Answers to Anonymous Referee #2

We thank the Anonymous Referee #2 for reviewing our manuscript and hope to clarify the questions raised in this review in the following text. Comments of Referee #2 are 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 word macromolecule is used a total of 61 times in the manuscript and assertions are made about their abundance and nature in different species. This paper however can hardly make assertions about macromolecules. The notion that macromolecules cause freezing is by reference to past work and in most places the authors simply speculate. All the authors studied were atomized birch pollen extract that was aged overnight inside a refrigerator. The IN activity may emanate from bacteria that are associated with the pollen, bacteria that grew in the extract overnight, contaminants that came with the purchased samples, or even dust that has settled on the pollen. It is interesting to mention what the authors believe to be true based on earlier work but in absence of clear evidence on the nature of the IN obtained via chemical analysis of the particle residues the discussion of the results and conclusions should limit itself to the simple fact on what was done (atomizing washed pollen extract).

We do not fully agree with the reviewer here, but instead will explain why we think that indeed we are entitled to use the word macromolecules when referring to the ice nucleating entity present in the pollen washing water.

The pollen samples we examined in our study were identical to the one examined by Pummer et al. (2012, ACP). This had not been said explicitly in the first version of the manuscript and was added to the revised version, together with some additional information on how the pollen samples were processed (see page 3, line 82 in the revised version and answer to question 2 of Referee one):

“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).”

Also, the generation of the washing water was identical to what has been described in Pummer et al. (2012, ACP) (Bernhard Pummer himself taught us how to prepare the solutions in our laboratory). Therefore it is safe to rely on the analysis and conclusion concerning the nature of the ice nucleating entity presented in Pummer et al. (2012, ACP). There it was clearly shown that the ice nucleating entities present in the pollen washing water did e.g. not originate from bacteria (where proteins are responsible for inducing the freezing), as the ice nucleation ability of the pollen washing water did hardly degrade when heated to temperatures above 100 °C (the bacterial ice nucleating proteins would loose their ice nucleating ability under these conditions).

Instead, the macromolecules (with a mass between 100 and 300 kDa) that we refer to so often were identified as causing the ice nucleation. Furthermore, contaminations with dust particles can also be excluded, as dust particles of sizes similar to those examined have been measured in LACIS and were found to start inducing freezing only at temperatures well below -25°C.

The authors claim that their model can explain the IN behavior of the samples (e.g. pg. 32920). However, all the authors are doing is fitting their data with a model. When the Northern Pine did not confirm to the model, they simply changed it to include another population of particles. A more accurate description of the methodology would be "we are able to fit the data assuming …

Thank you for pointing this out. The use of the word "explain" is certainly misleading, and we consistently changed it to "describe".

It was pointed out by referee #1 in the initial unpublished review that it is unclear that Figure 8 shows indeed two different slopes and hence two different IN populations. Perhaps there are two populations, but I find that the authors have too much faith in their data. For example DeMott et al. (2011, BAMS) report data from an ice nucleation workshop where all participants sampled the same aerosol using different methodologies. Examining their Figure on Sahara Dust should make it clear that small changes in f_ice or freezing temperature are unlikely to have meaningful physical interpretations until the community can substantially improve the accuracy of IN measurements. Perhaps LACIS is indeed superior to all of the other techniques, but the onus is on the authors to present a convincing argument why this would be the case. The observed second population may simply be due to a different size distribution and multiply charged particles (not accounted for here) in the sample, or different densities of IN active substances in the particle. It could be due to more unaccounted frost falling from the walls or the presence of more IN inhibiting substances in the matrix of the washing water. To my eyes the difference in the freezing temperatures are within a couple of degrees

Indeed, frozen fractions (f_ice) are prone to measurement uncertainties. However, these uncertainties can be quantified and propagated, and in the revised version of the manuscript we now included error bars e.g. in Fig. 9. It can be seen that our assumption that Southern birch carries one IN population while Northern birch carries two still holds. Additionally to this evidence, it also becomes clear when comparing Fig. 8 to Fig. 10 that the assumption of two IN populations for the Northern birch leads to a better agreement between modeled and measured frozen fractions over the whole temperature range. The revised version of our manuscript will additionally display error bars for the measurements (for all figures which display f_ice or nucleation rates). This gives additional information about the accuracy with which LACIS can measure.

As for the measurement artifacts you mention:

The whole measuring procedure (particle production and size selection as well as the freezing measurements with LACIS) was the same for both birch pollen samples. Measurements were

repeated more than once. When frost starts to fall from the LACIS walls, this is usually obvious and it denotes the end of a LACIS measurement. It is unreasonable to believe that measurements of the Northern birch should always have been influenced by wall effects while those of the Southern birch should not have suffered from this artifact.

Concerning different densities of IN active substances: wouldn't this mean that the Southern birch pollen did not have these different substances while the Northern birch pollen did, i.e. this argument rather goes along with our argumentation instead of against it.

Size distributions for particles generated from the pollen washing water of the Northern birch were measured (not shown in the manuscript) and maximum was observed at about 100 nm, i.e. multiply charged particles should not have played a large effect, and should, if at all, have affected both samples in a similar way.

In summary, we tried to give convincing arguments here and in the revised version (in the latter mainly based on added error bars), that LACIS measurements indeed are precise enough to distinguish between one and two separate IN populations in the Southern and Northern birch pollen washing water, respectively.

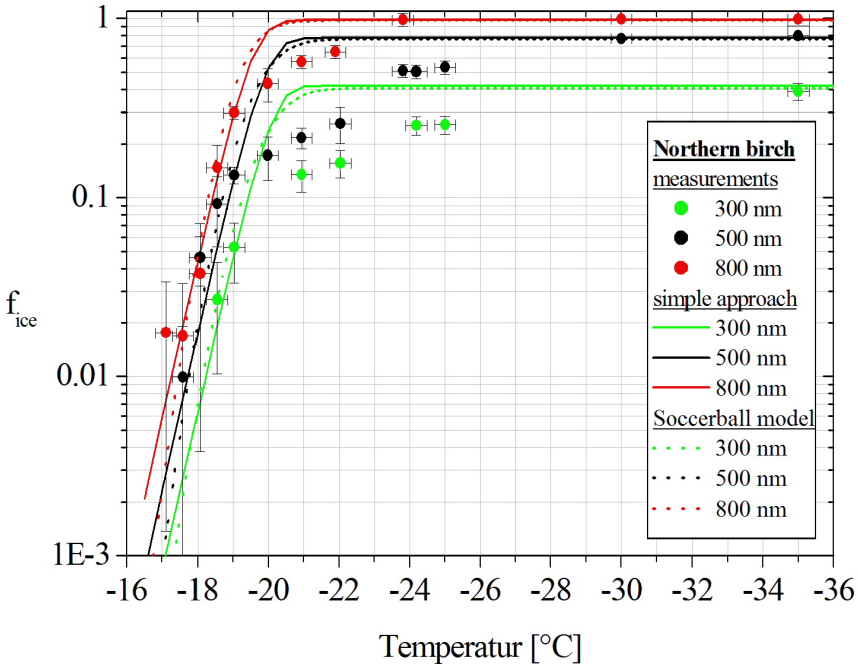

Figure 8: Ice fraction f_ice as function of temperature T for 300 nm, 500 nm and 800 nm Northern pollen washing water particles (dots) and model calculations for the different sizes (lines) assuming only one INA

macromolecules being present in the Northern birch pollen sample. The mean square errors between the measured and the calculated ice fraction for the 300 nm, 500 nm and 800 nm particles showed values of 7.22, 14.47 and 2.58 for the simple approach and 6.19, 14.70 and 2.50 for the SBM, respectively.

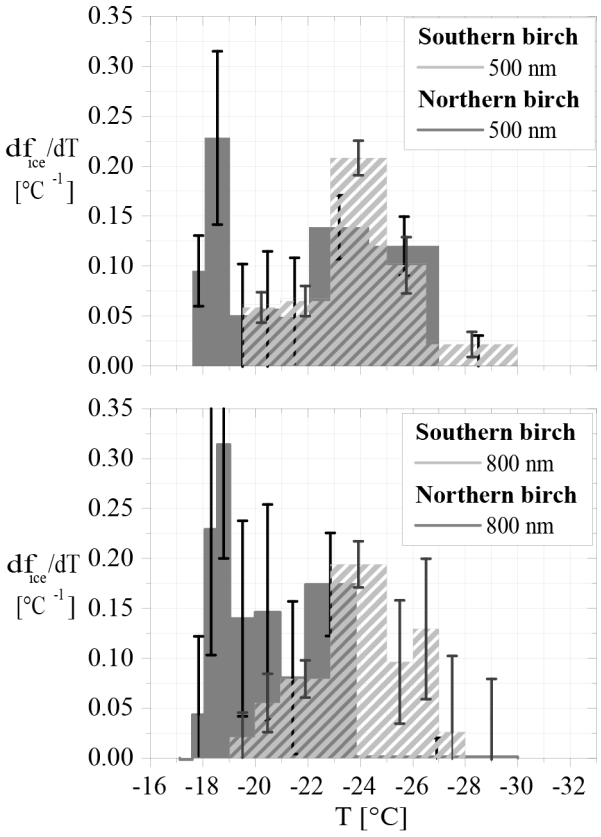

Figure 9: Change of the ice fraction f_ice} per temperature interval for the 500 nm (upper panel) and 800 nm (lower panel) particles of the Southern birch pollen washing water(light gray) and the northern birch pollen washing water (dark gray)

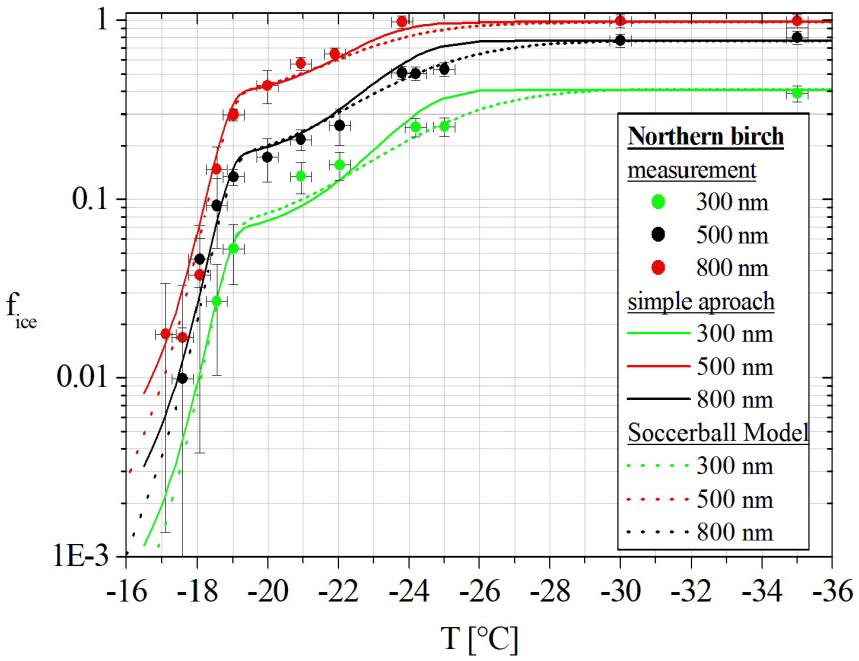

Figure 10: Ice fraction f_ice as function of temperature T for 300 nm, 500 nm and 800 nm Northern pollen washing water particles (dots) and model calculations for the different sizes (lines). The model calculation results from the combination of the Southern INA macromolecules and the Northern INA macromolecules. 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).