Answers to Anonymous Referee #1:

(In the following, Referee comments will be in italic, our answers in regular letters. Changed Figures are in the end of the main text and the labels are the same as in the manuscript)

The authors used the Leipzig Aerosol Cloud Interaction Simulator (LACIS) to study the freezing of washings from two different types of pollen: Northern birch and Southern birch. Freezing of biological material, such as pollen, may be important for ice cloud formation, and hence the current topic is an important one and well suited for Atmospheric Chemistry and Physics. However, I have concerns about the interpretation of the data. Also, the atmospheric implications of the new results are not developed much beyond Pummer et al. 2012. Because of this I don't think the manuscript, in the current state, meets the high standards of Atmospheric Chemistry and Physics. I feel this manuscript is more appropriate for a lower impact journal.

The authors of the manuscript thank the Referee #1 for useful remarks and suggestions. However, we were surprised to read that the impression arose that the work presented in this manuscript did not develop new results and implications beyond the work by Pummer et al., ACP, (2012).

Pummer et al. (2012) showed that washing water from different pollen can induce immersion freezing as good as the pollen themselves and that the IN are not bacterial material (as e.g. proteins) but ice nucleation active (INA) macromolecules. In contrast to that this new study used the information on the existence of INA macromolecules and quantified their ice nucleation behavior. Results are reported as heterogeneous nucleation rates for two different types of single INA macromolecules. These rates are generally valid and can be used in larger scale weather and climate models to describe the freezing of droplets containing these molecules.

Derivation of such a nucleation rate based on the data published in Pummer et al. (2012) is not possible. Oil emulsions in a cryo cell were used in those measurements. This method examines comparably large droplets which did contain large numbers of macromolecules, where these large numbers generally cause freezing to occur at higher temperatures. While the method used in Pummer et al. (2012) is still a powerful method, LACIS, which was used to derive the data for the manuscript under discussion here, offers a way to examine freezing of droplets for conditions closer to those in the atmosphere. The advantages of LACIS are that it allows for the examination of freely floating droplets, containing only one solid or probably dissolved particle. Furthermore the droplets are in the size range which can be expected to occur in the atmosphere. In this respect, it should be underlined that it was very important that not all of the generated particles (on which then the examined droplets were activated) contained an INA macromolecule (which shows up in the plateau regions where no further increase in ice fraction with further cooling was observed). This is a key point which only enabled us to derive the nucleation rate for single INA macromolecules, assuming the INA macromolecules to be Poisson distributed over the particles. As mentioned above, these highly useful nucleation rates cannot be obtained from the data presented in Pummer et al. (2012), due to the large and unknown number of INA macromolecules per droplet. Also a newly developed method (CHESS model) was applied to derive the nucleation rates. Hence our work is certainly new and carries

description of immersion freezing behavior of pollen (applicable for the atmosphere) one step further than it has ever been done before.

1) Abstract, Line 15. Heterogeneous nucleation rates do not explain the ice nucleation behavior. Please restate.

Thanks, we replaced “explain” by “describe”.

2) Material and methods. Page 32915, Line 10. The authors indicate that the sample contained pollen. Does it contain anything else? Did the company do any processing of the sample? More history of the samples would be useful.

We added the following to the text (page 3, line 82 in the new manuscript):

“The pollen samples were cleaned by the companies through size separation in a cyclone. The final product meets the purification degree necessary for pharmaceutical use (as these samples are usually used by allergologists). We requested that no further treatments (e.g. defatting) were carried out to keep the sample as natural as possible. The birch pollen considered in our study originated from the same badge as the birch pollen used in Pummer et al. (2012).”

3) Page 32917, Line 6. “Especially for 500 nm and 800 nm particles, the ice fraction was analyzed as a function of temperature in a range from -18C to -35 C.” What do the authors mean by “especially”? Did they do a different analysis for 500 and 800 nm particles? Why only focus on these sizes? Please explain.

“Especially” was used here only to highlight that for these sizes more measurements were done than for the others, but to eliminate sources for confusion, the word was deleted.

The choice of these two sizes happened by chance, to a certain degree. In general, it is easier to measure with larger sized particles, because the number of INA macromolecules and hence the frozen fraction increase with size, i.e. measurement uncertainties decrease. On the other hand, 800 nm is close to the upper size limit which can be selected with our instrumentation (DMA, differential mobility analyzer). Two different sizes were chosen to show that the nucleation rate we derive indeed agrees for differently sized particles (see Fig. 4 in the manuscript), i.e., that the same nucleation rate could be determined based on any particle size (provided that a plateau is observed below a certain temperature down to the homogenous freezing).

This is the strength of our study: the occurrence of plateau regions enables us to determine the average number of INA macromolecules in the particles / droplets by assuming Poisson distribution and this can then be accounted for when deriving the nucleation rate, i.e. the nucleation rate of a single INA macromolecule is obtained. With this, results should be and are independent of the particle size considered.

4) For Southern birch pollen the authors suggest that the ice fraction curves level off to constant values, indicating a saturation behavior of the immersion freezing process. However, I only see a leveling off for the 800 nm particles. The others sizes do not appear to reach a plateau.

Values of the frozen fractions for the 300 nm particles are 12.5% and 15.8% at -27°C and -35°C, respectively, and for the 500 nm particles they are 30.9% and 34.2% at these two temperatures. This difference is well within measurement uncertainty. The impression that there is a plateau for the 800 nm particles but not for the smaller sizes might be invoked by the logarithmic scale. Indeed, the values for the frozen fractions of the 800 nm particles at -27°C and -35°C are 63% and 70%, respectively. Also as ice nucleation is very sensitive to temperature a change in the ice fraction of 3-7% over a temperature range of 10 K is extremely small. So we can assume that there is no significant increase in freezing with decreasing temperature. However, error bars indicating the measurement uncertainty are now added to Fig. 2 (as well to all other figures displaying frozen fractions or nucleation rates). Additionally the following explanation was added to the new manuscript on page 5, line 128:

“Error bars given in Fig. 2 (as well as in all other figures) are standard deviations in those cases where ice fractions were measured at least on three separate occasions for a specific particle size and temperature. However, such a standard deviation could not always be obtained. In these cases, we report error bars based on counting statistics of TOPS-Ice (the number of droplets counted per measurement, which was at least around 2000), which, from our former experience, are equal to or smaller than those based on standard deviations from repeated measurements.”

5) Page 32918, Line 18-20. The authors should also point out that another possible explanation for their surface area dependence could be that there model (only a single IN) is incorrect.

We see two ways how to interpret your remark about “(only a single IN) is incorrect”, but both cannot be used to explain the observed surface area dependence:

i) Do you suggest that there might be more than one kind of IN present in our sample? The average number of INA macromolecules per particle / droplet (λ) is independent from the fact if there is only one or if there are many different kinds of IN. λ only reflects how many IN (more correct: INA macromolecules) are there per particle / droplet, on average. When something is Poisson distributed this basically means that a small number of “something” (INA macromolecules in our case) are distributed over a larger number of “something else” (in our case the particles / droplets). If there were several different kinds of IN, all of these would be randomly distributed, and all sizes of particles / droplets would have all of these different kinds of IN in them. Hence this cannot explain how λ depends on size.

The fact that we observe plateaus and that λ derived from them generally increases with particle size shows, that assuming a Poisson distribution in our case is valid. The fact that λ scales

slightly better with surface than with volume is an indication towards the fact that not the whole particle volume becomes “active”, which might come from the fact that the particle does not dissolve. This was carefully given as a possible explanation in the text (page 32918, line 16 in the original manuscript: “A possible explanation for the surface area dependence could be, …”), so that we don’t think that we need to weaken this statement further.

ii) Or alternatively, do you think we imply that each examined particle / droplet contains exactly one single INA macromolecule? This is not the case. Poisson distributed means that some particles / droplets contain no INA macromolecule, some contain one, some two etc. λ gives the average number, and from Poisson distribution it can be derived how many particles / droplets are there with none, one, two etc. INA macromolecules in them. Knowing λ, however, later on enables us to derive the nucleation rate for a single INA macromolecule, but this is independent on how λ scales with particle size.

However, the most important thing is that we could calculate the average number of IN per droplet which is necessary to determine the heterogeneous nucleation rate of single INA macromolecules.

6) Page 32920, Line 26. The data doesn’t show that the material does not dissolve incompletely. This is only one possible explanation. Please restate

We rephrased this sentence to:”Again, as for the Southern birch, one possible explanation for this behavior can be that particles produced from the Northern birch pollen washing water dissolve only partly when being activated inside LACIS.”

7) Page 32922, Line 17-18. “In our case we observe that the particles of the Southern birch pollen washing water show one mode.” For the 800 nm particles I see two possible modes, one at -24 C and one at -26.5 C. Furthermore If I look at Figure 5 it looks like a model with two different types of IN would describe the data better.

Error bars are now added to Fig. 9 in the revised version (as well as to all other figures displaying frozen fractions or nucleation rates). With this, Fig. 9 now more clearly shows the validity of our assumption concerning the occurrence of one mode for the Southern birch pollen washing water (and two modes for the Northern sample).

Also, when taking into account measurement uncertainties, comparison between measured and modeled ice fractions shows that they agree well, already when parameterizing the derived nucleation rates with a simple exponential equation (parameters A and B). Additionally, the fact that the parameterization for the Southern birch pollen could be used together with assuming only one additional kind of IN to describe the Northern birch pollen, further strengthen our assumptions (one type of IN for Southern birch, two types for Northern birch).

Additionally in the new version of the manuscript we added model calculations using a contact angle distribution (as introduced in the Soccerball model (Niedermeier et al., 2011)). With this

approach the ice fraction could be represented even more accurately especially in the temperature range between -24 °C and -28 °C (see Fig.5 in the new manuscript). So it can be said that the slight change in the slope of the ice fraction near the saturation range for the Southern birch pollen washing water is due to the width of the contact angle distribution and not due to a second kind of IN.

In principal, it is always possible to fit data the better the more free parameters are available, but one also should always prefer hypotheses that make the fewest assumptions (Occam's razor). Hence we prefer not to change our assumptions here.

8) Page 32923, Line 3. "This supports the assumption of two differently behaving INA macromolecules : : :" I think this should be changed to "this supports the assumption of at least two differently behaving IN macromolecules: : :" I would guess a model with three different types of INA macromolecules would also fit the data and probably better.

We did change the wording in the manuscript as you suggested (i.e. added "at least"). Otherwise, our answer to your remark 7) applies here as well. Additionally, we inserted some text responding to your remark 10) (see below), which explains why the existence of many different ice nuclei on one species, while there are none on other species, is unlikely.

9) 32924, Line 0-10. The authors discuss how the slopes of the heterogeneous nucleation rates are connected to the distribution of the ice nucleation related properties (e.g. contact angles) of the whole INA macromolecule population. I am confused by this discussion. For the Southern birch the authors assume only one type of INA. How can they have a distribution of ice nucleation related properties if the INA are all identical? Maybe I am missing something here?

Indeed we do assume that there is only one type of INA macromolecules present on the Southern birch pollen, however, these macromolecules do not all have to be all absolutely identical. Small variations might occur e.g. in their folding (see our discussion part which is topic also of your remark 10). We even already had said (right before equation (4) in the old version of the manuscript):" It should be noted that this nucleation rate represents an average over the individual nucleation rates of all macromolecules, present in the particle population".

To make it clearer that not all INA macromolecules on the Southern Birch pollen are identical, we added: page 6, line 189: "With this, J_het now represents a nucleation rate that can be interpreted as being representative for the kind of INA macromolecule which occurs on the Southern birch pollen."

Following one of your remarks, we also added model calculations to the manuscript which show the results for assuming a contact angle distribution (instead of using the simple exponential fit with parameters A and B, which is similar to assuming only one contact angle). For that, please check our answer to your remark 13).

10) 32924, Line 20. Please provide some justification for the statement "unlikely".

The respective entry in the text (page 11, line 342, in the new manuscript) was changed to:

"As the expression of certain macromolecules in a living being is the result of a long-term sequence of spontaneous mutations, it is in principal possible, but unlikely, that one species developed several completely different very active ice nuclei independently from each other. For example, bacterial ice nucleation can be tracked down to one specific type of a proteinaceous compound (Lindow, 1995). More likely an already existing possibility to express one type of INA macromolecule (i.e. a gene) was altered by additional mutation, leading to the expression of a somewhat different macromolecule which, however, still is ice active."

(Lindow, S. E.: Membrane fluidity as a factor in production and stability of bacterial ice nuclei active at high subfreezing temperatures, Cryobiology, 32, 247-258, 1995.)

11) Page 32925, Line 20-27. In your experiments did the pollen grains burst? If not, is this discussion relevant to your experiments? In the experimental section it sounds like it is only surface molecules that are dissolved in the washing waters. Please clarify what material is being incorporated into the washing water.

This paragraph tries to give a link to atmospheric conditions. It does not matter for our experiments if the pollen grains burst or not, and hence if the material in our pollen washing water is released from the pollen surface or from its interior. However, the fact that pollen can burst is important in the atmosphere.

We added to our experimental section (page 4, line 94): "Additionally, pollen grains may also burst upon contact with water (e.g. Schäppi et al. (1999)), so our suspensions might also contain material from the pollen interior."

12) Page 32927, Line 5. "From that we conclude that the INA North is more homogeneous with respect to the ice nucleating related properties than the INA South." I don't understand this statement. I thought the model for INA south assumed only one type of INA?

Please check our answer to your remark 9). Also, when looking at the derived contact angle distributions, the standard deviation σ of the distribution, which indicates how homogenous a particle population is with respect to its ice nucleating properties, is smaller for INA North than for INA South, which corroborates our statement here (σ_{South}= 4.6° and σ_{North}= 0.06°).

13) Could the data be fit equally well with other models, such as a distribution of contact angles?

Yes, this can be done, and we show this now. In the revised version of the manuscript, we now additionally use a contact angle distribution (in addition to using the simple exponential fit with

parameters A and B as we did previously, see new text passages below and changed figures). The measured ice fractions are described even better (see Fig. 5 and Fig.10). However, the introduction of contact angle distributions for parameterizing ice nucleation behavior implies a higher degree of complexity that may not always be required and useful. Therefore, we will continue applying the simple exponential fit as method for parameterization.

new text in lines 195 to 234 on page 7:

“Note that the expression given in Eq. (5) represents a simple way to parameterize the heterogeneous ice nucleation rate as a function of temperature assuming that all INA macromolecules feature the same ice nucleating properties. However, at low T (T < -22 °C, see Fig. 4), the actual nucleation rate is slightly over predicted by this simple parameterization (compare nucleation rate determined from Eq. (5) (straight red line) with the rate which is directly calculated from the measured f_ice (colored dots)). Additionally, we compared the temperature dependence of the derived heterogeneous ice nucleation rate parameterization (Eq. (5)) with that determined applying Classical Nucleation Theory (CNT, e.g., Zobrist et al., 2007) using a single contact angle (dark gray line in Fig. 4). We found slightly less pronounced temperature dependence for the parameterized nucleation rate. From Niedermeier et al. (2011b) it can be concluded that this is most likely due to the investigated INA macromolecules not having fully identical ice nucleating properties. This heterogeneity of the INA macromolecules could imply both, variations in size and/or structure. In other words, although we are investigating the ice nucleation behavior of single INA macromolecules that does not necessarily imply that all INA macromolecules are fully identical (a detailed discussion concerning possible macromolecule differences leading to slight different nucleating properties will be given in the discussion section). Therefore, the given parameterization for the nucleation rate has to be considered as an average over the individual nucleation rates of all INA macromolecules, present in the particle population.
We considered a more sophisticated parameterization based on the Soccerball model (SBM), which combines the CNT with the assumption of a contact angle distribution (Niedermeier et al.,2011b). In contrast to its original method presentation in (Niedermeier et al., 2011b), the number of sites per particle is replaced by the average number of INA macromolecules per droplet, namely λ. Then, with the assumption of the size of a single INA macromolecule to be Dp = 10 nm (Pummer et al., 2012 estimated its size to be about 300\,kDa in maximum), the Soccerball parameters, i.e., mean contact angle μ and standard deviation σ could be determined: μ = 58.2° and σ = 4.6°. The resulting parameterized nucleation rate is shown in Fig. 4, too. It can be seen that the Soccerball parameterization represents well the measurement based nucleation rate within the entire temperature range investigated. With the calculated average number of INA macromolecules λ and the parameterized heterogeneous ice nucleation rate J_het (by the simple exponential fit as well as by the SBM), we can now determine the ice fraction f_ice as a function of temperature for different particle sizes which means for different λ values. In Fig. 5, the results for 300 nm, 500 nm and 800 nm particles are shown as straight (simple exponential fit) and dotted lines (contact angle distribution). Both, the increase of the ice fraction with decreasing temperature and the different saturation ranges of f_ice* of the differently sized particles could be simulated by both parameterizations, whereas the SBM parameterization could represent the data slightly more accurate which is also shown by the least mean square (see caption of Fig.5). To summarize, we are able to consistently describe

the ice nucleation behavior of the Southern birch pollen washing water by assuming the number of INA macromolecules to be a function of initial particles size and by an ice nucleation rate, either parameterized by a simple exponential fit or by a more complicated distribution of contact angles"

lines 292 to 296 on page 9:

"Again also the Soccerball model was used in a similar way by including the parameterization for the contact angle distribution found for the Southern birch pollen washing water to represent INA South and combine this with another contact angle distribution for the INA North. Then Eq. (7) is also fitted to the measured ice fraction of the 800 nm and 500 nm particles.
The results and parameters are shown in Fig. 10."

lines 329- 334 on page 11:

"Additionally we used the Soccerball model as introduced in Niedermeier et al. (2011b) which includes a distribution of contact angles to fit our data and found differences in the contact angle distribution for INA South and INA North with INA North showing a lower mean contact angle and a much smaller standard deviation. That indicates that the INA North is a more ice active and a more homogeneous INA macromolecule concerning the ice nucleating properties than the INA South."

lines 437- 442 on pge 14:

"The application of the Soccerball model (Niedermeier et al., 2011b), which regards a more accurate, however, also more complicated parameterization method, showed a similar result. The contact angle distribution of the INA North showed a slightly lower mean value μ and a much smaller standard deviation σ than INA South. From that we concluded that the INA North is more homogeneous with respect to the ice nucleating related properties (i.e. contact angle) than the INA South."

And new figures 4, 5, 10 and 11

14) How would one use the heterogeneous nucleation rates determined in this paper to predict freezing in the atmosphere?

To tackle this question, we have added a paragraph at the end of chapter 4 (page 12, line 384) which describes in more details how the determined nucleation rates can be used in models.

"Concerning the possibility to use the here gained nucleation rates for atmospheric purposes, we want to point out that the presented nucleation rates themselves already allow for the dynamic simulation of heterogeneous ice nucleation induced by the INA macromolecules in cloud or larger scale models. The nucleation rates then would e.g. represent the source term in the differential equation describing the time evolution of the number concentration of frozen droplets. Alternatively, CHESS model and nucleation rate together could be used for describing ice

nucleation by INA macromolecules in larger scale models. This approach allows for the determination of frozen droplet fractions for a given temperature, time and λ without solving a differential equation.

In this context some additional remarks: The term (1-exp(-j_het*S_site*t)) in Eq. (4) represents the cumulative distribution function of the exponential distribution. This implies that τ_ice = 1/ (j_het*S_site) =1/ J_het corresponds to the characteristic time of freezing of a droplet population for a given nucleation rate J_het. As the nucleation rate J_het depends on temperature, this characteristic time for freezing is a function of temperature as well. For the birch pollen considered in this work, characteristic freezing times are as follows: In case of INA North (INA South) at T = -16°C, τ_North = 268s (τ_South = 68s); at T = -18°C, τ_North = 1s (τ_South = 13s) and at T = -20°C, τ_North = 0.003s (tau_South = 2.5s). As to be expected, characteristic times for freezing decrease with decreasing temperature. Such nucleation rate based characteristic freezing times could be considered in order to appropriately set the time-steps in atmospheric modeling applications and/or to judge, if for a given time step, a **time independent** (singular) treatment of the freezing process is possible. In the latter case, the frozen droplet fraction could be easily determined from the CHESS model by the limit of eq. 4 for time t to infinity, i.e., f_ice = 1-exp(-lambda). In other words, based on lambda only, the frozen droplet fraction can be determined if the nucleation process is fast compared to the model time step (τ_ice is smaller than approximately one third of the model time step).

Both approaches (nucleation rate alone and CHESS model together with nucleation rate) require information concerning the number of INA macromolecules present in the droplet population. This number could be easily determined having particle number and mass concentrations as model variables, and e.g. number of INA macromolecules per particle mass as a parameter. However, it should be noted that the number of INA macromolecules per particle mass is currently a parameter which is not known. But following what we said above, concerning the abundance of INA macromolecules, they might play a non-negligible role in atmospheric ice nucleation and hence research which helps to set limits on their abundance is strongly needed.”

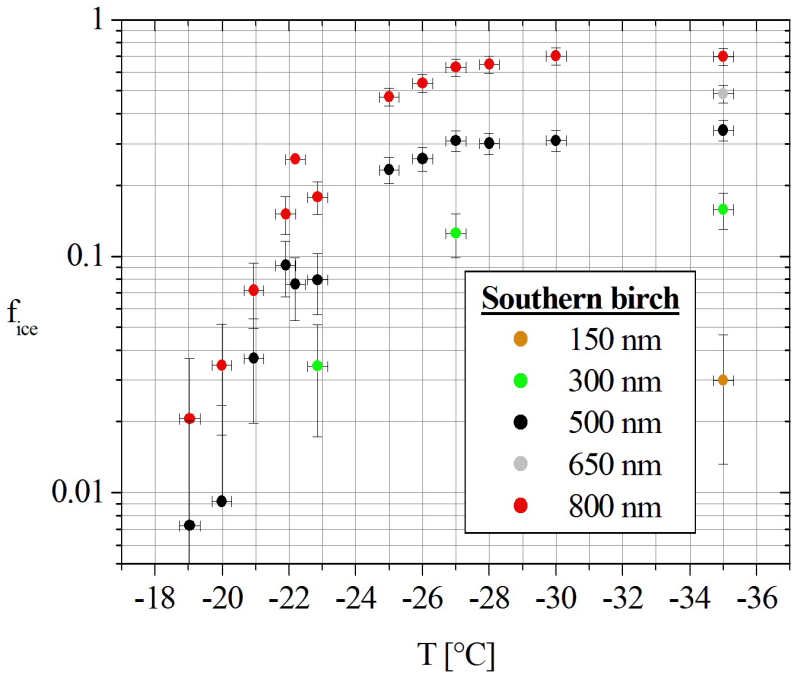

Figure 2: f_ice as function of temperature T for 150, 300, 500, 650, 800 nm Southern birch pollen washing water particles.

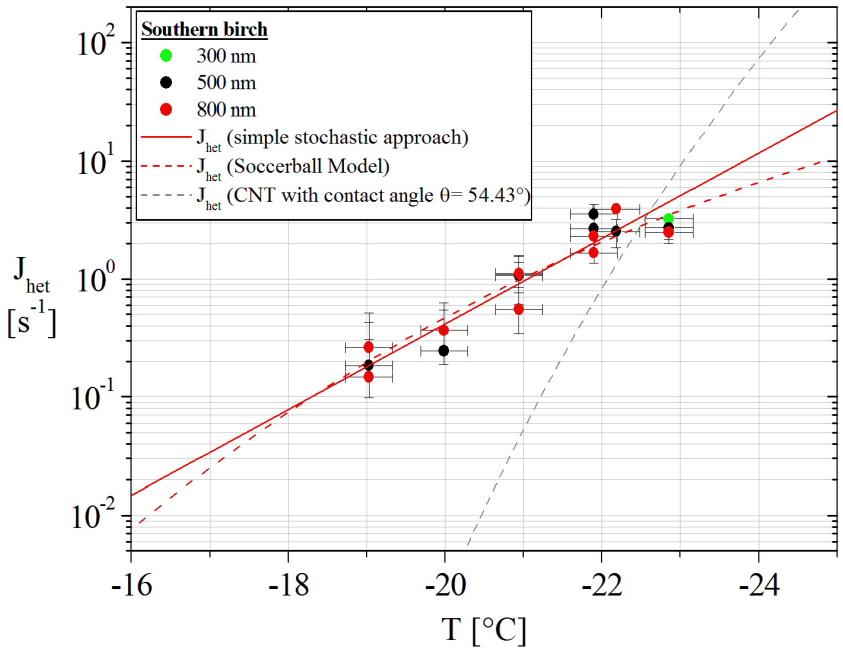

Figure 4: Heterogeneous ice nucleation rate (J_het) for 300 nm, 500 nm and 800 nm washing water particles of Southern birch pollen washing water as function of temperature T (colored dots) and parameterized J_het. straight line: simple stochastic approach with A=2.320 · 10^{-8} and B=-0.835 °C^{-1}, dotted line: SBM with μ= 58.2° and σ= 4.6°.

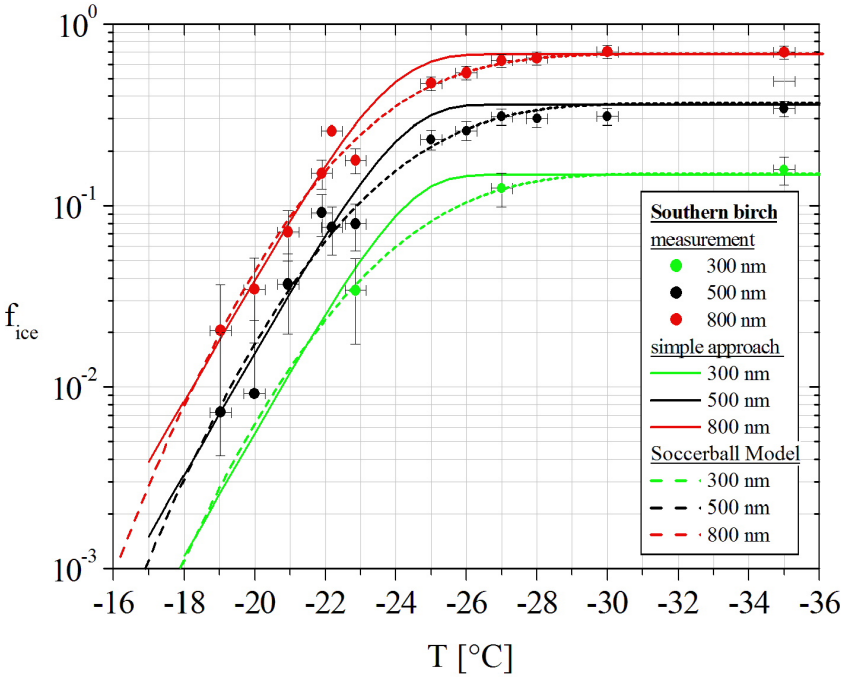

Figure 5: Ice fraction f_ice as function of temperature T for 300 nm, 500 nm and 800 nm Southern pollen washing water particles (dots) and model calculations for the different sizes (lines) thereby J_het is represented by a simple stochastic approach (straight line) and by the SBM (dotted line) (see Fig.4). Least mean square for 300 nm, 500 nm and 800 nm particles are 0.144, 1.180 and 0.760 for the simple stochastic approach and 0.004, 1.020 and 0.428 for the SBM, respectively).

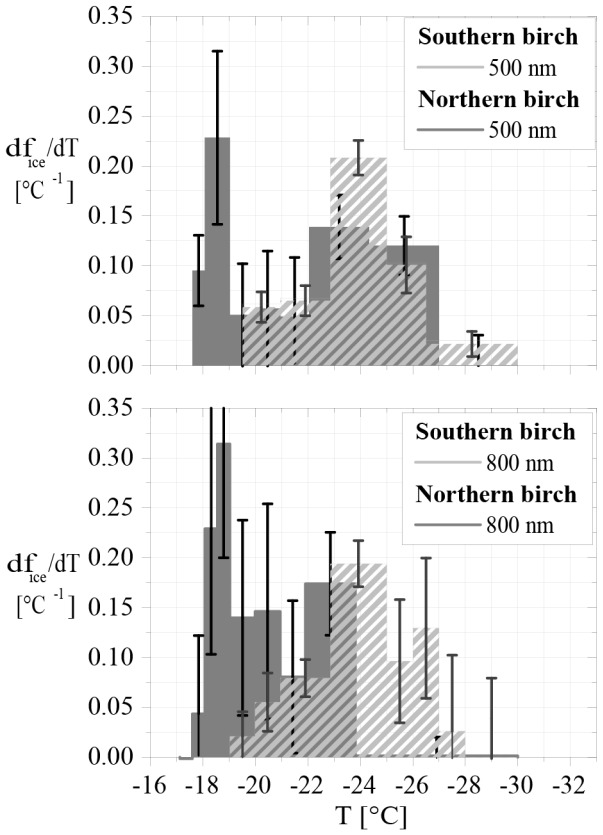

Figure 9: Change of the ice fraction f_ice} per temperature interval for the 500 nm (upper panel) and 800 nm (lower panel) particles of the Southern birch pollen washing water(light gray) and the northern birch pollen washing water (dark gray)

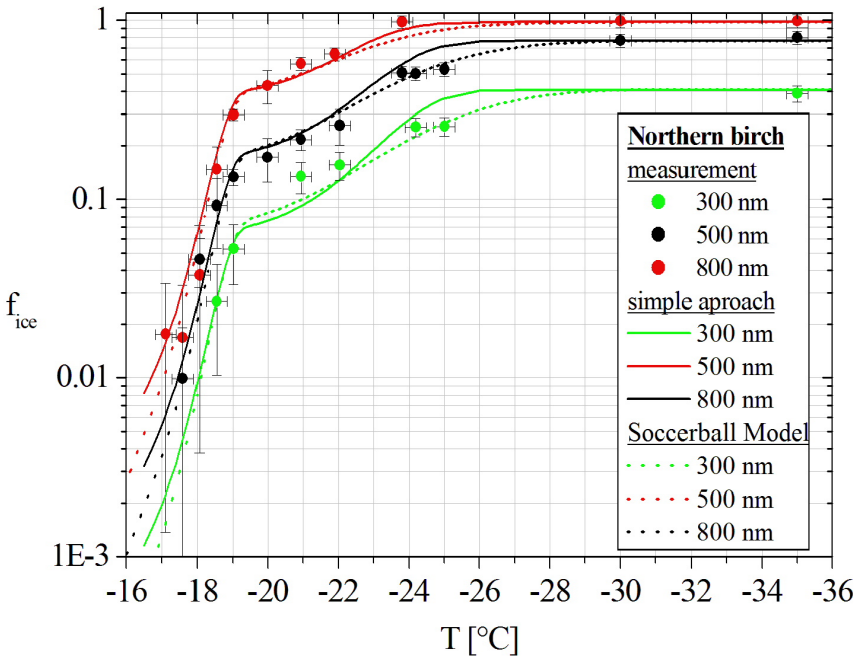

Figure 10: Ice fraction f_ice as function of temperature T for 300 nm, 500 nm and 800 nm Northern pollen washing water particles (dots) and model calculations for the different sizes (lines). The model calculation results from the combination of the Southern INA macromolecules and the Northern INA macromolecules. Therefore the parameterizations found for the Southern INA macromolecules (see Fig. 5) were used. The parameterization for the Northern INA macromolecules are: A=9.186 · 10^{-23} and B=-2.822 °C^{-1} for the exponential fit of J_het (straight lines), and μ= 47.8° and σ= 0.0573° for the SBM (dotted line).

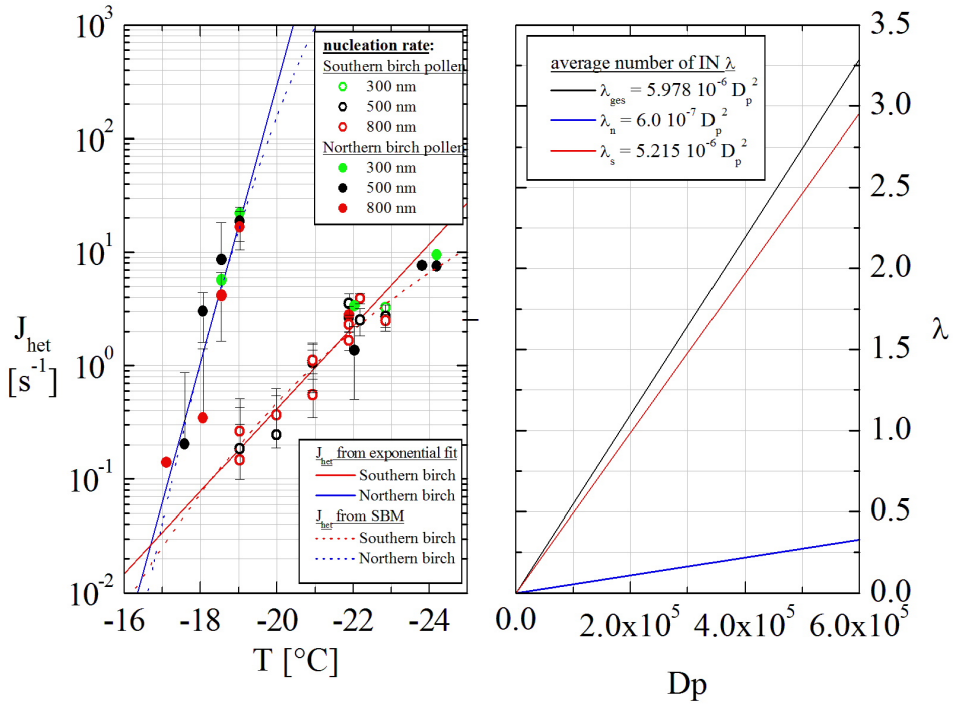

Figure 11: Left panel: parameterizations of J_het of INA North (blue lines, parameters in Fig. 10) and INA South (red lines, parameters in Fig.4). Additionally the J_het values from the measurements are plotted as colored dots and circles. right panel: λ_n, λ_s and λ_ges of the Northern birch pollen washing water.