonate chemistry is rapid in the Anthropocene in a geological time scale, but the rate is very slow compared to that observed in this culture experiment. For argument's sake, let's assume the condition of intensive coccolithophore blooms. This study investigate the response of coccolithophores to the abrupt change in carbonate chemistry. This sense is very important when the authors compare the results between this study and reported one.

3. The paper is poorly written in terms of English language, which often disrupt the proper understanding of this paper. No good English renders ineffective communication of the science. Some comments are listed below but many more unclear sentences could be seen. However, I do not correct everything because this is out of reviewer's work. English language should carefully be checked by a native English speaker or commercially available services before submission.

Specific comments

P. 676 L.3: The word "bioregion" indicates the area of interest. So, wording of organisms following "especially" is not appropriate. Please revise.

P. 676 L.10: Unclear sentence. Revise as follows: two Emiliania huxleyi and two Gephyrocapsa oceanica.

P. 676 L.13: grade indicates the degree of purity. Revise from grade to levels

P.676 L.16: Replace "in the process of cultivation" with "during the culture experiment"

P.676 L.17: Delete "(N-E)". Unnecessary here.

P.676 L.19–23: Hard to follow. What is "hypostatic difference"?

P.676 L.23–25: Hard to accept this conclusion from the above descriptions in Abstract.

P.677 L.2–18: Many sentences are no good. Please revise.

P.678 L.5: Do not brake line here.

P.678 L.18: Delete "(as in our research)". Unnecessary phrase.

P.678 L.20: ocean acidity is now increasing but increasing acidified ocean is unclear. Revise.

P.678 L. 26–28: Incorrect citation. Kottmeier et al. 2014 did not investigate the importance of naked E. huxleyi in the fields.

P.679 L.1–2: References concerning the long-term experiment (e.g. Jin et al. 2013, Lohbeck et al. 2012, 2014) and diploid/haploid tests (e.g. Müller et al. 2010, Fiorini et al. 2011a, Fiorini et al. 2012 Nature Geosci., Rokitta Rost 2012, Rokitta et al. 2012) should be introduced around here.

P.679 L.5: Cosmopolitan distribution of Gephyrocapsa oceanica is questionable because of their distribution is restricted in coastal region. See Okada Honjo 1973 Deep-Sea Res, 20: 355–374. If G. oceanica is truly cosmopolitan, please cite appropriate references.

P. 679 L. 4–5: Meaning unclear. Did the authors used only two strains; naked E. huxleyi and calcified G. oceanica? Please revise.

P. 679 L.5–14: Delete. These are methods. The authors should describe the purpose of this study here.

P. 679 L.19–20: Are there no accession number of N-E and C-G strains?

P. 679 L.26: f/2 medium

P. 679 L.26: Salinity have no unit. Recommend insertion of the information concerning salinity into L.24 as follows: filtered (0.2 um) natural seawater (salinity: 32) enriched

. . .

P. 680 L.2: The strains were maintained in sterilized . . .

P. 680 L.8: Hard to imagine the plant growth chamber, which is not common instruments. Xu et al. (2014) did not describe detail of it. Wrong citation! Please clarify such as material, volume and shape of chamber.

P. 680 L.15: Please describe the rate of bubbling which is a key to understand the effect of growth rate and calcification (e.g. Shi et al. 2009).

P. 680 L.18–19: Hardly understandable.

P. 680 L.20: When samplings were performed? Please clarify here.

P. 680 L.22: How to calibrate pH electrode? This is critically important in the ocean acidification study. Please see "Guide to best practices for ocean acidification research and data reporting". According to the Table S1, the measured pH values (or TA) were incorrect with respect to calculated pCO2.

P. 681 L.7, P. 682 L.8: Distinguish the character (C) of cell concentration from nitrate concentration. N/L is cell density (cells/L).

P. 681 L.19: Why not estimate ETR and/or NPQ?

P. 682 L.7: This equation is no good. Delete results and discussion concerning nitrogen uptake rate. Cell growth is exponential but the calculation and nitrate drawdown are linear. Please compare PON (preferably PN) production rate. Unify the unit between g and mol. If the authors calculate nitrogen requirement, (net) PON production rate is better.

P. 682 L.6: Because of extremely high nitrate concentrations in the culture media, precise measurement of nitrate should be difficult. How to overcome or please show the accuracy of nutrient measurement.

C77

P. 682 L.7: Why the authors fitted data by Michaelis-Menten equation? Are there any theory of enzymatic relationships between (net) nitrate uptake rate and CO2 conditions in the culture medium? Please clarify.

P. 682 L.24–26: This rate calculation is net rate. Please specify as "net production rate".

P. 682 L.21: In this analytical procedure, cellular inorganic nitrogen (NOx and NHx) is also measured. In my opinion, representing as particulate nitrogen (PN) is preferable rather than PON. Results. Please show the time course change in carbonate chemistry parameters, cellular C, N, and inorganic C quotas.

P. 683 L.16: What buffers added that was not described in Methods section. If the authors added chemical buffers into the culture media, it is hard to measure alkalinity accurately. Please clarify.

P. 683 L.17: If large biomass required to measure any parameters, the authors should increase the volume of culture, not biomass. This statement is a self-centered idea with respect to the ocean acidification study. In my opinion, this study has less implications for the future ocean ecosystem in terms of ocean acidification because of the extremely high biomass in a culture tank.

P.685 L.4: Unclear such as 0.32 ± 1.9

P.685–686: Delete section 3.4 as pointed out above.

Discussion

Unfortunately, because of improper experimental procedures, most discussion is hard to accept. Most of the previous studies were conducted using low-biomass batch-incubation, semi-continuous or continuous culture method, which quite different culture conditions of the rate of the change in carbonate chemistry compared with this study. The authors can simplify the manuscript by deleting the redundant descriptions of results in Discussion section. More comprehensive discussion may improve the quality

of this study.

It should be addressed before re-submission that how to produce high specific growth rate and net OC production with low chl-a and -c content and low photosynthetic activities under high CO2 conditions?

P. 687 L.10–12: Too speculative here. The results presented here cannot tell such a big picture.

P. 690 L.11–22: Hard to follow what the authors conducted. Please describe in the Method section.

P. 690 L.25–28: Concept described here seems incorrect and therefore give an inadequate impression on readers. The change in elemental composition of phytoplankton affect both on trophic interactions and biogeochemical cycling of nutrients. In my opinion, these interactions are so complex compared to the authors described here. Trophic interaction may partly relate biogeochemical cycling of nutrients, but great many other parameters should be taken into account to represent the biogeochemistry of nutrients. Please revise.

P. 691 L.5 and P. 692 L.15: Due to the wrong English representation, the calculated values seems incorrect. A rate of decrease (increase) differ from the word decrease (increase). Please clarify.

P. 691 L.12–13: Meaning unclear.

P. 691 L.15: The word "absorption" indicates chemically attached or aggregated on the surface of cell.

P. 691 L.18–19: Hard to follow.

P. 691 L.29: replace shell with coccolith

P. 692 L.1–2: "produced a ... coccolithophores." is uncertain. Please revise.

P. 692 L.17: What is "ecological factor" meaning? Incorrect phrasing.

P. 692 L.21: Langer et al. 2006 and Trimborn "et al." 2007 did not mention the relationships between calcification and pH/Ω-cal. I'm afraid that incorrect citations cheat readers.

P. 693 L.6–8: Meaning unclear.

P. 693 L.27–28: Unraveling the difference in the effect of the rate of change in carbonate chemistry on coccolithophores between this study and other reported studies may have a key to improve the quality of this study.

P. 694 L.3: Heredity is inherent. Therefore, inherent heredity is redundant.

P. 694 L.3–9: Too speculative. Hard to address such conclusions from the present study.

Thank you.

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