

Interactive comment on “Regulation of inorganic carbon acquisition in a red tide alga (*Skeletonema costatum*): the importance of phosphorus availability” by Guang Gao et al.

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Anonymous Referee #2 This manuscript reports results of experiments which aim to investigate the link between P availability and the C uptake by *S. costatum* diatoms. While apparently interesting interactions were observed, insufficient detail is provided about the methods, and I have reservations about the suitability of the statistical analysis employed.

Response: We appreciated these comments and have improved the manuscript by responding to the comments.

C1

Major Comments The introduction would benefit from adding hypotheses.

Response: We did state our hypothesis at line and it reads “Based on the connection between phosphorus and carbon metabolism in diatoms (Brembu et al., 2017), we hypothesize that phosphorus enrichment could enhance inorganic carbon utilization and hence maintain high rates of photosynthesis and growth in *S. costatum* under CO₂ limitation conditions.” at P6L85-89.

The methods section has a rather low amount of detail for each of the methods presented, with details of instrument manufacturers and models, and references frequently missing. In particular, there is no mention of how cells were counted, and normalizing this is an important aspect of many of the measurements.

Response: We appreciate these comments and have added more details to the Methods. In terms of cell counting, it has been clarified to “Cell density was determined by direct counting with an improved Neubauer haemocytometer (XB-K-25, Qiu Jing, Shanghai, China).” at P7L110-111.

I am also not convinced that 3 replicates of each treatment is sufficient, at least not for the parametric statistical testing that is employed.

Response: We agree that a higher replication would strengthen the study. However, we had to reduce the replication to obtain reliable data as we had 10 treatments for each measurement, indicating 30 samples (10×3) for each measurement. Three-replicate is fine for parametric statistical tests and it were used in many studies (Riebesell et al., 2007; Gao et al., 2012; Walworth et al., 2016; Hong et al., 2017).

Hong H, Shen R, Zhang F, et al. The complex effects of ocean acidification on the prominent N₂-fixing cyanobacterium *Trichodesmium*. *Science*, 2017, 356(6337): 527-531.

Riebesell U, Schulz K G, Bellerby R G J, et al. Enhanced biological carbon consumption in a high CO₂ ocean. *Nature*, 2007, 450(7169): 545-548.

C2

Gao K, Xu J, Gao G, et al. Rising CO₂ and increased light exposure synergistically reduce marine primary productivity. *Nature Climate Change*, 2012, 2(7): 519-523.

Walworth N G, Fu F X, Webb E A, et al. Mechanisms of increased *Trichodesmium* fitness under iron and phosphorus co-limitation in the present and future ocean. *Nature Communications*, 2016, 7: 12081.

The results section does not report what the actual values of the measured parameters were, only the results of statistical tests for differences between treatments.

Response: In our previous manuscripts, we were informed by some reviewers that the report of actual values was unnecessary as readers could see them from tables and figures. We anyhow added some actual values to the Results section at P13L226-P16L302.

There is rather limited discussion of the mechanisms behind each of the effects observed.

Response: We agree that we did not discuss the molecular mechanisms for the effects observed. We did not do it as our study did not refer to molecular measurements. Instead, we compared our results to those of similar studies, explained the meaning of our finding, tested our hypothesis by integrating the measured parameters, and finally produced take-home message “P enrichment could induce activity of extracellular carbonic anhydrase and direct utilization of HCO₃⁻ in *S. costatum* to help overcome the CO₂ limitation, as well as increasing photosynthetic pigment content and rETR to provide required energy.” in the Conclusion section. Honestly, we have no idea of how to improve the discussion of the mechanisms behind each of the effects observed and hope to hear more specific suggestions.

Minor Comments Not all of the figures are referred to in the text, or at least not in the correct order (there is no Fig. 3 reference between the first reference for Fig. 2 and that for Fig. 4).

C3

Response: We think all the figures were referred to in the text in order. We did refer to Fig. 3 between Fig. 2 and Fig. 4 at P14L251.

Line 10: Define rETR the first time it is used.

Response: It has been defined as “relative electron transport rate”.

Line 43: This should say ‘limiting’, not ‘limited’.

Response: Corrected.

Line 48: Give the name in full the first time it is used, and where it is used at the start of a sentence.

Response: Corrected throughout the text.

Lines 54-60: Please define all these acronyms the first time they are used.

Response: Corrected.

Line 88: I don’t think the units given here for irradiance are correct (micromoles per m squared).

Response: It has been corrected to “ $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ” at P7L103.

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