Reply to Referee #3

We are very grateful for your constructive comments to our manuscript. Following the helpful suggestions from you and the other reviewers, we believe that our manuscript has been modified significantly. Below are our point-by-point replies to your comments given in italics.

1. *Page 18106, lines 11-15. This is misleading! 1) "At the END of incubation, the relative contributions of diatoms to. . .". According to Materials and Methods, the incubation last for 7 days and therefore day 7 should be the END; however, Fig. 2A. only shows data collected on days 3 and 5.*

   In the revised manuscript, we have amended the incubation periods as 5 day for the control and 6 day for the Fe-added treatments.

2. *2) The contribution of diatom to total Chla biomass actually increased over the course of the experiment regardless of CO2 or Fe treatments (Fig. 2). It is the extent of this increase that was less at high CO2.*

   We have amended the sentence as follows:

   "At the end of incubation, the relative contribution of diatoms to chlorophyll *a* biomass was significantly higher in the 380 ppm CO_2 treatment than in the 600 ppm treatment in the controls, whereas minimal changes were found in the Fe-added treatments."

3. *Page 18106, line 21. No, it is not the "activity" – there is no RubisCO activity measurement in this study – it should be RubisCO transcription.*

   According to your kind suggestion, we have corrected the word.

4. *Page 18109, lines 2-4. Is the effect of CO2 and/or Fe availability on rbcL transcription in diatoms really COMPLETELY unknown? Here I just give two examples: Granum et al. 2009 J Phycol; Shi et al. 2013 Appl Environ Microb.*

   Thank you for your valuable comments. As you kindly suggested, the effects of iron and/or CO_2 (including low CO_2) on the diatom *Thalassiosira pseudonana* CCMP1335 were investigated. However, the diatom strain was isolated from Moriches Bay (New York, USA) and the cell size is rather small (4−6 μm: http://ncma.bigelow.org/ccmp1335) as compared with those observed in our experiments (see Sugie et al., 2013). We consider

that it is difficult to apply the results from laboratory experiments using the diatom strain to our study conducted in the oceanic Bering Sea. In the former manuscript, we intended that the effects of increased CO_2 and Fe levels were little known on natural diatom assemblages in HNLC waters. Therefore, we have amended the sentence as follows:

"In addition, there are no reports on the effects of CO_2 and Fe availability on *rbcL* transcription of natural diatom community in HNLC regions."

5. *Pages 18109-18110, "Experimental setup". More details on how trace metal clean techniques were applied should be provided. For instance, under what conditions and how was the seawater poured into 50 L carboys? Did the CO2 gas pass through 0.22 filters before being introduced into the incubation bottles?*

   We have added the detailed procedures for trace metal clean technique to the revised manuscript. We followed the procedures of Yoshimura et al. (2013).

6. *The authors discuss the roles of the CCM in the response of diatoms to CO2 and Fe. They first (page 18121, lines 4-5) suggest that CCM may have been down-regulated at high CO2, resulting in the decrease in biomass in both Fe-deficient and Fe-added bottles; however, later on (page 18123, line 26 to page 18124, line 1) they suggest that "diatoms can upregulate CCM activity at elevated CO2. . ., photosynthetic carbon fixation in diatoms could not be limited by CO2 availability as a consequence of the CCMs". These two statements are contradictory to each other. Please clarify! Without any direct experimental evidence it would be impossible to evaluate the roles the CCM may play in this paper.*

   We have deleted the paragraphs in pages 18121, 18123, and 18124, because we have no experimental evidence on it. In the revised manuscript, we have minimized the description on CCMs (see Lines 574–579 in the revised manuscript)

7. *Page 18124, lines 2-8. Fv/Fm indicates the maximum photochemical quantum yield of PSII. An increase in Fv/Fm doesn't necessarily mean more energy for CCMs.*

   In the revised manuscript, this sentence has been deleted.

8. *Figs. 1, 2, and 4. The time points at which the data presented in these figures were collected are inconsistent. Fig. 1 shows pigment data from the first and the last day (day 7,*

I presume), Fig. 2 shows data from days 3 and 5 for the Fe-deficient and days 4 and 6 for the Fe-added treatments, and Fig. 4 shows data from day 3 for the controls and day 2 for the Fe-added bottles. The authors need to clarify why the samplings/measurements were performed in such a way, which makes it difficult to compare the results among the treatments to arrive at conclusions.

We have added a supplemental table (Table S1) for clarifying the sampling points. In addition, figures 1 and 2 have been replaced by new graphs showing the temporal changes in fucoxanthin, 19'-hexanoyloxyfucoxanthin, and relative phytoplankton composition to Chl *a* biomass in order to exhibit the data from all sampling days in our experiment. We have also revised the chapters of results and discussion in accordance with the new data set.

Reference

Yoshimura, T., Suzuki, K., Kiyosawa, H., Ono, T., Hattori, H., Kuma, K., and Nishioka, J.: Impacts of elevated CO_2 on particulate and dissolved organic matter production: Microcosm experiments using iron deficient plankton communities in open subarctic waters, J. Oceanogr., 69, 601–618, 2013.