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## ***Interactive comment on “Response of bacterioplankton activity in an Arctic fjord system to elevated $p\text{CO}_2$ : results from a mesocosm perturbation study” by J. Piontek et al.***

### **Anonymous Referee #1**

Received and published: 24 September 2012

#### General comment

This study reports temporal variations of bacterial protein production and activity of three extracellular enzymes in the Kongsfjorden, Svalbard. This study was conducted in the frame of a mesocosm  $p\text{CO}_2$  perturbation experiment using a natural plankton community. The authors demonstrate that (1) bacterial community was initially limited by the availability of organic carbon, (2) activity of beta-glucosidase and leucine-aminopeptidase increased with decreasing seawater pH, (3) Q10 for beta-glucosidase, leucine-aminopeptidase and bacterial protein production decreased with increasing carbon exudation rate. They give two important suggestions: (1) future changes in

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seawater temperature and pH have a similar potential to increase extracellular enzyme activity, (2) future pH decrease have a potential to enhance consumption of labile organic matter by bacteria.

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.

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. 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 pCO<sub>2</sub> on BPP (see Fig. 2). They need to give rationale for this.

## 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 pCO levels (Fig. 6).

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

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.

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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.

P10471, L15-16: This sentence says that pCO<sub>2</sub> in the enclosed seawater was initially in a range of 250-1085  $\mu\text{atm}$ . But in fact the authors set up a pCO<sub>2</sub> gradient in a range of 250-1085  $\mu\text{atm}$  by the stepwise addition of CO<sub>2</sub>-enriched seawater during several days.

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.

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.

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.

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

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

P10474, L18-19: 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.

P10474, L23: Specify if "20  $\mu\text{mol/l}$ " is glucose concentration or carbon concentration.

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P10475, L3: It is unclear why the authors applied the diluted acid instead of CO<sub>2</sub> gas for pH adjustment in the acidification assay. This is apparently different from the pH modification in the mesocosm experiment.

P10476, L4-7: 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 CO<sub>2</sub> 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.

Results P10476, Enrichment assays: The limiting resource should be identified based on statistical analysis. The current ms shows the statistical analysis only on Lines 4-5, Page 10477. The result of the statistical test should be shown in the text and Figure 1.

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

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

P10478, L19-21: Fig. 6 shows that an elevated enzymatic potential was observed in the two highest pCO<sub>2</sub> mesocosms rather than "the three mesocosms of highest pCO<sub>2</sub>" during the first 20 days.

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.

P10479, L18-21: It is very difficult to understand that a substrate concentration of 200  $\mu\text{mol/l}$  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  $\mu\text{mol/l}$ . Isn't it 100  $\mu\text{mol/l}$ ?

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.

P10480, L12-13: Add reference.

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.

P10480, L17: 'revailing' -> 'revealing'?

P10481, L5&9: 'bPP' -> 'BPP'?

P10481, L15-16: Add 'Fig. 11' at the end of the sentence.

P10481, L13-19: It is interesting to mention the extent of difference between the use of pCO<sub>2</sub> 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 pCO<sub>2</sub> in order to analyze the effect of ocean acidification. The use of either pH or pCO<sub>2</sub> or both by all accompanying papers is recommended.

P10481, L24-28: It would be interesting to mention also the effect of acidification on time-integrated (bulk) enzyme activity as well. Explain how the bacterial abundance, which was used to calculate cell-specific enzyme activity (Fig. 10), was obtained.

P10482, L11-12: Add reference.

P10482, L14-15: Although BPP was defined as bacterial protein production (ng C/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.

P10483, L1-9: Indicate 'Fig. 12' for readers. The result of the statistical comparison

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should be shown in the text and Figure 12.

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?

P10486, L23-24: Add reference.

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?

P10488, L9-11: Add reference.

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?

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.

P10489, L24: Specify 'moderate acidification' using pCO<sub>2</sub> or pH values.

P10490, L13: 'its heterotrophic turnover' should be clarified.

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

P10491, L16-21: The authors mentioned that primary production was elevated under high pCO<sub>2</sub>. 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<sub>2</sub>. If the particulate primary production was dominant in total primary production and a classical food chain was active, one

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may assume the bulk organic carbon channelled mesozooplankton and then exported from the surface.

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

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

Fig. 11: The unit of bacterial protein production (BPP) is fgC/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.

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

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