**Biogeosciences
Discussions**

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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General comments:

The study presents new and interesting data on bacterial community structure in a coastal arctic system, and shows how environmental parameters, especially the organic matter availability, are important drivers for shaping microbial assemblages. The methods used (i.e., CE-SSCP and tag-sequencing) are innovative and thus, the work go beyond previous studies addressing this topic in the region by allowing a finer anal-

ysis of the diversity at the genus level for both the particle-attached and free-living bacteria. Results show a strong presence of particle specialists in areas of organic-rich particles, but a high degree of diversity overlap in areas of mineral-rich particles. These data are valuable because the detailed identification of bacterial groups gives a better picture of the microbial metabolism prevailing in this ecosystem, which is important for our understanding of the biogeochemical processes.

The work appears to have been done carefully and the statistical analyses are appropriate. However, I am not sure why the authors used salinity in their CCA analysis instead of DOC and CDOM. They did mention that salinity was a proxy for DOC and CDOM (p. 17403, lines 13-14), but since they have the data (presented in Table 1), I think it would be better to use directly these parameters. I would suggest redoing the CCA analysis, or justifying why using a proxy was more appropriate.

The paper is generally well organized, but several grammatical errors, as well as long and confusing sentences makes it difficult to understand at times. I have identified some of them in my specific comments. I would support publication of this manuscript in Biogeosciences with revisions.

Specific comments:

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

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."

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."

p.17404, line 15: Change for "Arctic rivers"

p.17404, line 17: Write "the occurrence of phytoplankton blooms" to avoid confusion

with ice algae

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

p.17405, line 13: Change for "subarctic regions"

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

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"

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"

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

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.

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

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

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

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).

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

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.

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.

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

p.17410, Line 6: Should be "fresh water"

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

p.17410, Line 12: Should be "ice meltwater"

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.

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

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

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

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

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.

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.

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?

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

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

p.17412, Lines 1-2: Reword as "Consequent axes accounted for less than 17% 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.

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

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)."

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

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

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 [. . .]"

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)."

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. . ."

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 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."

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

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 between the two sampling campaigns.

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?

p.17416, Line 27: To avoid ambiguity with river benthos, reword as: "non-active OTUs in surface waters of the river."

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

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.

p.17417, Lines 19-23: The sentence is too long, divide in two.

p.17418, Line 2: "have reported that bacteria attenuate"

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.