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## ***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.***

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Received and published: 25 December 2014

### General Comments

Frost flowers are an interesting ice-type both biologically and chemically. The authors collected data on the chemical and biological composition of frost flowers and various other snow/ice environments. This kind of data is much needed and could serve as fodder for an interesting analysis. There are, however, numerous issues with the analysis and the resulting manuscript that should be corrected before publication.

First, the authors are missing an opportunity to place their sequence results in the

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context of existing work on frost flowers (e.g. Bowman et al, 2013, EMIR; Bowman et al, 2014, FEMS; Barber et al, 2014, JGR Oceans) and snow (e.g. Hauptmann et al. 2014, Extremophiles, and Maccario et al. 2014, Frontiers in Microbiology among many others). In particular the three frost flower studies noted above describe two dramatically different frost flower communities. Where does the community described by this work fit in? As NGS technologies were used in all of these studies the authors should take the opportunity to compare and contrast these results. For example, are some of the same sequences described in more than one study? What might lead to the observed differences? Bowman et al (2014) specifically evaluated a frost flower metagenome for ice structuring genes, finding fewer genes in frost flowers than in the underlying sea ice. Some discussion of this in the context of the ice nucleation work presented here would be useful.

The results and discussion are presented somewhat haphazardly, making it difficult to determine what the authors have done and why. Some important data collected is not adequately discussed; why is the EDS data not discussed further, or presented in the results in a more quantified manner? Similarly, beyond some brief discussion of their ice nucleation potential the different microbial genera “identified” (at what confidence?) are not discussed. A lot of information was collected in this study, what does it tell us about these different environments?

I would encourage the authors to read the manuscript carefully for wording and grammatical errors. There are numerous errors which I did not document in detail.

#### Specific Comments

Suppliers of materials are inconsistently identified. For example the manufacture of the freezer is identified (not necessary), but the manufacture of the Ready-Lyse reagent is not (necessary). This should be corrected throughout.

What were the temperature and salinity of the collected frost flowers? How old do the authors estimate they are? How thick was the ice underneath? These are essential

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details for interpreting the community composition data.

What pore-size filter was used to collect cells for DNA extraction? Was a pre-filtration step used? Cyanobacterial reads were reported, were these chloroplasts? If so, from what eukaryote?

32095 Line 4 – citation needed

32095 Line 28 – there is a growing body of literature on bacterial survival in long-distance dust. Some of this work should be cited here (e.g. Smith et al. 2010, Aerobiologia).

32096 – I think the introduction needs to get more specific regarding the known and potential role of IN in high latitudes. Quite a bit of low latitude work is introduced that is not particularly informative.

32097 Line 4 – citations needed (e.g. Hauptmann et al. 2014, Maccario et al. 2014)

32098 Line 11 – how long did samples sit at -20 before analysis? Communities are not stable (particularly against loss) at these temps.

32099 Line 20 – what region is this amplifying? Important to identify.

32100 Line 2 – BLASTN is not an appropriate way to classify 16S sequences. The authors should use the RDP classifier, the classifier built in to Mothur, or a different tool and an appropriate database.

32100 Line 27 – the authors should more clearly describe the sequence analysis undertaken with Mothur.

32101 Line 14 – true, but I don't think the current study is addressing interactions at all...

32101 Line 15 – here and elsewhere, it is a little odd the way the authors refer to the sequencing methodology as the NGS technique. They relied on a sequencing platform

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(454), which is one of a suite of technologies that differ from Sanger sequencing. These technologies are very different from one another and are, collectively, now the standard technologies for obtaining environmental sequences. The authors should just state the platform used and move on. As currently used the authors run the risk of a reader perceiving the study as about NGS, not about the study of a particular environment. This further obscures interesting findings in this work.

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

ACPD

14, C10490–C10493,  
2014

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