Reply to Referee #2

Thank you very much for your helpful suggestions and constructive comments. Below are our point-by-point responses to your comments.

1. *The abstract states: "At the end of the incubation, the relative contributions of diatoms to chl a biomass decreased significantly with increased CO2 levels in the controls". This is misleading as the contribution of diatoms to chl a biomass increased over the course of the incubation in all bottles; it is the extent of this increase that is less at high CO2.*

   Thank you for pointing it out. 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."

2. *In addition, the sentence starts with "At the end of the incubation. . ..". This would be after 7 days when the bottles were clearly depleted of nutrients. Table 2 gives insufficient information to know when nutrient limitation occurred and I would also like information on how long it took the bottles to equilibrate with CO2 (this information is given in Sugie et al, 2013 but is not sufficiently discussed in this manuscript). In addition, it is confusing to know when the data points were collected. Table 2 and Figure 1 show data from the final day (7?) whereas Figure 2 shows data from days 3 – 6. This lack of clarification makes it difficult to draw conclusions to what is happening and raises question to whether the results are purely due to CO2 manipulation and not due to nutrient limitation.*

   Thank you for pointing them out. We have added the Figs. 1S and 2S showing the time courses of carbonate chemistry and macronutrients, respectively. In addition, temporal changes in fucoxanthin and 19'-hexanoyloxyfucoxanthin concentrations have been shown in Fig. 1 instead of the growth ratios of these pigments. We have also reconstructed the results and discussion in the revised manuscript in accordance with the new figures (see Lines 288–302, 390–392, and 420–423).

3. *The abstract further states "These results indicate that under Fe-deficient conditions, the growth of diatoms was negatively affected by the increase in CO2 availability". I would be careful with this statement. I would say their ability to compete is better at high CO2. I am*

interested in what is happening with the haptophytes. Like diatoms they also increase in abundance over the course of the incubation but this increase is less under high CO2. However, in Fig 2 it looks like they increase their contribution to total chl a at high CO2 in control bottles. Perhaps the story is more about the competition between diatoms and haptophytes under different CO2 rather than just interpreting everything in terms of diatom growth.

Following your kind suggestion, we have amended the sentence as follows:

"These results indicate that, under Fe-deficient conditions, the growth of diatoms could be negatively affected by the increase in CO_2 availability."

Furthermore, we have added the following sentences to the discussion:

"Another possibility is that the competitions between diatoms and other phytoplankton taxa could occur. For example, diatoms could become less competitive when silicic acid is exhausted, because Si-depletion significantly depressed the growth and could induce their cell death (Harrison et al., 1977; Jiang et al. 2014). However, concentrations of silicic acid were not significantly different among CO_2 levels in the Fe-added treatments (Fig. S2f). Moreover, in the control treatments, silicic acid was almost depleted in the low CO_2 treatment after day 5 but not in the high CO_2 treatment (Fig. S2e). These results suggest that availability of silicic acid little affected the decreases in relative diatom contribution to Chl *a* biomass."

4. *A lower expression of diatom rbcL normalized to rbcL gene number certainly implies the diatoms are less active. This finding supports other studies that show Rubisco is regulated at the expression level in diatoms. However, this has not been absolutely proven yet, and given the tight regulation of Rubisco protein activation in plants, it is hard to accept that rbcL expression equals photosynthetic rates in this paper without more study.*

We have added the following sentences to the end of the paragraph:

"Recently, Gontero and Salvucci (2014) pointed out that RubisCO activase plays a key role in the modification of RubisCO activity, and consequently in the capacity of carbon fixation, although the occurrence of RubisCO activase in diatoms is not well understood. Further studies must be needed for better understanding of the impacts of elevated CO_2 on photosynthetic physiology in diatoms."

5. *How do the authors reconcile that rbcL expression is lower in both Fe-added and Fe limited incubations whereas fucoxanthin concentrations are only lower in Fe-limited cultures?*

We have explained this in the chapter of discussion as follows:

"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 the 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."

6. *It is difficult to tell from the rarefraction curves whether they are approaching saturation. As such, it is difficult to say whether the number of OTUs are different between the treatments. I do not have a good understanding on whether the differences found in the Shannon Index and Simpson diversity are significant. More details would be appreciated.*

The difference between samples cannot be determined from the number of OTUs, because the rarefaction curves were not completely saturated. Alternatively, we have added the chao1 index as an indicator of OTU richness (Chao, 1984) (Table 2). This index was calculated based on the number of singleton OTUs (OTUs with only one sequence obtained) in the clone library. In addition, we showed the 95% confidence intervals (CI) for the Shannon and Simpson indices (Table 2) to clarify the statistical significance among treatments. According to these results, we have reconstructed the results and discussion on the manuscript (Lines 338–343 and 551–555).

7. *Significant differences were found in the cDNA libraries under different CO2 within the Fe-treated incubations. Are the authors certain that this is due to a change in diatom rbcL sequences rather than a change in the non-diatom rbcL sequences that were detected? (in the initial treatment it seems that _ 17 % of the rbcL cDNA library comes from other eukaryotes).*

As mentioned in the results on the revised manuscript, other eukaryotes contain diatoms that could be assigned to centrics and pennates. Actually, initial treatment contains only 11% of the sequence derived from eukaryotes other than diatoms. In addition, the other libraries consist of ≥92% sequences from diatoms. Therefore, we considered that the

differences between CO_2 treatments were primarily due to the changes in diatom *rbcL* sequences.

8. *The authors discuss the influence of Fe and CO2 on the CCM. However, I feel that their link between Fe and the CCM is tenuous. Fe is important for PSII, and Sugie et al (2013) found increased Fv/Fm with increased Fe, which is to be expected. However, speculating that the Fe limitation down-regulates the CCM through lack of energy provided by PSII seems tenuous. Without any further measurements it is difficult to draw any conclusions about the role of the CCM in this paper.*

   In the revised manuscript, the discussion on CCMs has been excluded.

9. *In the discussion about Chaetoceros, it should be noted that Trimborn et al, 2013 Limnol. Oceanogr., 58(3), 2013, 997-1007 | DOI: 10.4319/lo.2013.58.3.0997, found that Chaetoceros debilis increased growth rates under high CO2 and that Tortell et al 2008 GRL, 35 (4) DOI: 10.1029/2007GL032583 found Chaetoceros spp. to dominate shipboard incubations from the Ross Sea under high CO2.*

   According to your kind suggestion, the following two sentences have been added to the chapter of discussion:

   "However, Tortell et al. (2008) demonstrated that relative abundance of *Chaetoceros* spp. increased under elevated CO_2 levels in the Ross Sea."……."In contrast, Trimborn et al. (2013) showed a significant increase in the growth rate of *Chaetoceros debilis* under high CO_2 condition."

10. *I presume figure 1 is the concentration of fucoxanthin (µg/L) in the final sampling day (day 7?) divided by the fucoxanthin concentration (µg/L) in the initial bottle? This should be clearer.*

    We have revised figure 1 as temporal changes in phytoplankton pigment concentrations.

11. *Fucox is a strange abbreviation and I would keep the full word fucoxanthin.*

    We have changed the abbreviation "Fucox" to "Fuco".

12. *In abstract "To confirm this. . ." (that diatom growth is negatively affected by high CO2) seems to be too strong a statement as mRNA of rbcL is not a direct measurement of growth. "To further support this finding. . ." would be better.*

Following your kind suggestion, we have corrected this sentence in the revised manuscript.

13. *In the supplementary I would appreciate more information about how the conditions changed in the bottles over time. Perhaps a Table showing the measured values from every day so the readers can see when nutrient limitation occurs, how long it takes CO2 to equilibrate, the increase of total phytoplankton biomass (POC or Chla) over time and when different samples were collected.*

Following your kind suggestion, we have added the time courses of carbonate chemistry, macronutrients, and chl *a* in the supplementary materials (Figs. S1-S3). In this manuscript, we have dismissed POC, because Yoshimura et al. (2014) discussed this matter.

References

Yoshimura, T., Sugie, K., Endo, H., Suzuki, K., Nishioka, J., and Ono, T.: Organic matter production response to CO_2 increase in open subarctic plankton communities: Comparison of six microcosm experiments under iron-limited and-enriched bloom conditions, Deep-Sea Res. I, 94, 1–14, 2014.

Chao, A.: Nonparametric estimation of the number of classes in a population. Scand. J. Stat., 265–270, 1984.