Reply to Referee #1

[Referee comment] *The authors did not explain clearly in the title and the introduction of the manuscript that the experiment did not treat just about acidification effect on planktonic communities but also about the bloom effect on communities receiving acidification treatments. After reading the manuscript, we are quite confused about the results and their interpretations. We don't really know if the results obtained by the authors were caused by an increase of temperature during the experiment, by different incident light receiving at the mooring site in comparison with the sampling site (we don't know because it has not been measured), by the nutrient supply or all together. Furthermore, we do not know if the obtained results were due to the method used by the authors to measure the planktonic metabolism and how could have been the results if the authors had rather used for example the 14C method. Furthermore, the authors concluded about "significant" decrease of NCP with increasing pCO2 when for the whole experiment, phase 1 and 2, there is no significant NCP increase. I think that the manuscript can be very interesting but important data are missing to improve much better the manuscript (addition of a nutrient control mesocosm, measurement of the planktonic metabolism using a C-labelled method, daily planktonic metabolism, daily pH, pCO2, Chl, BA, temperature and etc measurements).*

[Author response] We changed the title and revised the introduction to clarify the design of our mesocosm experiment. The title is now: "Effect of increased pCO_2 on the planktonic metabolic balance during a mesocosm experiment in an Arctic fjord"

The increase in seawater temperature during the experiment was a natural event which occurred in open water as well as in all mesocosms. In our experiment, pCO_2/pH was only the only factor that differed significantly among the mesocosms (the nutrient addition was same in all mesocosms). The increase in temperature may have stimulated the plankton metabolism, however this effect would have been similar between the mesocosms.

^{14}C fixation was measured on pre-filtered (<200 μm) samples at another incubation site at which the irradiance might be different from our incubation site and the mesocosm site (see Engel et al., 2012). According to these authors, they measured a rate which was between net and gross primary production. Since we were interested in NCP only, we compare O_2-NCP with *C*t-NCP (Silyakova et al., 2012) and ^{13}C-NCP (de Kluijver et al., 2012).

We added the inorganic nutrients to all 9 mesocosms on t13, and therefore did not have nutrient control mesocosm in this experiment. The daily values of Chl-a have been added to

the revised manuscript. We do not think that there is a need to also add all data on C-based plankton metabolism, pH, pCO_2, bacterial abundance, and water temperature. It would be a lot of duplication. Instead, we refer to the relevant papers presenting these data.

[Referee comment] *You should introduce the nutrient bloom of your experiment on the text.*
[Author response] As suggested, we added a sentence in the introduction: "Nutrients were added to all mesocosms in the middle of the experiment in order to stimulate phytoplankton growth." (L99-101 in the revised manuscript)

[Referee comment] *Why did you choose to make your experiment with post-bloom communities? Don't you think that planktonic communities could respond differently to a pCO2 increase and nutrient supply with pre-bloom planktonic communities?*
[Author response] The post-bloom period was chosen for logistical reasons. It is impossible to precisely schedule such a large-scale experiment in pre-bloom conditions because the timing of the disappearance of the ice varies a lot from one year to the next. We could not risk arriving too early which would have had enormous financial and scientific consequences. Since different taxonomic groups in phytoplankton community responded differently to increasing pCO_2 (e.g., Aberle et al., 2012; Brussaard et al., 2012; Schulz et al., 2012), it is indeed possible that pre-bloom plankton communities respond differently to a pCO_2 increase and nutrient supply.

[Referee comment] *I recommend you to show in table and in a graph the daily evolution of pH, pCO2, Chl a, temperature, NO3 and PO4 concentration during the entire experiment.*
[Author response] These parameters are fully presented in Schulz et al. (2012). We agree that the data on chlorophyll *a* concentration are important to interpret NCP, CR, and GCP. We included chlorophyll data in Fig. 1 of the revised manuscript.

[Referee comment] *Please insert a reference for the nutrient concentrations that you used to simulate a phytoplankton bloom.*

[Author response] The following sentence has been added: "The nutrient concentrations were chosen to simulate an upwelling event (Schulz et al., 2012)." (L130 in the revised manuscript)

[Referee comment] *Why did you sample mesocosm water for the planktonic metabolism incubation in the afternoon when it is commonly made early in the morning?*

[Author response] The experiment involved a lot of sampling (typically several hours each day). Hence, two shifts were organized. The chemists sampled in the morning, while most biologists sampled in the afternoon. "Such a separate sampling program was used because of logistical constraints." (L137-138 in the revised manuscript)

[Referee comment] *You should precise in the text that the four bottles immediately fixed with Winkler reagent are considered as control for the DO measurement.*

[Author response] As suggested, this sentence now reads: "Four bottles were immediately fixed with Winkler reagents to determine the initial concentration of dissolved oxygen (DO), and served as a control." (L144-146 in the revised manuscript)

[Referee comment] *"NCP and CR were measured every 2 day and every 4 day", I do not really understand. Did you measure NCP and CR at t2 and t4 at each phase or at t2, t4, t6, t8, t10 and etc? Please could you make it clearer?*

[Author response] If the first incubation involved measurement of both NCP (24 h) and CR (48 h) on t0, the second incubation measured only NCP on t2, and the incubation measured both NCP and CR on t4. We added the following sentence: "That is, the incubation of both NCP and CR bottles and that of NCP bottles alone were done alternatively during the experiment." (L157-158 in the revised manuscript)

[Referee comment] *Please could you specify that planktonic metabolism was determined at 4m depth because "the incubation was at 4 m depth" means to me that the bottles were incubated at 4 m depth.*

[Author response] We modified to "The BOD bottles were incubated at 4 m depth." (L160 in the revised manuscript.)

[Referee comment] *How can you explain that CR measurements did not detect change in DO during 24h incubation in the dark? Did you use dark bottles and put them into dark plastic bags for the incubation? What can be the bottle effect or confinement effect after 48 h of incubation into 60ml bottles? How could those 48h of incubation bias the measured CR rates?*
[Author response] The initial water was characterized by low nutrients and low chlorophyll. The resulting low biological activity did not allow detecting statistically significant negative regression slope of DO concentration (i.e. CR) during preliminary 24 h incubation performed before the experiment started. We then decided to perform longer incubations throughout the experiment in order to avoid having non-significant slopes prior to the nutrient addition. We felt that the same duration should be used before and after the nutrient addition to avoid adding another factor (duration of incubation) which would have made the interpretation of the data difficult.
We agree that the description of the CR measurements was confusing and have revised the text as follows: "For the CR measurement, dark BOD bottles were incubated at the *in situ* mooring site until 18 June (t11), and clear BOD bottles were incubated in a dark laboratory incubator from 19 June (t12) onwards due to logistical constraints." (L164-166 in the revised manuscript)
While preliminary experiments with different time-point measurements showed linear decrease of DO concentration during 48 h, the DO concentration were determined only at the beginning and after 48 h of the incubation during the experiment. Hence, we cannot evaluate bias in the measured CR. We now acknowledge the potential bias of the bottle effect: "It has been reported that long incubation (24 h for NCP and 48 h for CR in this study) in bottles can result in important changes in the abundance, activity, and composition of the community, leading in turn to significant changes in the planktonic metabolism (Pomeroy et al., 1994; Calvo-Díaz et al., 2011)." (L376-380 in the revised manuscript)

[Referee comment] *Please could you also explain how did you calculate the SE of NCP and CR?*

[Author response] As mentioned in the manuscript, the rates of NCP and CR were determined by linear regression of DO against time (slope ± standard error: μmol O_2 l^{-1} d^{-1}). (see L175-176 in the revised manuscript)

[Referee comment] *Do you think that 7 days it was enough to observe any effect of pCO2 increase without any nutrient supply? Do you have any reference that supports your decision to start the phase 2 at t7? Why did you not measure the planktonic metabolism every day? How do you explain the "second chlorophyll minimum", its origin?*

[Author response] Not many but certain parameters showed significant responses to increasing pCO_2 in this experiment. The decision of the nutrient addition on t13 was somewhat subjective.

The frequency of NCP (every 2 days) and CR (every 4 days) measurements were decided by the necessary incubation time and logistical constraints. Of course, every day measurement of the plankton metabolism would have been ideal but was unfortunately impossible to perform (see above).

Please refer to Brussaard et al. (2012) and Schulz et al. (2012) for detailed information of phytoplankton dynamics during the same study.

[Referee comment] *Why did you use cumulative data rather than means? Any reference?*

[Author response] We examined the effect of increasing pCO_2 on the planktonic metabolism by analyzing the data on planktonic metabolic rate on daily basis (i.e. each measurement) and period basis (i.e. cumulative data), respectively.

With regard to the effect of increasing pCO_2 on plankton community and biogeochemistry, many papers report 'cumulative values'. For example, Riebesell et al. (2007, Nature, 450, 545-548), Bellerby et al. (Biogeosciences, 5, 1517-1527), Egge et al. (2009, Biogeosciences, 6, 877-885), Czerny et al. (2012, Biogeosciences Discussion, 9, 11885-11924), Engel et al. (2012, Biogeosciences Discussion, 9, 10285-10330), Piontek et al. (2012, Biogeosciences Discussion, 9, 10467-10511), Silyakova et al. (2012, Biogeosciences Discussion, 9, 11705-11737). Since this is common practice, we do not think that it is necessary to add a reference on the use of cumulative data.

[Referee comment] *Lines 190-192: Is it significantly higher? Please could you improve the different comparisons in the results section with statistical test?*

[Author response] We refer this to Schulz et al. (2012). We revised the text as follows: "After the addition of nutrients, the net consumption rate of NO_3 and PO_4 was statistically higher in higher pCO_2 mesocosms from t17 to t22, while the cumulative nutrient consumption became similar in all mesocosms toward the end of the experiment (Schulz et al., 2012)." (L213-216 in the revised manuscript)

[Referee comment] *How do you explain that you observed 3 times a peak of chlorophyll in all mesocosms? Which interpretation do you have for this phenomenon?*

[Author response] This is a result of the balance between phytoplankton growth and loss. We added the following description of these Chl-a peaks: "The first Chl-a peak during phase 1 was largely dominated by haptophytes, while, after the nutrient enrichment, the second was due to prasinophytes, dinoflagellates, and cryptophytes, and the third was due to haptophytes, prasinophytes, dinoflagellates, and chlorophytes (Schulz et al., 2012). Top-down control on nanophytoplankton by microzooplankton grazing and viral lysis was important especially during phase 1 (Brussaard et al., 2012). The Chl-a concentration at elevated pCO_2 was statistically higher during phase 2, but lower during phase 3 (Schulz et al., 2012)." (L220-226 in the revised manuscript)

Please refer to Brussaard et al. (2012) and Schulz et al. (2012) for detailed information of phytoplankton dynamics during the same study.

[Referee comment] *Lines 220-222: Did NCP increase significantly after nutrient supply? (statistical test)*

[Author response] Our main objective was to examine the effect of increasing pCO_2 on the planktonic metabolism. So we did not apply statistical test to compare NCP before and after the addition of nutrients.

[Referee comment] *Lines 226-232: Could you please clarify this section?*

[Author response] This paragraph has been revised as follows: "The cumulative NCP revealed that it was negative in only one mesocosm (M7) before the nutrient addition (phase 1)

(Fig. 2). It should be noted that M3 and M7 were treated as the control in the same way with regard to the CO_2 perturbation (i.e., no CO_2 enrichment). During phase 3, only the cumulative NCP in M9 was negative. The proportion of the cumulative NCP during the whole period was highest during phase 3 in all mesocosms except M9. The cumulative CR in all mesocosms tended to be similar between different phases. The proportion of the cumulative GCP was highest during phase 3 in all mesocosms except M1, 6, and 9." (L254-261 in the revised manuscript)

[Referee comment] *Why did you choose a photosynthetic quotient of 1 when the common conversion factor is of 1.25 molar stoichiometry between O2 and C (Williams 1979; Davies and Williams 1984).*

[Author response] As suggested, we recalculated using PQ of 1.25. However, this modification does not affect the conclusion.

[Referee comment] *Lines 266-267: Does the increase was significant? Please could you add some statistical results?*

[Author response] We do not think that the statistical test is necessary for temperature increase during the experiment, but the sentence has been revised as follows: "The water temperature increased gradually, especially in the upper 5 to 10 m, in all mesocosms during the experiment (2.7 to 5.5°C: Schulz et al., 2012)." (L294-296 in the revised manuscript) Please refer to Schulz et al. (2012) for details.

[Referee comment] *How do you explain the second chlorophyll minimum? It can be interesting to add some sentences about this chlorophyll minimum explaining why it occurred and how.*

[Author response] We have added the following sentence: "The first Chl-a peak during phase 1 was largely dominated by haptophytes, while, after the nutrient enrichment, the second was due to prasinophytes, dinoflagellates, and cryptophytes, and the third was due to haptophytes, prasinophytes, dinoflagellates, and chlorophytes (Schulz et al., 2012). Top-down control on nanophytoplankton by microzooplankton grazing and viral lysis was important especially during phase 1 (Brussaard et al., 2012). The Chl-a concentration at elevated pCO_2

was statistically higher during phase 2, but lower during phase 3 (Schulz et al., 2012)." (L220-226 in the revised manuscript) Please refer to Brussaard et al. (2012) and Schulz et al. (2012) for detailed information of phytoplankton dynamics during the same study.

[Referee comment] *It could be interesting to see (add a figure) the evolution of the chlorophyll concentration during the global experiment.*

[Author response] As suggested, we included chlorophyll data in Fig. 1 of the revised manuscript.

[Referee comment] *Could you please add some references for the different methods Ct, 13C, 14C.*

[Author response] The methods used to measure *C*t-NCP, ^{13}C-NCP, and ^{14}C-PP are described extensively in Czerny et al. (2012), Silyakova et al. (2012), de Kluijver et al. (2012), and Engel et al. (2012), respectively. Hence, we only provide a brief description of these methods in the discussion of our manuscript.

[Referee comment] *Lines 306-307: Do you mean that we can consider the POC from 14C as the NCP from O2 method and the DOC from 14C as the GPP from the O2 method? If you mean that, I am not agreeing with you about the comparison between the DOC and GPP. On contrary GPP could be closer to POC+DOC than POC alone or DOC alone. You should revise this sentence. I am also quite surprised that POC from 14C was higher than NCP from O2 method. Could you add please some statistical information (significant or not, test, P)?*

[Author response] We meant that the sum of production rate of ^{14}C-POC and ^{14}C-DOC is somewhat between net and gross primary production in the study by Engel et al. (2012). But we did not mean that the POC from ^{14}C as the NCP from O_2 method and the DOC from ^{14}C as the GPP from the O_2 method.

Engel et al. (2012) measured primary production using ^{14}C for the <200 µm pre-filtered community, while O_2-NCP, *C*t-NCP, and ^{13}C-NCP were measured for whole community (i.e. initially 3 mm mesh sieved: Riebesell et al., 2012). Engel et al. (2012) incubated ^{14}C-PP bottles at 1m depth at their mooring site, and we incubated BOD bottles at 4 m depth at our mooring site. *C*t-NCP and ^{13}C-NCP were determined without incubation of the integrated

water samples. Difference of irradiance between the ^{14}C-PP incubation site (1 m), the O_2-NCP incubation site (4 m), and the mesocosms (0–12 m) might vary temporally. Hence, we find it impossible to meaningfully compare the ^{14}C-PP data with the NCP.

[Referee comment] *Why didn't you measure the NCP with O2 method in the same way than with 13C and Ct (daily)?*

[Author response] We could not measure the O_2-based NCP every day due to constraints of time and manpower. Measuring NCP, CR, and bacterial respiration (see Motegi et al., 2012) involved 180 determinations of oxygen. With each titration lasting about 10 min, about 30 h were needed to process the samples. It was therefore impossible to perform daily measurements with one titrator and two staffs.

[Referee comment] *Lines 331-333: You should give more information about the irradiance, some data.*

[Author response] Before the experiment started, we confirmed that the irradiance at the 4 m depth at our incubation site corresponded to the average irradiance of the water column sampled in the mesocosms (0 to 12 m). Our irradiance sensor got flooded early in the experiment. We provided the irradiance information in the mesocosms: "In the mesocosms, photosynthetically active radiation at 14.5 m and 4.2 m depth varied in a range of 2–15% and 10–30%, respectively, in comparison to the surface layer (0.1 to 0.2 m), which was likely because of temporal changes of phytoplankton biomass (Schulz et al., 2012)." (L365-368 in the revised manuscript)

[Referee comment] *Lines 338-340: You don't know if the planktonic communities received a light-stressed at the mooring site comparing with their sampling site?*

[Author response] The irradiance at the depth of BOD bottles corresponded to the average irradiance of the upper 12 m in the mesocosms. We have no reason to suspect light stress: which if it occurred in our experiment, also occurred in the mesocosms. We added this discussion: "Gao et al. (2012) recently reported that the growth rate of three species of diatoms subjected to elevated pCO_2 is inversely related to light at irradiance levels above 22 to 36% of surface irradiance in the South China Sea, and the threshold of photoinhibition

occurs at lower irradiance in elevated pCO_2 compared to the ambient pCO_2. This demonstrates the confounding effects of the synergistic and antagonistic interactions of pCO_2 and irradiance conditions on the response of phytoplankton (e.g. Boyd et al., 2010)." (L368-374 in the revised manuscript)

[Referee comment] *Lines 359-363: Your conclusion about the decrease of NCP with increasing pCO2 is maybe a little bit too optimist when you actually see that for the global experiment, for phase 1 and/or 2, there is no significant decrease of NCP with increase pCO2. Finally, we can observe a NCP decrease just for the phase 3 that it does not allow us to conclude that NCP decrease with pCO2 increasing. I do not agree your conclusion.*

[Author response] The referee's interpretation is incorrect. The last paragraph of the discussion is in agreement with the view that s/he expresses in the comment: "In conclusion, the metabolic parameters (NCP, CR, and GCP) of planktonic communities based on changes of DO concentration at different pCO_2 levels showed insignificant response of NCP during phases 1 and 2 and a significant decrease of NCP as a function of increasing pCO_2 during phase 3. CR was relatively stable throughout the experiment in all mesocosms. As a result, the cumulative GCP significantly decreased with increasing pCO_2 only during phase 3. Similarly, the ratios of cumulative NCP to cumulative consumption of NO_3 and PO_4 showed insignificant response during phase 2 but significant decrease during phase 3 with increasing pCO_2. The results suggest that elevated pCO_2 influenced cumulative NCP and stoichiometric C and nutrient coupling of the plankton community in a high latitude fjord only for a limited period. Since there were some differences or weak correlations between O_2-NCP vs. C_T-NCP and ^{13}C-NCP during phases 1 and 2, this conclusion should be taken with caution."

[Referee comment] *Table 1: It could be more helpful to add a column on the left specifying which mesocosms are controls, which received low, intermediate and high pCO2. Please could you add also pH and pCO2 for phase 2?*

[Author response] In the revised manuscript, it is mentioned that M3 and M7 received no pCO_2 manipulation (i.e. control) (see the revised Table 1) and "While pH and pCO_2 changed in all mesocosms because of air/sea gas exchange and biological carbon uptake to different degrees, the gradients of pH and pCO_2 between the treatments remained until the end of the experiment (Bellerby et al., 2012; Schulz et al., 2012; Silyakova et al., 2012)." (L124-127 in

the revised manuscript) We therefore do not think that the data on pH and pCO_2 during phase 2 should be presented.

[Referee comment] *Table 2: I don't know if the table format changed when I loaded it but the heading columns are cut. Please could you remake this table and put in a same line entire words as "Parameter", "Temperature" or "umol Si l-1", 0.05 ± 0.01". How did you integrate the temperature data?*

[Author response] The article published in Biogeosciences Discussions, and openly accessible on the web site, correctly displays the Table 2. We have made one correction for the temperature in the footnote: "the mean of 0–12 m" for temperature.

[Referee comment] *Figure1. Please could you add in the figure some lines defining the beginning of the phase 2 and phase 3? How could you explain that you have more NCP data than CR data and why?*

[Author response] As suggested, we added vertical lines to define three different phases in Fig. 1 in the revised manuscript. Note that CR was measured less frequently than NCP (see also Materials and methods).

[Referee comment] *Figure 3. It is difficult to differentiate which line corresponds to which points. Could you please use different type of line for the different phases?*

[Author response] As suggested, we used different types of line for the different phases.

[Referee comment] *Figure 4. Could you please add for each graph line 1:1?*

[Author response] Added.

We thank Referee #1 for her/his thoughtful comments.