

Response to Anonymous Referee #2

We thank the Referee for a thorough review and for pointing out important gaps in our presentation and interpretation. We appreciate that the Referee thinks some of our findings are interesting and below we address how we are to respond to some of the comments made on our manuscript.

General comments:

Referee comment: The manuscript contains a substantial number of inconsistencies and typos. Some I listed below but the manuscript should be carefully checked.

Authors' response: The reworked manuscript was carefully checked.

Referee comment: It would be very helpful if the authors could also include the relative changes in inorganic nitrogen and phosphorus.

Authors' response: We included figures of a cumulative change in inorganic nitrogen and phosphorus on the final day of each phase (Figure 4 in the reworked manuscript).

Referee comment: How well do the changes in dissolved inorganic carbon and nutrients match with the changes in particulate carbon and nutrients? The authors should be cautious in their statements relating to particulate elemental ratios, since their own results are based on indirect measurements of the net uptake of inorganic carbon and nutrients. Also, any assumption on this aspect should be clearly indicated.

Authors' response: We agree that the statements on particulate elemental ratios are somewhat misleading and in the reworked manuscript we expanded the discussion on how well our calculations of NCP, C:N and C:P uptake ratios agree with the particulate and dissolved pools from the other relevant manuscripts (Czerny et al., 2012; Engel et al., 2012; Schulz et al., 2012).

Referee comment: How well do the estimates of NCP match with primary productivity estimates from Engel et al. 2012?

Authors' response: The primary production (PP) measurements, based on ^{14}C method, are not expected to agree very well with the integrated NCP measurements from samples from the mesocosms. To fully discuss the reasons for the differences between the PP and NCP in the reworked manuscript we added following sentences: "The mismatch between PP and NCP is a

result of i) the different methodological approaches to determine net carbon uptake, ii) the methods are designed to measure different variables and parameters. In short, ^{14}C method measures “new production” over periods of hours, whereas the integrated NCP measures the whole system carbon balance between two time steps. Most importantly, the Engel et al., 2012 PP data are derived from single depth incubations (1m) and received about 60% of incoming light, whereas NCP data captured productivity in the whole water column. Light in the mesocosms strongly decreased within the two meters, yielding a median light level of 23% for the whole water-column. Moreover, water for the incubations in study of Engel et al., 2012 was sampled in the mesocosms but pre filtered using 200 μm meshes. This may have lead to overestimation of phytoplankton productivity in the ^{14}C -incubations as grazing by larger zooplankton was excluded.”

Referee comment: The authors correctly state that the Arctic marine ecosystem may experience the greatest changes under ocean acidification. In this study, however, the authors did not test that particular hypothesis, as a detailed comparison with responses observed in other ecosystems is lacking. The authors should include such a comparison in more detail, and/or specify the aim of their study. Also, what did the authors expect to happen in the first place? This should be discussed more clearly in the introduction.

Authors' response: In the reworked manuscript we expanded our introduction discussing the response of other ecosystems to CO_2 perturbation, such as previous laboratory and mesocosm experiments of similar design in high latitude and temperate waters.

Referee comment: The termination of the first two blooms was included in the data analyses, whereas termination of the last bloom was not (i.e. day 28-31 is missing). Please discuss more clearly why this data was not included, and what the implications may be for the results.

Authors' response: Excluding the last bloom (t28-t30) from our study resulted in the lack of information about the decline of the bloom at the end of the experiment. The sampling after t27 was stopped for the majority of the experimental variables due to logistical constraints. We clarified this in the methods section of the reworked manuscript.

Referee comment: Regression analyses should include the variation of data on both x- and y-axis, as one point per mesocosm is obviously not sufficient to describe the impact of CO_2 on NCP and C:N and C:P uptake.

Authors' response: Fig. 5 and Fig. 8 were not included in the reworked manuscript, because

they repeat the information, which is presented in Table 2, namely the slope of linear regression.

Referee comment: The manuscript lacks a thorough discussion on most of the findings. The discussion is rather a more detailed repetition of the results.

Authors' response: In the reworked manuscript the results are discussed more thoroughly. The reworked discussion puts greater emphasis on taking the results out of the mesocosms and into the real-world. This encompassed greater integration with the other key results from the special issue and also it extrapolated the main findings to speculate on the consequences for Arctic biogeochemical cycling in the coming decades.

Specific comments:

Referee comment: Page 11708: Lines 19-21: Note that primary production was also shown to decrease upon elevated pCO₂ (e.g. Gao et al 2011, Nature Climate Change).

Authors' response: We referred to the study by Gao et al., 2012 discussing our results in the reworked manuscript.

Referee comment: Lines 25-26: Not a clear sentence, please specify what exactly is meant here with a dynamic ecosystem in terms of production and respiration of organic matter.

Authors' response: We changed this sentence to: “However, the ecosystem of the Arctic Ocean is characterized by significant abundance of heterotrophic bacterioplankton (Li et al., 2009), which is responsible for rapid turnover of carbon within the efficient microbial loop (Rokkan Iversen and Seuthe, 2011; Tremblay et al., 2012).”

Referee comment: Page 11709: Line 6: NCP is estimated from the net changes in dissolved inorganic carbon. Only in closed systems (like the mesocosms), this may be used to estimate net biological uptake of inorganic carbon. Please state this more clearly.

Authors' response: We modified this sentence stating that NCP was estimated based on a cumulative change in inorganic carbon in closed systems of mesocosms.

Referee comment: Page 11710: Line 21-23: The total experiment lasted 31 days, yet the NCP and C:N and C:P uptake ratios were measured only until day 27. Why?

Authors' response: The sampling after t27 was stopped for the majority of the experimental variables due to logistical constraints. We clarified this in the methods section of the reworked manuscript.

Referee comment: Line 24: Which nutrients were added, and to what final concentrations?

Authors' response: We added following sentence: “Nutrients (5 μM of nitrate (NO_3), 0.31 μM of phosphate (PO_4), and 2.5 μM of silicate (Si)) were added to mesocosms on day t13 to stimulate a phytoplankton growth.”

Referee comment: Page 11712: Line 18-20: NCP is based on the cumulative change of dissolved inorganic carbon. Why should NCP be cumulated as well? Please clarify what exactly is meant with cumulative NCP. Should it therefore also not be the ratio between NCP (corrected cumulative difference in dissolved inorganic carbon) and the cumulative difference in nitrogen and phosphate?

Authors' response: In the reworked manuscript we clarified that NCP is calculated based on a cumulative change of inorganic carbon. C:N and C:P ratios are the ratios between NCP and the cumulative change in inorganic nitrogen and phosphorus. In the reworked manuscript we do not use combination of words “cumulative NCP” to avoid confusion in our method.

Referee comment: Page 11713: Line 6-7: What is the difference between a ‘cumulative difference in nitrogen and phosphate’, and ‘a cumulative difference in nitrogen and phosphate uptake’?

Authors' response: They were used as synonyms. In the reworked manuscript we consistently used “a cumulative change in inorganic nitrogen and phosphorus”.

Referee comment: Lines 8-11: To what extent are NCP and the C:N and C:P uptake ratios autocorrelated? So what part of the changes in C:N and C:P uptake ratios are caused by changes in NCP? Please clarify.

Authors' response: By definition, NCP and C:N:P are to an extent correlated. Correlation coefficient (r) for NCP with C:N uptake ratio in phase II was 0.55; NCP with C:N uptake ratio in phase III was 0.75; NCP with C:P uptake ratio in phase II was 0.18; NCP with C:P uptake ratio in phase III was 0.28. However, we did not include this in the reworked manuscript, because we think it is unnecessary information.

Referee comment: Page 11714: Line 7-10: Please be more specific here. Changes in CT and AT are a result of the CO₂ gas exchange with the atmosphere, and of the community production and respiration. As it concerns a closed system, these changes can be used to estimate NCP.

Authors' response: Changes in CT are a result of CO₂ gas exchange with the atmosphere, and of the community production and respiration. Changes in AT are not a result of gas exchange but response to biological nutrient consumption and release from respired organic matter. Changes in AT could also be caused by processes of calcification or dissolution of calcium carbonate particles, however, there were no calcifying organisms in mesocosms, and there was no effect of calcification on AT change during the experiment. Mesocosms are closed systems, and therefore changes in CT and AT are exactly what we used in this study to estimate NCP. In the reworked manuscript we made it clearer that this was the method used.

Referee comment: Page 11715: Lines 10-26: Why are the C:N and C:P uptake ratios of phase II and III added? What does this exactly tell us?

Authors' response: It tells us about the relative nutrient and biogeochemical response of the pelagic ecosystem during the different phases.

Referee comment: How were the C:N and C:P uptake ratios of phase III obtained? According to the slopes in figures 6 and 7, and to the 'intercepts' in table 3 and 4, it seems that the values should be higher. If so, the difference in C:N and C:P uptake ratios between the two phases is substantial and deserves more attention.

Authors' response: In the reworked manuscript we clarified how C:N and C:P uptake ratios were obtained. We added the following description of the method: "A linear regression analysis was performed to define the relationship between NCP in each time period (phase) and corresponding cumulative change in inorganic nitrogen (ΔN) and phosphorous (ΔP). The cumulative change in inorganic nitrogen resulted from a sum of a cumulative change in nitrate, nitrite and ammonia. The relationships for each time period were defined with an equation type $Y = \alpha X + \beta$, where coefficient α corresponded to C:N or C:P uptake ratio. Tables 3 and 4 provide coefficients α averaged for low, intermediate and high pCO₂ levels (Slope), as well as standard deviations. Tables also provide regression coefficients (R^2) and p-values of F-test."

Referee comment: What exactly did change in the phytoplankton community, and how may

that explain the NCP, and the C:N and C:P uptake ratios?

Authors' response: In the reworked manuscript we stated that “Net community production, C:N and C:P uptake ratios during the experiment on ocean acidification in an Arctic fjord reflected the effect of increasing CO₂ on a distinct succession in phytoplankton groups occurred over the experimental period” and discussed this statement thoroughly with other key results from the special issue

Referee comment: Lines 15-17: What may explain the observed effect of CO₂ on NCP in the first phase?

Authors' response: We added the following explanation in the reworked discussion: “Phytoplankton growth in phase I was fueled by phosphate remineralised from organic matter and most importantly ammonia as an N-source, remaining after the spring bloom (Schulz et al., 2012). Remineralisation of inorganic nutrients from organic matter indicates that in a post-bloom situation in Kongsfjorden at the start of the experiment bacterial production and respiration were higher than primary production (Rokkan Iversen and Seuthe, 2011; de Kluijver et al., 2012). For the NCP it means negative values indicating net heterotrophy of the system. However, in phase I negative NCP was only observed in mesocosms with low pCO₂. In mesocosms with intermediate and high pCO₂ NCP was positive, indicating that production rates were higher than respiration rates, and most likely, were stimulated by elevated CO₂ (Engel et al., 2012). Positive NCP could also be caused by very low respiration rates in high CO₂ treatments, as there was an increased sedimentation of fresh organic matter with increasing CO₂ (de Kluijver, 2012). Zooplankton grazing decreased from low to high pCO₂ treatment (de Kluijver et al., 2012) and thus could also contribute to the NCP increase with increasing pCO₂.”

Referee comment: Lines 26-27: This should be tested with particulate carbon and nutrient data.

Authors' response: In the reworked manuscript we referred to the studies by Schulz et al., 2012 and Czerny et al., 2012 in the same issue, which report particulate carbon and nutrient data in the water column and sediments.

Referee comment: Page 11718: Line 3: How do the results show that the net uptake stoichiometry of carbon and nutrients varies regionally? Isn't it anyhow rather obvious that this varies in time and regionally?

Authors' response: We reworked the paragraph and deleted this sentence.

Referee comment: Line12-14: I would prefer 'the Redfield ratio' rather than 'Redfieldian'. What does this comparison of the 'post-nutrient period' with Redfield exactly mean? The C:N and C:P uptake ratios seem to deviate substantially from the Redfield ratio when the periods are analyzed separately. What causes this deviation?

Authors' response: We changed 'Redfieldian' to 'the Redfield ratio' in the reworked manuscript.

We compared ratios with the Redfield ratio in phase II and III as well as in the post-nutrient period (phase II+III) to show the great variability of C:N and C:P uptake ratios during short time periods. In the reworked manuscript we added the following sentences to discuss this variability: "C:N and C:P ratios in composition of particulate organic matter in the water column (Schulz et al., 2012) and in sediments (Czerny et al., 2012a, this volume) were close to the Redfield ratio during the whole experiment. C:N and C:P uptake ratios evaluated in this study were lower than Redfield ratio in phase II and higher than Redfield ratio in phase III. This discrepancy in uptake ratios could be explained by bacterial abundance, which significantly increased during the course of the experiment (Brussaard et al., 2012)."

Referee comment: Table 1, 2, 3, 4: It would clarify the tables if the period t14-t27 could be written as: Phase II+III.

Authors' response: As suggested by the Referee we changed t14-t27 with phase II+III in the headings of Tables 1, 2, 3, 4.

Referee comment: Table 1: Should it not be 'The dissociation constant for carbonic acid is based on: : :.'?

Authors' response: The papers cited in the heading of Table 1 report constants and coefficient, therefore in the reworked manuscript we used word 'adopted': "The dissociation constant for carbonic acid was adopted from Dickson and Millero (1987), for boric acid from Dickson (1990a), for sulfuric acid from Dickson (1990b); CO₂ solubility coefficient was adopted from Weiss (1974)."

Referee comment: Table 1: Was pH not measured? What may be the implications of a potential offset between calculations based on CT and AT, pH and CT, or pH and AT? Please see recent work by Hoppe et al. 2012 (BG, 9, 2401-2405). In particular, this may have consequences for the regression analyses.

Authors' response: pH was measured separately and is shown in the study by Schulz et al., 2012 in the same issue. However, if pH was needed to define the relationship with pCO₂, to be consistent, all papers in the same issue referred to calculated pH from measured CT and AT, reported in Bellerby et al., 2012 in the same issue. The offset between calculations of pH based on CT and AT and directly measured pH is out of scope of this study, because here we focus on net community production and stoichiometry of nutrient uptake. Regression analyses were done against pCO₂, which was calculated from measured CT and AT – not pH.

The work of Hoppe has been shown to only be relevant to experiments with high plankton and bacterial concentrations.

Referee comment: Table 3: Should the 'intercept' not read the 'slope', indicating the C:N and C:P uptake ratio (or actually, the N:C and P:C uptake ratios)? If so, it seems to be 1000-fold too high.

Authors' response: We changed 'intercept' with 'slope', because the slopes are shown in the tables, not the intercepts. The Slopes indicate C:N and C:P uptake ratio, in the reworked manuscript we added comma after first two digits, because commas were missing in tables.

Referee comment: Figure 3: The difference between the treatments seems to have primarily been caused by a lag period in the high pCO₂ treatments between t23 and t25. Please indicate what may have happened there, and how this affects the interpretation of the data.

Authors' response: In the reworked manuscript to describe and explain this lag period we added following sentence: "... in the beginning of phase III phytoplankton growth in mesocosms with high pCO₂ experienced a deficiency of inorganic nutrients, which was indicated by two days lag in chlorophyll a increase (Schulz et al., 2012) and caused the lowest NCP rates in these mesocosms.... The negative effect of elevated CO₂ on phytoplankton growth and NCP rates in phase III should be taken in caution, because at the time of maximum growth in mesocosms with low and intermediate pCO₂, biomass in mesocosms with high pCO₂ was still building up (Schulz et al., 2012)."

Referee comment: Figures 5 and 8: The figures should include the variability of the data, i.e. standard deviations on both x- and y-axes.

Authors' response: Fig. 5 and Fig. 8 are not included in the reworked manuscript, because they repeat the information, which is presented in Table 2, namely the slope of linear regression.

Referee comment: Furthermore, it would be more appropriate here to use the dissolved CO₂ concentration on the x-axis instead of the partial pressure.

Authors' response: Fig. 5 and Fig. 8 are not included in the reworked manuscript, because they repeat the information, which is presented in Table 2, namely the slope of linear regression.

Referee comment: Figures 6 and 7: Graphs do not show the ratios between N or P and CT uptake, but the cumulative uptake. Any slope in the graph will indicate a ratio. This ratio does not indicate the C:N or C:P ratio, but the N:C and P:C ratio. Please correct, also in the text. What exactly has been plotted on the x-axis? Is this cumulative net CT-uptake or cumulative NCP?

Authors' response: Figure 5 (previously Figure 6) in the reworked manuscript shows the relationship of NCP on y axes with cumulative change in inorganic nitrogen and phosphorus on x axes. Figure shows average slopes for low, intermediate and high pCO₂ treatments, as well as SD on y and x axes.

Referee comment: Figure 8: To what extent is the decrease in C:N uptake and C:P uptake due to the decrease in NCP?

Authors' response: Fig. 8 is not included in the reworked manuscript, because it repeated the information, which is presented in Table 2, namely the slope of linear regression analysis. By definition, NCP and C:N:P are to an extent correlated.

Referee comment: What is the impact of CO₂ on the cumulative N and P uptake?

Authors' response: Cumulative change in inorganic N and P concentrations reflected the effect of increasing CO₂ on a distinct succession in phytoplankton groups occurred over the experimental period. To demonstrate that we referred to study by Schulz et al., 2012, and also included figures of a cumulative change in inorganic nitrogen and phosphorus on the final day of each phase (Figure 4 in the reworked manuscript) and discussed the results.

Technical corrections:

Referee comment: Page 11707: Line 5: ‘bacterioplankton’.

Authors’ response: Corrected

Referee comment: Line 5: Was the volume 50 or 45 m³ (see material and methods)?

Authors’ response: In the methods sections of the reworked manuscript we provide a range between 43.9 and 47.6m³ with reference to Schulz et al., 2012. In the abstract of the reworked manuscript we provide the approximate number of 50m³ volume according to technical note of Riebesell et al., 2012.

Referee comment: Line 10: ‘carbon and nutrients’.

Authors’ response: Corrected

Referee comment: Line 21: The data on NCP, and on C:N and C:P uptake ratios was collected until day 27, not 31.

Authors’ response: Corrected.

Referee comment: Page 11713: Line 7: ‘uptake’.

Authors’ response: Corrected.

Referee comment: Page 11714 Line 8: Also include ‘(AT)’ after total alkalinity.

Authors’ response: Added

Referee comment: Lines 12: As the values indicate a mean and standard deviation, what does the ‘about’ sign add?

Authors’ response: We removed ~ sign.

Referee comment: Line 18 and 23: Please include ‘predominantly’ or ‘net’ when referring to the autotrophic mesocosms.

Authors’ response: We included 'net'.

Referee comment: Line 29: Was the P value truly 0, or just a very small number?

Authors’ response: P was a very small number. We changed it to $p < 0.001$.

Referee comment: Page 11715: Lines 6-7: Earlier, the C:N and C:P uptake ratios were referred to as ‘utilization ratios’, please make consistent.

Authors’ response: We changed all synonymous expressions to C:N and C:P uptake ratios for consistency in the reworked manuscript.

Referee comment: Page 11716: Lines 12 and 13: Correct sentence to, for instance, ‘The mean cumulative NCP: :with increasing CO₂’.

Authors’ response: We changed it as suggested by the Referee

Referee comment: Page 11717: Line 10: Remove ‘a’ before ‘a phosphate’.

Authors’ response: We removed it.

Referee comment: Line 17: ‘low nutrients’.

Authors’ response: We do not find words 'low nutrients' on Page 11717 Line 17