

Author Response to Reviews of

Measurement report: The ice-nucleating activity of lichen sampled in a northern European boreal forest

Ulrike Proske, Michael P. Adams, Grace C. E. Porter, Mark Holden, Jaana Bäck, and Benjamin J. Murray
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RC: Reviewer Comment, AR: Author Response, Manuscript text

1. Reviewer Comment # 5, from Reviewer # 4

RC: *Proske et al. have significantly revised the manuscript, improving its clarity and strengthening the conclusions. The manuscript now presents a cohesive narrative, and the new additions have made the findings more robust. This is highly engaging work, and I believe the manuscript is now suitable for publication. I have only a few minor suggestions for the authors to consider before final acceptance.*

AR: *We thank the referee for reviewing the manuscript a second time and again offering constructive feedback.*

RC: *The novelty of this study lies not only in its location but also in its detailed investigation of INP size. While Kieft and Ruscetti (1990) examined only 0.2 μm INPs, this study expands on that by presenting data from filtration across multiple size ranges. Understanding the size distribution of INPs is undoubtedly valuable information. The authors might consider emphasizing this aspect more explicitly in both the abstract and the conclusion of the introduction.*

AR: *We agree that the size filtration is a valuable addition of this study. While we believe the abstract highlights this aspect sufficiently (“Successive filtration to smaller sizes removes some of the most active INPs in suspension, but substantial activity remains even when filtering to 0.1 μm .”), we have added the point to the conclusion of the introduction as you suggested:*

In this paper we tackle the first part of the hypothesis, namely the question of if the lichens in the forest during the winter of HyICE-2018 contained ice-nucleating entities. Eufemio et al. (2023) have made this possibility obvious since they showed that lichen from across Alaska harbour INPs. It remains to us to confirm this for the Hyytiälä boreal forest, and to investigate the size of the lichenous INPs that we find.

RC: *Line 11: Typo: “Hyytiäläre” should be corrected.
Line 12: Consider adding “previous” or “existing” before “evidence” for clarity.*

AR: *Thank you for pointing us to these oversights, which we have corrected accordingly.*

RC: *Line 114: I assume you mean above $-24\text{ }^{\circ}\text{C}$ here?*

AR: *No, in fact Vogel et al. (2024) specifically report INPs below $-24\text{ }^{\circ}\text{C}$.*

RC: *Lines 444-445: The statement, “Many of the lichenous INPs were found to be between 0.1 and 2 μm in size when immersed in water, and those active at temperatures higher than 18 $^{\circ}\text{C}$ were heat-labile,” raises an*

interesting point. Could some of these particles be bacteria living symbiotically with the lichen? Bacterial cells typically range around $\approx 1 \mu\text{m}$ and are often heat-labile. While the authors mention that Kieft (1988) did not find cultivable bacteria in lichen samples, it's important to note that not all ice-nucleating bacteria are easily cultured. The introduction touches on how bacteria, among other things, can contribute to the INPs from lichen samples. Given the size range and heat sensitivity, the authors might want to discuss the potential bacterial connection to their findings, specifically, further in their discussion.

AR: Thank you for this suggestion. We have taken up your discussion idea in the Conclusions section by adding:

Alternatively, the INP may also be bacteria living symbiotically with the lichen. As mentioned in Sec. 1, the bacteria that Kieft (1988) cultivated from lichen showed no ice-nucleating activity, but not all ice-nucleating bacteria are easily cultured.

References

- Eufemio, Rosemary J, Ingrid de Almeida Ribeiro, Todd L Sformo, Gary A Laursen, Valeria Molinero, Janine Fröhlich-Nowoisky, Mischa Bonn, and Konrad Meister (2023). “Lichen Species across Alaska Produce Highly Active and Stable Ice Nucleators”. In: *Biogeosciences* 20. DOI: 10.5194/bg-20-2805-2023.
- Kieft, Thomas L (1988). “Ice Nucleation Activity in Lichens”. In: *Applied and Environmental Microbiology* 54, p. 5.
- Vogel, F. et al. (2024). “Ice-nucleating particles active below -24°C in a Finnish boreal forest and their relationship to bioaerosols”. In: *EGUsphere* 2024, pp. 1–25. DOI: 10.5194/egusphere-2024-853. URL: <https://egusphere.copernicus.org/preprints/2024/egusphere-2024-853/>.

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