Response to the interactive comment by Referee #3:

We thank referee #3 for his or her thoughtful comments and feedback. Please find below our responses and suggestions for the manuscript revision, with the referee comments in black, our answers in red, and suggested changes or additions to the manuscript in blue.

In the manuscript titled “The seasonal cycle of ice-nucleating particles linked to the abundance of biogenic aerosol in boreal forests”, Schneider et al. describe results from a year-long measurement campaign of ice nucleating particles (INPs) over a forested site in Finland. The data are unique and provide an additional constraint for INPs present over a boreal environment, which are of value to the aerosol-cloud interaction community. I have several comments, most of which I think can be addressed by the authors and I hope to support the publication of this manuscript once these comments have been adequately addressed.

SECTION 1 INTRO The introduction is concise and has room to address two current missing items: 1) a description of the goal of the manuscript or study; and 2) because one of the main deliverables of this paper is parameterized INP concentrations at this location, it is important to describe the physical mechanisms that INPs may be generated locally or other regional aerosol sources that may impact the INP populations - this is important in creating a physically-based parameterization that may one day be expanded to modeling studies.

We agree with the referee that the paper would benefit from a stronger definition of the objective of the study. Therefore, we have added a description of the objectives of this study at the end of the introduction:

The main objective of this study is to investigate and describe the variability and seasonal trends in INP concentrations and INP temperature spectra in a boreal forest environment. The absence of anthropogenic and/or dust aerosol sources in the boreal region motivates the additional investigation of biogenic ice nucleation activity and reveals the relevance of boreal forest areas as an important INP source. The comprehensive instrumentation provided at the measurement site at the SMEARII station allows comparisons between INP measurements with simultaneous measurements of many meteorological variables. These measurements are complemented by measurements characterizing the sampled aerosol number concentrations, size distributions and chemical compositions in order to elucidate the potential origin and nature of the INPs. Heat treatments of the suspensions prior to INP analysis also help identifying the nature of INPs. We aim to improve the parameterizations describing atmospheric INP concentrations in the boreal forest by considering seasonal dependences in the formulations. Finally, this study provides motivation for further continuous long-term studies of INP in different environments across the globe.

General information about the characteristic aerosol particle population is already given in the introduction. Here, we state that boreal forests are generally far from anthropogenic or dust sources, which consequently are not expected to contribute to the generation of boreal INPs. As the main source of aerosol particle in boreal forest areas appeared to be the forest itself, it is also likely, that the INP populations are mainly influenced and generated by this source. As we present indication for abundant biogenic particles in the INP population, this expectation is underlined. We cannot give more precise description on INP generation processes in the boreal forest, as we did not directly measure the nature of the INP in our samples. Of course, different biogenic aerosol particles are also

generated differently, but in general, we state the forest with its vegetation as the main INP source, releasing biogenic particles like pollen and fungal spores e.g. through wind dispersion.

SECTION 2 METHODS The methods describing measurements of INPs is quite thorough.
I would like the authors to expand on the uncertainty description for INPs - it is not clear where the systematic error percentages were derived. Were blanks collected?

Thanks for this suggestion. We have added a more detailed description of the determination of the systematic errors to Section 2.2 "INSEKT":

A systematic error due to the preparation process and flow measurements is added. Applying a simple linear error propagation to the formulas given in Vali (1971) and inserting the error-containing parameters like the pipetted suspension volumes and the flow rate systematic errors of 4% for the undiluted suspension, 5% for the first dilution step, 8% for the third dilution step and 11% for the fourth step, are calculated. The systematic error increases with each dilution step because the additional pipetting step adds uncertainty.

In the following sentence, it is mentioned that blank filters have also been collected and the resulting INP temperature spectra have been subtracted from the INP temperature spectra of the "normal" samples.

The inlet for the "additional instrumentation" was heated and "RH remains below 40%"
– how does this compare to the inlet used for the INP filter collections? Without knowing the exact RH at this site, I suspect RH can get quite high and may increase collection efficiencies of large particles.

Thanks for this comment, this is a good point to consider. We do not expect the collection efficiency to be impacted by the relative humidity. However, it is possible that the measured surface area distribution is somehow shifted, which is a common problem when using different inlets. This adds uncertainty to the INAS densitiy parameterization. Currently, we are not able to quantify this uncertainty, but it is important to be aware of the potential bias. It is important to note that the INSEKT is only measuring ice-nucleating particles, which are not soluble.

For the other measurements, there seems to be a few missing details: - What exactly was measured by the L-ToF-AMS? What sizes are detectable by this measurement? - Was the WIBS connected to a similar inlet as the other instruments? Where large super micron particles measurable? Could the authors include a comparison between the scattering particle size distribution measured from the WIBS and the APS data?

We agree with the referee that the paper would benefit from a more detailed explanation on the additional instrumentation used for this study. For both instruments, the WIBS and the L-Tof-AMS, we added a more detailed description, which addresses your questions and also the associated questions of the other referees.

We added a more detailed description on the WIBS instrument to Section 2.3. "Additional Instrumentation at SMEARII":

The WIBS-NEO (Droplet Measurement Technologies, Longmont, CO, USA) is a bioaerosol sensor that provides information on the fluorescence properties, size and asphericity ratio of individual aerosol

particles. It operates with an inlet flow of 0.3 l min^{-1} and detects particles with diameters between 500 nm and 30 µm. From 11 March 2018 to 2 April 2018, the WIBS was located about 50m from the aerosol filter sampling line used for the INP analysis. There, it was attached to a total aerosol inlet, which is characterized in Vogel (2018). On 3 April 2018, the WIBS was moved and installed directly next to the filter sampling line and attached to a PM10 inlet, which is described in Schmale et al. (2017). For the WIBS data analysis, particles from 0.5 µm to 10 µm were considered. To analyse the fluorescence of the particles, the WIBS sensor utilizes two xenon flashlamps as excitation light sources (optically filtered at wavelengths of 280 nm and 370 nm) and two emission detection channels (wavelength bands 310 – 400 nm and 420 – 650 nm). Optical size information is acquired utilizing elastic scattering from a continuous wave laser with a wavelength of 635 nm and a photomultiplier tube located orthogonally with respect to the laser. The excitation pulses are fired into the sample volume at different times and both detection channels record the emission(s) from both excitations, leading to three distinguishable excitation-emission combinations (the 370 nm light saturates the 310 – 400 nm detection channel and therefore does not provide any information). Thus, the fluorescence can be divided into 7 unique fluorescence groups based on the excitation-emission wavelength pairs and their combinations, after Perring et al. (2015) and Savage et al. (2017): A (only FL1: excitation 280 nm, emission 310 – 400 nm), B (only FL2: excitation 280 nm, emission 420 – 650 nm), C (only FL3: excitation 370 nm, emission 420 – 650 nm), AB (FL1 + FL2), BC (FL2 + FL3), AC (FL1 + FL3) and ABC (FL1 + FL2 + FL3).

The WIBS performs an empty-chamber background signal check every 8 hours, during which the excitation pulses are fired into the optical chamber without any present particles. The background check collects a multitude of emission intensities that form a baseline for particle fluorescence. In this study, a particle is considered fluorescent, if the associated emission peak intensity is larger than $FT + 9\sigma$. FT is the mean value of the forced trigger intensities and σ is their standard deviation.

A more commonly used method would be to compare the emission peak intensity to $FT + 3\sigma$. However, some non-biological particle types such as wood smoke, African dust and black carbon are weakly fluorescent and therefore might satisfy the lower threshold value, leading to an overestimation of biological particle concentration. Furthermore, the stricter threshold only marginally affects the detection efficiency of biological particles, because they tend to have stronger fluorescence (Savage et al., 2017). More detailed descriptions on the WIBS are also available in Savage et al. (2017) and Perring et al. (2015).

We also added the following text to the description on Figure 4 (old Figure 3b) to Section 3.2 "Comparison to meteorology ad aerosol properties" to explain the characteristic of the different excitation-emission wavelengths pairs:

The time series of the number concentration of particles with a fluorescence signal in other fluorescence groups is shown in the Appendix in Fig. A2. In this Figure, the strongest seasonal increase in the transition period from winter to summer is observed in the group ABC. Consequently, this fluorescent group correlates best with the measured INP concentrations (see Figure 4). The characteristics of each fluorescence group are comprehensively investigated and reported in Savage et al. (2017), who examined the fluorescence emissions of different types of pollen, fungi, bacteria, biofluorophores, dust, HULIS (humic-like substances), PAH (polycyclic aromatic hydrocarbons), soot and brown carbon. Using the $FT + 9\sigma$ threshold for defining a particle as fluorescent, nearly all dust and HULIS types show no fluorescence signal at all. Some of the soot and brown carbon types only show weak signals in A, and B, BC and A, respectively. Nearly all of the bacteria types show fluorescence only in group A. The fluorescence of fungal spores are also mainly detected in group A, but also in AB and ABC. The investigated biofluorophores show mainly fluorescence in the groups BC (Riboflavin, NAD), A (Pyridoxamine), AB (Tryptophan) and ABC (Ergosterol). PAHs show fluorescence

mostly in groups ABC and A. Finally, most pollen types show fluorescence in groups ABC and AB. Some pollen types also show a fluorescence signal in groups A and B.

We also adjusted the discussion on the comparison of WIBS data to INP in the same Section as follows:

Such a correlation is also supported by the peaks of pollen and PBAP concentrations observed in snow-free periods in spring and in autumn, and by the increases of the organic aerosol mass concentration and fluorescent particle numbers of group ABC observed in spring. According to the study of Savage et al. (2017), we assume particles of fluorescence group ABC to be mainly pollen, particles containing PAH or Ergosterol, or fungal spores. ~~Fluorescent particles are expected to be primarily of biological origin except for a few percent, which could arise from non-biological materials (Pöhlker et al., 2012; Savage et al., 2017).~~

We also added a more detailed description on the L-ToF-AMS instrument to Section 2.3. "Additional Instrumentation at SMEARII":

The size-resolved chemical composition of ambient aerosol was measured with the L-ToF-AMS. Its application in the same campaign has been described in Paramonov et al. (2020). It builds on the functionality and characteristics of the high-resolution ToF-AMS (DeCarlo et al., 2006). However, due to the longer time-of-flight chamber, the L-ToF-AMS, has a better resolution (8000 M/ΔM) than the standard ToF-AMS (2000 M/ΔM in V-mode). Detailed descriptions of the instrument, measurements and data processing are available in other publications (Canagaratna et al., 2007; DeCarlo et al., 2006). In general, the L-ToF-AMS measures the size-resolved, non-refractory composition of submicron aerosols, including organic, sulfate, nitrate, ammonium and chloride. The aerodynamic lens has a 100% transmission range of 75-650 nm (in vacuum aerodynamic diameter; Liu et al. (2007)) and focuses particles into a narrow beam that impacts the surface of a porous tungsten vaporizer heated to 600°C, followed by ionization by a 70eV electron source. Ions are detected by a long time-of-flight mass analyzer (Tofwerk AG). The sample flow of 0.09 l min^{-1} is extracted from an extra suction flow (3 l min^{-1}) that is used to avoid aerosol losses in the inlet line. A PM2.5 cyclone mounted at the inlet removes large particles to avoid clogging the critical orifice (100µm), and before entering the L-ToF-AMS, the samples are dried by a Nafion dryer to keep the RH below 30%.

The L-ToF-AMS data were analyzed using standard ToF-AMS data analysis toolkits (Squirrel V1.61B and PIKA1.21B) using Igor Pro software (V6.37, WaveMetrics Inc.). To calculate mass concentrations an ionization efficiency (IE) was determined using 300 nm, size-selected, dry ammonium nitrate particles, and a relative ionization efficiency (RIE) for ammonium of 3.7 was determined. The default relative ionization efficiency (RIE) values of 1.1, 1.2, 1.3 and 1.4 for nitrate, sulfate, chloride and organics, respectively, were applied. A composition-dependent collection efficiency (CE) was applied based on the principles proposed by Middlebrook et al. (2012).

We also added the following text to the discussion on Figure 4 (old Figure 3b) to Section 3.2 "Comparison to meteorology ad aerosol properties" to give more information about the measured organics:

The non-refractory organic components measured by the AMS include the commonly observed primary organic aerosol (POA) and oxygenated organic aerosol (OOA).

WIBS vs. APS: We have included a new Figure in the Appendix (Figure A3) showing the size distributions measured with the APS/DMPS (red) and the WIBS (blue) from 11 March 2018 to 13 May 2018. In each panel, one size distribution per month is highlighted to better show the agreement between WIBS and APS/DMPS measurements.
A description of this Figure has been added to section 2.3 "Additional Instrumentation at SMEARII":

Daily size distributions measured by the DMPS and APS combination are compared with the size distributions measured by the WIBS from 11 March 2018 to 13 May 2018 and are shown in Figure A3. Note, that the WIBS only measures particles larger than 0.5 µm. In summer, WIBS tends to measure slightly more particles with diameters larger than about 3 µm compared to the APS. However, the size distributions agree well for the other time periods and the smaller size ranges.

SECTION 3 RESULTS AND DISCUSSION Figure 1 is nearly impossible to read, consider expanding this figure to take up the full page and also increase font sizes for the axes.

We have increased the size of the axis description in Figure 1.

L200-204 – are the increases in INPs, organic aerosol, and fluorescent particles statistically significant? I'm not sure I would consider the organic and fluorescent particle concentrations to demonstrate "clear increases".

We agree that the increase in organic and fluorescent particle concentrations are not as pronounced as the increase in INP concentration. We have therefore reformulated the description:

Both, the organic aerosol mass concentration measured by L-ToF-AMS and the number concentration of PM10 fluorescent particles measured by WIBS, tend to increase ~~show clear increases~~ during the transition period from winter to summer (Fig. 4a and b).

Figure 3 – It looks as though the INP data are smoothed significantly in Figure 3a. This is especially clear when one compared the subfigure (Figure 3b) with the rest of the timeline. what kind of curve is fit to the INP data in Figure 3a? If there is no curve, why is it so smoothed? The daily data or monthly averages as points with standard deviations would be a better, more clear, way of visualizing the data.

INP data are represented as monthly averages in Figure 3a, as are the other data (NPF events, pollen and other PBAP). We have adapted the suggestion to not draw a smoothed line, but to draw points of the averaged INP concentrations with the standard deviation as error bars. Thanks for this suggestion!

L208 – This section is title" Comparison to meteorology and aerosol properties", yet only temperature and snow cover are compared. Are there other ambient variables that are correlated, for example relative humidity & winds? It is possible that relative humidity or winds may impact emissions?

Thanks for this comment. Other referees are also interested in comparisons with other meteorological variables, and therefore we have extended Figure 4 (in the new version, this is Figure 5) by including three additional panels showing the time series of RH (Fig. 5c), wind speed (Fig. 5d) and precipitation (Fig. 5e) and have ajusted the description accordingly. We have included the following section in the manuscript:

Figure 5c shows the comparison of the INP time series with the time series of relative humidity (RH) measured 35 m above ground. Over the entire time period, no clear relationship between RH and INP concentrations is observed. For a shorter time period from June 2018 to September 2018, there seems to be some correlation of the INP concentration with the RH, during which the peaks in INP concentration in June corresponds to a RH peak. In Figure 5d, we compare the time series of INP

concentrations with the time series of wind speed measured 34 m above the ground, which is also above the forest canopy. A relationship between measured INP concentrations and wind speed is not observed. In Figure 5e, the time series of INP concentration is compared to the occurrence of precipitation. Although other studies like Prenni et al. (2013), Huffman et al. (2013) and Iwata et al. (2019) report increasing INP concentrations during and after rain events in forested sites, we do not consistently observe this behavior. In this respect, increased INP concentrations are observed only during two of the strongest precipitation events in June and September 2018. However, it should be noted that in the cited studies INP concentrations were measured with higher time resolutions from minutes to hours. Huffman et al. (2013) reported increased INP and biological particle concentrations during rain events and up to one day after rain events. With our sampling (and therefore averaging) time of 24 hours or more, rain-induced enhancements of INP concentrations may have been missed. Therefore, this type of sampling strategy may not be appropriate to deterministically link INP concentrations with rain events.

Finally, when considering the full time period of this study, we did not find clear correlations of the INP concentrations with other parameters like the relative humidity, the wind speed or precipitation events. We do not exclude that the INP concentrations measured in the boreal forest are influenced by precipitation events for the whole year. However, our sampling set up is not appropriate to investigate the relationship more precisely.

L258 – While heating does remove a significant portion of the ice nucleation activity, I think it is important for the authors to also acknowledge that the heat-resistant residual is still associated with significant INP numbers. That is, the INP population is not just heat-labile and may not be entirely biological. I think the way the manuscript is currently written indicates that all INPs are biological, but that is not supported by Figure 5, where there are still significant INPs remaining after heating the samples.

Thanks for this comment. As the INP concentrations are significantly reduced after heat-treatment (about two orders of magnitude lower), we concluded that the majority of INPs in our samples are heat-sensitive, what indicates biogenic origin. However, we agree that this does not mean that **all** INPs have to be of biogenic origin. We therefore choose not state that **all** INPs are biogenic, but that the **majority** seems to be of biogenic origin or that boreal forest INP populations **seem to be dominated** by biogenic particles. To address this more clearly in the manuscript, we have added the following text to Section 3.3 “Heat treatment tests”:

As a significant number of residual heat-resistant INPs is still remaining after heat treatment, this indicates that not all measured INPs are associated with heat-labile biogenic materials. However, the majority of the INP population seems to be dominated by heat-labile materials, which is shown by the systematic shift in INP-temperature spectra.

L272 – “: : : as they do not include seasonal dependencies.” – this is not necessarily the case. Seasonal variability in INP abundances is accounted for by being linked to aerosol amount (in these cases n500 or nFBAP), which have seasonal variability.

That is correct, thank you. We have removed the second part of this sentence:

The predictions of DeMott et al. (2010) and Tobo et al. (2013) overestimate INP concentrations especially in wintertime~~, as they do not include seasonal dependencies~~.

L267 - I understand the WIBS was used to determine the fluorescent biological aerosol particles (FBAPs); How does this method compare to the UV-APS used in the Tobo et al. (2013) study? Given the number of uncertainties associated with measuring fluorescent measurements, can the authors describe the possible differences between the UV-APS and WIBS and how that would impact the performance of the Tobo et al. (2013) parameterization? Also, how does the Boreal forest in this study differ from that measured in Tobo et al. (2013)?

In general, the UV-APS operates with only one light source (excitation wavelength of 355 nm) and one detection channel (wavelength range of 420-575 nm), which reduces the classification power compared to WIBS. The excitation wavelengths of WIBS are 280nm and 370nm, with detection channels at 310 – 400 nm and 420 – 650 nm and thus the wavelengths of the two instruments also do not agree. For the application of the Tobo et al. (2013) parameterization, we used the WIBS data with excitation wavelength of 370nm and detection channel of 420 – 650nm (FL3), as this comes closest to the set-up of the UV-APS used in Tobo et al. (2013). Besides the elastic light scattering and fluorescence, the UV-APS measures the Aerodynamic particle size (which WIBS does not). Further, UV-APS does not perform any scheduled background "empty chamber" fluorescence check, instead it must be manually checked and calibrated using manual laser power and PMT gain adjustments. Furthermore, the UV-APS is discontinued and TSI no longer supports the instrument.

Concerning the forest around the measurement sites, both are dominated by pine trees and are far from anthropogenic sources. In both studies, it is assumed that the abundant aerosol population is mainly influenced by the forest and emissions from the vegetation. Differences are that the location in Tobo et al. (2013) is in Colorado, America and therefore at lower latitude than the Finnish forest. The dominant pine species in this lower latitudes is ponderosa pine. The Finnish forest is dominated by a different pine species called Scots pine.

Finally, potential differences in the Tobo et al. (2013) and our study, which could impact and explain the performance of the Tobo et al. (2013) parameterization, are the different wavelengths used in the UV-APS and the WIBS, the different latitudes, the different pine species or simply the season in which the measurements have been conducted. The parameterization in Tobo et al. (2013) is based on measurements conducted in the North American monsoon season from July to August 2011. The HyICE-2018 campaign took place from March to May 2018. In this time period, we do not observe a relation of INP concentrations to FL3 fluorescent particle concentrations. It cannot be excluded that this relation becomes stronger in the summer months, as it is observed in Tobo et al. (2013).

Figure 6 – I think it would be helpful to mention that only data from the HyICE-2018 period were possible to use in the Tobo et al. (2013) (2) parameterization and therefore that panel has fewer points.

Thanks for this suggestion. We have added the following text to Section 3.4 Parameterizations:

Note for the application of the Tobo et al., (2013) parameterization using FBAP concentrations, only data from the HyICE-2018 time period could be used, as the fluorescence measurements from the WIBS are only available in this period. Therefore, the number of data points in Fig. 7c is lower than in the other panels.

L295 – Should clearly state that this parameterization is based on ground-level ambient temperature, not "ambient temperature". If T is described as ambient temperature, a model will implement this as

the temperature predicted at any level, which I do not think is the intention. Additionally, while I understand the impressive correlation between
INP and ambient temperature is inviting for a parameterization, I caution the authors in publishing this as an INP parameterization given that it is highly specific to this location and has not been tested for other years, or is not really supported by a physical mechanism.
For example, aerosol-based INP parameterizations have a physical mechanism
– an aerosol particle that is seasonally variable with an assumed ice nucleation density.
The second parameterization presented in this paper has more physical meaning, linking INP abundance to the physical process of snow melting and exposed surface emissions. Without a clear physical basis for a "ground-level temperature-based" parameterization, I would recommend removing this.

The "ground-level temperature-based" parameterization is an empirical description of the INP concentration at ground level. It is not a theory, which tries to describe the physical mechanisms and processes behind the observed INP. Thus, this parameterization is not an explanation of the complex processes that result in observed INP occurrences. This formulation can still be useful as a technical basis for atmospheric models, which could help to improve the representation of atmospheric INP concentration by using the ground-level ambient temperature. The models will not manage to describe the real physical processes behind aerosol particle behaviors as this is very complex and any model cannot resolve the involved time scales. We therefore suggest this parameterization to technically describe INP concentrations in models and to test it and develop it in further studies.

We added "ground-level" to all passages where we discuss the ambient air temperature to make clear, that we do not use ambient air temperature in other higher altitudes. Thanks for this comment!

L311 – What is T in this equation?

Thanks for this question. T is the activation temperature of the ice-nucleating particles. As this explanation is missing in the manuscript, we have added the following text right after Eq. (2):

[…], where T is the activation temperature of INPs.

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