Jensen et al. present a valuable study on INPs in soil and stream water samples and the microbial community composition in soil from multiple sites in Arctic Greenland. Their research includes size filtering to assess the different types of INPs in the samples. Compared to earlier studies, the INP concentrations in the soils were found to be somewhat lower. The authors also explore the potential linkage between INPs in soils and streams, aiming to test the hypothesis from previous research that soil-derived INPs may become airborne in the Arctic via the water-atmosphere interface. The authors conclude that the INPs detected in the soil are likely of fungal origin, specifically from species such as *Mortierella sp.,* and suggest that the INPs found in the streams may be linked to those in the soil. While the study is interesting and merits publication, several critical issues should be addressed prior to its acceptance.

General comments:

"Permafrost" INPs: My primary concern with this manuscript is the claim that permafrost samples were evaluated, which is inaccurate. The authors collected surface soil samples from the active layer, not permafrost. Active layer soil can differ significantly in composition from permafrost, as it is generally "younger" and largely composed of deposited loess. Thawed permafrost soil is typically located between the active layer and underlying frozen permafrost table, with the exception of coastal and freshwater shoreline erosion. The authors should avoid referring to these samples as permafrost, as this characterization is misleading.

Blank correction: The manuscript mentions the use of filtered Milli-Q water as a negative control, but were the samples blank-corrected using these spectra? It is essential to use the blanks to correct the spectra, given that Milli-Q water was involved in the sample preparation process.

Spectra error bars: In Figures 2, 3, 4, 6, 7, as well as in the INP spectra and aerosol size distribution figures in the SI, error or uncertainty bars should be shown.

Comparison of onset temperatures: Onset temperatures from different ice nucleation analytical techniques cannot be directly compared due to subtle differences that may affect the detection limits of each instrument. For instance, nanoliter-sized droplets have a lower detection limit compared to microliter- or milliliter-sized droplets (see Figure 4 in Tobo, 2016). The authors should avoid comparing onset temperatures with those reported in previous studies throughout the manuscript.

INP sizes: The conclusion that INPs were either bound to soil particles or microbial membranes at certain locations, while other sites displayed a variety of soluble INPs with different molecular sizes, is particularly interesting given the heterogeneity across sites. In the results, the authors suggest that fungal INpro are the most likely candidates (based on the discussion starting at line 297). However, are there other possible materials that could serve as INPs? For example, carbohydrates (polysaccharides) can range from 100-1000 kDa, although it's unclear if these sizes are typically found in soils or streams, and they have been shown to nucleate ice (e.g., Alpert et al., 2022). Furthermore, considering the sieving and comminution using a mortar, could cellular material have fragmented into smaller pieces? How easily do these proteins detach from cell walls? It would be advantageous for the authors to rule out other potential ice-nucleating materials based on size to support their claim that these particles are most likely INpro, either on their own or attached to soil particle surfaces.

Conclusions not supported by results: Some conclusions in the manuscript are not fully supported by the results. For instance, on lines 515-516, the authors state, "The findings for the first time describe parallel measurements of INP concentrations in Arctic soil and stream systems and open the necessity for more studies investigating these environments." However, this is not technically the first time such measurements have been made, as Barry et al. (2023a) compared freshwater outflow INPs with soil INPs.

On lines 463-464, the authors claim, "The presence of high INP concentrations in Arctic streams has implications for cloud formation and regional climate," and in the conclusions, they assert that "In this way, the highly active INPs could impact cloud formation and climate, implying that bioINPs from soils and streams play a significant, yet complex, role in the Arctic climate system." This conclusion is somewhat overstated, given the current evidence, especially since INPs in aerosols were not measured or linked to the soil and stream water source samples. The authors should avoid such claims and instead focus the intent on the characterization of potential local Arctic sources of INPs.

Additionally, the statement in the conclusions, “Stream INP concentrations demonstrated a positive but not significant correlation with INP concentrations in soil, which indicates that INPs are transported from soil into adjacent streams but are not the sole source for stream INPs,” raises questions. Why were 16S and ITS analyses not performed for the stream water samples? Without this information, it is challenging to draw meaningful connections between the soil as a source of INPs and the processes that facilitate their transfer to streams.

Specific comments:

Line 47: “…ice nuclei to form ice particles…” should be “ice nucleating particles to form ice crystals…”

Lines 47-48: This statement is inaccurate. Interest in bioINPs dates back to the 1970s, with pioneering studies by Schnell and Vali (1976) and Vali (1976). The authors should acknowledge these foundational works. Additionally, more recent reviews, such as Huang et al. (2021), should be cited in this context.

Line 49: It would be best to update to the newest IPCC report.

Lines 74-75: “Ice nucleation below -15°C is initiated by abiotic INPs…” and ““…while the only known INPs that are active above -15°C and present at relevant concentrations are of biotic origin…” are both inaccurate statements. See Kanji et al. (2017) and Murray et al. (2012). Certain minerals have been shown to nucleate ice above -15°C, although in low concentrations (e.g., Harrison et al. (2019)).

Lines 76-77: This statement on the types of bioINPs should be cited.

Line 77: This statement exhibits significant self-citation. The authors should consider incorporating several key papers on Arctic bioINPs, such as Bigg (1996), Bigg and Leck (2001), Creamean et al. (2022), Hartmann et al. (2020, 2021), Ickes et al. (2020a, b), Jayaweera and Flanagan (1982), Porter et al. (2022), etc. to provide a more comprehensive perspective. Some of these could also be used for the statement on lines 78-79.

Lines 89-90: The statement, "Aerosolization of INP by bubble bursting in freshwater bodies is more likely than in the ocean since more bubbles are produced by frequent small waves…" overlooks other factors like fetch and salinity that influence bubble concentration. Studies such as Cartmill and Yang (1993) have found higher bubble concentrations in saltwater, and Zinke et al. (2022) observed lower particle number fluxes in fresher water compared to saltier water. These factors should be considered for a more accurate interpretation.

Line 107: What is the classification of the underlying permafrost (e.g., thick, continuous, discontinuous)? Additionally, was the ground completely free of snow and ice? More details about the sites are useful for context.

Line 121: When referring to airflow, do the authors mean clean air or dry nitrogen? Additionally, are there any concerns regarding the evaporation of the droplets at a sheath flow rate of 15-20 lpm?

Lines 137-139: Why were the samples freeze-dried overnight? Could the use of a desiccator potentially stress the microbial cells in the samples, possibly affecting their viability? Additionally, does the mortaring process lead to any degradation of the INPs in the samples? Finally, what is the rationale behind sieving the samples? It is unclear why this preparation method was chosen over simply freezing, suspending, and testing the soil samples. Although the authors cite Conen and Yakutin (2018), their methodology differed slightly, as they used air drying rather than freeze-drying and did not employ a mortar. An explanation justifying the chosen steps in this study would be helpful.

Fig 3: This is a well-constructed summary figure; however, could the authors also include the other figures referenced on line 237 (Creamean et al., 2020; Schnell and Vali, 1976)? Incorporating these would provide a more comprehensive overview.

Line 242: Since the authors note that the Tobo et al. study focused on glacial outwash sediment, it would be helpful to specify that the Barry et al. study pertains to permafrost.

Lines 243-244: How do these TC values compare to others in the literature for similar soils?

Lines 245-246: More biomass and soil carbon content than what?

Lines 251-253: Other factors contributing to the large variations may stem from the sample preparation methods. Conen et al. used sieving but did not employ mortaring, while Tobo et al. utilized neither technique. While differences in microbial community composition or soil properties could influence the results, the impact of the varying sample preparation methods should not be overlooked.

Lines 254-255: While Santl-Temkiv et al. provides a valuable review on aerobiology, the authors should include other relevant papers as mentioned in previous comments.

Fig 3: The spectra from Conen et al. are somewhat difficult to distinguish. I recommend using a different color for clarity. Additionally, it would be beneficial to assign different colors and/or markers to the spectra from the 2011 and 2018 studies for better differentiation.

Lines 271-277: This text would be better placed in the methods section.

Figs 4 (and 7): Technically, the sample should not be labeled as "bulk" as indicated on the x-axis. The authors should refer to this as "≤ 63 μm" instead.

Line 287: This analysis focuses on INP size and inferred composition, rather than direct composition. Additionally, it would be helpful to mention whether other studies, such as Barry et al. (2023a, b), observe significant variations in the INPs present in soil. The Barry et al. studies investigated concentrations and the effects of heat and peroxide treatments on composition (2023a and b), along with size filtering (2023b only).

Line 304-205: The statement, "The gradual loss of INA during filtration at the different locations suggests a mixture of different-sized INPs, predominantly originating from fungi," needs clarification. Where is this information presented in the manuscript, or what other evidence supports this claim?

Lines 308-318: This is a nice summary, but would fit better in the conclusions section.

Line 316: Regarding the "upward fluxes," was the surface marshy or dry? Positive fluxes from the surface would depend on the surface aridity. This is an example of how describing the landscape of the sampling locations would be beneficial. Additionally, on line 390, wind erosion is mentioned; however, this also depends on surface aridity, which may not be realistic if the sampling locations were marshy.

Lines 337-344: The authors conclude that the abundances of known INP-producing species are very low for both 16S and ITS, with only sequences affiliated with *Acremonium* (at one location) and *Mortierella* (at most locations) present in their dataset. They suggest that the observed taxa might be INP producers that have not yet been recognized as such. However, could the INPs be derived from other organic materials, rather than exclusively from cellular or proteinaceous sources?

Lines 417-445: The authors should summarize and directly compare their findings to those of Barry et al. (2023a), as their INP results are derived from freshwater thermokarst lakes in the Arctic and are likely the most relevant for comparison with the Arctic stream water analyzed in this study. Barry et al. also included comparisons with locally sampled permafrost and active layer soils, while the other studies cited are focused on temperate regions.

Lines 425-427: Huang et al. (2021) discuss how local Arctic sources can be rich in INPs, yet the concentration of aerosol INPs remains low. Given this context, is this finding truly surprising? Additionally, on lines 527-528, the authors state that “In streams, INP concentrations defied conventional expectations, exhibiting elevated concentrations contrary to the typical decrease towards polar regions.” Is this assertion accurate?

Lines 434-445: If these are all possible explanations, why would they apply specifically to the stream samples and not to the soil? This suggests that the INP populations in the two environments are not the same.

Lines 487-489: Missing some key references here that looked at INPs in snowmelt, such as Brennan et al. (2020), Creamean et al. (2019), Stopelli et al. (2015, 2017). It would be useful to compare values to more than just Christner et al., (2008) and Santl-Temkiv et al. (2018).

Lines 536-537: The statement, “...future research should focus on deciphering the contributions from various sources such as soil, runoff, and marine emissions to fully elucidate their roles in cloud formation and climate processes,” should acknowledge the work of Barry et al. (2023a), who investigated a wide range of potential sources, including those mentioned, and linked their findings to INP data collected upwind and downwind of thermokarst lakes. They should receive appropriate credit for their contributions in this context.

Supplemental Figs 3 and 4: These figures seem central to the main takeaways, why are they not shown in the main text?

References:

Alpert, P. A., Kilthau, W. P., O’Brien, R. E., Moffet, R. C., Gilles, M. K., Wang, B., Laskin, A., Aller, J. Y., and Knopf, D. A.: Ice-nucleating agents in sea spray aerosol identified and quantified with a holistic multimodal freezing model, Science Advances, 8, eabq6842, https://doi.org/10.1126/sciadv.abq6842, 2022.

Barry, K. R., Hill, T. C. J., Nieto-Caballero, M., Douglas, T. A., Kreidenweis, S. M., DeMott, P. J., and Creamean, J. M.: Active thermokarst regions contain rich sources of ice-nucleating particles, Atmospheric Chemistry and Physics, 23, 15783–15793, https://doi.org/10.5194/acp-23-15783-2023, 2023a.

Barry, K. R., Hill, T. C. J., Moore, K. A., Douglas, T. A., Kreidenweis, S. M., DeMott, P. J., and Creamean, J. M.: Persistence and Potential Atmospheric Ramifications of Ice-Nucleating Particles Released from Thawing Permafrost, Environ. Sci. Technol., 57, 3505–3515, https://doi.org/10.1021/acs.est.2c06530, 2023b.

Bigg, E. K.: Ice forming nuclei in the high Arctic, 1996.

Bigg, E. K. and Leck, C.: Cloud-active particles over the central Arctic Ocean, J. Geophys. Res., 106, 32155–32166, https://doi.org/10.1029/1999JD901152, 2001.

Brennan, K. P., David, R. O., and Borduas-Dedekind, N.: Spatial and temporal variability in the ice-nucleating ability of alpine snowmelt and extension to frozen cloud fraction, Atmospheric Chemistry and Physics, 20, 163–180, https://doi.org/10.5194/acp-20-163-2020, 2020.

Cartmill, J. W. and Yang Su, M.: Bubble size distribution under saltwater and freshwater breaking waves, Dynamics of Atmospheres and Oceans, 20, 25–31, https://doi.org/10.1016/0377-0265(93)90046-A, 1993.

Creamean, J. M., Mignani, C., Bukowiecki, N., and Conen, F.: Using freezing spectra characteristics to identify ice-nucleating particle populations during the winter in the Alps, Atmospheric Chemistry and Physics, 19, 8123–8140, https://doi.org/10.5194/acp-19-8123-2019, 2019.

Creamean, J. M., Barry, K., Hill, T. C. J., Hume, C., DeMott, P. J., Shupe, M. D., Dahlke, S., Willmes, S., Schmale, J., Beck, I., Hoppe, C. J. M., Fong, A., Chamberlain, E., Bowman, J., Scharien, R., and Persson, O.: Annual cycle observations of aerosols capable of ice formation in central Arctic clouds, Nat Commun, 13, 3537, https://doi.org/10.1038/s41467-022-31182-x, 2022.

Harrison, A. D., Lever, K., Sanchez-Marroquin, A., Holden, M. A., Whale, T. F., Tarn, M. D., McQuaid, J. B., and Murray, B. J.: The ice-nucleating ability of quartz immersed in water and its atmospheric importance compared to K-feldspar, Atmospheric Chemistry and Physics, 19, 11343–11361, https://doi.org/10.5194/acp-19-11343-2019, 2019.

Hartmann, M., Adachi, K., Eppers, O., Haas, C., Herber, A., Holzinger, R., Hünerbein, A., Jäkel, E., Jentzsch, C., Pinxteren, M., Wex, H., Willmes, S., and Stratmann, F.: Wintertime Airborne Measurements of Ice Nucleating Particles in the High Arctic: A Hint to a Marine, Biogenic Source for Ice Nucleating Particles, Geophys. Res. Lett., 47, https://doi.org/10.1029/2020GL087770, 2020.

Hartmann, M., Gong, X., Kecorius, S., Van Pinxteren, M., Vogl, T., Welti, A., Wex, H., Zeppenfeld, S., Herrmann, H., Wiedensohler, A., and Stratmann, F.: Terrestrial or marine – indications towards the origin of ice-nucleating particles during melt season in the European Arctic up to 83.7° N, Atmos. Chem. Phys., 21, 11613–11636, https://doi.org/10.5194/acp-21-11613-2021, 2021.

Ickes, L., Porter, G. C. E., Wagner, R., Adams, M. P., Bierbauer, S., Bertram, A. K., Bilde, M., Christiansen, S., Ekman, A. M. L., Gorokhova, E., Höhler, K., Kiselev, A. A., Leck, C., Möhler, O., Murray, B. J., Schiebel, T., Ullrich, R., and Salter, M.: Arctic marine ice nucleating aerosol: a laboratory study of microlayer samples and algal cultures, Aerosols/Laboratory Studies/Troposphere/Physics (physical properties and processes), https://doi.org/10.5194/acp-2020-246, 2020a.

Ickes, L., Porter, G. C. E., Wagner, R., Adams, M. P., Bierbauer, S., Bertram, A. K., Bilde, M., Christiansen, S., Ekman, A. M. L., Gorokhova, E., Höhler, K., Kiselev, A. A., Leck, C., Möhler, O., Murray, B. J., Schiebel, T., Ullrich, R., and Salter, M. E.: The ice-nucleating activity of

Arctic sea surface microlayer samples and marine algal cultures, Atmos. Chem. Phys., 20, 11089–11117, https://doi.org/10.5194/acp-20-11089-2020, 2020b.

Jayaweera, K. and Flanagan, P.: Investigations on biogenic ice nuclei in the Arctic atmosphere, Geophysical Research Letters, 9, 94–97, https://doi.org/10.1029/GL009i001p00094, 1982.

Kanji, Z. A., Ladino, L. A., Wex, H., Boose, Y., Burkert-Kohn, M., Cziczo, D. J., and Krämer, M.: Overview of Ice Nucleating Particles, https://doi.org/10.1175/AMSMONOGRAPHS-D-16-0006.1, 2017.

Murray, B. J., O'Sullivan, D., Atkinson, J. D., and Webb, M. E.: Ice nucleation by particles immersed in supercooled cloud droplets, Chem. Soc. Rev., 41, 6519–6554, https://doi.org/10.1039/C2CS35200A, 2012.

Porter, G. C. E., Adams, M. P., Brooks, I. M., Ickes, L., Karlsson, L., Leck, C., Salter, M. E., Schmale, J., Siegel, K., Sikora, S. N. F., Tarn, M. D., Vüllers, J., Wernli, H., Zieger, P., Zinke, J., and Murray, B. J.: Highly Active Ice-Nucleating Particles at the Summer North Pole, Journal of Geophysical Research: Atmospheres, 127, e2021JD036059, https://doi.org/10.1029/2021JD036059, 2022.

Schnell, R. C. and Vali, G.: Biogenic Ice Nuclei: Part I. Terrestrial and Marine Sources, J. Atmos. Sci., 33, 1554–1564, 1976.

Stopelli, E., Conen, F., Morris, C. E., Herrmann, E., Bukowiecki, N., and Alewell, C.: Ice nucleation active particles are efficiently removed by precipitating clouds, Sci Rep, 5, 16433, https://doi.org/10.1038/srep16433, 2015.

Stopelli, E., Conen, F., Guilbaud, C., Zopfi, J., Alewell, C., and Morris, C. E.: Ice nucleators, bacterial cells and Pseudomonas syringae; in precipitation at Jungfraujoch, Biogeosciences, 14, 1189–1196, https://doi.org/10.5194/bg-14-1189-2017, 2017.

Tobo, Y.: An improved approach for measuring immersion freezing in large droplets over a wide temperature range, Sci Rep, 6, 32930, https://doi.org/10.1038/srep32930, 2016.

Vali, G., Christensen, M., Fresh, R. W., Galyan, E. L., Maki, L. R., and Schnell, R. C.: Biogenic Ice Nuclei. Part II: Bacterial Sources, Journal of the Atmospheric Sciences, 33, 1565–1570, https://doi.org/10.1175/1520-0469(1976)033<1565:BINPIB>2.0.CO;2, 1976.

Zinke, J., Nilsson, E. D., Zieger, P., and Salter, M. E.: The Effect of Seawater Salinity and Seawater Temperature on Sea Salt Aerosol Production, JGR Atmospheres, 127, https://doi.org/10.1029/2021JD036005, 2022.