Reply to Referee #2

[Referee comment] *However, there are additional discrepancies and oversights which may further weaken such conclusion. The discrepancies between the present results and those reported by Engel et al. need to be resolved, as well as possible methodological biases in the calculation of NCP to nutrient consumption rations.*

[Author response] ^{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).

[Referee comment] *There should also be more connection with the results reported in other articles of the same issue, regarding for instance elemental composition of particulate matter.*

[Author response] We improved this aspect by referring to the accompanying papers such as Aberle et al. (2012), Brussaard et al. (2012), and Riebesell et al. (2012).

[Referee comment] *As with all short-term experiments looking at the biological effects of high CO2, all the papers in this special issue suffer from the same limitation: to which extent do short- term responses to sudden changes in pCO2 (hundreds of ppms in 5 days) inform us about the real effects of high CO2, which is increasing at a rate several thousand times slower? The authors should acknowledge this limitation in the discussion of their results.*

[Author response] It is now known that some phytoplankton strains can adapt to higher CO_2 levels during short-term (Barcelos e Ramos et al., 2010, Biogeosciences, 7: 177-186; Lohbeck et al., 2012, Nature Geoscience, 5: 346-351). These experiments were performed in the laboratory and it is important to recognize that our experimental design (9x50 m^3 mesocosms for 5 weeks) is state-of-the-art. There is simply no better experimental design for investing the response of plankton communities described in the literature.

[Referee comment] *There are well-known issues with long (24-48h) in vitro incubations, which however are not mentioned in this article: it has been shown that important changes in the abundance, activity and composition of phytoplankton and bacteria take place during in vitro incubations (e.g. studies by Pomeroy et al AEM 60, 1994, Calvo-Diaz et al. AEM 77, 2011 and references therein), which are likely to affect the estimates of both photosynthesis and respiration. The authors should discuss the possible impacts of these artefacts for their results and conclusions.*

[Author response] We acknowledged the potential bias of long in vitro incubation in the revised manuscript: "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)

On the other hand, we highlight the fact that 24 h incubation was used for determining NCP.

[Referee comment] *What are the main differences/similarities with the paper by Silyakova et al in the same issue, which seems to address a very closely related topic using data from the same experiments? This should be clarified at the end of Introduction.*

[Author response] In the revised introduction, we mention that "We also compare NCP estimated by different methods based on dissolved oxygen (this study), stable carbon isotope (de Kluijver et al., 2012), and total carbon (Silyakova et al., 2012)." (L104-106 in the revised manuscript) The main differences and similarities with the paper by de Kluijver et al. (2012) and Silyakova et al. (2012) are explained in the discussion.

[Referee comment] *It is not clear if the different temporal phases used to analyse the results can be interpreted ecologically. Certainly the authors do not interpret them ecologically. If the different phases do not represent clear ecological situations (e.g. the onset of a bloom, or a post-bloom phase), they are arbitrary and therefore it becomes difficult to translate the conclusions to the real world.*

[Author response] The three different phases used in this study are based on the manipulations and the temporal changes of phytoplankton biomass (see Riebesell et al., 2012). We added a description of phytoplankton dynamics in each phase: "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)

[Referee comment] *The authors calculate ratios of NCP to consumed nutrients in units of molC:molN and molC:molP. To do so, they converted the original NCP data (in O2 units) to C units, using a PQ of 1 (page 11023). PQ is strongly dependent on the nitrogen source (e.g. nitrate vs ammonia), which means that using a constant PQ may have led to significant biases, since nutrient concentrations changed widely during the experiment, and perhaps even among treatments. For instance, as added nutrients become exhausted, the importance of ammonium as a nitrogen source is likely to increase relative to that of nitrate, leading to a decrease in PQ. So to which extent the NCP decrease relative to N and P consumption (Fig. 3) has been, at least in part, caused by the use of a constant PQ? This is vaguely referred to in the Discussion but a more careful discussion of the issue is required.*

[Author response] According to the suggestion by Referee #1, we recalculated O_2-NCP in carbon unit using a PQ of 1.25. We agree that PQ depends on the nitrogen source. In this experiment we have no information on temporal changes of PQ in response to nitrogen availability. Instead, we discussed on changes in ratio of cumulative NCP to cumulative consumption of NO_3 and PO_4 using a PQ of 1.8 (e.g. Platt et al., 1987; Laws 1991).

[Referee comment] *If pCO2 does affect the stoichiometry of organic matter production and remineralization, this should be observed in the chemical composition of particulate and dissolved matter. However, and rather surprisingly, there is no mention to measurements of C, N and P in organic matter in the Results, and only very briefly in the Discussion. The data seem to be available (Schulz et al.) and should be discussed here in more detail.*

[Author response] Agreed. The manuscript was revised accordingly: "During phase 2, a statistically significant, positive correlation with increasing pCO_2 was found for the

concentrations of DOC, POC, PON, and POP, but not for any stoichiometric ratio (Schulz et al., 2012)." (L418-421 in the revised manuscript)

[Referee comment] *Another article in the same issue (Engel et al.) reports a positive relationship between pCO2 and C14-based productivity. The present article does not really address the reasons for this discrepancy, but limits itself to discuss why 14C-based production is higher than NCP. The fact that opposite CO2-dependent trends are observed in NCP and 14C-based production must be addressed here. At first sight, the explanation given by Engel et al (that high CO2 induces high 14C-PP but also high release of DOC which in turns enhances microbial respiration, thus leading to lower NCP) is not convincing, since CR seemed to be independent of pCO2.*

[Author response] 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] *The title can be improved.*

[Author response] Agreed. We modified the title as "Effect of increased pCO_2 on the planktonic metabolic balance during a mesocosm experiment in an Arctic fjord".

[Referee comment] *Somewhere (end of Introduction, beginning of Methods) a brief description of the study site is needed, so that the reader knows the context (especially, as far as seasonal variability is concerned) of the experiments. This is also important to assess whether the nutrient addition mimics something that does happen in this system, or else if is not realistic.*

[Author response] As suggested, we improved the brief description of the study site: "In Kongsfjorden, the spring phytoplankton bloom occurs in April, which results in low

concentration of dissolved inorganic nutrients (Rokkan Iversen and Seuthe, 2011; Hodal et al., 2012). Nine Kiel off-shore mesocosms (KOSMOS: thermoplastic polyurethane 0.5 to 1 mm thick, 17 m long, and 2 m in diameter, approximately 50 m^3 volume) were deployed on 31 May 2010 (t–7). The site of the mesocosm mooring was ice-free during the experiment except for a few occasions when ice floats needed to be pushed out from the site (Riebesell et al., 2012)." (L113-119 in the revised manuscript)

We also provided information on the nutrient addition: "The nutrient concentrations were chosen to simulate an upwelling event (Schulz et al., 2012). The addition of CO_2 (t–1 to t4) and inorganic nutrients (t13) were performed in the upper 13 m of the mesocosms using the dispersal device in order to assure an even distribution in the water column (Riebesell et al., 2012; Schulz et al., 2012)." (L130-133 in the revised manuscript)

[Referee comment] *A more complete description of mesocosms is needed: material, volume, dimensions, etc. Was there any recirculation/stirring of water? Sedimentation must have been intense, particularly after nutrient addition, and vertical gradients very sharp. The vertical profile of irradiance must have changed dramatically after the onset of the nutrient- induced bloom – however BOD samples were incubated at a constant depth of 4m.*

[Author response] As suggested, we extended the description of mesocosms: "Nine Kiel off-shore mesocosms (KOSMOS: thermoplastic polyurethane 0.5 to 1 mm thick, 17 m long, and 2 m in diameter, approximately 50 m^3 volume) were deployed on 31 May 2010 (t–7). The site of the mesocosm mooring was ice-free during the experiment except for a few occasions when ice floats needed to be pushed out from the site (Riebesell et al., 2012)." (L115-119 in the revised manuscript), and

"The addition of CO_2 (t–1 to t4) and inorganic nutrients (t13) were performed in the upper 13 m of the mesocosms using the dispersal device in order to assure an even distribution in the water column (Riebesell et al., 2012; Schulz et al., 2012)." (L131-133 in the revised manuscript)

We agreed that the irradiance varied in the water column of the mesocosms and the incubation site: "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). 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)." (L365-374 in the revised manuscript)

[Referee comment] *Chla levels are important to understand what's happening in the different mesocosms and in fact are used to define temporal phases. They should be shown in a plot similar to Fig. 1.*

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

[Referee comment] *Given that NCP measurements based on 13C-accumulation and DIC changes are also used in the Results section, they should be briefly mentioned in Methods.*

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

[Referee comment] *Table 3, Fig. 4. Three methods are used to determine NCP, not four as stated.*

[Author response] Corrected.

We thank Referee #2 for her/his thoughtful comments.