Reply to Referee #1 Dr. D. Campbell,

Thank you very much for your positive and constructive comments on our discussion paper. Below are our point-by-point responses to your comments.

1. *Materials & Methods: Given the importance to the findings, I think the authors should include a diagramatic figure of the standards, the amplification primers, and the amplicons used for the DNA and cDNA quantitations. From the text, I infer that the standard is only 113 bp long, for the DNA quantitations, but that a different standard was used for cDNA (length?). The primers 5'- GATGATGARAAYATTAACTC-3', reverse primer: 5'-TAWGAACCTTTWACTTCWCC-3'. are 19-20 bases long, leaving an amplified region of only 60 bp between the primers. It appears (but I am not sure) that the same primers are used for both DNA and cDNA quantitation. If so, why would you use two different quantitation standards?*

   We used the same region (same length and same sequence) for both DNA and cDNA quantifications. However, double stranded DNA and single stranded cDNA standards were used for DNA and cDNA samples, respectively, because these samples should be quantified as copy numbers. According to Smith et al. (2006), standard curves must be constructed from single stranded cDNA for the accurate determination of RNA transcript numbers, because cDNA exists as a single stranded form in the samples. We consider that the diagrammatic figure is not necessarily to explain our qPCR method, since we followed the general procedures described in Smith et al. (2006) and John et al. (2007). Alternatively, we have added the following sentence to the revised manuscript:

   "Following Smith et al. (2006), we used double-stranded DNA and single-stranded cDNA standards for DNA and cDNA quantification, respectively."

2. *Discussion: "Our study indicates that the decrease in diatom biomass given elevated CO2 levels was unique to the Bering Sea basin." No. Unique would mean that this response is only present in the Bering Sea, and we do not know that yet. In fact a preceding sentence mentions similar responses in the Okhotsk Sea.*

   This sentence has been deleted.

3. *"However, we speculate that CCMs in the diatoms might not be active in the control treatments because Fe deficiency could reduce the functionality of algal CCMs due to a reduction in their light energy-harvesting ability (Giordano et al., 2005)." This needs to be better explained. It is unlikely that Fe deficiency would limit CCM simply through a limitation on light energy harvesting.*

According to the valuable comments from you and other reviewers, we have excluded the discussion on CCMs and reconstructed the corresponding paragraph as follows:

"The negative effects of increasing CO_2 on diatom biomass were not severe in the Fe-added bottles relative to Fe-limited control bottles (Figs. 1a and b), whereas *rbcL* transcription decreased with increased CO_2 regardless of Fe availability (Fig. 4). This suggests that the diatoms could overcome the decrease in RubisCO activity in the Fe-added treatments. According to our cloning data (Fig. 6), a shift in phylogenetic composition of the diatoms actively transcribed *rbcL* was observed in the Fe-added bottles. In addition, F_v/F_m values increased significantly with Fe enrichment in our incubation experiments (Sugie et al., 2013), indicating an increase in photochemical quantum efficiency of photosystem II for the diatoms. Therefore, the photosystem II activity might compensate for the decrease in RubisCO expression under Fe-replete conditions."

4. *"However, because carbon fixation in diatoms is controlled not only by RubisCO activity but also by CCMs (Rost 20 et al., 2003)," Actually, in the discussion you raised the issue of RuBP regeneration as a limiting factor under elevated CO2 as well.*

We have amended the sentence as follows:

"However, photosynthetic carbon fixation in diatoms can be controlled not only by RubisCO activity, but also other processes such as carbon concentration mechanisms (CCMs) and/or RuBP regeneration (Rost et al., 2003; Onoda et al., 2005). More detailed studies on molecular mechanisms are required to clarify the physiological responses of the diatom community to CO_2 and Fe enrichments."

5. *Technical corrections: Table 1: "Macronutrients and Fe parameters are the values at the initial or final sampling days." Is the final sampling day 4? or day 6? Or either depending upon the particular treatment? I think this needs to be defined.*

We have modified the caption of Table 1 as follows:

"Macronutrients and Fe parameters are the values at the initial or final sampling days (i.e., day 5 for the control and day 6 for the Fe-added treatments)."

In addition, we have added a supplemental table (Table S1) showing the sampling times for each parameter.

6. *Given the large drops in NO3-, PO43- and silicic acid, what is the time course? By the final sampling points the cells were likely limited by macronutrients.*

Macronutrients were depleted after days 4 or 5 in the Fe-added treatments (see Lines 270–271 in the revised manuscript), suggesting that the phytoplankton cells were limited by nutrient availability at the final sampling day. We have added the time course of macronutrients in the supplementary (Fig. S2).

7. *Figure 1 legend: define the basis of the normalization (g pigment/g chlorophyll a, I think).*

In the previous manuscript, the pigment concentration on the final sampling days was divided by initial concentration of the same pigment. However, in the revised manuscript, figure 1 has been replaced by the graphs showing temporal changes in the concentrations of fucoxanthin and 19'-hexanoyloxyfucoxanthin, following the suggestions from the other reviewers.

8. *Figure 3 & Results: Fucox This is not a standard abbreviation. Why use it? Why not just write Fucoxanthin? Fucox also has an unfortunate pronounciation in English. Discussing the 'Fucox' graph is going to make people think of rude behaviour with neutered male cattle ;).*

Following the kind suggestions from you and the reviewer #2, we have amended the abbreviation "Fucox" to "Fuco".

9. *Dinoflagellates, not dinoflagillates*

Corrected

10. *"and diatoms that were neither centrics nor pennates" Do you mean diatom sequences that could be assigned to centrics or pennates? Or diatoms that are actually something other than centric or pennate? I did not know about any.*

We intended that “diatoms which could be assigned to centrics and pennates”. We have amended the sentence.

11. *“A significant correlation between rbcL copy number in diatoms and Fucox concentration was found in this study (Fig. 3), suggesting the usefulness of the rbcL gene fragment as a proxy for diatoms as well as Fucox.” I think, rather: “A significant correlation between diatom rbcL copies per litre and Fucox concentration was found in this study (Fig. 3), suggesting the usefulness of the rbcL gene fragment as a proxy for diatom biomass.”*

    Corrected. Thank you for your kind suggestion.

References:

1. Smith, C. J., Nedwell, D. B., Dong, L. F., and Osborn, A. M.: Evaluation of quantitative polymerase chain reaction-based approaches for determining gene copy and gene transcript number in environmental samples, Environ. Microbiol, 8, 804–815, 2006.
2. John, D. E., Patterson, S. S., and Paul, J. H.: Phytoplankton group specific quantitative polymerase chain reaction assays for RuBisCO mRNA transcripts in seawater, Mar. Biotechnol., 9, 747–759, 2007.