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***Interactive comment on* “Spatial variability of particle-attached and free-living bacterial diversity in surface waters from the Mackenzie River to the Beaufort Sea (Canadian Arctic)” by E. Ortega-Retuerta et al.**

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We first want to thank M-É Garneau for her careful reading and her constructive comments to the previous version of our manuscript. We explain our corrections and changes made below.

Specific comments:

p.17404, line 2: Change “On one hand” for “On the one hand”

Done

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p.17404, line 8-10: Reword to something as “This last fact will likely increase the importance of riverine nutrient inputs, which will in turn sustain primary and secondary production in an area that already receives 10 % of the global river discharge.”

Done: “This last fact will increase the importance of riverine nutrient inputs, which will in turn sustain primary and secondary production in an area that already receives 10% of the global river discharge”

p.17404, line 12-14: Reword to something as “This area receives approximately 128 tons of sedimentary particles per year from the Mackenzie River, which is the main source of particles and brings 95 % of the shelf sediment supply.”

Done: “This area receives approximately 128 tons of sediment particles per year from the Mackenzie River, which is the main source of particles and brings 95% of the shelf sediment supply”

p.17404, line 15: Change for “Arctic rivers”

Done

p.17404, line 17: Write “the occurrence of phytoplankton blooms” to avoid confusion with ice algae

Done

p.17405, line 3: Change for “than with mineral particles”

Done

p.17405, line 13: Change for “subarctic regions”

Done

p.17405, line 18: Specify as “different particle fractions (i.e., particle-attached vs. free-living)” to avoid confusion with size fraction

Done “different particle fractions (i.e., particle-attached vs. free- living)”

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p.17406, lines 14-15: Reword to something as “The Mackenzie River maximum discharge usually occurs at the end of spring (June–July), decreasing thereafter”

Done

p.17406, line 17: Change for “surface waters from 15 stations were collected along two transects from the Mackenzie River mouth to the open sea”

Done: “surface waters from 20 stations were selected along two transects from the Mackenzie River mouth to the open sea”

p.17406, line 21: Change for “from a zodiac (top to surface, 0 - 0.5 m deep) using clean 5-liter carboys”

Done

p.17406, line 23-24: Change for “12 and 24 liters each”. Rephrase to avoid the redundant word “equipped”. Please specify at which depth in meters samples were collected.

We have rephrased as “water was collected from 3 to 3.5m using PVC bottles (12 liters each, Ocean Test) mounted on a SBE Rosette equipped with a SBE911-plus CTD profiler”.

p.17407, line 5: Define acronym here: “Bacterial production (BP) was measured”

p.17407, line 18: Change for “of DAPI stained samples”

Done “Bacterial production (BP), was estimated from bacterial activity measures of 3H-leucine incorporation”

p.17407, lines 24-25: Specify the primers sequence here, as the AME journal is not open access.

We have included them: primers w49F (5'-ACG GTC CAG ACT CCT ACG GG-3') fluorescently 5'-labeled with phosphoramidite (TET, Eurogentec) and w34R (5'-TTA CCG CGG CTG CTG GCA C-3')

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p.17408, lines 4-12: The primers Gray28F and Gray519r are not used in Dowd et al. (2008). Did you also use the same thermal protocol? Please specify. For the details of the method, I would rather refer to Sun et al. 2011 (Sun, Y., R. D. Wolcott, and S. E. Dowd. 2011. Tag-encoded FLX amplicon pyrosequencing for the elucidation of microbial and functional gene diversity in any environment. *Methods Mol. Biol.* 733: 129–141).

We have changed the reference for Sun et al 2011. The protocol was exactly the same than in their paper.

p.17408, Line 9: use “Gray28F” instead of “28F”

Done

p.17408, Lines 10-13: Reword to something as “Tag-encoded FLX amplicon pyrosequencing analyses were completed using the Roche 454 FLX instrument with Titanium reagents and procedures were performed at the Research and Testing Laboratory (RTL, Lubbock, TX) based upon RTL protocols.

Done

p.17409, Line 21: Remove the reference to Table 1 because it does not present statistical results. How did you test the difference in the environmental parameters between the “zones” presented in Table 1? ANOVA, Kruskal-Wallis? Please specify.

We performed non-parametric Mann Whitney U tests. This is specified in the table caption

p.17410, Line 4: Change for “in both eastern and western transects in the Mackenzie River”

Done

p.17410, Line 6: Should be “fresh water”

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We changed it

p.17410, Line 11: You wrote a salinity unit here “stations had salinity higher than 26‰. IA’, whereas no units were used in lines 5 and 10.

We deleted the unit

p.17410, Line 12: Should be “ice meltwater”

Changed

p.17410, Lines 15-16: “Bacterial production was significantly higher”, please specify how you tested that. I would suggest that you indicate by an asterisk the values in Table 1 that are significantly different. Also, I wonder what you found for DOC, POC, and all nutrients listed in Table 1. Any other relevant significant differences? Please specify. Finally, Table 1 contains data that are not discussed in the text, and most acronyms are not defined. I would remove NO₃, NH₄, PO₄, DON, TDAA, DOP, PON, POP from Table 1 since you don’t use refer to them in the text.

We now include the results of non-parametric Mann Whitney U tests to check for differences in environmental variables between river and coast and between coast and open sea samples, specifying the different p levels by asterisks. We agree with the referee that we don’t refer to some of the data presented in Table 1 in the text. However, since we use all these variables in the CCA analyses, we find informative to show absolute values and we decided to maintain them in the table

p.17410, Lines 16-17: Should be “The proportion of bacterial production due to PA bacteria”

Changed

p.17410, Line 24: The value R²=0.63 is not in Table 2, which is confusing. Please clarify.

True. We did direct comparisons (i.e. ANOSIM tests) between river and sea, coast and

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sea, and river and coast and included the information only in the text

p.17410, Line 26: How does this compare with the ANOSIM R2 for sea vs. river?

This is the $R^2=0.63$ mentioned above

p. 17411, Lines 3-4: Rewrite as “distance to the coast in each cluster, i.e., river, coastal and open sea.”

Done

p.17411, Lines 6-9: I would place this paragraph at the end of this section to match the order of analysis shown in

Table 2. Also change the R2 from 0.7 to 0.71, and refer to Table 2.

Done

p.17411, Lines 12-13: I don't understand this: “to the rest of samples lower than 60% in DNA-based profiles and lower than 50 % in RNA-based profiles”. It does not seem to fit with the previous part of the sentence.

We mean that PA samples are separated from FL ones forming single clusters in both dendrograms (DNA and RNA-based)

p.17411, Lines 18-19: Since you don't present the data, complete the information by giving the correlation coefficients, the sizes of the sample (n) and the significance levels. Also, define the acronyms CDOM and DOC. But more importantly, why didn't you use directly DOC and CDOM in your CCA analysis? These are the actual factors that influence the structure of bacterial community. I suggest that you present the results of the CCA using these factors separately. I think this would be more accurate. Have you also performed CCA with SPM? POC?

SPM and POC were included in the CCA analysis. The information about DOC, CDOM and Si (r, p levels, n) is provided in the revised version.

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p.17411, Line 23: Replace by “we used only DNA-based profiles”

Done

p.17411, Line 27: Standardize the number of digits. Should be “47.0% and 22.5% [. . .] and 44.1% and 29.5%”

Done

p.17412, Lines 1-2: Reword as “Consequent axes accounted for less than 17% of the variance each, and thus were no longer considered.”

Changed for “Consequent axes accounted for less than 16.5% of the variance each, and thus were no longer considered”

p.17412, Lines 10 and 13: You wrote, “we selected three samples” and then you wrote, “reads were obtained for the six samples analyzed”. Please clarify.

We have changed it into “six” (three locations, two fractions each)

p.17412, Line 17: Present Fig. 4 before Fig. 5, or change the numbering of the figures.

Done

p.17412, Lines 27-28: I don’t understand this sentence. Rephrase. p.17413, Lines 1-3: This is also unclear. Reword to something as “Conversely, a large percentage of OTUs that were exclusive to either sea or river samples were also exclusive to the PA fraction; in other words, 43.3 % of OTUs from the PA fraction were unique to the sea samples, whereas 32.9 % were unique to river sample (Fig. 5).”

We have rephrased the paragraph as: “Sixty percent of OTUs shared between sea and coast samples and 78% of OTUs shared between coast and river samples were found in both PA and FL fractions. Conversely, a large percentage of OTUs that were exclusive from either sea or river samples were also exclusive from the PA fraction. In other words, 43.3 % of OTUs exclusive of the sea sample and 32.9% of OTUs exclusive

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of the river sample were also exclusive of the PA fraction”

p.17413, Lines 11-12: Change to “the PA open sea sample was dominated”

Done

p.17413, Lines 19-20: Change to “Actinobacteria were more abundant”

Done

p.17414, Lines 1-7: This sentence is too long. Rephrase in two sentences as: “The MALINA Arctic sampling campaign of summer 2009 in the Mackenzie Delta-Beaufort shelf area revealed an ecosystem characterized by its oligotrophy, i.e. low primary production (Ortega-Retuerta et al., 2012b), dissolved amino acids, and labile organic matter (Shen et al., 2012). This was partially maintained by Mackenzie River inputs that structured [. . .]”

Done

p.17414, Lines 15-16: Reword as: “in a scenario of increased particle loads to the system via river discharge (Peterson et al., 2002) and increased primary production (Arrigo and van Dijken, 2011).”

Done

p.17414, Line 25 to p.17415 line 4: This sentence is too long. Divide in two sentences as: “[. . .] reported by Galand et al. (2008). We report here. . .”

Done

p.17415, Lines 12-16: This sentence is confusing, and I don’t understand the reasoning because it seems redundant. Which factors of which study are you referring to? From this present study, or from Ortega-Retuerta et al. (2012b)? I think there’s a misuse of the word “given” in the following: “[. . .] the significant role of these factors in structuring bacterial communities given the importance of bacterial community structure on the

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bulk bacterioplankton activity.” Maybe it should be something like: “the significant role of these factors in structuring bacterial communities, and very likely these factors will also have an impact on the bulk bacterioplankton activity given that the community structure affects its activity.”

We have changed the paragraph to: “We have previously shown that bacterial abundance and activity (production and respiration) was controlled by the availability of labile organic matter, with DOC, amino acids and primary production as proxies (Ortega-Retuerta et al., 2012b). This supports that these factors (DOC, amino acids, labile organic matter) control both bacterioplankton activity and community structure in this region, an important fact given that the community structure affects its activity (e.g. Obernosterer et al., 2010)”

p.17415, Line 22: Replace by “Contrary to expectations”

We have replaced it by “unexpectedly”

p.17415, Lines 24-26: I think you need to be careful when citing Garneau et al. (2009). These authors observed “differences between the particle-associated and free-living bacterial assemblages at the estuarine stations with highest POM content”, and not at the stations with the highest total particle concentration, as you mentioned in the previous sentence. Garneau et al. rather refer to “particle liability” than particle concentration, as they haven’t found any correlation with SPM. So this is in line with, and not contrasting to, what you suggest “that particle quality, rather than their quantity, would play a major role structuring bacterial communities” (p. 17416 lines 2-1). This assertion was already mentioned by Hollibaugh et al. (Aquat. Microb. Ecol. 21, 2000), Garneau et al. (2009), and many other authors. Your results are contrasting to the ones of Garneau et al. because the POM-rich stations were then located in the estuary, whereas in your study, POM-rich stations were located in the open sea (as you mentioned on p. 17416, lines 4-6). Water movements are very likely to happen in such dynamic system as the Mackenzie Shelf, and this may explain these spatial differences

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between the two sampling campaigns.

We have changed the sentences to clarify the distinction between particle abundance, particle quality and location (river vs sea) as the referee comments: “This result contrasts to previous observations in the Mackenzie River- Beaufort Sea system, where highest differences in PA vs. FL fractions were found in the Mackenzie River estuary” “Therefore, the particulate organic substrates were likely influencing the bacterial communities growing on them, similar to the results of Garneau et al. (2009) that showed differences between PA and FL bacteria at locations with highest POM content”

p.17416, Lines 12-13: I think there is a misused of the adverb “indeed”. This sentence starting with “indeed” should corroborate the previous sentence, but as I understand the context, it doesn’t. The “indeed” sentence discusses about cosmopolitan OTUs, whereas the previous sentence indicates “the presence of particle specialists.” Also, the sentence is unclear: “[. . .] within the shared OTUs (i.e. cosmopolitan OTUs) there was a higher proportion of OTUs found on both PA and FL fractions.” A proportion higher than what?

We have changed the sentence to “On the other hand, within the OTUs that were shared between river, coast and open sea samples (i.e. cosmopolitan OTUs) there was a higher proportion of OTUs found on both PA and FL fractions than within OTUs exclusive from a certain location (river, coast or open sea)” p.17416, Line 27: To avoid ambiguity with river benthos, reword as: “non-active OTUs in surface waters of the river.”

Done

p.17416, Line 29: “higher diversity in the PA fraction”

Changed

p.17417, Lines 12-13: Be more accurate by mentioning that it’s the availability of organic material (i.e., CDOM, DOC, amino acids, SPM) that was the main structuring

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factor, and not the location.âĀĀ

We changed the sentence to “Despite the outcome that location (river, coast, open sea) and their associated differences in salinity, DOC, SPM or amino acids were the main structuring factor”

p.17417, Lines 19-23: The sentence is too long, divide in two. p.17418, Line 2: “have reported that bacteria attenuate”

Done

Figures and Tables: Please use the same terms to refer to particle-attached bacteria and free-living bacteria throughout the manuscript. In the text, you use PA and FL, whereas you used “attached” and “free” in Table 4. In Fig. 2, the OTU name codes used A and F, whereas in the legend you wrote PA and FL. Also in the legend of Fig. 2, specify the colors for each area.

We have made all suggested changes in tables and figures and standardized all terms into “PA” and “FL”

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