Author's response to Referee #1

We thank reviewer #1 for the thoughtful comments on our manuscript. Below we provide detailed answers to the individual point giving in black the original comments, in red our answer and in blue the changes in the manuscript.

General comments

1) The manuscript lacks discussion on contrasting results from previous research, such as the study by Prenni et al., 2013, which indicates a correlation between increased biological INP concentration and high RH. The field study by Prenni et al., 2013 was conducted in a forest in Colorado, USA with the presence of pine trees as well. Since the authors argue in their manuscript that pine forests in Colorado and Finland can be compared (parameterization comparison), I miss the discussion about these contrasting results.

We agree with the reviewer, that a discussion on these contrasting results is missing (although we did discuss related studies to that of Prenni). Also reviewer #2 commented on that, and we included a discussion on that in the manuscript. This is part of the discussion mentioned under point (3) and the updated manuscript can be found there.

2) Paramonov et al., 2020 measured INPs at the same research station in the same year and proposed that INPs were influenced by long-range transport, a result that is different from the outcome of this study. A more thorough discussion on the differences in outcomes between studies would enhance the manuscript.

The study of Paramonov et al., 2020 presents INP measurement from the end of February until beginning of April at a temperature of -30°C. With that the overlapping period was 3 weeks. Both studies consider the entirety of their measurements, meaning that for Paramonov et al., 2020 there is a significant contribution to the total INP spectrum before our measurements started and our manuscript contains more than one month additional data for a time period not covered in the other study. Paramonov et al., 2020 says that 'No single dominant local or regional sources of INPs in the boreal environment of southern Finland could be identified', from which they conclude that the INP population at SMEAR II originates from long-range transport. In our study, we also did not identify one single dominant local source, however we provide evidence for periods of a more local influence of INPs, such as a correlation between INP and time over land of 0.39 and a connection of the INP concentration to fluorescent particles. Therefore, we draw a different conclusion for our study. We already pointed to this difference in the conclusions, but extended the statement as follows:

L.381 ff: In a previously published study from HyICE-2018 (Paramonov et al. 2020), using data from a thermal gradient diffusion chamber, it is suggested that INP were from distant sources. However, the positive correlation with time-over-land and the connection of the INP concentration to fluorescent particles detected in the F3 channel of WIBS, which can be assigned to NAD(P)H, a co-enzyme connected to the energy metabolism of cells, suggest that the boreal forest serves as a source of biogenic INP. It should also be borne in mind that (Paramonov et al., 2020) made measurements from the end of February until beginning of April at a temperature of -30°C, hence

only overlapping with our measurements by 3 weeks. In the future we should aim to make INP measurements over a full annual cycle with PINE as the sources of INP will likely change with season.

3) The authors speculate that the INPs originate from lichens. However, their argument, primarily based on the negative RH correlation and lichens not being covered by snow, warrants further elaboration. In general, biosystems are rather complex and the emission of bioparticles from the biosphere to the atmosphere depends on multiple factors (Pasanen et al., 1991). It is worthwhile noting that also certain fungal spores, like those from the genus Cladosporium, are released into the atmosphere at low RH (Sabariego et al., 2000). Further, Cladosporium have been documented to nucleate ice at temperatures consistent with those presented in this study (Iannone et al., 2011). Additionally, besides lichens being not covered by snow, INPs could also originate directly from trees. Seifried and Reyzek et al., 2023 discovered biological INPs on the surface of Scots pines (bark and branches) capable of nucleating ice even below -25°C. Kokkila et al., 2002 mentions that Scots pine are the dominant tree species at the Hyytiälä research station. These points should be included in the discussion.

Indeed, the emission of bioaerosol is quite complex and is based on multiple factors. As also mentioned be referee #2, we extended our discussion around the negative correlation of INP and RH and included a new subsection. Also, the referee suggested the example of Cladosporium from Sabariego et al. We decided not to include this particular example in part because it is not clear how relevant Cladosporium is for Boreal forests (Saberiego et al. is a study in Spain), but also while there was a correlation reported with temperature, the correlation with RH was actually rather weak (and mixed sign, depending on type). We added the following new section:

3.4.2 The link between high INP concentrations and low relative humidity

In Section 3.4 we discuss the observation of a negative correlation between the INP concentration and the relative humidity (RH), which is potentially linked to an increased release of bioaerosol from lichen (Armstrong, 1991; Tormo et al., 2001). To better understand potential connections between the INP concentration, relative humidity and aerosol sources, the data are grouped based on precipitation into the three categories snow fall, rain fall, and no precipitation (snow fall and rain fall). The data of the last category are further split into five different RH ranges: > 99.8 % (which considers saturated conditions including the uncertainty of measurement), 99.8 % > RH > 80 %, 80 % > RH > 60 %, 60 % > RH > 40 %, 40 % > RH > 20 % (Fig.8a). (Fig.8a) reveals no substantial change in the INP concentration between snow fall, rain fall, and no precipitation. However, when dividing the data points without precipitation into the RH categories, the lowest INP concentrations with a median of 5 L−1 are measured for the highest RH. For the following groups the median INP concentration increases until it reaches 20 L−1 at a RH of 60 % and lower. A similar trend with RH is observed for the aerosol concentration in the accumulation mode and the WIBS F3 channel. The WIBS F3 channel can point to increased concentration in NAD(P)H (Savage et al., 2017), a co-enzyme linked to energy metabolism in cells and is an indication for living biological organisms. In contrast, the total fluorescent particles concentration of WIBS showed no clear increase in the mean concentration with decreasing RH (Fig.8b). In other locations fluorescent bioaerosol concentrations were found to increase markedly with increasing RH, which may be related to RH-dependent fungal spore release mechanisms (Toprak and Schnaiter, 2013; Gabey et al., 2010; Timothy P. Wright and

Petters, 2014). Hence, the bioaerosol released in the Boreal forest of Southern Finland appears to be different to other locations.

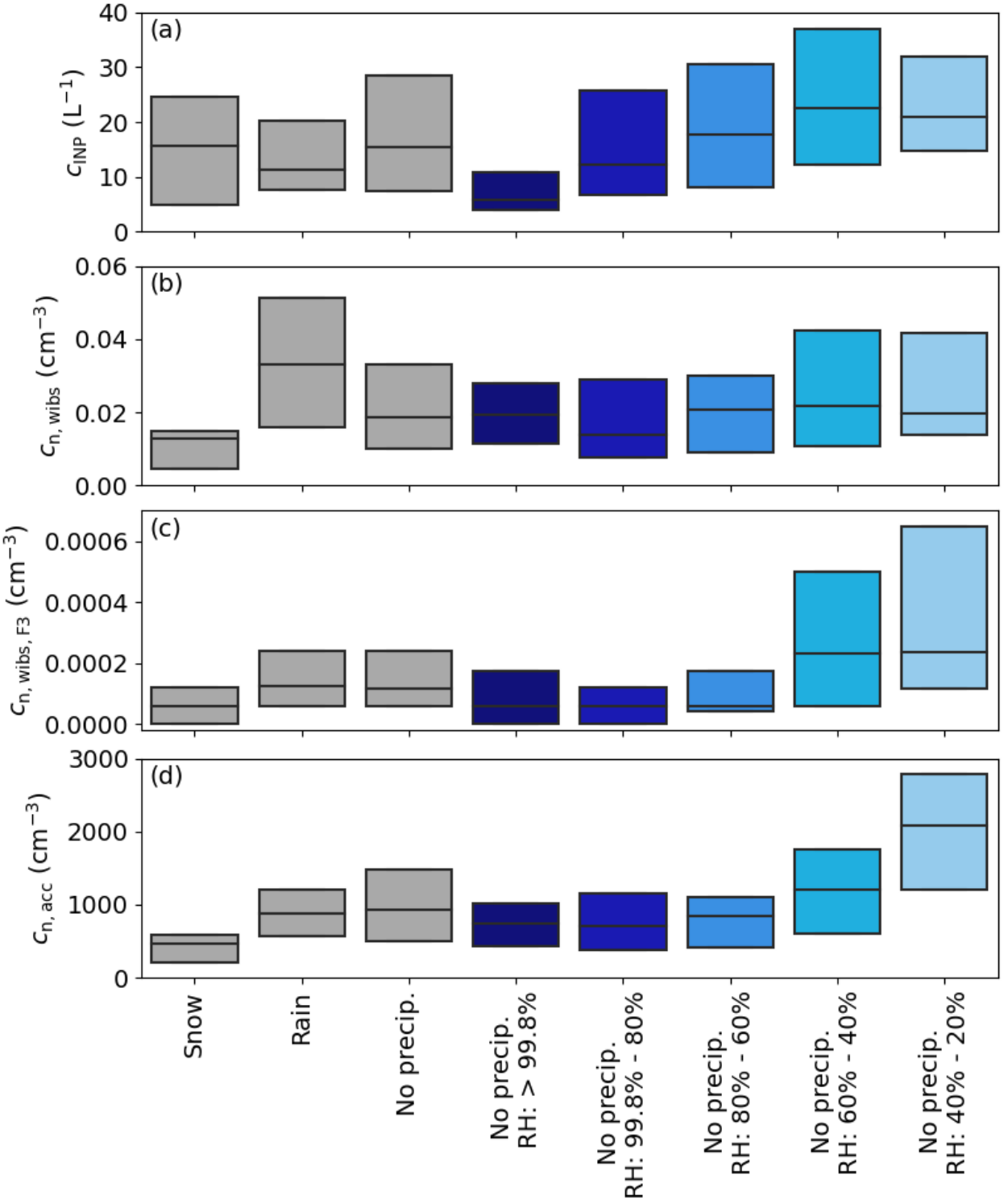

Figure 8. The relationship between aerosol, precipitation and humidity. (a) The INP concentration, (b) fluorescent particle concentration measured by WIBS, (c) fluorescent particle concentration measured in the F3 channel of WIBS and (d) the particle concentration in the accumulation mode are divided into several precipitation and relative humidity categories. The precipitation is split into cases of snow fall, rain fall and no precipitation (including also no snowfall). The data of the 'no

precip.' category were again divided into different relative humidity (RH) classes, which are RH > 99.8 % (saturated conditions, considering some uncertainty of the measurements), 99.8 % > RH > 80 %, 80 % > RH > 60 %, 60 % > RH > 40 %, 40 % > RH > 20 %. The boundaries of the boxes are the 25th and 75th percentile. For clarity, whiskers and outliers are not shown.

The WIBS results and the correlation with RH are consistent with release of bioaerosol particles from lichens. Lichens can reproduce asexually through the production of diaspores. These diaspores contain living components of lichen, the mycobiont and photobiont, that can then colonise new locations (Hale, 1974). These diaspores would therefore contain NAD(P)H since they are metabollically active. In addition, as mentioned above, release of diaspores has been shown to be negatively correlated with RH (Armstrong, 1991; Tormo et al., 2001). Hence, the hypothesis that lichen derived bioaerosol contribute to the INP population is consistent with both the F3 channel (NAD(P)H of WIBS and the negative RH dependence.

A remarkable feature of Fig.8 is that for the fluorescent particle concentration, the WIBS F3 concentration and the aerosol concentration in the accumulation mode, the lowest values are detected during snowfall, while for the INP concentration this is not the case. This implies that many aerosol types are preferentially removed during snow fall events, but the INP concentration is not affected to the same extent. When snowing, the ambient RH would be expected to be well below 100% (with respect to water), for example, if the air were at ice saturation the RH would be about 90% at -10°C, conditions under which we might expect to start to see lichens producing aerosol. However, the low bioaerosol concentration from the WIBS is inconsistent with this idea.

During rain fall, an enhanced concentration of fluorescent particles is measured by WIBS, however it is not reflected in the INP concentration. Previous studies reported an increase of INPs during and after rain events during measurements in Colorado in summer. The highest increases in fluorescent particle and INP concentrations were observed during intense rain events with rain rates more than 3 mm/5min (Tobo et al., 2013). Less pronounced increases were observed during less intense events and at lower measurement temperatures, which are equal to our covered temperature range (Huffman et al., 2013; Prenni et al., 2013). The overall rain rate during our measurement period was about 0.03 mm/5min and by that two orders of magnitude lower than during the measurements in Huffman et al. (2013); Prenni et al. (2013); Tobo et al. (2013). Moreover, they report measurements in summer, when rain fell on dry soil and by that released the biological particles. During the HyICE-2018 campaign, the soil was always covered by snow or wet, hence release of biological particles might have been hindered.

We further followed the suggestion of including the discussion of more bioaerosol sources and adjusted our manuscript as follows:

L. 306: Another study suggests the surface of Scots pine trees, the predominant tree species in Hyytiälä (Kokkila et al., 2002) as a potential source of INPs (Seifried et al., 2023). Since lichens and the bark and branches of trees are one of the few biological entities L. 386: Potential reservoirs of INP that were exposed to air during HyICE-2018, despite the snow cover, are tree dwelling lichens (Proske et al., 2024) and the surface of Scots pine trees. L. 390 Also biological INPs emitted from the

surface of Scots pine trees, the dominant tree species at SMEAR II (Kokkila et al., 2002), were found to nucleate ice below a freezing temperature of -25 °C and could contribute to the INP population (Seifried et al., 2023).

Specific comments

4) Line 6: To provide a clearer overview, I recommend inserting numbering (i), (ii), (iii) before each argument supporting the hypothesis that INPs below -24°C originate from biological sources.

We added the numbering before the three key arguments.

...this location are also from biological sources: (i) an INP parameterization developed for a pine forest site in Colorado, where many INPs were shown to be biological, produced a good fit to our measurements; a moderate correlation of INP with aerosol concentration larger than 0.5 µm and the fluorescent bioaerosol concentration; (ii) a negative correlation with relative humidity that may relate to enhanced release of bioaerosol at low humidity from local sources such as the prolific lichen population in boreal forests. (iii) The absence of correlation...

5) The Introduction is well written and the state of the art is well described.

We are pleased the referee found the intro well-written

6) Line 37: Typo “relay”

Changed to ‘rely’

7) Line 50: Typo “bioaerosols” instead of bio aerosols

Changed to ‘bioaerosols’

8) I suggest including a brief description or equation explaining how c_INP (L^{-1}) is calculated to ensure that readers understand it is measured per litre of air rather than per litre of liquid volume, as reported for some immersion freezing assays.

We inserted an equation showing how the INP concentration is calculated and a respective description in L. 142ff.

The resulting INP concentration is calculated per liter of air following equation 1:

$$c_{n,INP} = V_{sol} / V_{air} * c_{n,INP,sol} = - V_{sol} / V_{air} * d / V_{well} * \ln(fliq(T)) \quad (1)$$

d is the dilution factor, which is for this analysis 1, 10 or 100, Vair is the volume of the sampled air, that passed the filter and fliq is the fraction of liquid wells at a certain temperature of the measurement.

In L. 144, we added an additional sentence for the µL-NIPI.

The obtained INP concentrations are given per standard liter of air

9) Line 103: Typo “ETH” instead of EHT

Changes to ‘ETH’

10) Line 122-124: missing space between units “L min -1 ”

Space added between ‘L’ and ‘min-1’ in all three lines

11) Line 125: missing space between number and unit “6 min”
Space added

12) Line 140: What is nanopure water? Please provide more details about the water used in the offline ice nucleation assays
NanopureTM water is filtered and deionized water. Typically, the purity of water is given with its conductivity, so we added this value in the text in L. 136.
... 8 ml of nanopureTM (conductivity of approximately 0.056 - 0.057 µS cm−1) water ...

13) Line 140: The freezing data is presented in °C in all graphs, so I recommend maintaining consistency by using the same temperature unit throughout the manuscript, including for the cooling rate. In addition, there is a space missing between the units “°C min-1 ”
We changed the units of the cooling rate in L. 140 and L. 143 from K min-1 to °C min-1

14) Line 141: Consider rephrasing the sentence as not the well itself freezes but the content. Maybe: “[…] to a temperature at which all droplets freeze.”
We agree that this sentence sounds misleading. We rephrased it in the text.
... to a temperature at which all solution droplets inside the wells freeze.

15) Line 143: change unit for cooling rate
We changed the cooling rate as suggested

16) Table 1: Last column, last cell: What do you mean by “end”? May 11, 2018?
We intended to say that it was measuring until the end of the campaign, which was after May 11, 2018. However, we agree to change to the end date of PINE measurements to avoid misunderstandings.

(17) Line 156: add excitation and emission wavelengths
We added the two excitation and detection band wavelengths in L. 156
... measures particle fluorescence with two excitation lasers (wavelength of 280 nm and 370 nm), and emission is monitored in two detection bands (310 nm to 400 nm and 420 nm to 650 nm).

18) The months April and May are renowned for their elevated pollen concentrations, with species such as Betula pendula exhibiting notably high levels, especially towards the end of May in the southern regions of Finland (see e.g. Manninen et al., 2014). Could these pollen have contributed to the INP population?
The winter in Hyytiala in 2018 lasted rather long and trees started having leaves only at the beginning of May. The concentration of fluorescent particles increased already towards the end of March as shown in Schneider et al. 2021. However, the INP concentration did not show any significant increase. It is possible that the pollen contributed to the INP population, but we do not

have any further poof for that. Since pollen is not sensitive to the wet heat test, the heat sensitivity in droplet freezing assays is indicative of something other than pollen (but does not rule out pollen).

19) Figure 2, caption: Put listing items (a), (b), etc., before the sentence to match the style used in Figure 3.
We moved the listing items in the figure caption from the end of the sentence to the front of the sentence

20) Figure 6 and line 284: It is not explained in the manuscript what FP3 for WIBS measurements is referred to. Please add the details.
We added an explanation of the FP3 channel and gave indications which type of biological particles can be detected in this specific channel.
The p values indicate that these correlations are significant. An aerosol particle entering WIBS gets excited by two lasers with a different wavelength and can be detected in two different detection bands. Depending on its composition, fluorescence is detected in only one or more laser detection band channels. The F3 channel is fluorescence triggered by the 370 nm laser and detected in the 420 nm to 650 nm band. Particles detected only in this channel can be assigned to NAD(P)H, which is a tracer for the viable biological fraction (Savage et al., 2017).

21) Line 285: missing space between the comma and “which”
We rephrased L.285 ff based on the comment before. Therefore, this part with the missing comma was deleted

22) Line 295: consider rephrasing from “large concentrations” to “high concentrations”
Changed as suggested

23) Line 316: Do you mean at around 14:00 (2 pm)? If so, use style hh:mm (same for time in line 335)
Changed as suggested

24) Line 352: Typo “Figure 9” instead of “figure 9”
Changed as suggested

25) Are the aerosols after PINE collected and could be measured in the future with offline spectrum to obtain the entire freezing spectrum from the same aerosol population?

Aerosol particles exiting PINE are currently not collected on filters or as a bulk. This is something that would be interesting to work on in the future, perhaps even with a virtual impactor.