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.

Thank you for highlighting this important distinction. We acknowledge that the samples we collected were from the active layer, not from permafrost itself, and we regret any confusion caused by this mischaracterization. We have carefully revised the manuscript to refer to these samples as "active layer soil" throughout the text to ensure accurate representation. We appreciate your observation

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.

Thank you for your comment. We understand the importance of blank corrections, especially given that Milli-Q water was used during sample preparation. Milli-Q water was used as a negative control, and its freezing behavior has been added to the fraction frozen plots (Supplementary Figures 1 and 7). These controls consistently show onset freezing temperatures below -15°C, which do not overlap with the temperature range of primary interest (0°C to -15°C).
Given this clear separation, background corrections were deemed unnecessary for this study. However, we acknowledge that applying such corrections may be crucial in cases where there is a significant overlap between control and sample data.

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.

Thank you for your comment. We appreciate your suggestion to include error or uncertainty bars. The INP spectra presented in Figures 2, 3, 4, 6, and 7 does not involve binning the data into temperature intervals, as we rely on cumulative freezing spectra derived from individual droplets or wells. This approach provides precise freezing temperatures for each droplet, without aggregating the data into bins. Consequently, there is no statistical variation across temperature intervals to calculate error bars.
Furthermore, each sample is analyzed using 80 droplets, instead of analyzing smaller numbers of droplets in replicates, as has been recommended by Polen et al (2018) (Polen et al., 2018). This also means a high coverage of the freezing temperature distribution within the sample. This high number of droplets allows us to capture the variability of freezing events across the sample population with high confidence, minimizing the need for additional statistical representation such as error bars.

Regarding the size distribution of soil particles included in the Supplementary Information, these data are based on four independent measurements, each obtained from different sampling sites. These samples are used to investigate potential differences between sites and not merely to provide an average for the locations. Hence, we believe that it would be appropriate to keep the measurements separate.
We hope this explanation addresses your concerns, but we are happy to provide further clarification if needed.

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.

We agree with this concern regarding the comparability of onset freezing temperatures across studies using different droplet volumes or different input amount of soils in freezing assays. We have revised the manuscript to explicitly discuss the potential implications of methodological differences. Specifically, we added a section that acknowledges that larger droplet volumes are more sensitive to detecting rarer INPs. Many of the studies we compare our results to, used larger volumes and therefore their sensitivity is higher than in our assays, supporting the statement that INPs observed in our study indded show a higher onset temperature than other studies. In these revisions, we now emphasize both the methodological context and the unique INP activity observed in the Arctic soils we studied. By addressing these points, we believe the discussion now provides a balanced interpretation of the results while maintaining scientific rigor.

"Our results showed higher onset temperatures (between -1.5 °C and -4.7 °C) compared to previous studies of Arctic soils (Fig. 3). Methodological differences, such as droplet volume used in freezing assays, must be considered when interpreting this trend. Studies using smaller volumes (e.g. 5 µL in Tobo et al., 2019), have a lower sensitivy and cannot be directly compared to our study. However, several studies used larger droplet volumes

(e.g., 50 µL in Conen et al., 2018; Barry et al., 2023b, and 100 µL in Conen et al., 2012), which have a higher sensitivity than the micro-Pinguin asssay and a comparable potential to detect rare highly active INPs. Therefore, the higher freezeing onset that we observe does not seem to only be linked to methodological differences but reflects differences in the INP popoluations in these environments. INPs active at such high temperatures are generally proteinaceous (Santl-Temkiv et al., 2022) and are often associated with microbial sources, including bacteria and fungi (Barry et al., 2023b; Tobo et al., 2019; Conen et al., 2011). The presence of higher onset temperatures in this study may indicate differences in either the identity or in the activity of their microbial producers across Arctic terrestrial environments."

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.

Thank you for your insightful question. Studies of defined carbohydrates, such as cellulose and lignin have shown that these nucleate ice at temperatures below -13°C (Bogler and Borduas-Dedekind, 2020; Hiranuma et al., 2015), while fungal and bacterial proteins nucleate ice at temperatures between -1 and 13°C (Schwidetzky et al., 2023; Hartmann et al., 2022). The freezing profiles we recorded are thus closer to known proteinaceous INPs than with those of carbohydrates. Alpert et al., (2022), who studied algal exudates, consisting of a mixture of proteinaceous and polysaccharidic compounds and found that their activity is typically lower than that of the terrestrial proteinaceous INPs active above -13°C.

While we cannot entirely rule out the possibility that some INPs might have been detached from the cells during the mortaring process, it is important to note that mortaring was conducted as a standard soil preparation step prior to sieving. This process was essential for breaking down soil aggregates that had formed during freeze-drying, ensuring effective sieving and sample consistency. Given that the mortaring was not performed with a high intensity to specifically degrade cells or INPs, we do not expect significant degradation or loss of INPs within the samples.

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.

We appreciate the reviewer’s feedback and would like to address the concern regarding our statement on lines 515-516, where we refer to our study as the "first time" describing parallel measurements of INP concentrations in Arctic soil and stream systems.
While we acknowledge that Barry et al. (2023a) surveyed potential INP sources in permafrost and adjacent water bodies in active thermokarst regions, we believe the distinction between our work and theirs warrants clarification. Barry et al. focused on the broader permafrost zone, specifically analyzing water from thermocast lakes, lagoons, and the ocean, while our study specifically investigates streams and adjacent soils. Furthermore, our study uniquely incorporates microbial community analysis, providing direct connections between microbial taxa and their respective INP contributions - an aspect that was not explored in Barry et al.'s work. Barry et al. did not perform microbial sequencing, and thus their findings did not delve into the potential biological sources of INPs at the level we present in our manuscript.
We have further added this to the manuscript:

“This study presents a detailed analysis of soil and freshwater INPs in High Arctic Greenland, offering critical insights into their sources and diversity. The findings for the first time describe parallel measurements of INP concentrations in Arctic soil and stream systems and incorporates microbial community analysis, providing direct connections between microbial taxa and their respective INP contributions which opens the necessity for more studies investigating these environments.”

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.

We appreciate the reviewer’s concern and agree that, given the current scope of our study, the conclusions may be overstated, particularly as we did not measure or directly link aerosolized INPs to the soil and stream water samples. In response, we have revised the manuscript to tone down the language and focus on the characterization of potential local Arctic sources of INPs. We now emphasize the observed high INP concentrations in Arctic streams as a significant finding in understanding the potential role of these

freshwater systems as contributors to atmospheric INPs, without overreaching into claims about their broader impact on cloud formation and climate.

"Our findings indicate that streams with high INP concentrations could contribute to atmospheric INP levels, similar to observations in other freshwater systems (Larsen et al., 2017; Knackstedt et al., 2018). The presence of high INP concentrations in Arctic streams suggest their potential role as local sources of atmospheric INPs particularly in the context of Arctic amplification and increased freshwater discharge (Mankoff et al., 2020). However, further studies are needed to explore the linkage between these sources and aerosolized INPs, as well as their broader implications for cloud formation and regional climate. "

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.

We appreciate the reviewer's insightful comment. We fully agree that performing 16S, ITS, 18S, or metagenomic analyses on stream water samples would have provided valuable insights into the microbial community in the streams. Ideally, we would have included these analyses in this study. However, due to logistical constraints, we were unable to collect stream samples for DNA analysis.
That said, we believe meaningful conclusions can still be drawn based on the data we have. Our results, which show the presence of INPs in both soils and streams, along with their size distribution and activity, suggest a transfer of bioINPs from the soil to the stream environment. Although we were not able to directly identify the microbial contributors in the streams, the positive correlation (albeit not significant) between INP concentrations in soil and streams supports the idea that soils are a potential source for stream INPs. Additionally, the size distribution patterns observed in both environments suggest that microorganisms which produced these INPs were related and that similar processes influenced the INPs size distribution in both soils and streams, further supporting the possibility of bioINP transfer between these two environments.
Moreover, it is important to note that the INPs transferred to the streams are predominantly in the soluble fraction, as suggested by our filtration experiments. This could indicate a decoupling between the microbial producers and the actual INPs present in the stream environment. The processes facilitating this decoupling, such as cell lysis or the release of extracellular INPs, may further obscure the direct link between soil microbial communities and INPs in the streams.
While identifying the exact microbial sources in the streams would have been ideal, we believe our conclusions remain valid and meaningful based on the available data. We acknowledge this as a limitation of the study

Specific comments:

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

Thank you for this comment, which has been implemented in the new version of the manuscript.

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.

Thank you for this comment, which has been implemented in the new version of the manuscript.

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

Thank you for this comment, which has been implemented in the new version of the manuscript.

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)).

We have revised the wording to addresses your concern.

"Ice nucleation below -13°C can be initiated by abiotic INPs, such as mineral particles, soot, or by incidental INA from biomolecules, such as carbohydrates (Kinney et al., 2024). In contrast, proteinaceous INPs are the predominant type of INP that are active above -13°C, shown by both laboratory studies and in-situ measurements involving inactivation by heating (Murray et al., 2012; Cornwell et al., 2023; Kanji et al., 2017; Daily et al., 2022)."

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

Thank you for this comment, which has been implemented in the new version of the manuscript.

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.

Thank you for this comment, which has been implemented in the new version of the manuscript.

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.

We appreciate the reviewer’s insightful comment highlighting the complexity of factors influencing bubble concentrations and aerosolization processes in freshwater and saltwater environments. Upon further consideration, we have decided to remove this comparison from the manuscript. Since no quantitative measurements were conducted in this study to support a detailed comparison, we concluded that this discussion is not essential to the manuscript’s focus.

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.

Thank you for this comment. We have now added a brief section in the methods about the sampling site, along with relevant references. Unfortunately, we do not know whether the permafrost at the collection sites was thick, continuous, or discontinuous.

“The study streams are located in the Northeast Greenland National Park, near the Zackenberg Research Station (74°28’N, 20°34’W). Streamflow in the region is predominantly derived from melting snow and glaciers, with additional contributions varying by stream. For example, Kærelv A, Kærelv C, Grænseelv, and West 1 receive water from small, seasonal snow patches, while Aucella, and Jurassic1 is partly fed by larger ice aprons adhered to mountainsides (Docherty et al., 2019; Hasholt and Hagedorn, 2000). This region has a polar tundra climate and is underlain by continuous permafrost with an active layer thickness of 0.4 - 0.8 m (Christiansen et al., 2008; Hollesen et al., 2011). Geologically, the area is divided into crystalline complexes to the west and sedimentary successions to the east, with Quaternary sediments covering the valley floor and slopes. For a broader overview of the region’s climate, geology, and vegetation, see Riis et al. (2023).”

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?

We thank the reviewer for this comment. We have now specified that the air supplied was HEPA filtered clean air, and that the tower first was flushed for 5 minutes at a constant flow rate of 20 LPM, while turning down the flow during the experiment.

"The tower was first flushed with HEPA filtered clean for 5 minutes at a constant flow of 20 L/min before. Then, the samples were cooled at 1K min^{-1} down to -30°C while supplying a constant HEPA filtered clean airflow between 5-10 L/min to keep the relative humidity low, avoiding condensation."

Evaporation of the droplets due to airflow was tested and was insignificant.

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.

We appreciate the reviewer's concerns. Below, we provide clarification and rationale for each of the points raised.

1. **Freeze-Drying vs. Air Drying**:
   The decision to freeze-dry the samples was based on our aim to minimize the time required for soil drying, as opposed to air-drying used by Conen and Yakutin (2018). Prolonged air-drying might support microbial activity during its initial stages when water availability is still high, potentially leading to the production or degradation of INPs, which could alter the ice nucleation properties of the samples. Freeze-drying ensured rapid drying, thereby preserving the original INP content and composition.
2. **Viability of Microbial Cells**:
   The concern about microbial cell viability is noted; however, this is not a critical factor for our analyses. The primary goal of the preparation was to retain intact cells, soil particles, and INPs bound to these components. Viability is not required neither for the INP analyses nor for the community composition analysis performed in this study.
3. **Mortaring**:
   Mortaring was conducted as a standard soil preparation step prior to sieving. This process ensured the breakdown of soil aggregates formed during freeze-drying, enabling effective sieving. We do not expect mortaring to cause significant degradation or loss of INPs within the samples.
4. **Sieving**:
   Sieving was employed to isolate particles smaller than 63 µm, which are more representative of those aerosolized directly from the soil. Analyzing bulk soil samples without sieving would not have accurately reflected the aerosolizable fraction of the soil.

We have incorporated the following justification into the manuscript:

"We prepared soil samples for ice nucleation analysis as previously described, with slight modifications (Conen and Yakutin, 2018). The soil samples were placed in a small petri dish and freeze dried overnight (Edwards Micro Modulyo Freeze Dryer). Freeze-drying was chosen instead of air-drying to minimize the potential for microbial activity during the drying process and preserving the original composition of INPs, as prolonged air-drying could induce microbial activity, potentially altering INP concentrations due to production or degradation. The freeze-dried samples were kept in a desiccator to prevent rehydration and subsequently comminuted in a mortar by hand. Mortaring was performed to break down aggregates formed during freeze-drying and ensured effective sieving. Samples were dry sieved with a 125 µm and 63 µm sieve for two minutes using a vibratory sieve shaker (Analysette 3 PRO, Fritsch). The <63 µm fraction was collected for analysis, as this size range represents particles most likely to aerosolize (Fröhlich-Nowoisky et al., 2016). Hundred mg of dry <63 µm soil particles was weighed into an Eppendorf tube. For many samples, there was less than 0.1 g soil after sieving. Instead, all the sieved soil was added to the Eppendorf tube and the weight was noted. 1 mL filtered Milli-Q (0.22 µm PES) was added to the Eppendorf tube, then vortexed for two minutes and afterwards allowed to settle for 10 minutes. 0.5 mL was withdrawn from the top of the suspension and added to a falcon tube with 9.5 mL of filtered Milli-Q (0.22 µm) creating a 1:20 dilution. "

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.

We thank the reviewer for acknowledging the value of Fig. 3 and for suggesting the inclusion of additional figures from Creamean et al. (2020) and Schnell and Vali (1976). Unfortunately, the data from Creamean et al. (2020) are not publicaly accessible via the provided data availability statement https://stack.iop.org/ERL/15/084022/mmedia. Thus, we could not add them to the summary figure.

Regarding Schnell and Vali (1976), we note that their study focused primarily on leaf litter as a source of ice nucleating particles (INPs) rather than Arctic soil. While their findings are insightful in broader contexts, they are not directly relevant to our study of Arctic soil INPs. Therefore, to maintain the figure's focus on Arctic-specific sources, we have opted not to include this reference.

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.

Thank you for this comment, which has been implemented in the new version of the manuscript.

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

soils?

We thank the reviewer for this question. The TC values in our samples were relatively low, with 9 out of 11 samples containing <5% w/w TC. We have compared these values to the literature and observed that they are lower than those reported by Conen and Yakutin (2018) for soils from Central Yakutia. However, our TC values are comparable to the glacial outwash sediments studied by Tobo et al. (2019). This comparison has been included in the revised manuscript to provide the necessary context.

"A possible explanation for the lower concentration could be the rather low carbon content of the soils measured in this study with 9 out of 11 samples <5 % w/w TC, which is less than what Conen and Yakutin (2018) found in soils from Central Yakutia, but similar to the glacial outwash sediment that Tobo et al. (2019) investigated (Conen and Yakutin, 2018; Tobo et al., 2019)."

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

Thank you for this comment, this has now been clarified in the manuscript

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.

We refer to the reviewer's earlier comment (Lines 137-1) and our response.

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.

Thank you for this comment we have now added Kanji et al. (2017) and Huang et al. (2021) to provide a broader overview.

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.

Thank you for this comment. The spectra have now been updated with slightly different grey colors for Conen et al 2011 and 2018, respectively, together with differing symbols.

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

Thank you for this comment. We have now specified the statistical approach in the methods section and shortened the text lines 271-277

“To further characterize INPs within the Arctic soil, we used filtration analysis as different microorganisms produce INpro of different molecular sizes, which can either be firmly bound to the cells or easily removed resulting in soluble proteins (O'sullivan et al., 2015; Santl-Temkiv et al., 2022). A similar approach has previously been used to study the origin of INP in environmental samples (Conen and Yakutin, 2018; Fröhlich-Nowoisky et al., 2015). A Kruskal-Wallis test, indicated significant differences between the filtration treatments (p-value = 0.0001) (Fig. 4). A Wilcoxon rank sum test showed a significant difference between the bulk sample and the 300-100 kDa fraction (p = 0.0046). Subsequently, we analyzed the samples from individual locations to identify specific patterns. “

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.

Thank you for your comment. We have updated Figure 4 and supplementary Figure 1 to reflect that the "bulk" category refers to particles < 63 µm. However, we have retained Figure 7 as originally presented, as the water samples were not pretreated in this case.

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).

Thank you for this comment. We have now added the word “inferred in front of the word “composition”. Additionally, we discuss the results from Barry et al. 2023 a & b:

Our findings somewhat align with Barry et al. (2023b), who demonstrated that the majority of INPs in soil were larger than 0.2 µm and primarily of biological origin. Furthermore, their heat treatment experiments revealed that INPs in permafrost soil are predominantly heat-labile, further supporting their biological nature. Interestingly, Barry et al. (2023b) reported a limited presence of soluble INPs smaller than 0.2 µm in permafrost soil, suggesting a scarcity of such low-molecular-weight biological INPs in their study system. This contrasts with the observed presence of INPs spanning a large range of sizes in our samples, including <100 kDa and aggregates >1000 kDa (Fig. 4). Such discrepancies might reflect differences in the environmental conditions, microbial communities, or soil composition between their study sites and ours. Additionally, Barry et al. (2023a) highlighted that INP concentrations in permafrost soil are influenced by particle size and composition, observing that larger particles (>10 µm) were significant contributors to INA in younger permafrost samples. While their findings pertain to larger particle fractions, our study emphasizes the role of smaller clay-bound particles (<5 µm) in transporting INPs into the atmosphere. This difference may reflect distinct mechanisms of INP atmospheric rentention time: larger particles contribute primarily to undisturbed permafrost soils and while quickly settle after aerosolization, while smaller,

clay-bound INPs being more prone to a longer atmospheric residence time after aerosolization.

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?

We thank the reviewer for this comment and agree that further clarification is necessary. In the revised manuscript, we have explicitly referenced Figure 4, which shows the data supporting this conclusion. Furthermore, we expanded the discussion to better explain the basis of this interpretation:

The gradual loss of INA during filtration at the different locations suggests a mixture of different-sized INPs, predominantly originating from fungi (Fig. 4). This interpretation is supported by the fact that fungal INPs are known to span a wide range, including small <100 kDa (e.g., 5 kDa), and medium-sized molecules (100-300 kDa and 300-1000 kDa), and can bind to clay particles, resulting in INPs >1000 kDa and >0.2 µm (Kunert et al., 2019; Schwidetzky et al., 2023; O'sullivan et al., 2015; Conen and Yakutin, 2018). The size distribution, combined with the observed solubility and INA, aligns with the characteristics of fungal INPs, further supporting their major contribution to the INP pool in these soils.

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

We appreciate the reviewers suggestion to move the summary (Lines 308–318) to the conclusions section. However, we believe this section is essential within the Results and Discussion because it integrates our findings with their broader implications, linking them directly to existing literature. The interpretation of fungal INPs as contributors to the observed ice-nucleating activity in Arctic soils builds on the presented evidence, such as size distribution, solubility, and INA characteristics. This placement allows us to connect our observations - such as the role of clay-bound particles and fungal INPs - to atmospheric processes and contextualize these results against previous studies (e.g., O'Sullivan et al., 2016; Kanji et al., 2017; Tobo et al., 2019). Moving this discussion to the conclusions risks detaching these connections from the results, which could dilute the integration of evidence and interpretation.

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.

Thank you for pointing out the relevance of surface conditions, such as aridity, to upward fluxes. While surface aridity is indeed a critical factor in aerosolization processes, we argue that the period of sampling in the Arctic is equally important when discussing

upward flux potential. In the Arctic, environmental conditions, including soil moisture and aridity, can vary substantially over the course of the melt season. Even if the surface appeared marshy on the specific day of sampling, it does not necessarily reflect the overall potential for aerosolization throughout the different seasons. As the seasons progresses, the soil surface in the Arctic often transitions from wet to increasingly dry due to diminishing snow and ice, coupled with higher evaporation rates and minimal precipitation. These drying phases are key drivers of upward fluxes, as drier surfaces are more prone to aerosolization under wind or disturbance.

As previously mentioned we have now included a section describing the sampling area, with reference to Riis et al. (2023), to provide further context.

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?

Thank you for the thoughtful observation. While we suggest that the observed taxa might include INP producers that are not yet recognized, we also acknowledge that INPs in the environment are not exclusively derived from cellular or proteinaceous sources. Organic materials such as polysaccharides, humic substances, and other macromolecules are known to exhibit ice-nucleating activity under certain conditions.
As discussed in a previous response, carbohydrates like cellulose and lignine have been shown to nucleate ice (Bogler and Borduas-Dedekind, 2020; Hiranuma et al., 2015); however, their nucleation activity generally occurs at significantly lower temperatures than what we observed in our study. The onset freezing temperatures and the freezeing profiles in our samples are indicative of proteinaceous INPs.

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.

Thank you this suggestion. In the revised manuscript, we have expanded the discussion in Section 3.4 to provide a direct comparison with Barry et al., as their work on Arctic thermokarst lakes is indeed the most relevant reference for our Arctic stream water data. This expanded discussion should provide a more robust comparison of our findings to Barry et al. (2023a) and other relevant studies, addressing the reviewer's concern:

"In addition to characterizing soil INPs and their potential sources, we investigated the linkages between soil-freshwater INPs. The freezing onset was > -10°C for all twelve water sampling locations (Supplementary Fig. 7). The highest onset temperature was found in

Aucella (-5.9°C) and lowest in West 4 (-9.1°C). The high freezing temperatures indicate that the INPs are of biological origin (Kanji et al., 2017). The ice nucleation site density per volume of freshwater (N_V) as a function of temperature is shown in Fig. 6. The INP_{-10} concentration measured in our study (average: 1005 mL^{-1}; range 24-4,880 mL^{-1}) (Table 1) are significantly lower than those reported by Barry et al. (2023a) for Arctic thermokarst lakes (average: 34300 mL^{-1}; range 1360-242,000 mL^{-1}). One potential explanation for this difference is the significantly higher soil INP concentrations reported in Barry et al.'s study compared to our measured soil INP concentrations. If soil is a major source of INPs to freshwater systems, as suggested by both studies, then lower soil INP concentrations in our sampling locations may directly contribute to the lower INP concentrations observed in Arctic streams relative to thermokarst lakes. "

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?

Thank you for your valuable feedback. We agree with your assessment, and we have revised the manuscript to clarify these points. In response to your first comment, we have revised the discussion section (Lines 425-427) as follows:

"The high concentrations of INP_{-10} in Arctic streams align with findings that several Arctic environments are rich in INPs, emphasizing their potential contribution as a regional source. However, despite these abundant local sources, aerosol INP concentrations in the Arctic atmosphere remain relatively low, possibly due to transport and deposition dynamics (Huang et al., 2021)."

Regarding your second comment on the assertion about INP concentrations in streams, we have updated the conclusion section to state:

"In streams, INP concentrations were similar to those observed in temperate region rivers, such as the Mississippi and Gwaun Rivers. However, these concentrations were lower than those reported for other Arctic freshwater systems like thermokarst lakes."

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.

Thank you for this question. These explanations refer to the insoluble fraction of the INP population in the streams and hence not the soluble part. When looking only at the soluble part of INPs, which is likely the ones being transported from the soil to the streams it seems that the filtration experiment is in line with each other for both soil and stream INP as stated in lines: 445-449:

Statistical analysis using the Kruskal-Wallis test showed a significant difference among the treatments (p-value = $1.721 \cdot 10^{-8}$). Post hoc Wilcoxon rank sum tests revealed a

significant change from bulk to the 300-100 kDa category (p = 0.0021). This was also observed in soil samples (Fig. 4), indicating that similar INPs are present in soil and streams which further imply the possible transfer of INPs from soil into the streams.

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).

Thank you for this comment, we have updated the text accordingly to show more examples of INP concentrations in snowmelt

"Precipitation is unlikely to be a significant source of INPs in the studied streams, as INP concentrations in snow are typically much lower than those measured in the streams. Previous studies report INP_{-10} concentrations in snow ranging from as low as $1.2 \cdot 10^{-2}$ INP_{-10} mL^{-1}. to approximately $8 \cdot 10^{1}$ INP_{-10} mL^{-1}, which are significantly lower than the values observed in stream water (Christner et al., 2008; Santl-Temkiv et al., 2019; Creamean et al., 2019; Brennan et al., 2020). "

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.

Thank you for the comment, we have now implemented the acknowledgement in the conclusion:

"While the direct quantification of aerosolization was beyond the scope of this study, future research should focus on deciphering the contributions from various sources, such as active layer soil, runoff, and marine emissions, combining the approaches used in this study with those employed in studies like Barry et al. (2023a, 2023b), to fully elucidate their roles in cloud formation and climate processes."

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

We appreciate the reviewer's suggestion regarding the inclusion of Supplemental Figures 3 and 4, which display the phylum-level bacterial and fungal relative abundances. We agree that these figures provide valuable context for understanding the microbial community composition. However, we believe that they serve primarily as an overview and do not directly contribute to the central conclusions of our study. The main focus of our manuscript lies in linking specific microbial genera to INP activity, which is more effectively illustrated by Figure 5, showing the significant correlations between microbial taxa and INP concentrations.

Including supplementary Figures 3 and 4 in the main text could distract from the key findings and potentially overwhelm the reader with less central information. Therefore, we have chosen to retain these figures in the supplement, where they provide additional context without detracting from the main narrative. We hope this approach maintains the clarity and focus of our message.

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