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Atmospheric Chemistry and Physics Discussions

Interactive comment on "A next generation sequencing of Arctic bacteria in snow and frost flowers: identification, abundance and freezing nucleation" *by* R. Mortazavi et al.

Anonymous Referee #1

Received and published: 24 December 2014

Atmospheric Chemistry and Physics Title: A next generation sequencing of Arctic bacteria in snow and frost flowers: identification, abundance and freezing nucleation

This paper provides insights into the microbial composition of frost flowers and other snow sources as well as the ice nucleation capability of culturable bacteria from these samples. This seems to be a novel dataset that is worthy or publication. The paper would greatly benefit from including more details on the methods that were used, particularly with regards to the pyrosequencing and how the samples were prepared and processed in MOTHUR (see specific notes below). The paper would also benefit from being substantially edited and reviewed by a microbial ecologist to improve the flow

of language and grammar to more clearly communicate the results and background information (see specific comments below). The paper also mentions more than once the possibility of microbes in snow and ice in contributing to ecological processes in the Arctic but this is not returned to in the discussion of the results with respect to the microbial composition revealed by sequencing. Although this type of discussion requires a bit of speculation, it would be interesting to discuss some hypotheses for future research to build upon.

Abstract: Line 1: Seems like there should not be hyphens between “Ocean, Atmospheric Sea Ice Snowpack”

Line 2: unclear what is meant by “population”. What about the “population” are you examining?

Line 3: What is a “snow type”?

Line 5: Awkward to say ”conventional culture-based PCR identification approach”. Suggested revision: “In addition to culturing and gene sequence-based identification”

Line 6: Suggest replacing “deployed” with “utilized”

Line 7: suggest replacing “diverse” with “diversity of”

Line 7: Do not start a sentence with numbers.

Line 8: replace “identified” with “detected”

Line 8: eliminate “great”

Line 9 and 10: Phylum names are not italicized

Line 10: suggest replacing, “at the genus level, 101-245 different genera” with “The number of genera detected ranged from 101-245.”

Line 11: eliminate “”in cultured samples”

Line 14: eliminate “Complementary”

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Line 16: “isolate” should be “isolates”

Line 18: “An isolate belonging to the Bacillus species” should be “An isolates belonging to the genus Bacillus”.

Line 20: How do you know that the microbes in the Arctic snow originated from distinct ecological environments.

Lines 21 and 22: It is not clear what is meant by “microbial snow”. It seems unlikely that snow would exist that does not have microbes in it. Furthermore, how would the presence of microbes in snow influence the freezing and melting processes of the snowpack in the Arctic.

Introduction:

Page 32095 Lines 4-5: It is not clear what “bio-organic” molecules are and microogan-isms (spelled with no hyphen) are not molecules, they are organisms comprised of molecules.

Line 5: Is there are difference between freezing nucleation and ice nucleation? Both phrases are used and it is not clear what the difference is, if there is one.

Line 6: there should be a period after “understood” rather than a comma.

Line 14: “microbial” should be “microbes”?

Line 18: “and survived” should be eliminated.

Page 32096 Line 9: “/z” should be “h” Line 15: “microbial” should be “microbes” or “microorganisms” Line 16: “as an” should be eliminated Line 20: Not clear what natural and anthropogenic sources are contributing....ice nucleation particles? Please be more specific.

Page 32097 Line 3-4: need to cite the studies that indicated diverse bacterial popu-lations exist in snow. Line 10: I think “number density” should be either “number” or

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“density”

Experimental Methods: Line 21: Is “hoar” a typographical error? Line 21: It is not clear what frost flowers are. Is there a reference that can be cited here or can a brief description be provided? To some people frost flowers refer to this: http://www.kuriositas.com/2012/12/frost-flowers-natures-exquisite-ice.html

Page 32098 Line 3: Spell out what HDPE means before using the acronym. Line 3: When citing the company where a particular instrument or supply was purchased, put the full company name, city and country in parethesesâĂŤnot just “(Fisher)”. Do this for the rest of the experimental methods section. Line 24: a citation or the full recipe needs to be written into this section for “mycological agar”

Page 32099 Lines 1-13: for the drop-freezing assay, how many 10 uL drops were examined per sample or bacterial culture?

Section 2.3: says “used to lyse the cell”. I am pretty sure that more than one cell was lysed. Please specify what material the DNA was extracted from (e.g. liquid culture, filtered environmental sample).

Please cite the original source and names of the PCR primers used, unless these were custom made by the authors. Also please state the region of the 16S rRNA gene that is being amplified and how long the fragment is that is being amplified. Please state the specific PCR mixture components, their manufacturer and the volumes and final reaction concentrations of each that wee used. Please state the make a model of the thermalcycler used for PCR. Please state how the PCR products were purified and prepared for DNA sequencing.

How many bacterial isolates were selected from each culturing effort for each sample? How many were tested for ice nucleation ability? How many isolates were sequenced?

Page 32100 Section 2.4 should be split into separate sections: one for pyrosequencing and one for the electron microscopy.

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How was the DNA extracted from snow? Please describe this in detail. Was the snow filtered and then extracted from a filter? Please explain.

Please provide the details of how MOTHUR was used to trim and process the libraries.

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Page 32101 Results and Discussion

L11-14: What are photobiological reactions? How is this compare with photochemical reactions? L14-15: knowing how microbes interact in snow will not necessarily "reveal the role these play in alterin the Arctic environment and climate". Is there any reasonable evidence that suggests bacteria in snow pack are indeed altering the "Arctic environment and climate"? If so, it is necessary to cite those sources here. L19-21: The following should be included in the materials and methods section, not the results and discussion section, "NGS analysis was performed using 1/8 of sequencing 20 plate of GS FLX Titanium (454/Roche) for reading. Individual sequences were based on the V1–V3 primers."

The first paragraph of the results and discussion section needs to be broken up into multiple paragraphs and different topics are in there.

L22: are you referring to the average number of reads per sample? If so state that. Otherwise, it is not clear what you mean by average reads.

The following should be eliminated as it is not needed nor usually reported, "The lowest and highest number of reads was obtained for windpack, WP, (13 831) and urban snow, US, (24 968) respectively. The total number of bases was 32 284 312. The 25 lowest and highest number of bases belonged to blowing snow, BS, (4 164 307), and urban snow, US, (7 966 589) respectively. The average raw read length varied among the samples and was obtained as: 542_23 bases (urban snow, US), 422_21 bases (blowing snow, BS), 542_23 bases (snow hoar, SH), 533_23 bases (windpack, WP), and 556_24 bases (frost flower, FF)."

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Page 32102 During the trimming process, all sequences should have been trimmed to the same length. It should not vary from sample to sample as described in the following section, “After trimming and passing through quality control, the final read length was recovered as: 319_18 bases (urban snow, US), 299_17 bases (blowing snow, BS), 401_20 bases (snow hoar, SH), 385_20 bases (windpack,5 WP), and 419_20 bases (frost flower, FF) (Table A1).”

L6-8: Rarefaction Metric is not a way to measure bacterial diversity—it is a way to measure bacterial richness.

Page 32103 L8: “most abundance” should be “greatest abundance”

L11: “genus” should be genera

L22: “microbial” should be “microbes” or “microorganisms”

Page 32106 L6: it is not accurate to say that most aerobic bacteria can grow on TSA. Considering only 0.01% of bacteria can be cultured at this point, you cannot say this about any medium type.

L11: “cultural” should be “culture”

Page 32109 L16: “We observed much more insight” should be “We gain more insight”

Conclusions: This section is an exact repeat of the abstract. Are there any deeper conclusions or implications of the work that can be discussed here?

Table 1 is not clear in the information that it contains. What do the percentages refer to ? It seems that abundance should be reported in actual numbers of colony forming units. The caption is not clear; it says that “ice nucleation/melting properties as detected by Roche 454 GS-FLX Titanium”. One cannot determine ice nucleating properties using next generation sequencing tools.

Does Table 2 contain all of the bacteria that were isolated in this study?

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The information in table A1 is not typically presented. When NGS data are trimmed, all sequences, regardless of what sample they are from, should be trimmed to the same length and aligned together to ensure the ability to compare the microbial composition across the samples as was desired in this study.

Table A2 would be more useful to the reader if it were converted into a bar chart where the reader could quickly identify the differences in microbial composition among the different sample types.

Figure A1. The inverse Simpson index should not be graphed vs the number of sequences sampled. The Simpson index is a dominance index and continued sampling of "rare" types or singletons, is not going to significantly change this index as more sequences are sampled. Hence the rather flat "curves". The inverse Simpson index should be presented as an endpoint number at the same number of sequences for each of the samples that one wishes to compare. For instance, if the greatest common number of sequences for all samples is 10,000 sequences, then the inverse Simpson index should be reported for 10,000 sequences for all of the samples.

Figure 2. The legends are too complicated and are not readable even when the figure is blown up on my computer screen. It is not clear if the bars in the bar graphs each represent a different genus or something different.

Figure 3. Do the bars represent averages? If yes, please state in the legend how many replicates were averaged. Are the error bars, standard error or standard error? Please state which one.

Interactive comment on Atmos. Chem. Phys. Discuss., 14, 32093, 2014.