Interactive comment on "Effect of CO_2 enrichment on bacterial production and respiration and on bacterial carbon metabolism in Arctic waters" by C. Motegi et al.

We thank the referees for their constructive comments. As described below, we will accommodate the referee's suggestions and modify the Results and Discussion sections accordingly once the open discussion of the comments is terminated and pending whether we are invited to submit a revised manuscript.

Anonymous Referee #1

1. *the manuscript is rather descriptive since many relationships, e.g. between the bacterial parameters and those of virus, grazers and phytoplankton, should have been tested by the appropriate statistics. Therefore, I rate large fractions of the discussion highly speculative.*

Response: According to the suggestion, we will add more information on background data and add the statistical test result on the relationship between bacterial and environmental parameters including viruses and phytoplankton. In the revised version of the manuscript, we will modify the discussion based on the result.

2. *Secondly, the separation between bacterial parameters of free-living and attached bacteria needs some clarification. If I understand it right, bacterial respiration always refers to that of free-living bacteria (0.8 um prefiltered)? Whereas for BP both fractions have been measured... In conclusion also BGE and BCD only include those of the free-living bacteria? Also with flow cytometry only the free-lving bacteria can be counted...*

Response: Sorry for the confusion. In this study, we investigated bacterial production (BP_{TdR}) of both free-living and total community and bacterial respiration of free-living community. Therefore, we estimated BGE_{TdR} and BCD_{TdR} of free-living community. However, to estimate BGE_{Leu} and BCD_{Leu}, we used bacterial production (BP_{Leu}) of the total community. We realize that we cannot estimate BGE and BCD using BP and BR of different fractions of the community, therefore we will remove this result (BGE_{Leu} and BCD_{Leu}) from the revised version of the manuscript. Particularly for bacterial respiration, there is no way to estimate attached bacterial respiration, since without filtration, we measure community respiration. This is basically true for all such experiments and measurements. In the revised version of the manuscript, we will be more precise about how to separate 2 fractions (total and free-living) and to estimate each bacterial parameter in the material and method.

3. *Third, I do not see the ratio of seperating between BP-TdR and BP-Leu. In particular, it has been shown by Perez et al. 2010 (EMI 12:74-88) that uptake of thymidine and leucine is highly species-specific... Hence this ratio also changes with a changing bacterial community composition. The centrifugation method for bacterial production measurements (due to its small volume) is rather insensitive if the activities are low (as I assume they are at such low temperatures and at the rather short incubation time of 1 hr). This is also reflected by a CV of up to 41%!*

Response: As the referee point out that previous study shows that uptake of thymidine and leucine is highly species-specific in freshwater system. However, another study showed that all major phylogenetic groups of bacteria in aquatic systems assimilate both thymidine and leucine and that there is no difference in single cell activity for bacterial groups (Cottrell and Kirchman 2003). Our results do not allow us to identify the difference of thymidine and leucine uptake in each phylogenetic community. We will make this statement in the discussion of the revised version of the manuscript.

Prior to the mesocosm experiment, we determined the best incubation time of TdR incorporation in this environment. Briefly, triplicate samples were incubated at in situ temperature (2^{o}C) and collected after 1, 2, 3, 4, 5, 20, 24 and 27.5h. We assumed that the 1h incubation was the adequate period of incubation because 1) the variation between replicates was relatively small (CV: 9%) and 2) there was a clear difference in TdR conc. measured between blanks and incubations. Longer incubation times have the problem that the TdR label can be diverted from bacterial biomass (e.g. grazing and lysis), thus resulting in incorrect BP estimations. We will state this in the method section and add a result of changes in TdR incorporation over time in the appendix of the revised version of the manuscript.

In addition, we thank the referee for pointing out high CV of triplicate measurements. We checked our data carefully and found a mistake. The maximum CV of triplicate measurements of TdR incorporation rate was 28.3%. We will be corrected this in the revised version of the manuscript. As point out by the referee, the centrifugation method for bacterial production measurements may rather insensitive if the activities are low. However, the bacterial production rate we observed is within the range of values previously reported in the Arctic region. Thus, we think that the determination of BP was done correctly.

4. *Further, the day to day comparison to better resolve for dependencies between bacterial parameters and pCO2 seems to be a bit problematic for me, since the*

statistical procedures to test for significance are rather limited.

Response: As mentioned above, we will add the statistical test result of relationship between bacteria and environmental parameters in each phase. In addition, we will correct the figures accordingly.

5. *Finally, I think the discussion includes a lot of speculations. To better put the obtained results into context I suggest that the authors give a brief overview on the results of other working groups on viruses, grazers and phytoplankton. As a reader I find it hard to obtain the necessary information from the cited and yet not published references... I strongly recommend some more statistical approaches to proof for dependencies of bacterial parameters on the cited environmental parameters!*

Response: We agree with referee that we need to present background data. We will provide a brief summary paragraph in the introduction (or maybe as start of the discussion) of the paper. In addition, we will add statistical test result and will reconstruct and modify the discussion based on the result in the revised version of the manuscript. However, many papers are now published or are available as BG discussion papers. It is the nature of a special volume that there are strong cross-references and repetition of data presentation should be avoided.

Specific comments:

6. *P15214, L26: ...conditions...*

Response: Will be collected.

7. *P15215, LL8ff: references Grossart et al. and Allgaier et al. 2008 are cited in a contrary manner. hence the statement should be more precise.*

Response: We will be more precise with this statement.

8. *P15215, L21: For nitrification you may also refer to the pCO2 dependence of cyanobacteria (Wannicke et al. Biogeosciences, 9, 2973–2988, 2012)*

Response: We will refer to the above citation.

9. *P15125, LL21ff: pCO2 influence on anabolic and catabolic processes has been studied by the references given above (hence the statement needs some rephrasing...).*

Response: We will rephrase the sentence.

10. *P15216, LL3ff: Leu vs. TdR uptake may differ with community composition (Perez et al. 2010)*

Response: Please see response 3 to the corresponding comment.

11. *P15216, LL19ff: Taking integrated water samples for activity measurements could be problematic since incubation conditions may not sufficiently well reflect in situ conditions??? Please comment.*

Response: This is true but an integrated water sample was the only way how sampling could be accomplished (e.g. there was logistically no way to analyze samples from two depths). So, we have do live with that. Also, there is no a priori reason why this should mask pCO_2 effects.

12. *P15217, L12: low temperatures and short incubation times may lead to a high CV of up to 41%. This should be mentioned in the method section.*

Response: Please see response 3.

13. *P51217, LL14ff: Seperation between free-living and attached bacterial parameters is unclear. When was the filtration done? Before or after the tracer incubation? Did you yield negative values when substracting free-living activities from total activities? Please be more precise in the method description!*

Response: The sample was filtered before tracer incubation. See also response 2 to the corresponding comment. When free-living activities are subtracted from total activities, 12 out of 62 measurements are negative value. Higher free-living activities than total activities were potentially due to prefiltration, e.g. due to DOM release from protistan cells or changes in the organic matter continuum (well-known but unavoidable facts). About effect of prefiltration, please see response 14. In the revised version of the manuscript, we will describe the method more precisely and state the methodological concern.

14. *P51217, LL22ff: Die the 0.8 um prefiltration affect your respiration rtaes, e.g. due to shear stress during filtration? Please specify if this is only the respiration of the free-living bacteria?!*

Response: We used prefiltered water (<0.8μm pore size and low pressure filtration) to estimate free-living bacterial respiration and production. We agree with referee that prefiltration might have affect bacterial respiration and production. Shear stress is unlikely at this scale for cells, but it is known that prefiltration can disturb organic

matter and nutrient condition, destroy some protists (DOM production) reduce bacterial abundance and change in community structure (e.g. Gasol and Moran 1999). In the revised version of the manuscript, we will state this methodological concern. However, particularly for bacterial respiration, there is no way around filtration, since without filtration, we measure community respiration. This is basically true for all such experiments and measurements. In addition, it is preferable to measure BP also in the <0.8 µm fraction, since the error is then same as for BP and BR (thus, BGE and BCD data are more consistent).

15. P51217, LL22ff: Cell specific activities should be given as cs instead of s

Response: We will be revised as suggested.

16. P15218, LL4ff: Did you only include BPfree?

Response: Please see response 2.

17. P15218, LL10ff: problems, particularly with the TdR incubation, see Perez et al. 2010

Response: Please see response 3.

18. P15218, L14: With flow cytometry you only measure the free-living bacteria... Please also mention the high nucleic acid bacteria...

Response: Since we did not use the high nucleic acid bacterial data, we will reference Brussaard et al. (2013) who provide a detailed description of bacterial abundance and flow cytometry subgroups.

19. Discussion: it is difficult to put the results into a context without having a brief overview on the most important environmental parameters such as viruses, phytoplankton etc.

Response: Indeed. Please see response 5. Accordingly, we will add more information on the environmental parameters in the revised version of the manuscript.

20. P15220, L25: see my comment below, how did you measure these? P15220, LL25ff:... suggestion for viral lysis comes out of the blue!

Response: Indeed. Viral lysis and grazing on bacteria were measured in the same experiment. However, the authors of those data have not finished data analysis. So, we will remove this sentence and will discuss the data based on the data shown in the

result in the revised version of the manuscript.

21. *P15221, LL5ff: relation between bacteria, viruses and phytoplankton should be tested by statistical approaches...*

Response: Please see response 1.

22. *In general, the discussion has lots of speculations!*

Response: Please see responses 1 and 5.

23. *For the figures I suggest not to use the day to day approach. It would be better to seperate between certain phases during the mesocosm development as has been done in the other publications of the same experiment!*

Response: Please see response 4.

Anonymous Referee #2

1. *The authors point out correctly in the introduction that there is already a large variability in documented effects of higher pCO2 on natural bacterial communities. However, this is obviously related to the phytoplankton community and the planktonic trophic interactions which occur in those mesocosms, and which depend on various factors, particularly how the phytoplankton is affected by increased pCO2, control by grazers, nutrient limitation etc. Therefore it needs a detailed view on these factors if we really want to learn something new, that might also be generalised,how increased pCO2 affects the bacterioplankton. From the data presented in the ms it is not possible to deduce what was going on in the mesocosms. Even the relationship to the development in phytoplankton is not shown, nor the impact of grazers on bacterial development. Altogether the pattern in BP is astonishingly similar between all mesosocosms (Fig. 1A), already indicating that the impact of pCO2 cannot be significant (considering that also other factors such as phytoplankton biomass and bacterial grazing differ between the treatments). This might be already a major message of the paper! I do not think that it makes sense to interpret positive or negative correlations of BP with pCO2 for selected time points if the main, directly driving factors are not shown. This gives the (probably wrong) impression that pCO2 is directly acting on BP. In this study I miss mainly the following aspects:*

Response: We will provide a brief summary paragraph in the introduction (or maybe as

start of the discussion) of the paper. Indeed, a major message is that elevated pCO_2 levels had no major impact on BP and BR.

- Clear hypothesis on the effects of increased pCO2 on bacteria that are being tested in the experiment

Response: Will state the hypothesis in the revised version of the manuscript.

- An illustration how bacterial development is related to the development in phytoplankton (biomass and primary production) and preferably also to bacterial loss processes (if available)

Response: The phytoplankton data and the bacterial data have been shown in other papers. So, we do not want repeat that, we can only refer to these publications, but we will write a summary paragraph in the introduction.

- A discussion which is focussed on the results shown and not referring to data not accessible to the reader of this article

Response: This sentence is unfortunately incomplete and therefore we cannot provide an answer. We hope though that the answer is provided in the comments provided above.

2. *I general, I think too few background data from the mesocosm experiment are shown to be able to interpret what is happening at the bacterial side. Probably the authors expect that one has to consult the other publications resulting from this mesocosm experiment (on phytoplankton, viruses etc.). But this is not what I expect from a research paper, which has to be informative also by its own. I think it is not a problem to repeat some of the major data here, which are essential to understand the bacterial development (all factors contributing to bottom-up and top-down control), even if they are already published.*

Response: Indeed. Please see response 5 to the corresponding comment of referee #1. We will refer to the major findings of the published papers. There is no need to repeat showing all these data as they are in the same a special volume. Also, a summary paragraph will be included in the introduction (or maybe as start of the discussion).

Specific comments

3. *Introduction p.15215, l. 1-2: I think there are 100s of studies that examined diverse environmental conditions on bacterial activities! Be more specific!*

Response: We will be more precise.

4. *Results Fig.1: give an explanation for the variability shown in the plots*

Response: We will discuss the variability of bacterial production and respiration in the revised version of the manuscript.

5. *Fig. 4: axes and figure legends are hard to read*

Response: We will correct that.

6. *Discussion p.15221: I cannot follow these speculations on the role of viral lysis as no data are shown. Or do the authors expect that all other papers on the mesocosm experiments have to be consulted?*

Response: Please see response 20 to the comment of referee #1.

7. *Conclusion: From the data shown I cannot follow the authors conclusion that "changes in pCO2 potentially influence bacterial production and growth balance. . ."*

Response: We will add statistical test result and will reconstruct and modify the discussion based on the result in the revised version of the manuscript.

Anonymous Referee #3

1. *the manuscript must be reexamined after evaluation of data presentation by authors. The authors used simple correlation analyses between real pCO2 value and BPTdR, the Leu : TdR ratio, BGE or BCD obtained from different mesocosm tanks at the same day. Although data obtained from same day were compared, biological and chemical environments surrounding bacteria will be varied among the mesocosm tanks.*

Response: Indeed. Please see response 1 to the corresponding comment of referee #1.

2. *Moreover, elevated pCO2 could affect bacterial community production and respiration both by direct (change in pH, etc.) and indirect pass way (change in DOM release, food web structure etc.). Delay in bacterial Response to increase of pCO2 through indirect pass way in a few tanks could make true effects blur in the present analysis. Thus the simple correlation analysis might not extract effects of value of pCO2 on bacterial metabolism. I am not sure whether aim of this comparison is to extract possible factors which affects bacterial metabolism or to extract variability of metabolic rate under different pCO2 condition. Authors should state their purpose of analysis and should discuss variability of their analysis. (But I*

recommend addition of other biological and chemical parameters to statistical analysis.)

Response: The aim of correlation analysis was to see whether differences in pCO_2 levels affect the variability of bacterial metabolism. In the revised version of the manuscript, we will also add statistical test result which incorporate biological and chemical parameter, state the purpose of analysis, and discuss variability of the analysis.

3. *Further, in the comparison between pCO2 and BP, significant correlation was found only discrete two days. This result is very week to discuss long-term trend in bacterial production under elevating pCO2 in the ocean because effects of elevated pCO2 seems to be disappeared within 2 days. As describe above, single day comparison could make effects of elevate pCO2 blur out. Comparison in commutative values of bacterial parameters for all experimental period or 4 phases like other papers in this special issue may be more effective. Authors discuss the balance and imbalance growth of bacteria as interpretation of the change in the Leu : TdR ratio. If authors relate the change in the Leu : TdR ratio with bacterial growth condition, authors should compare the Leu : TdR ratio not only with real pCO2 value but also with sBP, sBR and BGE.*

Response: We will re-organize the data presentation and will show the variation of bacterial parameters in the 4 phases as done in other papers in this special issue. We will also be added a comparison between pCO_2 values and bacterial parameters in each phase.

4. *Detailed comments: P15218 2.4 Bacterial growth efficiency (BGE) and bacterial carbon demand (BCD): Whether were BGE and BCD estimated for only free living bacteria or for total (free living + attached) bacteria? Please clarify it in this section.*

Response: Please see response 2 to the corresponding comment of referee #1.

5. *P15220 Line 24-P15221 Line 2: Does the evidences about BPTdR and HDNA suggest that viral lysis is dominant factor of bacterial mortality?*

Response: Brussaard et al. discuss this issue in the recently published paper (*Biogeosciences*, 10, 719–731, 2013). Although some viral lysis data are presented in Brussaard et al. (2013), the manuscript on viral lysis and grazing of bacteria is still in preparation. Therefore, in the revised version of the manuscript, we will remove the sentence about viral lysis.

6. *P15222 Lines 3-6 "Although BPLeu was positively correlated with primary production in. . .": Does "phytoplankton" mean abundance? Or primary production? Or both? Please clarify it.*

Response: Piontek et al. (*Biogeosciences*, 10, 297–314, 2013) shows that there is a positive relationship between cell specific BP_{Leu} and primary production. This will be clarified in the revised version.

7. *P15223 Lines 14-15. "In particular, the Leu:TdR ratio decreased with increasing pCO2 concentration at t5 and t7 but this trend changed at end of the experiment." And following discussion: Although regression lines in Figure 5B, C, K and L show this trend, this trend seems to be based on increase in low pCO2 tank (the ratio in high pCO2 tanks seem to be constant relative to low pCO2 tanks.). Figure 5B shows that the most unfavorable condition during whole of experimental period low pCO2 condition. Authors should compare not only slope of regression line in each panel but also fluctuation of the ration in each tank.*

Response: It is true that the Leu:TdR ratio in high pCO_2 mesocosms (we did not use tanks) seems to be constant relative to low pCO_2 mesocosms. However, we believe that this trend is not only based on the variations of low pCO_2 mesocosms. The Leu:TdR ratio in medium pCO_2 mesocosms also varied during the experiment, and thus showed a significant negative gradient with pCO_2 level at the end of experiment (t24 and t26). As suggested, in the revised version of the manuscript, we will also discuss fluctuation of the Leu:TdR ratio.

Anonymous Referee #4

1. *Since no clear direct effect on BP and BR could be investigated, the authors should include data which refer to potential indirect effects (phytoplankton development, substrate availability, food web structure, . . .). Although other results of this mesocosm study are and will be presented in this special issue of BG, I have concerns regarding the ability of interpretation due to the lack of background information in this manuscript. As a reader it is hard to understand how the ecosystem reacts to the induced environmental changes within the mesocosms from the data presented here. For a research paper I would expect to read a kind of a stand-alone manuscript. I recommend including some of the data the authors referring to in the discussion section (including more statistical verification).*

Response: We agree with the referee that it is preferable to present more background data in the sense of a brief summary as well as more statistics. See also response 5 to

the corresponding comment of referee#1. However, it is the nature of a special issue (and a mesocosm experiment of this broad approach) that other papers are cited instead of repeating data in all papers. This has also been done in other papers.

2. *Furthermore the data obtained in this study do not allow calculating BGE and BCD, since the factions of BP and BR seem to be not the same. It is not clear whether BGE and BCD were calculated for total or only for free-living bacteria. Please clarify in the material and method section.*

Response: To estimate BGE and BCD (i.e. BGE_{TdR} and BCD_{TdR}), we used BP_{TdR} and BR of free-living fraction. However, to estimate BGE_{Leu} and BCD_{Leu}, we used bacterial production (BP_{Leu}) of the total community and BR of free-living fraction. We realize that we cannot estimate BGE and BCD using BP and BR of different fractions of the community, therefore we will remove this result (BGE_{Leu} and BCD_{Leu}) from the revised version of the manuscript.

3. *Despite the fact that this manuscript needs some major changes and that these kind of data is still very difficult to interpret in the context of future environmental changes, I would like to emphasize that the scientific community has also the need for datasets which shows no or only small effects regarding ocean acidification. So far a large variability of increased pCO2 effects on bacterioplankton dynamics and activities has been shown, which reflects the importance of studies dealing with these complex interactions of the ecosystem's community and their indirect effects, especially in cases where no significant direct effects can be determined.*

Response: We agree with referee that we need to present background data. In addition, we will add statistical test result to see potential indirect effects. Accordingly, we will change data presentation, reconstruct and modify the discussion in the revised version of the manuscript.

Specific c*omments:*

4. *Title (1) The title is very long and could be more precise. (i.e. the study was investigated in an arctic environment, in the Kongsfjord and not in general in "Arctic waters".)*

Response: According to the referee's suggestion, we will be modified the title.

5. *Abstract (1) Please add one sentence about the scientific context of your study (introduction to topic).*

Response: We will add phrase about the scientific context of our study in the revised version of the manuscript.

6. *Introduction (1) Please provide a clear objective/hypthesis of this study. p. 15215 l. 21-23: in contrast to p. 15215 l. 7-13. p. 15215 l. 27ff : please state a clear objective/ hypothesis.*

Response: We will state the specific goal of our research in the revised version of the manuscript. Our objective in this interdisciplinary study was to assess whether BP, BR, and other bacterial parameters would response to changing levels in pCO_2 in mesocosm experiments with natural communities.

7. *Material and methods p. 15216 l. 15 & 16: be consistent with your abbreviation (". . . pCO2 levels. . ." vs. ". . .levels of CO2. . .").*

Response: Will be done.

8. *p. 15216 l. 21: Nalgene*

Response: We apologize for this mistake and thank the referee for noting this. We will be corrected.

9. *p. 15217 2.2 BP: Separate more clearly between BPLeu and BPTdR. The method you describe here can be easily mixed up with the method Piontek et al. (2012) used. Furthermore 1h of incubation time is rather short for a cold environment! Did you test the bacterial incorporation of 3H-thymidine by means of a time kinetic?*

Response: We will clearly describe the differences between BP_{Leu} and BP_{TdR}. Leucine incorporation was only measured by one group in this experiment. So the leucine incorporation data presented in this manuscript and Piontek et al (2012) is the same one. About incubation time of BP_{TdR}, please see response 3 to the corresponding comment of referee#1.

10. *p. 15217 2.3 BR: Did you only determine the bacterial respiration rates from the free-living fraction (<0.8µm)?*

Response: Yes. Please see response 2 to the corresponding comment of referee#1. There is no way to estimate attached bacterial respiration.

11. *p. 15218 2.4 BGE and BCD: Which BP rate did you use for this estimation?*

Response: Please see response 2.

12. *Discussion (1) In general the discussion section is too speculative, along with a lack of statistical verification of the relationships discussed. p. 15223 l. 9-15: belongs to the result section*

Response: We will modify the discussion section. Please also see response 1 to the corresponding comment of referee #1.

13. *Figures (1) Please provide legends and refer to treatments.*

Response: Will be done.

14. *(2) The caption of a figure should have enough information to allow it to be understood in isolation.*

Response: Will be corrected.

15. *(3) It would be helpful to distinguish in which phase the experiment was at which time point.*

Response: We will do so.

16. *(4) Do you show mean values?*

Response: Yes. We show average value of triplicate measurement. Error bars are standard deviations for triplicate measurement. We will be mentioned this in the figure legend in the revised version of the manuscript.

17. *Fig. 3: Why did you choose different tick label for the x-axes? (Figures on the left hand side)*

Response: Tick label for the x-axes will be corrected.

18. *Fig. 4: Pleas give more information about the figure (i.e. colour bar, etc. . ..)*

Response: Will be explained more precisely.

19. *Technical corrections: Please check for language and style. I recommend to ask a native-English speaker or to use professional help to improve the English in this manuscript.*

Response: We will send the revised version of the manuscript to a native speaker for checking the language.