

Response to EGUsphere-2026-2699 reviews for RC2

We thank Reviewer #2 for their effort and feedback on our manuscript EGUsphere-2026-2699. In response to the suggestions and questions, please find our answers and corrections listed below: **Reviewer #2 comments are extracted in bold from original review supplement**; our responses are given directly below in normal font; *the original text in previous manuscript is repeated in red italic* and *revised text is typed in blue italic*. We also list our revisions after proof-reading but not commented by reviewers at the end of our response document.

General comments:

The manuscript by Gao and co-authors presents an interesting dataset and state that bioaerosols contribute significantly to immersion-mode INPs in central Europe. The study is well written and the usage of different methods to investigate INP types and sources will be interesting to the reader of ACP. However, the conclusion that bioaerosols outcompete dust is not unambiguously supported by the methods, which rely on indirect attribution (heat sensitivity and correlations). Key claims should therefore be moderated to better reflect the level of evidence.

Major comments

- 1. Heat treatments have been shown to alter dust samples, while some biological particles were not affected by wet heat treatment. Thus, heat treatment alone is not necessarily an indication that the ice-active material is biogenic (Daily et al., 2022). Minerals (e.g., quartz) can lose their ice-nucleation activity during wet heating, which can mimic biological signatures.**

R: Thank you for this insightful comment. We appreciate the opportunity to clarify the interpretation of our heat-treatment results regarding biological vs. mineral ice-nucleating particles (INPs).

- For heat treatment effects on biological particles

While Daily et al. (2022) observed that heating in boiling water (~96°C) only slightly deactivated birch pollen (*Betula pendula*) washing water, more recent work by Wieland et al. (2025) demonstrated that heat treatments applied to birch pollen washing water at temperatures exceeding 40°C, 78°C, and 98°C disrupt the aggregation of ice-nucleating macromolecules (INMs). This leads to a stark drop in ice nucleation efficiency, particularly for temperatures warmer than -15°C. Specifically, while the 40°C treatment preserved the macromolecular structure, higher temperatures (78°C and 98°C) physically disrupted and broke down large, highly efficient INM aggregates into smaller subunits. This loss of structural aggregation directly caused a significant drop in their ability to catalyze freezing at warmer subzero temperatures, effectively eliminating activity warmer than -15°C. Therefore, heat sensitivity remains a strong indicator of biological INPs in this warm temperature regime.

- For heat treatment effects on mineral particles

Regarding the potential for wet heating to deactivate minerals (such as quartz or plagioclase) and mimic biological signatures, we would like to highlight two key differences between the experimental conditions in Daily et al. (2022) and our study: (1) Temperature differences: Daily et al. (2022) utilized boiling water (~96°C), which is higher than the 90°C threshold tested in our study. This temperature difference may lead to varying degrees of mineral surface alteration. (2) Sample mass concentration: Daily et al. (2022) tested high quartz concentrations (1 wt% in liquid solutions), which are substantially higher than the typical mineral mass loadings found in our ambient atmospheric samples. This high concentration explains why they observed an onset freezing temperature for quartz as warm as -6°C, whereas atmospheric minerals typically exhibit freezing activity at temperatures colder than -15°C (Murray et al., 2012). Moreover, we presented evidence from comparisons between dust and non-dust groups. Our results in Figure 7 showed that heated samples during Saharan dust events preserve more INPs in comparison to the non-dust group. This indicates that our heating treatment had a negligible deactivation effect on mineral dust ice nucleation.

We believe heat sensitivity remains a valid test to identify biological INPs at temperatures warmer than -15°C, whereas it may deactivate a part of atmospheric mineral particles like quartz or plagioclase at lower temperatures. Considering that quartz or plagioclase could be relevant components in local soil dust from Payerne or in transported Saharan dust within our samples, we have updated the Methodology section to include recent literature on the heating effects of both biological and mineral particles to address this uncertainty. Furthermore, we have revised the Discussion section to address the potential contributions and heat sensitivities of these mineral phases, adding necessary nuance to our interpretation of the biological signatures. Also, we note that the potential heating effect on dust does not alter our

conclusions regarding the primary contribution of biological particles to the observed INPs. Our data demonstrates that even during intensive Saharan dust events, INP concentrations did not increase, further confirming that mineral dust was not the dominant driver of ice nucleation in this study. We list our revisions as below:

(a) We added the following sentences in the Methodology section:

“While heat treatment is a widely used diagnostic tool for biological INPs, Daily et al. (2022) observed that boiling (~96°C) only slightly deactivated birch pollen (Betula pendula) washing water, while suppressing the median freezing temperature of highly concentrated quartz by 4°C. However, more recent work by Wieland et al. (2025) demonstrated that heat treatments exceeding 40°C, 78°C, and 98°C disrupt the aggregation of ice-nucleating macromolecules, significantly suppressing pollen ice nucleation at $T > -15^{\circ}\text{C}$. Consequently, heat sensitivity remains a valid indicator for identifying biological INPs for $T > -15^{\circ}\text{C}$, whereas it may affect minerals like quartz or plagioclase forming ice at lower temperatures.”

(b) We revised the following sentences in the Discussion section as follows:

“This may indicate important contributions of PBAPs to INPs (Murray et al., 2012; Kanji et al., 2017) and heat-sensitive dust like quartz or plagioclase (Daily et al., 2022).”

The original sentence was as below in the original manuscript (Line 301-302):

“This indicates important contributions from PBAPs to INPs (Murray et al., 2012; Kanji et al., 2017).”

(c) We revised the following sentences in the Discussion section:

“The reduction may result from deactivation of soil dust containing heat-labile biological material (O'sullivan et al., 2013) or dust samples containing quartz or plagioclase (Daily et al., 2022).”

The original sentence was as below in the original manuscript (Line 310-311):

“The reduction may result from deactivation of soil dust containing heat-labile biological material (O'sullivan et al., 2013).”

(d) We revised the following sentences in the Discussion section:

*“For $T < -12^{\circ}\text{C}$, the dN_{INP} fractions of T90 samples are consistently more than 85% (**Error! Reference source not found.c**), suggesting the contribution of heat-sensitive INPs of biological origin and/or IN active but heat-sensitive quartz or plagioclase (Daily et al., 2022).”*

The original sentence was as below in the original manuscript (Line 403):

*“For $T < -12^{\circ}\text{C}$, the dN_{INP} fraction of T90 samples are consistently more than 85% (**Error! Reference source not found.c**).”*

(e) We revised the following sentences in the Discussion section:

*“**Error! Reference source not found.**i compares the fraction of heat-resistant INPs in dust and non-dust influenced groups. Overall, heat-resistant INPs constitute $<10\%$ in both groups. It also shows that dust events only slightly enrich heat-resistant INPs for $T \leq -20^{\circ}\text{C}$ but not for warm Ts. This minimal impact of dust events eliminates minerals like quartz or plagioclase as major contributors to the observed IN activity, thereby supporting the discussion on heat sensitive tests in Section 3.4 and highlighting the importance of PBAPs. Therefore, our results demonstrate that heat-labile FBAPs are the dominant source of INPs, irrespective of dust presence.”*

The original sentence was as below in the original manuscript (Line 469-472):

*“**Error! Reference source not found.**i compares the fraction of heat-resistant INPs in dust and non-dust influenced groups. Overall, heat-resistant INPs constitute $<10\%$ in both groups. It also shows that dust events only slightly enrich heat-resistant INPs for $T \leq -20^{\circ}\text{C}$ but not for warm Ts. In other words, our results demonstrate that heat-labile FBAPs are the dominant source of INPs, irrespective of dust presence.”*

2. Many of the correlation coefficients are < 0.4 , which does not indicate a strong relationship, even if the correlations are statistically significant. It may be more insightful to focus on the higher correlation coefficient values.

R: We agree with the reviewer that correlation coefficients below 0.4 indicate a relatively weak linear or monotonic relationship, even when statistically significant. In our analysis, we deliberately avoid over-interpreting isolated, weak correlations. Instead, our approach focuses on identifying correlations that are not only statistically significant ($p < 0.05$) but also persistent across **consecutive temperature intervals**. Because ice nucleation activity is a temperature-dependent physical process, a true physical link between an aerosol property and INP concentrations should manifest across a temperature range rather than at a single, isolated temperature. Observing consistent significance over consecutive temperatures greatly reduces the likelihood of false positives and makes the underlying physical conclusions far more reliable, even if the individual correlation coefficients are modest.

To clarify this, we have added a statement in Section 3.3 to explicitly state our criteria for interpreting these correlations, emphasizing that consistency across consecutive temperatures is used as the primary metric for reliability. The new statement is as below:

“However, ABC_{WIBS} particles, a subclass of FBAPs, show significant correlations ($\rho_{Pearson}$ and $\rho_{Spearman} \sim 0.4$) with INPs for $-19^{\circ}\text{C} < T < -9^{\circ}\text{C}$. This persistence across consecutive temperature intervals strongly supports the role of PBAPs as important INPs, reflecting the temperature-dependent nature of IN activities.”

The original sentence is as below in the original manuscript (Line 343-344):

“However, ABC_{WIBS} particles, a subclass of FBAPs, show significant correlations ($\rho_{Pearson}$ and $\rho_{Spearman} \sim 0.4$) with INPs for $-19^{\circ}\text{C} < T < -9^{\circ}\text{C}$.”

3. Most samples were collected in May and June, which may bias the correlation analysis, as biological INPs might indeed dominate the INP population during this period. It would be useful to assess whether biological particles are present at the site throughout the entire year and at what concentrations.

R: Thank you for this valuable point. We agree that extending the sampling period across all seasons would provide a more comprehensive view of annual INP variability and further strengthen the statistical analysis. Due to logistical constraints, our intensive field campaign was specifically focused on May and June. We proactively recognized this potential seasonal bias in the original manuscript and explicitly stated (Line 281-283):

“We note that 80% of samples in Fig. 2g were collected during the summer (Supplement Table S1), which may bias the statistical analysis of seasonal patterns. Therefore, we do not pursue further in-depth discussion of INP seasonality.”

While we cannot characterize the full annual cycle, a total of 103 distinct aerosol samples were analyzed for this study. The dataset is substantially larger than many comparable INP field studies in literature. Given this extensive dataset, we believe the correlation analysis remains highly reliable for evaluating the relationships and driving factors during the late spring and summer periods.

Minor comments

1. General: I recommend the use of "e.g." for references when they do not represent the only examples supporting the statement.

R: Agreed and added “e.g.” to some in-text citations in the introduction section in the revised manuscript.

a. The sentence is revised as below in the revised manuscript:

“The largest source of uncertainty regarding cloud impacts on climate is related to low clouds, largely stemming from uncertainties in processes that affect their life time (e.g., Mülmenstädt et al., 2021), glaciation state (e.g., Lohmann, 2002) and optical thickness (e.g., McCoy et al., 2016) in which the cloud phase plays a key role (Korolev and Milbrandt, 2022).”

b. The sentence is revised as below in the revised manuscript:

“In the atmosphere, INPs can originate from different sources (Kanji et al., 2017), including mineral dust particles from deserts (e.g., Brunner et al., 2021; Gao et al., 2024), soil dust (e.g., Steinke et al., 2016; O’Sullivan et al., 2013), marine organics and sea spray aerosols, volcanic ash, biomass burning aerosols from wildfires and/or anthropogenic fuel uses, as well as primary biological aerosol particles (PBAPs) from ecosystems (e.g., Tobo et al., 2013; Schneider et al., 2021; Gao et al., 2024).”

2. Lines 113 – 114: What is the collection efficiency for larger particles, e.g., 10 μm and 20 μm ?

R: Based on the known collection efficiency for smaller particles (50%, 80%, and 94% for 0.5, 2.0, and 5.0 μm particles, respectively) as provided in the original manuscript, the collection efficiency of the sampler for particles larger than 10 and 20 μm should be more than 94%. Unfortunately, we did not test the collection efficiency of the sampler for particles larger than 10 and 20 μm .

3. Line 114: „water vapor evaporation“is doubled, water vapor is produced by evaporation?

R: We now revised the sentence to make it more concise.

The original sentence is as below in the original manuscript (Line 114-115):

“Due to water vapor evaporation, the cyclone was refilled to 15 ml for every 10 min.”

Now, it is revised as below in the revised manuscript:

“To compensate for evaporation, the cyclone was refilled to 15 ml for every 10 min.”

4. Lines 118 – 119: It might be useful for the reader to explain the sampling strategy briefly here; e.g., was one sample taken each day, at the same time? Also, I recommend to be transparent that most samples were taken in May and June.

R: Agree. We now added new sentences as below:

“Aerosol samples were collected almost every day during the intensive observation period of the campaign before the end of June in 2023 and at least once a week from July to December 2023.”

5. Lines 122 – 123: Please provide a reference for the statement „to quantify the contribution of heatlabile particles, such as proteins or biological macromolecules, to the INP population“ and specify the method of heat treatment.

R: Agreed. The sentence is revised as below in the revised manuscript:

“Also, 79 of the 103 liquid samples were heated at 90°C (maintained stable in an oven) for 40 minutes prior to DRINCZ experiments (termed T90 samples), to quantify the contribution of heat-labile particles (Li et al., 2023), such as proteins or biological macromolecules, to the INP population.”

6. Figure 1: Please check if the reference for using a picture from google maps is correct; in my understanding, the watermarks and copyright notices need to be visible, and the description in the caption should include at least the year the picture was taken.

R: Agree. Figure 1 and its caption are revised as below:

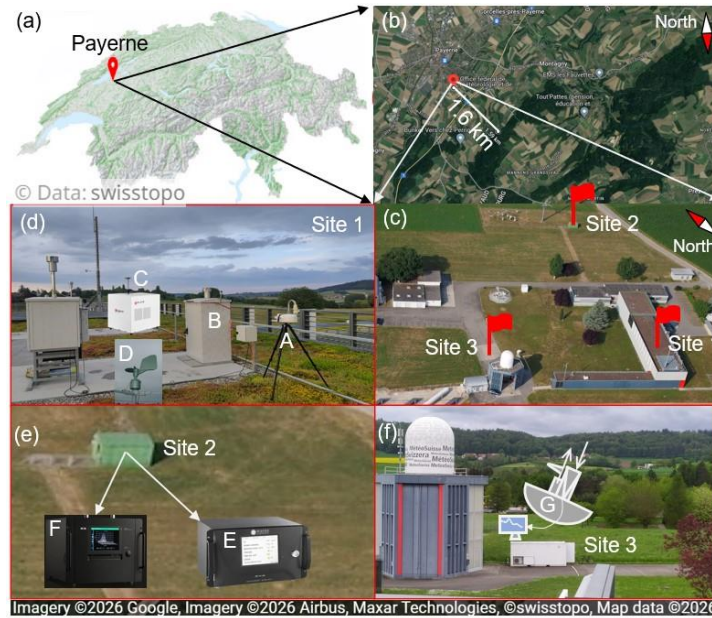


Figure 1. Overview of the instrumentation setup for PERICLES campaign. (a) The location of MeteoSwiss station at Payerne, Switzerland (©Data: swisstopo). (b) Satellite aerial view of the station. (c) The top view of the station and the location of three observation sites. (d) Site 1 for Coriolis aerosol sampler (A), Wideband Integrated Bioaerosol Sensor (WIBS, B), Rapid-E (C) and Hirst (D). (e) Site 2 for total carbon analyzer (E) and aethalometer (F). (f) Site 3 for remote sensing measurements using a UV-Fluorescent-Mie lidar (G) to observe the spatio-temporal evolution of aerosol particles. Panel (b) to (f) credit: Imagery ©2026 Google, Imagery ©2026 Airbus, Maxar Technologies, ©swisstopo, Map data ©2026.

7. **Figure 2:** The labels and text are very small, and the visibility of the light blue color is not good. E.g., the variability in the INP concentration in panel a is not easy to depict given the scale of four orders of magnitude; in general, I find it hard to see the described behaviour of the data in section 3.1.

R: We changed the color scale and maximized the font size of axis labels in Figure 2 to improve the visibility. The variability of INP concentrations at the same fixed temperature is approximately within one order of magnitude. Across -10 and -20 °C, the INP concentration varies between 3 and 4 orders of magnitude. Our results here show the temperature dependence of INP concentrations. We briefly described the main features of INP abundance in Figure 2a in the original manuscript (see below).

“Error! Reference source not found.a shows that INP concentration (N_{INP} at -10, -15 and -20°C) increases by roughly one order of magnitude for each 5°C decrease, showing the temperature dependence of INP abundance in the atmosphere. N_{INP} values at -10 and -20°C across samples generally vary within one order of magnitude while N_{INP} values at -15°C approximately span two orders of magnitude.”

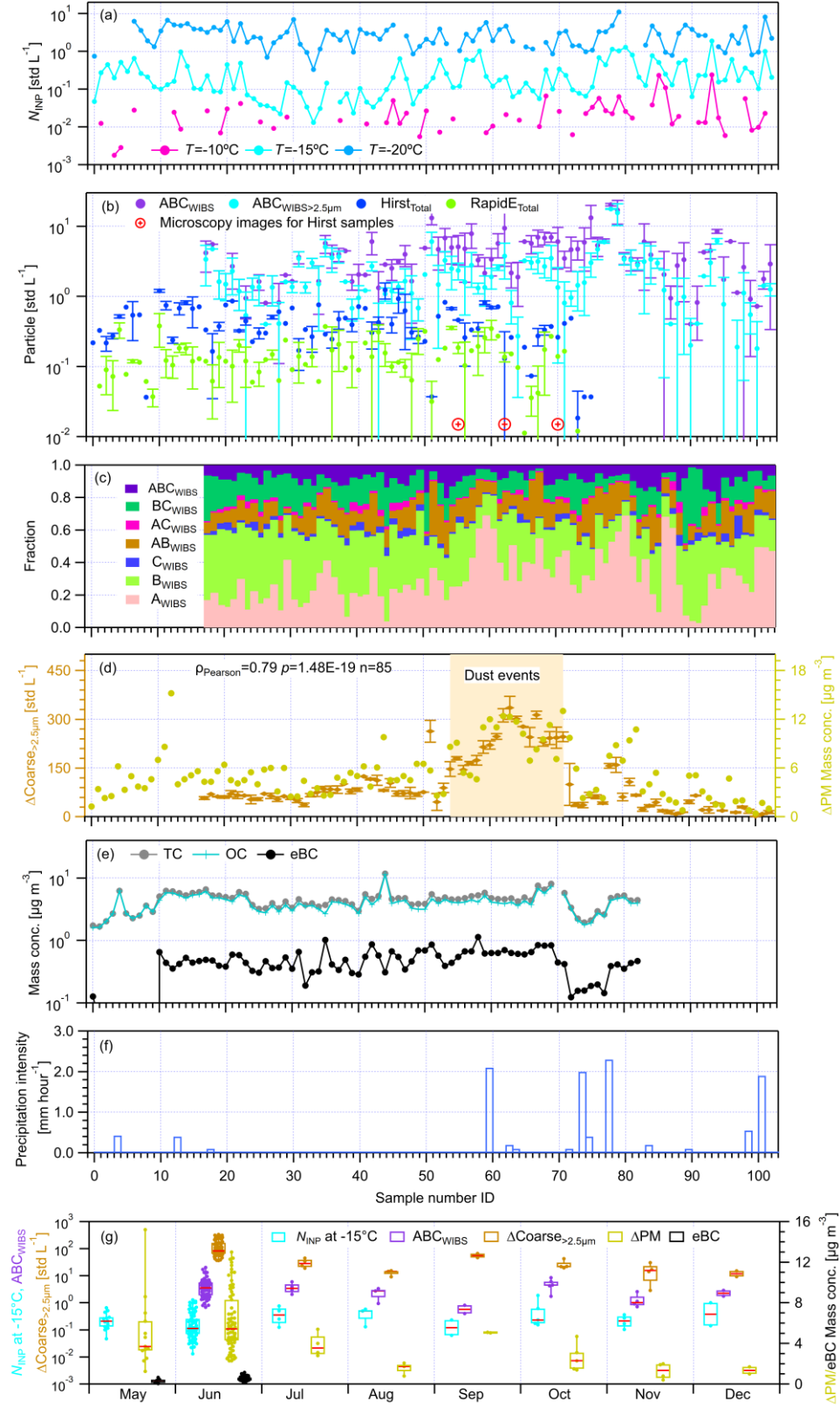


Figure 2. Overview of N_{INP} at three fixed temperatures and different aerosol properties for all IN samples. Detailed information on samples as a function of sample number ID is provided in the Supplement. (a) INP number

concentration (N_{INP}) at -10 , -15 and -20°C . (b) The number concentration of ABC_{WIBS} and $\text{ABC}_{\text{WIBS}>2.5\mu\text{m}}$, $\text{RapidE}_{\text{Total}}$ and $\text{Hirst}_{\text{Total}}$ particles. Also, three Hirst samples with exemplary microscopy images are indicated. (c) The fraction pattern of different types of fluorescent particles classified by WIBS. Different types of particles are indicated in the legend. (d) The number concentration of $\Delta\text{Coarse}>2.5\mu\text{m}$, i.e., the number concentration difference between $\text{Total}_{\text{WIBS}>2.5\mu\text{m}}$ and $\text{ABC}_{\text{WIBS}>2.5\mu\text{m}}$, on the left axis, and the mass concentration of ΔPM , i.e., the mass concentration difference between PM_{10} and $\text{PM}_{2.5}$, on the right axis. (e) The mass concentration of TC, eBC and OC (the difference between TC and eBC). (f) The precipitation intensity for the period of aerosol sampling. (g) Monthly box plots of number concentrations of N_{INP} at -15°C , ABC_{WIBS} , and $\Delta\text{Coarse}>2.5\mu\text{m}$ (on the left axis), and mass concentrations of ΔPM and eBC (on the right axis). The red central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. All concentration values were corrected to equivalent atmospheric standard condition (i.e., per standard volume of sampled air, std). The error bars for bar plots represent 1 standard deviation of corresponding measurements. N_{INP} values are weighted averages of at least three measurements per sample, calculated following Wieder et al. (2022). Blank spaces indicate data gaps resulting from instrument unavailability.

8. Section 3.1: You reference Fig. 1 here, but I assume you mean Fig. 2.

R: Thanks. We corrected all in-text figure citations for Figure 2 and all other figures.

9. Line 241: At -15°C , the variability in INP concentration is greater than one order of magnitude.

R: Agreed. The sentence is revised as below in the revised manuscript:

“ N_{INP} values at -10 and -20°C across samples generally vary within one order of magnitude while N_{INP} values at -15°C approximately span two orders of magnitude.”

10. Lines 260 – 267: This section could be moved to the methods.

R: We already defined those properties in the Methodology section (2.3.1). We prefer to retain these sentences here to keep the parameter definitions immediately accessible to the reader. Additionally, this text directly supports the discussion of the results in Figure 2d, which are analyzed in this section.

11. Line 278 – 279: Are there no ABC_{WIBS} data for May? And what are the correlation coefficients for the relations between the monthly INP concentration at -15°C and WIBS_{ABC} , $\Delta\text{Coarse}>2.5\mu\text{m}$, ΔPM and eBC? It is hard to see this in Fig. 2g given the size (see previous comment).

R: No, unfortunately there is no WIBS data available in May because of unavailability of the instrument. As stated in the original manuscript, *“we note that 80% of samples in Fig. 2g were collected during the summer (Supplement Table S1), which may bias the statistical analysis of seasonal patterns. Therefore, we do not pursue further in-depth discussion of INP seasonality.”* Therefore, we did not calculate the correlation coefficients as suggested by Reviewer 2.

12. Lines 301 – 302: If the INPs were biological particles, wouldn't you expect that the first freezing would occur at higher temperatures, or that the freezing onset would shift after the heat treatment? It might be useful to reference typical onset temperatures of some biological particle types.

R: We agree with Reviewer #2 that biological particles with active ice nucleation abilities usually result in ice activation at warmer temperatures (O'sullivan et al., 2013; Wieland et al., 2025). However, some pollen (birch) particles only nucleate ice close to -15°C (Wieland et al., 2025; Augustin-Bauditz et al., 2016). The onset ice nucleation temperature for sample in Fig. 3a is also close to -15°C and its relevant INP source may be birch pollen particles (Hirst data) that is similar to the pollen taxa reported by Augustin-Bauditz et al. (2016) and Wieland et al. (2025). We now add a new sentence to the revised manuscript as below:

“The onset INP data in Fig. 3a for the untreated sample is at -13°C which is close to the onset ice nucleation temperature of birch pollen particles reported by Augustin-Bauditz et al. (2016) and Wieland et al. (2025).”

13. Line 409: Do you mean „The limiting role...“?

R: Thanks. Now, the section title is revised as below:

“The limited role of dust events in the observed INP abundance”

14. Section 3.5: It might be helpful to give a short overview of atmospherically relevant dust types and their onset temperature; as your freezing method is limited to $T > -24\text{ }^{\circ}\text{C}$, you might not activate some clay minerals (e.g., Pinti et al., 2012), which are a major component of airborne mineral dust.

R: Laboratory ice nucleation data on atmospherically relevant dust in the previous literature was generally reported in terms of active site density concentration normalized to sample surface (n_s). However, we could not directly calculate n_s for field samples tested in this study due to the unavailability of particle total surface area. In addition, we did not conduct mineralogical analysis of our samples to identify specific dust types. Instead, we rely on established literature indicating that biological particles predominantly contribute to INPs active at temperatures above -15°C , whereas mineral dust particles become the more relevant INPs at temperatures below -15°C (Murray et al., 2012; Kanji et al., 2017). We have already included a statement on this effect in the Introduction, please see below:

“It is reported that PBAPs play a dominant role for the ice formation in MPCs with $T > -15^{\circ}\text{C}$ (Coluzza et al., 2017; Cornwell et al., 2023; O’sullivan et al., 2018) where secondary ice production also occurs and can be strongly amplified, while dust generally acts as INPs at lower temperatures (Murray et al., 2012; Kanji et al., 2017).”

15. Lines 464 – 465: It might also be that the air mass is depleted in long-range transported INPs at the site due to an earlier activation.

R: Agreed. We added a new sentence to the revised manuscript as below:

“Another possibility is that these highly active dust INPs were activated and scavenged via cloud formation and lost due to precipitation during transport, preventing them from reaching the sampling site.”

16. Line 515: You could briefly explain what BIC is or how it is calculated.

R: We have already included a statement on this in the caption of Figure 8 (please see below):

“BIC is a value calculated by applying the Bayesian information criteria to evaluate the goodness of parameterizations based on the same dataset (Schwarz, 1978).”

17. Lines 552 – 553: As the effect of precipitation on the INP population is discussed in the SI only, I would not include it in the conclusion section; or move it to the results in the main manuscript.

R: While we appreciate the suggestion, the manuscript's length prevents us from moving the full precipitation analysis to the main text. We have already introduced this analysis at the beginning of Section 3 (Line 221 in the original manuscript) to guide interested readers to the Supplement. However, we believe retaining a brief mention in the Conclusion is necessary to highlight the overarching impact of precipitation on the INP population.

Revisions after proof-reading (but not suggested by reviewers):

In addition to revising the text in the manuscript, we did a double check for our dataset used in this paper given that many measurements were included. We listed these extra revisions at the end of our responses to Reviewer #1.

References:

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