**Response to Referee #1**

We greatly appreciate the time the referee spent on a detailed review of our manuscript. He/she gives a number of good suggestions to improve text and presentation. Please find our specific answers below (*in italics*):

**General comments:**
I think that the presentation of the 'fjord' data (outside the mesocosms) does not fit to the scope of this ms. Hence, it appears to me that the current ms is unfocused. Once the mesocosm experiment starts, the plankton community inside and outside the mesocosms are under different conditions. Provided the scope of this study and of the EPOCA experiment, I had the difficulty to understand the importance of (for example) the comparison of bacterial production between the mesocosms and in situ on day 20 in this ms. I recommend to reduce the presentation of in situ data.

We think that the Kongsfjorden data are an important baseline to assess the effect of nutrient addition on the development of the plankton community in the mesocosms. Since all manuscripts in the special issue will include the analysis of fjord samples also data on bacterial activity should be of interest to the readers to obtain a complete description of the Kongsfjorden system during the EPOCA study. However, we agree with the referee that the presentation of the Kongsfjorden data in our manuscript needs some restructuring. Therefore, we will summarize the results on the Kongsfjorden in a short paragraph at the beginning of the results section instead of combining the description of fjord and mesocosm data for the different parameters.

Q10 of bacterial protein production (BPP): I think that the water temperature at the in situ incubation site was not constant during each incubation. If the incubation temperature varied during 24 h incubation for BPP, the authors should mention the range of temperature variation and explain how the daily change of temperature was taken into account for the calculation of Q10 of BPP.

We agree with the referee that the calculations of Q10-values need a more precise description in our Material and methods section and we further agree that the calculations of Q10 for BPP from in situ incubations includes temperature variation over 24 hours. Therefore, we will modify our descriptions (in M+M, p. 10475) as follows:

*“To test the effect of temperature on bacterial activity, Q10 factors were calculated as

$$Q_{10} = \left( \frac{r_2}{r_1} \right)^{10/(T_2 - T_1)}$$

where $r_2$ and $r_1$ are rates at high ($T_2$) and low ($T_1$) temperature, respectively. $T_1$ and $T_2$ were 2°C and 5.5-6.5°C, respectively, for enzyme assays (see also section 2.2). BPP at elevated temperature was derived from in situ incubations (see also section 2.3). In situ temperature increased during the mesocosm study, and it must be assumed that these incubations were exposed to slight temperature changes during incubation time. Accordingly, temperature sensitivity of BPP is estimated from

$$Q_{10}^* = \left( \frac{r_{in situ}}{r_{2°C}} \right)^{10/(T_{in situ} - 2°C)}$$

where $T_{in situ}$ ranged from 3.2°C for incubations on day 14 to 7.7°C on day 22, and $r_{in situ}$ and $r_{in situ}$ integrate the temperature variability of a day-night cycle.”*

The authors conclude the significant effect of water temperature on BPP, however, they present the data of BPP at 2°C (not at in situ temperature) for analyzing the effect of pCO2 on BPP (see Fig. 2). They need to give rationale for this.
We conclude significant temperature effects on BPP from the comparison of incubations at 2°C and in situ temperature. Q_{10}\* factors of >1 were determined for 8 out of 9 incubations and Q_{10}\*>3 in 4 out of 9 incubations (Fig. 9). Based on Q_{10}\* values it could be estimated that “rising in situ temperature alone increased BPP in the mesocosms by a factor of 1.3-1.7, corresponding to 27-61 mg C l^{-1}.” (p. 10481, l. 8/9). In order to separate these temperature effects from CO_{2}-related effects, rates determined at 2°C were used for regression analyses shown in Figs. 10 and 11.

Other comments
Abstract P 10468, L9-10: It appears that this sentence means that the extracellular enzyme was highest at moderate acidification level in this experiment. But the results suggest that the extracellular enzyme was highest at the higher pCO2 levels (Fig. 6).

We agree that this sentence in the abstract is misleading. “Moderate” was used as a general description for acidification that decreases pH moderately in comparison to many physiological studies that change the pH by several pH-units to test for maximum and minimum conditions of activity. We will delete the word since it is specified in the next sentence that a decrease by 0.5 pH units doubled enzymatic rates.

Introduction P10469, L4: Iversen and Seuthe -> Rokkan Iversen and Seuthe

Will be changed.

P10469, L4-6: Neither Rokkan Iversen and Seuthe (2011) nor Seuthe et al. (2011) report that bacteria were subject to intense grazing by heterotrophic dinoflagellates and ciliates during the vernal bloom in April. Please specify the reference.

Our statement was derived from the following sentence by Rokkan Iversen & Seuthe (2011) on p. 740: “For example, low integrated bacterial biomass, in conjunction with high bacterial specific growth rates, implies that heterotrophic bacteria were heavily subjected to removal processes, such as protozooplankton predation (e.g. Sherr et al. 1989) or viral infections (e.g. Weinbauer 2004). In fact, high biomass and potential grazing rates suggest that heterotrophic dinoflagellates and ciliates probably were the principal grazers under the spring bloom in Kongsfjorden (Seuthe et al. accepted).”

It seems that we misunderstood these sentences. Seuthe et al. (2011) explain that protozoan grazing in spring mostly affected phytoplankton in Kongsfjorden (although it is also stated that algaevory alone “does not reflect the true composition of the diet of ciliates and dinoflagellates”): “…protozooplankton could have grazed equivalent to 100% of the daily primary production in April. This calculation assumed complete algaevory, which most probably does not reflect the true composition of the diet of ciliates and dinoflagellates. Nevertheless, it illustrates the great potential of protozooplankton to cycle a significant fraction of primary production even under phytoplankton bloom conditions. The higher biomass of protozooplankton compared to metazooplankton in April, in combination with an order of magnitude greater specific ingestion rates of protozoans than copepods (Hansen et al. 1997 and references therein), suggests further that heterotrophic ciliates and dinoflagellates were the principal grazers in Kongsfjorden under these conditions” (Seuthe et al., 2011, p. 763).

Therefore, we will change our sentence to: Intense grazing by heterotrophic dinoflagellates and ciliates leads to cascading effects on carbon transfer through the microbial food web during the vernal bloom in April.

P10470, L10: It is not clear which period “past marine research” means. However, it should be noted that, as the authors cited in the ms, for example Liu et al. (2010) did meta-analysis of the
published papers about the effect of ocean acidification on the structure and functioning of microbial communities.

With “past marine research” we intended to describe the last decades before acidification of natural marine systems by anthropogenic emissions became evident. During this period, pH was almost not studied as a factor that potentially co-determines biological activity in the ocean. In contrast, effects of temperature were intensively investigated by field studies in earlier times and these studies give valuable suggestions for potential impacts of global warming. We will clarify this in the revised version of the manuscript.

P10471, L15-16: This sentence says that pCO2 in the enclosed seawater was initially in a range of 250-1085 µatm. But in fact the authors set up a pCO2 gradient in a range of 250-1085 µatm by the stepwise addition of CO2-enriched seawater during several days.

“Initially” will be omitted.

P10471, L20-21: Specify if whole plankton community or certain groups developed, and what the nutrient deplete condition mean here (e.g. concentration, type of nutrients). Add relevant reference.

We will add information on initial nutrient concentration in the M+M section (“Mesocosm set up and sampling”). The development of the plankton community is part of another manuscript in the special issue (Schulz et al., 2012). The reference will be added.

P10372, L17-18: It would be useful for readers to mention how different pH levels affected the calibration factor of MUF and AMC fluorescence. It is suggested to mention how the authors measured blank fluorescence.

Information on calibration factors will be added. Blank incubations were done with boiled mesocosm water and did not show any increase in fluorescence over time.

P10472, L25-26: Enzymatic rates were given as nmol/l in the ms, so that the unit of mean and standard deviation of rates should be nmol/l. “a standard deviation of 9%” should be rewritten.

We prefer to give the standard deviation in % in order to average standard deviations for rates at all concentrations. After transformation into % a single number valid for rates at all concentrations can be given instead of 8 individual values in nmol L⁻¹ for the different substrate concentrations.

P10473, L4-5: Specify the number of live samples and killed-control samples.

Three replicate mesocosm samples and one TCA-killed control were incubated. Information will be given in the revised manuscript.

P10473, L11-12: Give the information of the light condition in a temperature-controlled walk-in room and in situ during the incubation.

Both incubations were accomplished in the dark. Information will be added.
The authors argued water temperature and labile organic carbon as the important bottom-up control on bacteria in the Arctic marine system in the introduction. However the importance of inorganic nitrogen on bacterial activity and growth was not mentioned. Hence it is hard to understand the experimental setting.

We wanted to test whether ammonium addition affected/increased the assimilation of labile carbon. ANOVA and pairwise post-hoc multiple comparisons revealed that this was not the case in our experiment. We will clarify this in the results section of our revised manuscript. Since the results of our incubation assay are in agreement with earlier studies that identified labile carbon as the prevailing limitation of bacterial activity in the Arctic Ocean and testing carbon vs. mineral nutrient limitation was not the major goal of our experiment we do not want to address this aspect in more detail in our introduction.

Vadstein (2011) showed that mineral nutrient limitation can occur at coastal sites in the west of Spitsbergen and we included these findings in our discussion:

"Enhanced carbon consumption was evident in incubations of coastal samples, while mineral nutrient deficiency limited bacterioplankton production in fjord samples of high phytoplankton biomass (Vadstein, 2011). Our incubations were accomplished during the initial days of the mesocosm experiment when low levels of phytoplankton biomass and primary production prevailed (Engel et al., 2012; Schulz et al., 2012). Therefore, it can be assumed that regeneration of nutrients was high and prevented mineral nutrient limitation, while the input of fresh and labile organic compounds was low and thus limiting at that time." (p. 10486, l. 24 and the following).

Specify if "20 µmol/l" is glucose concentration or carbon concentration.

Incubations were supplemented with 20 µmol glucose L-1. The sentence will be modified for clarification.

It is unclear why the authors applied the diluted acid instead of CO2 gas for pH adjustment in the acidification assay. This is apparently different from the pH modification in the mesocosm experiment.

Ocean acidification induced by increased atmospheric CO2 can be understood as changing DIC at constant TA with concomitant increases in CO2 and HCO3- at decreasing seawater pH. It has been shown that in the context of experiments on ocean acidification DIC manipulation by CO2 aeration and TA manipulation by acid addition yield similar changes in all parameters of the carbonate system. Hence, no systematic differences in biological responses are expected between the two approaches (Schulz et al., BG, 6, 2145-2153, 2009).

The aim of our acidification assay was to test whether changes in pH can explain differences in enzymatic rates determined in the mesocosms (p. 10482, l. 21), so that the direct manipulation of seawater pH by acid addition was straightforward.

It is unclear why delta-hydrolysis potential was calculated by the difference in enzyme activity between the acidified mesocosms and the two control mesocosms (M3 and M7) on each day. The two mesocosms (M3 and M7) were control in terms of CO2 manipulation but received nutrient enrichment on day 13. The response of plankton community may be different even between the control mesocosms during one month incubation. In this context, it would be better to show the integrated values of enzyme activity in the two control mesocosms as well.

In l. 4-7 on p. 10476 the calculations for Fig. 6 are described. The purpose of Fig. 6 is to show the strong temporal variation of the hydrolytic potential of the acidified mesocosms relative to the non-acidified controls that is induced by differences in bacterial cell numbers developing after
day 20. This picture does not emerge if integrated rates of all mesocosms (including the controls) are shown because cell number increased over time in all mesocosms but at different rate. We agree that variability between the two non-acidified controls needs to be discussed and we will add a small paragraph on that to our discussion (as also suggested by reviewer #2). We will also clarify the purpose of our calculations in the M+M section.

Results P10476, Enrichment assays: The limiting resource should be identified based on statistical analysis. The current ms shows the statistical analysis only on Lines4-5, Page 10477. The result of the statistical test should be shown in the text and Figure 1.

We agree with the referee that the statistical analysis of the enrichment assay and its presentation in the results section need to be improved. We performed ANOVA with post-hoc comparison for all parameters given in Fig. 1. Results confirm our interpretation that labile carbon limited bacterial activity in the fjord at the beginning of the mesocosm study. ANOVA results will be presented in a table and section 3.1 will be revised.

P10478, L7-8: Figs. 4-5 do not support the description 'activities remained rather constant between days 12 and 20'.

The referee is right. The description will be corrected.

P10478, L16-17: The method used in this study measured not only 'bacterial enzymes released into seawater' and also bacterial enzymes in particulate fraction.

In our incubations of whole seawater samples activities of enzymes released into seawater and of enzymes associated with the outer cell ("ectoenzymes") are assessed but not the activity of intracellular enzymes. We will specify this in the revised manuscript.

P10478, L19-21: Fig. 6 shows that an elevated enzymatic potential was observed in the two highest pCO2 mesocosms rather than "the three mesocosms of highest pCO2" during the first 20 days.

The referee is right. This will be corrected in the revised version.

P10479, L14-17: It seems that the data on DOC and DON are (or will be) published in an accompanying paper. If yes, add the reference. The same for Fig. 7 legend.

The data on DOC and DON concentrations will be shown in the manuscripts by Engel et al. and Schulz et al. in the special issue (both manuscripts are published in BGD). References will be added.

P10479, L18-21: It is very difficult to understand that a substrate concentration of 200 µmol/l did not saturate alkaline phosphatase activity, so that the data are shown based on the measurement of alkaline phosphatase activity at a non-saturation substrate concentration of 10 µmol/l. Isn't is 100 µmol/l?

In fact, many concentration kinetics do not show a saturation curve but an almost linear increase of rates up to a substrate concentration of 200 µmol L-1. We can only speculate about the reasons. We found two potential explanations in the literature:
1. Also microzooplankton (>90 µm) can be a producer of extracellular phosphatase. Gambin et al. (1999) show that these phosphatases may not follow Michaelis-Menten kinetics. Hence, zooplankton phosphatases may have affected kinetics in the mesocosms.

2. Sebastian & Niell (2004) showed that MUF-phosphate addition in excess of saturation can induce artefacts. However, in this case the kinetics typically show a reduction in reaction velocity at substrate concentrations that exceed the saturation level.

We will briefly discuss both potential explanations in our revised manuscript. Furthermore, we will change the sentences: “Concentration kinetics of extracellular phosphatase showed that substrate concentrations of 200 µmol l⁻¹, the maximum applied in our incubations, were not saturating at several time points, so that V_max was not reached. Therefore, rates derived from a non-saturating substrate concentration of 10 µmol l⁻¹ are presented (Fig. 8). “

to:
“Extracellular phosphatase activity did not follow Michaelis-Menten kinetics at several time points. Therefore, phosphatase rates achieved at 10 µmol l⁻¹ substrate concentration (V_{10µM}) are shown instead of V_max. V_{10µM} represents the enzymatic turnover of organic phosphates at a non-saturating concentration (Fig. 8).”

P10479, L24-26: The production of alkaline phosphatase is generally enhanced under low concentration of phosphate. It would be interesting to compare the relationship between alkaline phosphatase activity and phosphate concentration.

There is no significant correlation between phosphate concentration and phosphatase activity over time and phosphatase activity is not consistently higher before nutrient/phosphate addition than afterwards. We think that these observations do not allow proper conclusions on the influence of inorganic phosphate on phosphatase in this experiment, complicated by the fact that concentrations were manipulated after 12 days.

The temperature development is shown by Schulz et al., 2012. The reference will be added.

P10480, L13-16: Specify if no significant differences of water temperature, Q10 for extracellular enzyme, and BPP between the mesocosms were tested by statistical analysis.

This was confirmed by ANOVA (β-glucosidase) and ANOVA on ranks (leu-aminopeptidase, BPP, temperature). Information will be added.

P10480, L17: ‘revailing’ -> ‘revealing’?
P10481, L5&9: ‘bPP’ -> ‘BPP’?
Thanks for careful reading. Will be corrected!

P10481, L15-16: Add ‘Fig. 11’ at the end of the sentence.
The sentence says: “The range of mesocosm pCO₂ from 175 to 1085 µatm corresponded to pH values of 7.63–8.34”, while Fig. 11 shows the correlation of BPP with primary production. This figure/correlation is explained on p. 10482, l. 12-19.
P10481, L13-19: It is interesting to mention the extent of difference between the use of pCO2 and pH for the regression analysis. The necessity to use delta proton concentration instead of proton concentration is unclear. The use of delta proton concentration indicates that the relative pH value is more important than the absolute pH value. In addition, most of the accompanying papers use pCO2 in order to analyze the effect of ocean acidification. The use of either pH or pCO2 by all accompanying papers is recommended. 

There is no difference in correlation coefficient and significance level between correlations of bacterial activity with pCO2 and pH for our data after transformation of pH to a linear scale. We have decided to correlate with the difference in proton concentration between acidified mesocosms and the controls to set the control mesocosms to zero in the graphs. However, the same picture emerges, if absolute proton concentrations were used. We think it is reasonable to use both units in our manuscript because pCO2 was manipulated but likely the corresponding change in proton concentration induced direct effects on extracellular enzyme activities:

"Physiological performance and metabolic activity of heterotrophic microbial organisms like bacteria may not be directly affected by changes in seawater pCO2 but by co-occurring changes in pH." (p. 10481, l. 13-15)

P10481, L24-28: It would be interesting to mention also the effect of acidification on time-integrated (bulk) enzyme activity as well. The effect of acidification on bulk enzymatic rates is investigated in Fig. 6. As explained in the discussion, acidification effects on bulk rates are strongly affected by differences in the temporal development of bacterial cell numbers at different CO2: “This top-down regulation of bacterial cell numbers and thus enzyme producers strongly affected the bulk hydrolytic potential of beta-glucosidase and leu-aminopeptidase in the mesocosms (Fig. 6).” (p. 10490, l. 19-21). For this reason, regression analysis of time-integrated rates was carried out with rates normalized to cell numbers (Fig. 10,11).

Explain how the bacterial abundance, which was used to calculate cell-specific enzyme activity (Fig. 10), was obtained. Bacterial abundance was analyzed by flow cytometry as described on p. 10474, l. 23 and the following. The description will be slightly changed according to the suggestions of reviewer #2.

P10482, L11-12: Add reference. Both sentences in l. 11-13 refer to Engel et al., 2012, as mentioned in l. 13.

P10482, L14-15: Although BPP was defined as bacterial protein production (ng C/l d) (P10473, L21), BPP in the text as well as in Fig. 11 (upper panel) was shown as bacterial cellular carbon content (fg C/cell). Please correct this discrepancy. We will change “BPP” into “cell-specific BPP” in Fig. 11 and in sentences that describe cellular rates. The definition of cell-specific BPP is given on p. 10473, l. 17/18.

P10483, L1-9: Indicate ‘Fig. 12’ for readers. The result of the statistical comparison should be shown in the text and Figure 12.
We will refer to Fig. 12 in the text and add the information that the significance of difference between rates at in-situ pH and lowest pH was tested by means of Wilcoxon signed rank test.

Discussion P10486, L9-15: Although the first peak of alkaline phosphatase activity coincided with that of chlorophyll, it does not necessarily suggest that phytoplankton were limited only by inorganic phosphorus. How about the possibility of N-limitation (or N and P co-limitation) of phytoplankton before the nutrient addition?

We do not assume that phytoplankton was P-limited. However, very low initial phosphate concentrations of 0.06-0.09 µmol L-1 were determined. Furthermore, phosphate concentrations decreased faster than ammonium concentrations before nutrient addition. It must be assumed that intense recycling activity as shown by high phosphatase rates supported phytoplankton growth during the first 12 days of the experiment.

Both sentences in l. 23-27 refer to Vadstein, 2011 (mentioned in l. 27).

P10487, L1-4: If regeneration of nutrients is high (i.e. nutrient supply is high), one can expect enhanced phytoplankton biomass and primary production. How about inorganic nutrient concentration at the start of the experiment?

We will add information on initial nutrient concentration in the M+M section ("Mesocosm set up and sampling").

Both sentences in l. 9-13 refer to Engel et al., 2012, as mentioned in l. 13.

P10488, L13-16: This sentence should be rewritten. Fluorescent markers were added at different concentrations. So, the differences in Q10 of enzyme activity might be induced by direct substrate-enzyme interaction?

The Q_{10} values for enzymatic reactions were calculated from V_{max}. Although V_{max} was derived from a kinetic model that includes reaction velocities at eight substrate concentrations, V_{max} is based on rates at saturating concentration. Rates at substrate saturation are not affected by temperature effects on the enzyme’s substrate affinity.

P10488, L21-25: Because the authors added fluorescent markers at different concentrations, it would be possible to mention if they observed biphasic or multiphase kinetics of enzyme activity during the experiment.

We could not find any indication for biphasic or multiphasic kinetics. However, eight different substrate concentrations are probably still not sufficient to identify biphasic or multiphasic kinetics. A better resolution of reaction velocities at non-saturating/low substrate concentrations would be necessary.

P10489, L24: Specify ‘moderate acidification’ using pCO2 or pH values.
The sentence will be modified: “A comparison with the mean $Q_{10}$ values for $\beta$-glucosidase and leu-aminopeptidase activity in the Kongsfjorden reveals that the rate increase after reduction of seawater pH by 0.3-0.5 units equals the rate increase at a temperature elevated by 3°C.”

P10490, L13: ‘its heterotrophic turnover’ should be clarified.

The sentence will be modified: “Furthermore, a direct relationship between primary production and BPP shows that increased primary production under elevated pCO$_2$ not only enhanced the hydrolysis of organic matter but also its assimilation into bacterial biomass.”

P10490, L28-29: It is unclear what kind of situation was considered to result in ‘increased competitive relationships’.

*Increased competitive relationships at high diversity can result in a negative effect on the activity of communities when competitively strong species or groups do not contribute a large part to the community function of interest. We will clarify this in our revised manuscript and will choose a more suitable reference (Jiang et al., Oikos 117, 488-493, 2008 instead of Becker et al., 2012).*

P10491, L16-21: The authors mentioned that primary production was elevated under high pCO$_2$. This result suggests a possible increase of labile organic carbon for bacteria. However the authors should clarify the proportion of exudate production to total primary production in response to increasing pCO$_2$. If the particulate primary production was dominant in total primary production and a classical food chain was active, one may assume the bulk organic carbon channelled mesozooplankton and then exported from the surface.

*The percentages of extracellular release (PER) ranged from 21-23% before nutrient addition on day 12 and between 14 and 18% after nutrient addition. Despite lower PER the amount of DOC released by autotrophs was higher at high CO$_2$ due to absolute higher primary production. Both particulate and dissolved primary production were elevated in mesocosms at high pCO$_2$ (Engel et al., 2012). We agree with the referee that parts of the particulate primary production were likely processed by zooplankton/protistan grazing. Our statements do not contradict this but we will address this point more specifically in our revised discussion. Nevertheless, the direct relationship of primary production and bacterial protein production over the applied CO$_2$ range strongly suggests that also bacterioplankton benefited from enhanced primary production in mesocosms at high CO$_2$. The bacterial substrate supply is not exclusively derived from exudation. Also sloppy feeding and the viral lysis of phytoplankton cells contribute. Therefore, correlation analysis was carried out with total primary production.*

Fig. 1: The upper panel shows BPP and bacterial abundance. The legend should mention bacterial abundance as well.

“Abundance” will be changed into “bacterial abundance” in the legend.

Fig. 2: The lower panel indicates specific growth rate ($\mu$) on the right axis. The legend should mention specific growth rate as well.

*We see no need to change the legend but propose a change in the caption to better explain the figure: “Temporal development of bacterial protein production (BPP$_{2\mathrm{C}}$) and specific growth rate ($\mu$) at 2°C in mesocosms of low (175–250 µatm; blue symbols), medium (340–600 µatm; grey symbols), and high pCO$_2$ (675–1085 µatm; red symbols).*
Upper panel: BPP$_{2°C}$ normalized to volume (ng C l$^{-1}$ h$^{-1}$), green lines represent chlorophyll a concentrations (Chl a, µg l$^{-1}$). Lower panel: BPP$_{2°C}$ normalized to bacterial abundance (cell-specific BPP$_{2°C}$, fmol C cell$^{-1}$ d$^{-1}$) and corresponding specific growth rate ($\sigma$ d$^{-1}$).

Fig. 11: The unit of bacterial protein production (BPP) is fg C/cell which does not correspond to the first definition. In the legend, primary production should be time integrated primary production. The integration period should be added.

The y-axis label “BPP” will be changed into “cell-specific BPP” (upper panel). We will mention in the figure caption that rates are time-integrated over the whole experiment.

Fig. 13 is not referred in the text. I do not think that this figure is useful for the ms.

We think that Fig. 13 helps the reader to follow our concluding remarks on p. 10491, l. 5-27. The reference will be added to the text.