

Interactive comment on “High growth potential and activity of 0.2 μm filterable bacteria habitually present in coastal seawater” by Yumiko Obayashi and Satoru Suzuki

Anonymous Referee #2

Received and published: 13 February 2017

General comments: This manuscript examines the growth, protease activity, and taxonomic composition of small bacteria filterable through 0.2 μm pore size filters. The authors provide excellent background information on the importance of heterotrophic bacterial activities in the ocean, and contextualize the research from the perspective of ultra-small bacteria being examined in the open ocean realms. However, in explaining the novelty of their examinations that focus on coastal systems, they do not provide evidence of why these coastal ecosystems are important in the first place. Further, it is not evident how these small bacteria are potentially influential substrate processors if their presence is only enabled when larger bacteria, which are not classified in the manuscript as competitors, pathogens, or commensals, and grazers are removed. It

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is intriguing that the identity of these putatively starved cells are not seemingly different than that of the original community, but the application of DGGE to make this conclusion likely introduces a large level of uncertainty in truly elucidating differences in community composition between and among the manipulations, particularly in light of the current use of next generation sequencing and metagenomic approaches that can identify even rare members of the microbial biosphere, including a new phyla of ultra-small bacteria (Candidate Phyla Radiation).

Specific comments: Page 3, Lines 13-17 – More details on the microscopy counts are required. Were the filters replicated, how many fields of view were counted, minimum number of cells counted per field of view, and approximately how many total cells, etc.?

Page 4, line – 13 – Has it been shown that PCR-DGGE is comparable to other, more recent community composition assessment techniques, e.g. versus iTag, or even versus TRFLP, clone libraries, or others?

Page 5, Line 2 – How many bands were excised? Why were many ignored? There were several bands in figures 4 & 5 that are present in one sample but not in the other. A comprehensive analysis of the community must be performed if 16S iTag sequencing is not performed.

Page 5, Line 15 – Specific numbers are needed to state exactly how low the counts in FSW are.

Page 5, line 20 – If you assume a minimal influence of grazers on growth rate, you must also make an assumption on viruses. Are these assumptions valid? This requires a citation or other explanation.

Page 7, lines 4 – 8 – It is unclear what this section is attempting to support and/or conclude. There should be a citation regarding the influence of the grazer, another section discussing the potential influences of viruses, and a discussion of why the Li & Dickie 1985 paper is mentioned and what it means (particularly since this paper

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seems to have already shown what the authors say they are reporting for the first time!). Lastly, it is difficult and likely incorrect to assign a bacterial community growth rate since organic matter, viruses, competition, and a multitude of other abiotic factors affect growth of single cells comprising that community. If the authors believe a potential growth rate is warranted here, a more in depth discussion with citations are needed.

Page 7, lines 17 – 18 – The potential for PCR bias and how it potentially alters the interpretation of community diversity should be mentioned and discussed. Also, is there a statistical method to compare the Shannon indices at each time point?

Page 7, lines 22 – 28 – It is interesting that these cells seem pervasive in coastal waters. However, there is no convincing evidence that they meaningfully contribute to biogeochemical cycles under natural conditions. There must be a discussion outlining what types of conditions the 0.2 μm filtering mimicked to facilitate increased growth and activity of these cells. This is particularly needed since DAPI counts were not completed everyday, as was the case for the enzyme activity measurements.

Page 8, lines 7 – 8 – If the enzyme activities of the particulate fraction are going to be mentioned, the methods involved with this measurement and the associated data must be included.

Page 8, line 26 – What about the effect of bacterial cell breakage?

Page 9, lines 1 – 2 – As mentioned previously, more evidence is needed to show that 0.2 μm filterable bacteria are significantly contributing to organic matter biogeochemistry. This is difficult to reconcile with the fact that the DGGE-based conclusions that state the small bacteria are no different than the unfiltered community toward the end of the microcosm experiments. How can the community be the same yet occupy a different biogeochemical niche? The results seem to suggest a succession in community composition that is dictated by organic matter availability and/or competition at the early stages among the small bacteria. In any case, more thought out conclusions must be formulated that include a mechanistic model of how these manipulated condi-

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tions reflects actual microscale events that are likely to influence microbial ecological control of biogeochemistry.

Technical corrections Page 2, lines 19 – 20 – This sentence should be rewritten for clarification.

Page 4, line 28 – Change to Arabic numerals.

Page 5, lines 15 – 16 – Rewrite for clarification.

Page 7, lines 29 – 30 – Rewrite for clarification.

Page 8, lines 3 – 5 – Rewrite both sentences for clarification.

Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-560, 2017.

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