Anonymous Referee #1

[Comment] Besides, the specific growth rate and nutrient drawdown were only based on chlorophyll a, which is affected by iron status. The presentation of POC data is necessary here to aid our understanding of the results. The change in pCO2 usually causes changes in several parameters, including CO2 availability for photosynthesis, pH of seawater and iron availability due to the change in pH. Each parameter has different effects on phytoplankton physiology. The authors should try to tease apart these effects in the discussion.

[Response] Particulate nutrient data has been shown in the Result section as suggested.

In this study, we would like to present our finding, especially the changes in particulate Si:N ratio under iron-limited, high-CO_2 condition. Possible mechanisms changing elemental compositions were discussed in sections 4.1 and 4.2 with considerations of the effects of CO_2 availability, pH or iron availability on phytoplankton processes (e.g., P.4345 L.22– P.4346 L.11). In addition, to distinguish the effect of CO_2 from that of pH accurately, at least two manipulation experiments in terms of carbonate chemistry are required. At this point, however, we cannot discern the individual effect of CO_2 availability and pH further. We discussed the effects of carbonate chemistry and iron on phytoplankton ecophysiology individually. Our responses to your comments were also described in the specific comments below.

Specific comments:

[Comment] P4341, 2nd paragraph and Fig 3. As mentioned above, it would be helpful if the growth rate based on POC were presented here.

[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 μmol L^{-1}, respectively. POC concentrations increased to 40.1 and 23.8 μmol 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 μmol L^{-1} on day 5 without statistically significant difference among CO_2 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] P4343, 2nd paragraph and Fig 7. Again, data on nutrient drawdown per unit of POC would be more informative and reflect the real phytoplankton biomass here.

[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_2 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] Page 4343, 3rd paragraph and Fig 8. It is not clear how PDMPO fluorescence was quantified and normalized. If cells in iron-replete treatments have higher growth rate, shouldn't cells have higher fluorescence? Was there any change in cell size during incubation? In some cases, cells become smaller under iron limitation. Is it possible that the higher fluorescence in *Neodenticula seminae* is due to the difference in cell size compared to other species?

[Response] Thank you for your kind attention.

The 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] Page 4344, 2nd paragraph and Fig 9. What about the actual values of POC, PON and Si? In Fig 9, Si:C and Si:N ratios were higher in control treatments than in iron added treatments. However, in Fig 8, there was no significant difference in PDMPO fluorescence between control and iron added treatments, which is not consistent with Fig 9. Why?

[Response] Actual values of POC, PN and BSi have been presented in the Result section. Possible mechanisms for changing elemental compositions were described over the Discussion 4.2 section.

[Comment] Page 4345. 1st paragraph. Since in this particular incubation experiment, the coastal diatoms dominated the phytoplankton community, it may not reflect the response of the original phytoplankton community in HNLC region to pCO2 and iron, which is often dominated by oceanic species. The authors should keep this in mind in their discussion.

[Response] Coastal diatom species such as resting spore-forming *Chaetoceros* subgenus *Hyalochaete* spp. was initially observed as shown in Fig. 5. We acknowledged that seawater used in this experiment is seemingly different from the typical open ocean HNLC waters as discussed in the first paragraph of our Discussion. Comparisons between our results and those of the other CO_2 manipulation experiments using open ocean HNLC waters were also made in the last paragraph of the section 4.2.

[Comment] Page 4345, 2nd paragraph. The conclusion drawn at the end of this paragraph is not

well supported by data presented in this study. First, the change in species composition was very subtle and only occurred in minor species. Second, the change was already seen between day 2 and day 4 when nutrients were not depleted yet.

[Response] We believe the results observed in this study are important when understanding other manipulation experiments or predictions for the future environment. In addition, it should be noted that comparing community composition among treatments was made at each time point. We need to consider the mechanisms for changes in community composition during experiment at each point in time. Although the reviewer indicated is the alternation of the community after the collection of seawater from *in situ*, which could be caused due to the bottle effect and the absence of mesozooplankton in the incubation bottles.

[Comment] Page 4348, 1st paragraph. Again, it would be clearer if POC and PON values were presented in results. If indeed the higher Si:N and Si:C ratios were caused by the decrease in PON and POC content under iron limitation, it seems surprising and puzzling that POC and PON in iron replete treatments were 3 to 4 folds lower that that in iron limited treatments.

[Response] We are afraid that the reviewer probably misunderstood our results. If BSi content was constant as described in the manuscript, “higher” POC and PN (i.e., iron-replete) should produce lower BSi:POC and BSi:PN ratios, respectively.