Dr. Akob, we would like to thank you for your great suggestions for improvement on our manuscript, which we have revised further based on your feedback. Please find our responses to your comments and changes to the manuscript below in blue text. A track changes version is also included.

Specific Comments:

1. Title: reviewer #2 suggests amending the title by replacing "Haloarchaea" for Archaea. I think this would be a valuable change and would highlight the focus of your study.

Great suggestion, done.

2. L. 15-16: consider revising to "nucleation activity of 4 species in the class Haloarchaea".

Done.

3. L. 10: change to "plants"

We were not sure what you wanted changed to "plants". We ended up removing "plants" and included some additional microorganisms that we have in Figure 4 for consistency.

4. L. 57, 72 and elsewhere: when referring to Bacteria (and Archaea and Eukaryotes) as a domain please capitalize. There are a number of instances where this change is needed for accuracy. Also, if Haloarchaea is a formal taxonomic group name it should always be capitalized.

Done, for when referring to their domains / classes.

5. L. 95: change to "these four"

Done.

6. Tables 1: It's not clear from the table and the methods text at which time the cell counts were made. In response to Reviewer 2, text was omitted on shipping the cultures from SC to CO. I disagree with this decision. The reviewer is pointing out a key methodological consideration -- even though you shipped the samples on ice it is important to know if the cultures were still in log-phase growth. It is super important to know what the cell abundance was at the time the experiments were started and how cell abundance changed during shipping. Even though you shipped the samples on ice there could be cell growth or death. Line 130 talks about the cultures being measured for ice nucleating ability within a few days of reaching log-phase but was this before or after shipping? Were the cultures still in log phase? More detail is needed.

Thank you for clarifying how we should handle the issue of transport. Samples were monitored for growth at the College of Charleston. They were shipped overnight on ice to Colorado State where they were stored for up to 48 hours at 4°C. They were then checked for growth again and no change in cell density was observed. We have updated the text to better communicate our efforts at maintaining consistent culture environments. The text now reads: "Cells were counted and monitored for growth until mid-log phase at which point, they were shipped on ice overnight to Colorado and stored for up to 48 hours at 4 ˚C. In the event that cell densities were too high to achieve an accurate count, cultures were diluted to a countable level. Cultures were checked a final time for cell density immediately prior to ice nucleation assays to ensure that no appreciable growth had occurred during transport and storage. Table 1 provides the cell concentrations and salinities of all four prepared cultures immediately prior to ice nucleation assays."

7. Table 1: why didn't you dilute the cultures in media to count them? That would have prevented cell lysis and give you an accurate cell count. Or you could have fixed the cells before diluting for cell

counts as suggested by Reviewer #3. Clearly you can't go back and redo this but consider this for future experiments.

Cells were diluted to a factor of 1:6 for counting (except for H. morrhuae which could readily take a dilution in excess of 1:15). Since the microscopy check upon full dilution was primarily to assess whether cells remained intact, at the time we didn't think it was necessary to fix the cells or further dilute with a saline solution or media to protect against lysis. In retrospect, a second check on cell densities would have been helpful and we will certainly consider this for future experiments.

8. Tables 1 and 2: I wonder if you really need 2 tables? The culture medium is already stated in the methods so could be omitted. You could combine the information into a single table and have columns of initial and diluted cell concentrations and salinities. I also suggest putting information in order of action, e.g., you diluted before knowing the cells were intact so switch the column order; otherwise it makes it sound like you diluted lysed cells.

Good idea, done.

9. L. 105-112: I think this paragraph is of value and should be retained. The authors do a nice job here of identifying how the lab cultures can be environmentally relevant.

Thank you!

10. L. 137-142: a lot of the reviewers' comments were around the different treatments and use of lysed vs. intact cells. The text here is a great justification on why the treatments make sense and that the different cultures behaving differently has environmental relevance. But, this message really isn't stated in the introduction. It would improve the paper if the statement about testing cell lysis having environmental relevance was included somewhere in the end of the intro.

Done. We have added the following sentence towards the end of the introduction when first mentioning cell lysis: "Assessing a variety of cells that lyse or remain intact is relevant for ice nucleation because cell fragments of other microorganisms have been shown serve as INPs and (Anderson and Ashworth, 1986; Du et al., 2017; O'Sullivan et al., 2015; Šantl-Temkiv et al., 2015) and archaea might lyse naturally once exposed to atmospheric water vapor in the aerosol phase."

11. L. 144-148: the added text is very helpful.

Glad to hear, thank you.

12. L. 142: I'm not sure if I get what is meant by "threshold" and "serially dilution" here – Table 2 doesn't show data for serial dilutions, just a few select samples. If the cultures were serially diluted that would indicate to me that there is a whole series of dilutions that were assessed for cell survival and the highest dilution that didn't show lysis was the threshold. Are you really just presenting the final dilution selected based on those factors? Or did you only select these dilutions based on tests with saline solution controls? Please clarify.

Thank you for bringing this to our attention. For clarification, we removed "serially" since these were indeed the only dilutions we created and tested. We also changed "Cell lysis thresholds were determined…" to simply "Cell lysis was determined…".

13. L. 152: please define the abbreviation CSU

Done (Colorado State University).

14. Section 3.2: I found the start of this section to be overly focused on the dilutions taking away from the study aim. I suggest revising to start with the main finding and not the methodology. Also, the use

of “dilution” seems a bit mis-leading since the focus is really on the cell density and not the salinity of dilution of the sample.

We moved some of the text at the beginning of section 3.2 to the methods. We now discuss the findings and try to avoid using dilution as much as possible by clarifying that increasing dilution = decreasing cell density. When intercomparing the dilutions, we changed “dilution” to “sample” (e.g., “1:6 dilution” to “1:6 sample”).

15. L. 216 and elsewhere: the original paper did not have statistics presented. Adding the fisher’s exact test was really helpful for showing the differences in the treatments. Consider adding a supplemental table of the statistics results to compliment the narrative.

We have now compiled a table for statistics for the treatments and will upload as a supplementary file and referenced to in the text (Table S1).

16. L. 266: change to “range tested” as it would be a stronger statement

Good suggestion, done.

17. L. 269: cite the references on H. morrhuae cells not lysing here.

Done.

18. L. 270-272: the statement on the DUF3494 protein domain in the Archaea does not belong here. The reference is not from any of your authors, and you don’t show any data. If you want to include this observation the results and methods need to be included.

Vance et al. (2019) indicated that archaea can contain this protein, but perhaps this is too vague to make a link to Haloarchaea. We also did not conduct any such observations, so we have removed the reference to DUF3494.

19. L. 294: based on this acknowledgement it sounds like the organisms were cultured in Colorado and not just in SC. If they were preserved does that indicate cells were fixed before counting? Clarification would be really helpful per the comments above about the methods for culturing and shipping.

This reflects their efforts on a previous iteration of the experiment that is not discussed in the manuscript. This acknowledgement has been removed.

20. Table 2: correct the spelling of pharaonis in the footnotes

Fixed.

21. Figure 3: the revised, track changes ver

sion of the paper shows 2 graphs with different data presented. According to a response to Reviewer #1 the data in Figure 3 were originally mislabeled but are now fixed and the text is correct. However, please double check this for accuracy during revision.

Reviewer 1 was correct in that we did mix up the symbols and colors originally, which we fixed for the revision. We have gone back to the raw data files and confirmed that the data shown in the revision are correct.