

Anonymous Referee #2

[Comment] a) Title: The study presents much more than just the effects on the nutrient consumption ratio. I realize the focus is on the nutrient dynamics but the changes in community composition are just as important. Therefore, I would suggest the following revised title: "Synergistic effects of pCO<sub>2</sub> and iron availability on the phytoplankton community and nutrient consumption ratio dynamics in the Bering Sea".

[Response] Thank you for your constructive comments. We recognized that phytoplankton dynamics we observed was also interesting. Unfortunately, we feel that mechanisms altered their composition was difficult to discuss without some speculations. Furthermore, we believe that title should be as simple as possible. Therefore, we have left the title untouched.

[Comment] b) Particulate nutrients: In addition to the ratios, it would be nice for the authors to present the actual concentrations and how they changed over time. If not incorporated in the discussion, this data could be included in the supplemental.

[Response] Concentration of POC, PN, and BSi values have been described in the result section as follows; "At the beginning of the experiment, the concentrations of POC, PN, and BSi were 10, 1.5, and 3.8  $\mu\text{mol L}^{-1}$ , respectively. POC concentrations increased to 40.1 and 23.8  $\mu\text{mol L}^{-1}$  in the C-380 and C-600 treatment, respectively on day 6. In the Fe-added treatments, POC concentration increased to 66–89  $\mu\text{mol L}^{-1}$  on day 5 without statistically significant difference among CO<sub>2</sub> variations and it increased further after the nutrient depletions (suppl. Fig. 1). Net specific growth rate calculated from the POC data showed the same trend as estimated from Chl-a. The increase in PN and BSi was closely followed by the amount of nutrient drawdown described below (suppl. Fig. 1)". Figure has been made as a supplementary material.

[Comment] c) Pg. 4340: Is the PDMPO fluorescence intensity normalized to a specific area? In other words, are the measurements independent of the actual size of the cell?

[Response] Thank you for your kind attention.

Fluorescence data represented in this paper is normalized with cell size. Therefore, we have added one sentence to the method section as follows; "To minimize the difference in cell size of each diatom species and among treatments, cellular fluorescence intensity was normalized with the area of fluorescent frustules."

[Comment] d) Pg. 4342: I found the lack of response by the Rhizosoleniaceae diatoms in the Fe-amended high CO<sub>2</sub> treatments rather intriguing. However I was left a bit unsatisfied as there is little discussion as to why they did not respond. Could the authors speculate how these diatoms may have been negatively affected by high CO<sub>2</sub> compared to the other diatoms groups that seemed to be less affected? Is it because they are much larger cells?

and

[Comment] f) Pg. 4347: The authors claim the main effects of CO<sub>2</sub> levels on species composition are under Fe-replete but (macro)nutrient-depleted conditions. This is an important result, but a bit confusing. How does the species composition still change after macronutrients are depleted? Shouldn't this terminate growth? That is, unless there is significant nutrient storage right? Do the authors think CO<sub>2</sub> could be affecting their nutrient storage capacities? I would be interested in further discussion about the actual mechanism behind this affect.

[Response] The reviewer tasked us to describe a possible mechanism for the decline in the dominance of Rhizosoleniaceae in the Fe-replete condition after nutrient depletions. We could not construct a proper logic to respond against the above comments. Probably, large cell can storage nutrients in their vacuole but we lack the information to date to support the hypothesis as reviewer pointed out that alternation of stored nutrients against the change in environmental conditions such as CO<sub>2</sub> and pH. Other plausible mechanisms are also difficult to represent here without a leap of logic. Therefore, we only can disseminate the information from our findings to readers that we need further investigation under nutrient-depleted conditions.

[Comment] e) Pg. 4343 (and Figure 7): Overall I find this figure not very informative and a bit misleading. Through plotting the changes in nutrient concentrations over chl a, I do not get the sense this ratio will provide a measure of the nutrient requirements per unit phytoplankton biomass. This is due to the significant changes in cellular chl a after iron addition. As shown in the figure, this implies a much larger consumption of nutrients (per unit phytoplankton biomass) in the controls versus the iron amended treatments, which I don't think is actually the case. I realize POC concentrations are not algal specific however the differences from the initial concentrations to day 4 are likely to be dominated by increases in the phytoplankton biomass. Therefore, to get a sense of the changes in cellular Chl a quotas, could the ratios of Chl a/POC provide a good measure? Also, did the authors try plotting changes in nutrient drawdown normalized to POC concentrations?

[Response] We avoid presenting our data on nutrient drawdown per unit POC because (1) substantial part of POC was probably composed of heterotrophs such as bacteria and micro-zooplankton which could grow rapidly as fast as phytoplankton (Rose and Caron, 2007, *Limnol. Oceanogr.* 52, 886); and (2) carbon content per unit phytoplankton biomass might also change due to the change in external environment (Sugie and Yoshimura, 2013, *J. Phycol.* in press. In this respect, our analysis based on Chl-a have a clear advantage because Chl-a is derived solely from phytoplankton. The decrease in Chl-a quota in response to iron deficiency has now widely been recognized as the reviewer pointed out and we have discussed the phenomena of chlorosis under low iron availability in the revised manuscript. Further, fluctuations of intracellular carbon content against CO<sub>2</sub> or iron variations have rarely examined, but the POC per cell or cellular C concentration can be significantly changed due to the effects of them (e.g. Sugie and Yoshimura, 2013). Therefore, we normalized the amount of nutrient drawdown by chl-a.

[Comment] Although it is important to note that the dramatic changes in phytoplankton species composition in response to iron addition need to be considered as well as changes in individual cell physiology.

[Response] The changes in diatom community composition in the Fe-limited controls were slower than that of the Fe-added treatments. However, the algal compositions between the controls and the Fe-added treatments were rather similar to each other until the nutrient depletions.

[Comment] g) Pg. 4347: In addition to the mechanisms listed, the authors also cannot rule out variations in the phytoplankton composition (and their intrinsic nutrient requirements) between the control treatments as an explanation for differences in the Si:N and Si:C ratios. As shown in figure 5, although very similar, the diatom composition of the control treatments was not exactly the same. In addition, there is no description of possible differences in the non-diatom plankton communities that could be affecting the nutrient consumption ratios (although likely in a very minor way).

[Response] We found that the community compositions between the two iron-limited controls were similar to each other. However, it would be very difficult to explain the underlying mechanisms altering such a very small variations. In our results, the dominance of autotrophs other than diatom was very small and it was difficult to discuss the variations of them among treatments. Therefore, we estimated the effects of CO<sub>2</sub> and iron on the diatoms solely.

A few technical comments:

[Comment] a) Pg. 4341, Line 28: Use “lower” instead of “small”

[Response] Revised as suggested.

[Comment] b) Pg. 4346, Line 26: Use “less” instead of “minor”

[Response] Revised as suggested.

[Comment] c) Pg. 4348, Line 26: Instead of “Our recent study reported...”, should this be “Our other recent studies reported...”

[Response] Revised as suggested.

[Comment] d) Pg. 4349, Line 25: This sentence is a bit awkward. What does “when the nutrient remained in conditions” mean? Please rephrase.

[Response] We revised it follows; “In the PDMPO incubation experiment conducted before nutrient exhaustions, the silicification...”