Response to referees and short comments

We thank the two anonymous referees and Dr. Vali for their careful reviews. We also thank Dr. Jaenicke and Dr. Grothe and co-workers for their valuable short comments. This document contains responses to the reviews and short comments and details how the manuscript has been altered to address the comments. The remainder of this document is structured as follows.

Referee #1 (Gabor Vali) pg. 2-5

Referee #2 (anonymous) pg. 6-18

Referee #3 (anonymous) pg. 19-25

Short comment #1(Jaenicke) pg. 26

Short comment #2 (Grothe) pg. 27-30

Revised Figures 3-8 pg. 31-38

We have identified the key issues raised in each comment and sequentially number them Issue #1 – Issue #n for each set of comments. Editorial comments are sequentially numbered as E#1 – E#n. Original comments are in *italic font*, our response is in roman font, and changes made to the manuscript are in **bold font**. We also provide a manuscript version with **changes tracked in bold font** that will be uploaded to the Copernicus manuscript system.

Response to referee comment by Gabor Vali:

Vali Issue #1: Although the paper deals specifically with pollen samples, the many procedural and conceptual factors that need to be considered in such work are well illustrated in this work and have broader applicabilities. Assessments of the background, and of the impacts of variations of the drop volumes have been done with care. Assumptions made in the analysis procedure and with respect to particle sizes are clearly stated.

The statement that pollen grains do not initiate freezing at temperatures warmer than -10C is, of course, relative to the concentration range observed. There is no indication of a significant change in the slope of the spectra at -10C so that more active ones can be expected to exist in yet lower numbers. Where would that extension stop is an interesting question. It is also an open question whether the foregoing statement regarding extrapolation is really valid or not. All of the spectra in Fig. 6 of the paper show somewhat steeper spectra at the warm end of the range of observations. This is to some extent a consequence of limited sample sizes (1 or 2 drops per temperature interval) but may also be a true pattern.

Response: We thank Dr. Vali for the positive feedback regarding the methodology and background corrections. Of course it is correct to note that our original statement: *Notably, pollen grains do not appear to initiate freezing at temperatures warmer than -10°C* (Pg 31688, Line 6) is relative to pollen surface area/volume probed in a particular experiment. It is an interesting and unresolved question whether pollen contain active sites that can in principle initiate freezing at warmer temperatures. We therefore revised the statement to account for that possibility:

Revision: Notably, pollen grains do not appear to initiate freezing at temperatures warmer than -10 °C for the range of experimental conditions probed in current studies.

Vali Issue #2: Assessment of what concentrations of pollen grains would have atmospheric relevance due to their ice nucleating ability can only be approached through cloud models and will have rather different answers depending on the cloud conditions assumed. Similar problems are associated with the conversion of measured concentrations of ice nucleating particles to concentration in the air from which the rain fell. Neither the many possible pathways for particles to get inside raindrops, nor the breakup, aggregation or dissolution of particles in the rain, can be considered at this level of analysis. The many qualifying words (rough, approximate, etc.) in the text are indications of these problems and are, unfortunately, truly needed.

Keeping in mind the various limitations of measurements of atmospheric ice nucleation activity, the correlation cited with large particles also need to be taken with caution.

Response: We fully agree that correlations based on size without information on particle surface composition and/or morphology may not be sufficient to prognose IN in global models and thus that caution is warranted. In this work we simply state that (1) the correlation has been observed, and (2) that it is currently used in climate models. It is only used as motivation why particles with $D > 500$ nm are important to capture with the freezing method. We revised the wording to state this more clearly (Pg 31691, Line 16).

Revision: Furthermore, supermicron size particles can be collected with the SAC and analysed in the cold-stage freezing assay. The ability to sample supermicron particles is desirable since particle number concentrations with $D > 0.5$ µm have been shown to correlate with IN (Geogii and Kleinjung, 1967) and are currently used to parameterize IN in global models (DeMott et al., 2010).

Vali Issue #3: So are comparisons between the measured values and 'typical' ones worldwide. The lack of increases during the pollen period in comparison with other times is a weak argument without the same technique being applied over extended time periods. Short-term correlations are also hard to validate or exclude with many other variables uncontrolled.

Response: We agree that the argument we present is not a definitive proof that the process of pollen bursting does not play a role anywhere around the globe. This cannot be achieved with a one month study and the limited scope of the experiments presented here. We do believe, however, that our approach is valid to test the hypothesis in principle, and that in our case the results are inconsistent with pollen providing a large source of IN in this particular case. We wholeheartedly agree that uncontrolled variables can muddy the picture. In fact, one of the sub-texts of our manuscript is some evidence for IN production following rainfall which interferes with our ability to cleanly separate between pollen bursting leading to copious IN production and fresh biological IN being released during rainfall. Clearly more studies are needed to fully account for the source and mechanism of biological IN.

Revisions: We clarified the background concentration and variability of IN at T = -20 ºC and further restrict the scope of the tested hypothesis. The revised paragraph is:

Pummer et al. (2012) acknowledge that pollen is typically rejected as a significant source of ambient IN concentrations. Emissions are episodic, concentrations are typically less than 1 L-1 and they strongly decrease with height so that only a few grains are entrained in updrafts that penetrate the mixed-phase cloud regime. However, Pummer et al. (2012) suggest that the impact of pollen on atmospheric clouds might have been underestimated due to the ejection of IN active macromolecules from the pollen grain. Our results, restricted to a single ecosystem with limited variation in meteorological conditions, are inconsistent with this hypothesis. For pollen or pollen-derived IN to be important in cloud processes they must contribute significantly to IN number concentrations beyond background levels. At T ~ -20 ºC a large number of observations around the world suggest that IN concentrations range between 0.5 L^{-1} and ~ 30 L^{-1} (Mossop, 1963; DeMott et al., 2010). Our values measured at the beginning and end of April, corresponding to before and after the peak pollen season, are well within that range. At ambient pollen grain concentrations of 1 L^{-1} and anticipating the release of 10s to 100s of macromolecules per grain, one would estimate maximum IN concentrations of 100-1000 L^{-1} at T ~ -20 ºC. Our measurements show that at the peak of the pollen season near a source of pollen there appears to be no such increase in IN concentrations. The absence of an elevated IN signal near a strong source at the peak of the pollen season underscores the fact that pollen emissions were likely too small to dramatically augment atmospheric IN spectra during the 2013 pollen season in Raleigh, NC. Similar methods need to be applied over extended time periods to further validate this finding and to account for the potential influence of other uncontrolled factors.

Revision required the addition of the following citation:
Mossop, S. C.: Atmospheric ice nuclei, Zeitschrift für Angew. Math. und Phys. ZAMP, 14(5), 456–486, doi:10.1007/BF01601253, 1963.

Vali Issue #4: *Comments in the Discussion about contact nucleation may need to be made more specific.*

Response: We clarified the section starting on Pg 31692, Line 10.

Revision: During measurements particles inside the drops can migrate to the water/oil interface via Brownian motion or gravitational settling and induce contact freezing from the inside. Inside-out contact nucleation has been hypothesized as a mechanism whereby the nucleation rate increases through surface crystallization when the IN moves into contact with the edge of the water droplet (Shaw et al., 2005; Durant and Shaw, 2005). It is not possible to distinguish between immersion and inside-out contact freezing modes of ice nucleation within our current experimental set-up when performing single run measurements. Evidence that this phenomenon could be occurring in our set-up has been documented in the past with experiments that included repeated freeze-thaw cycles (Wright and Petters, 2013; Wright et al., 2013). Since particles that induce freezing by the inside-out contact mode do so at a warmer temperature than identical particles that induce freezing by the immersion mode, contributions from the contact mechanism may lead to our results having a slight warm bias in the spectra (Durant and Shaw, 2005).

Revision added following citation to reference list:
Shaw, R. A., Durant, A. J. and Mi, Y.: Heterogeneous surface crystallization observed in undercooled water, J. Phys. Chem. B, 109(20), 9865–8, doi:10.1021/jp0506336, 2005.

Vali Summary: In all, the authors are on much more solid ground with respect to their measurements than in evaluating the atmospheric impacts of their results. This problem is not unique to this work and will only be improved with studies that are much more comprehensive, and go well beyond the likely possibilities of small groups. Within its limited scope, the paper is a good contribution and a useful contribution to the growing body of ice nucleation studies.

Response: We thank Dr. Vali for the overall positive feedback of this manuscript.

Response to Anonymous Referee #2

R2: General comments

The above mentioned article presents recent ice nuclei (IN) measurements during the pollen season 2013 in Raleigh, North Carolina, USA. Ambient samples were collected using a swirling aerosol collector and rainwater collection for analysis with a droplet freezing array. The authors investigate possible correlations between pollen concentration and ambient IN spectra obtained from experiments to compare with previous bioaerosol studies concerning this topic. Additional reference experiments with local long leave pine pollen as a major source and ATD are presented to support their technique.

Assuming an abundance of pollen in the ambient sample, such a clear correlation could not be observed. Furthermore a source of pollen multiplication processes is suggested to be unlikely for this study. The authors conclude the contribution of pollen on a global scale to be relatively low compared to background signals in such IN experiments. A low atmospheric relevance of pollen as IN is expected to be due to the low pollen number concentrations, the seasonality and the vertical distribution in the atmosphere.

The present paper addresses the need to investigate the atmospheric relevance of pollen as natural ice nuclei, which is of increasing interest in the community. Beside the novel experiments on ambient pollen as potential IN in the immersion mode, I question the procedure used to test the hypothesis the authors put forward. The reason for this concern is the processing of the ambient samples prior to measurement (for details see below). Thus, I only recommend this paper for publication in Atmospheric Chemistry and Physics after the following remarks are included:

Response: We thank the referee for the careful review and constructive critiques. We believe that our responses below clarify the procedures used to test the hypothesis.

Specific comments

R2 Issue #1: Abstract, Line 2-5: Do you suggest that IN active macromolecules are being dispersed during drying as well?

Response: Yes. We expanded the introduction to provide more details about the various proposed dispersal mechanisms (Pg 31676, Line 1).

Revisions: Grass pollen can produce cytoplasmic debris on contact with water by osmotic shock that can be separated from the pollen grain as micron- and submicron-sized starch granules and produce up to 700 starch granules per pollen grain (Suphioglu et al., 1992). A wetting-drying cycle consisting of changes in relative humidity from ~60% to >90% back to ~60% produces aerosolized fragmented cytoplasm (Taylor et al., 2002). The approximate size ranges of the released particles are between 0.2 and 5 µm. Similar mechanisms may release submicron particles from birch, alder, and hazel pollen grains (Grote et al., 2003). Because the ice nucleating activity may emanate from suspendable macromolecules that can be extracted from the pollen grain, Pummer et al. (2012) hypothesize that the aerosolized fragmented cytoplasm may lead to significant heretofore underestimated ice nuclei emissions.

Revision added following citation to reference list:
Taylor, P. E., Flagan, R. C., Valenta, R. and Glovsky, M. M.: Release of allergens as respirable aerosols: A link between grass pollen and asthma, J. Allergy Clin. Immunol., 109(1), 51–56, doi:10.1067/mai.2002.120759, 2002.

R2 Issue #2: Abstract, Lines 6-8: The 2013 pollen season How do you define the local pollen season (peak number concentration, pollen species,...)?

Response: We assign the pollen season to the time period between Apr-4 and Apr-27 during which pollen number concentrations undergo a clear cycle. The peak of the season is between Apr 8 and Apr 16 when pollen concentrations are near their maximum value. This was not defined in the manuscript. Historically in Raleigh, NC, during April the pollen counts during April increase by 50 fold with the majority of the pollen coming from trees (NC Division of Air Quality).

Revisions: Modified abstract: "Rainwater samples were collected at times when pollen grain number concentrations were near their maximum value and analysed with the drop freezing assay to compare the potentially enhanced IN concentrations measured near the ground with IN concentrations found aloft."

Modified sentence starting on Page 31676, Line 12: "Rainwater samples were collected during rain events near the time of peak pollen grain concentrations and analysed similarly."

Added the following sentences in the paragraph starting on Page 31679, Line 1: "…during the peak of the pollen season. We define the pollen season as the period of time when the NC Division of Air Quality operates the pollen sampler, which is typically from late-February through mid-November. This month has historically contained the peak of the tree pollen season for central NC with pollen concentrations increasing

approximately fifty fold over the course of a week (North Carolina Division of Air Quality, 2010)."

Modified sentence starting on Page 31679, Line 13: "Two rain events occurred near the time of peak pollen grain concentrations."

Modified sentence starting on Page 31688, Line 24: "The highest pollen grain concentrations observed occurred between April 10 and 14…"

R2 Issue #3: Abstract, Lines 24-26: Please explain the self-regulated feedback cycle between the atmosphere and biosphere via the release of cloud forming particles.

Response: As requested by referee #3 we removed this part from the manuscript (Page 31674, Line 24 and Page 31697, Line 22).

Revisions: Removed statement from abstract and conclusions.

R2 Issue #4: Page 31677 Line 4: How does a change of the cooling rate affect the experiment and why was this cooling rate chosen?

Response: The cooling rate of 1 K per minute was chosen because it approximates cooling rates in moderate updrafts in convective clouds while providing sufficiently fast processing of samples in the lab. For example, a 2.5 m/s updraft and moist adiabatic lapse rate of 6.6 K km^{-1} leads to a cooling rate of 1 K min^{-1}. Furthermore varying the cooling rate between 0.01 and 1 degree K per minute results in a minimal change in the median freezing temperature of the population of drops. We added more explanation to motivate our choice of cooling rate (Page 31677, Line 4).

Revisions: The dish is cooled at a rate of 1 K min^{-1} and the freezing of droplets is observed via sequential imaging of the slide at 1 frame per 10 sec using a stereomicroscope. The cooling rate of 1 K per minute was chosen because it approximates cooling rates in moderate updrafts in convective clouds while providing sufficiently fast processing of samples in the lab. For example, a 2.5 m/s updraft and a moist adiabatic lapse rate of 6.6 K km^{-1} leads to a cooling rate of 1 K min^{-1}. Furthermore, freezing spectra derived from cold-stage experiments are only weakly dependent on the cooling rate (e.g. Wright et al., 2013). An illustrative image …

R2 Issue #5: Page 31677 Line 13: Better to explicitly define 'median [freezing] temperature' and add the word 'freezing' between 'median' and 'temperature' for consistency.

Response: Done

Revision: The addition of ATD to the sample leads to a shift of the median freezing temperature of the population.

R2 Issue #6: Page 31677 Line 25: Please add that the larger droplets freeze at higher temperatures because of the increase in ice nucleation active sites.

Response: Done

Revisions: As expected the median freezing temperature is warmer for the nanodrops due to their larger droplet volume because of an increased number of ice nucleation active sites present.

R2 Issue #7: Page 31679, Line 6-8: Can you rule out that the ice activation of pollen is via macromolecules as suggested by Pummer et al., 2012 and Augustin et al., 2013 if you restrict yourself to sampling above 200nm?

Response: Of course we cannot rule out that some IN may be smaller than 0.2 μm and thus not be captured by our sampling method. However, previous studies clearly demonstrated that particles formed from pollen grain rupture range between 0.2 and 5 μm in diameter, and these are effectively sampled with the SAC. We now provide the size range in the body of the manuscript.

Revisions: Please see revision in response to R2 Issue #1.

R2 Issue #8: Page 31679, Line 13; Table 1: Sample periods are summarized in Table 1, where the reader can find sampling at different conditions and also variations in the diurnal time of sampling. In contrast to this, the ambient pollen concentrations are reported as 24h average pollen concentration. Can you exclude diurnal variations in pollen concentrations?

Response: No we cannot. The NC Division of Air Quality only reports a 24 hour average and we are limited by this data. Based on past measurements we believe that actual pollen concentrations were higher during the times when we sampled with the SAC. If true, our conclusions regarding the absence of a large impact of pollen as IN are strengthened. We added discussion regarding the diurnal cycle to the manuscript.

Revisions: The program reports 24 h average pollen concentrations (# of grains m^{-3} air) for the city of Raleigh on weekdays and differentiates between tree, grass, and weed pollen. Consequently, diurnal variations in pollen concentration are not captured. Pollen emissions typically peak during daytime (Ogden and Hayes, 1969), coinciding with the SAC measurements (Table 1). Consequently, the reported 24h average pollen concentration likely underestimates the actual pollen concentration during the sampling period. Raleigh is located …

Revision added the following citation to the reference list:
Ogden, E. C. and Hayes, J. V.: Diurnal Patterns of Pollen Emission in Ambrosia, Phleum, Zea, and Ricinus, American Journal of Botany, 56(1), 16, doi:10.2307/2440389, 1969.

R2 Issue #9: Page 31679, Line 17-20: If you freeze and thaw the samples, could that cause pre-activation? Or could that destroy the viable biological particles?

Response: First, this phenomenon would only apply to the rain samples that were frozen before they were processed (the rain samples from 4/19). The data do not show dramatic differences between the rainwater sampled on 4/12 and 4/19. Second, the sample was warmed to room temperature before conducting the experiment. Retention of unmelted ice in cavities of the nuclei seems unlikely. Finally, experiments where rainwater undergoes repeated freezing and thawing does not show systematic active site modification on all nuclei. The following was added:

Revisions: …two weeks later. Freezing and thawing of this rain water sample is unlikely to cause systematic active site modification for any nuclei in the sample (Wright et al., 2013).

R2 Issue #10: Page 31679, Line 22-25: The authors describe the process of filtering and re-suspending rainwater samples for which a 0.2 um filter has been used and the filtrate removed before analysis. This is in contrast to previous immersion freezing studies on pollen (Pummer et al., 2012; Augustin et al., 2013) where the IN active particles were found in the filtrate of commercial pollen grains and therefore did not contain the whole pollen grains. How can the authors conclude that no correlation between pollen and IN concentration is found when excluding parts of the sample which might contain potential IN?

Page 31680, Line 1-2: How did you estimate the re-suspended fraction?

Response: The point of the referee is well taken and studies on the filtrate would have been desirable. We removed all discussion of the filtered and resuspended rainwater sample.

Revisions:

Removed paragraph starting on Page 31679 Line 21, from "When enough sample water…" to "...For ATD the recovered fraction was 100%"

Modified Page 31685, Line 9: To identify whether the premature freezing is due to impurities in the water or defects in the glass slide, pure water was filtered through a 200 nm Nuclepore filter (Whatman). The material trapped on the filter was then resuspended to produce a 10:1 concentration of any particles that would be in the ultrapure water.

Modified paragraph beginning on Page 31686, Line 1:

The method to infer IN concentration in the liquid was validated by inverting the measured raw data for the 0.01 wt% ATD suspension (Fig. 1c and d). Both the pico- and nanodrop raw freezing spectra were inverted using Eq. (4). Inferred ice nuclei concentrations pL^{-1} of liquid are presented in Fig. 4. There appears to be satisfactory overlap between the picodrops and nanodrops. Combined, this demonstrates that the method is able to quantify IN concentrations ranging between 10^{-8} and 10^{-2} pL^{-1} of liquid.

Modified sentence beginning on Page 31687, Line 6:
Based on these results we conclude that the methods using picodrops and nanodrops can reliably quantify the IN concentrations in liquid solutions.

Removed following sentences beginning on Page 31687, Line 12:
Due to the low water volume (~10–20 mL) used to operate the sampler, filtering and resuspension of the SAC water was not possible. For this reason, SAC data only contains unfiltered results.

Removed paragraph beginning on Page 31693, Line 3: "An interesting observation…"

Modified sentence beginning on Page 31693, Line 15 to: "The above data are suitable to…"

Modified Table 2 caption on Page 31704 to: "Summary of rain water collection times"

Modified Table 2 removing column with heading "Concentration"

Modified Fig. 4 and 8 to remove references to filtered data in both figure and captions.

R2 Issue #11: Page 31680, Line 21-24: Only particles with diameter >10um are considered as pollen here. Multiplication processes (release of IN active particles e.g. by rupturing) are not taken into account to estimate the pollen concentration. Is the pollen grain number concentration thus identical to the particle concentration that has to be taken into account to estimate its IN ability? How do the authors conclude this? Can you rule out a possible influence of other IN?

Response: We agree. As we point out in the same paragraph: "…not all particles counted were necessarily intact pollen grains. Agitation of the suspension through vortexing and shaking (in order to achieve a well-mixed solution) may have caused breakup of cellular debris." What was missing is the statement that the estimate is a *minimum* particle concentration.

We specifically addressed the referees concern in the latter part of their question when discussing the results starting on Page 31688, Line 14:
"… it is important to note that not all of the particles that induced freezing were necessarily pollen grains. Bacteria, dust, fragments of the pollen sac itself, or other species of pollen present on the pine pollen cone could have served as IN in these experiments. The significant excess of nucleation sites per grain at $T < -22$ ◦C suggests that there are IN active particles among the plant debris."

Revisions: From these measurements we estimate a minimum particle concentration of ~ 20 particles μL^{-1} of suspension, averaged over 4 samples.

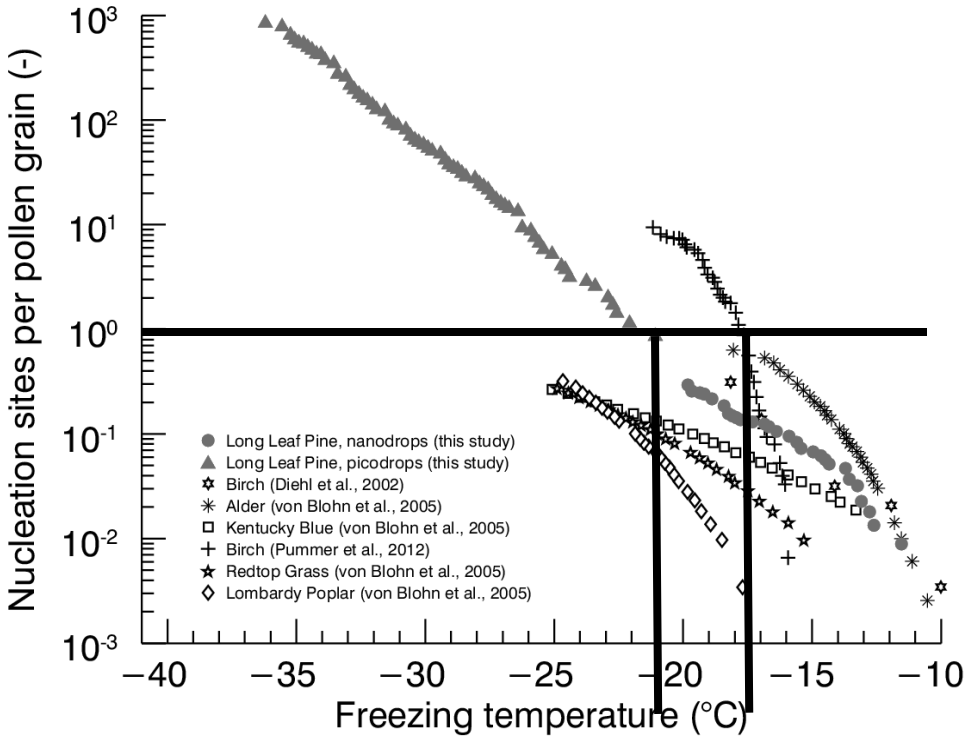
R2 Issue #12: Page 31682. Varying cooling rates....only leads to a shift of a few degrees K in the population median observed freezing temperature is a lot. Please justify why this is negligible.

Response: First it is important to specify *few degrees* to be quantitative. Typical shifts range from 0 to 2K for an order of magnitude change in cooling rate (Wright et al., 2013). If residence times in clouds were an order of magnitude larger than our cold-stage (~30 min for the 1 K min^{-1} cooling rate), the effective IN spectrum would be shifted by 0 to 2K toward warmer temperatures. Whether or not that is important for realized ice crystal concentrations in clouds will depend on a number of factors – e.g. updraft speed, entrainment rates, and secondary ice processes – that are beyond the scope of this work. We revised the paragraph to clarify

Revision: Although ice nucleation is fundamentally cooling-rate dependent, data for a wide range of different types of ice nuclei (including those found in rainwater) indicate that varying the cooling rate by an order of magnitude leads to shifts ranging from 0 to 2K in observed population median freezing temperatures (Vali, 1994; Wright et al., 2013; Knopf and Alpert, 2013). Assuming deterministic or singular freezing behaviour will lead to error commensurate with the deviation from the cooling rate of 1 K min^{-1} used in this study.

R2 Issue #13: Page 31688, Line 9: '...at least one nucleation site per grain...' is only true for Birch pollen measured by Pummer et al., 2012 shown in Figure 6, but not for any other pollen measurement.

Response: We thank the referee for the suggesting the need to rephrase the paragraph to be more precise. The temperature where at least one nucleation site per grain nucleates ice is at T = -17.5 °C for birch pollen (Pummer et al), and T = -21 °C for pine pollen (see illustration). Although alder pollen technically does not cross the one nucleation site per grain line, the idea that alder pollen efficiently nucleate ice at T = -18 °C this is still approximately correct. We revised the corresponding paragraph accordingly.



Revision: Notably, pollen grains do not appear to initiate freezing at temperatures warmer than -10 °C for the range of experimental conditions probed in current studies. At T ~ -10 °C between 1:1000 and 1:100 grains are able to serve as IN. Birch pollen grains studied by Pummer et al. (2012) and alder pollen grains are expected to nucleate ice (approximately one nucleation site per grain) by T ~ -18 °C. Pine pollen examined in this study has, on average, one nucleation site per grain at T ~ -21 °C. Only 20-30% of Kentucky blue, Redtop grass, and Lombardy poplar pollen nucleate ice at T ~ -25 °C.

R2 Issue #14: Page 31688, Line 11: Please correct 'IN activity of pine pollen .... are well within the range of results obtained from previous studies,...' Name the studies which you are referring to as Figure 6 does not contain reference data for pine pollen.

Response: We were not referring to previous studies of pine pollen but rather we were comparing the results of our tests on pine pollen to the results of studies presented in Figure 6. We rephrased the sentence accordingly.

Revision: The IN activity of the pine pollen tested is unremarkable and is well within the range of results obtained from the previous studies of different pollen species presented in Fig. 6.

R2 Issue #15: Page 31688, Line 20: How can the authors conclude from Figure 6 the 'generic pollen grain' induces ice formation at T<-20 C? This only seems to hold true for your measurements and thus should be stated as such.

Response: The referee is referring to the following statement: *The main result from Fig. 6 is that as a first order approximation a generic pollen grain is expected to induce ice formation at T ~ -20 °C.* He/she is correct in pointing out that this order of magnitude approximation may be too sweeping. We rephrased the sentence.

Revision: The main result from Fig. 6 is that some pollen species are sufficiently IN active such that each grain can induce ice formation at T ~ -20 °C. If one were to assume this to be true for all species and not assert any multiplication process due to bursting, the maximum contribution of pollen to ice nuclei concentrations at T ~ -20 °C is obtained from the pollen grain number concentration in the air.

R2 Issue #16: Page 31693, Line 22-25: The authors conclude that 'pollen only accounted for a fraction of the observed IN concentration'. What else do the authors expect to be activating as IN in their samples?

Response: We cannot say with certainty what the make-up of the IN within our samples. The principle particle types that nucleate ice are mineral dust, volcanic ash, black carbon, and biological particles (bacteria, fungi, plant fragments, etc.). Which of these contributed to the IN concentrations we observed is difficult to ascertain without composition measurements.

Revision: We therefore believe that pollen and pollen-derived particles only accounted for a fraction of the observed IN concentration, with the remainder being controlled by other natural and/or anthropogenic sources.

R2 Issue #17: Page 31696, Line 12: The authors should explain why the observed IN burst in their study is based on biological origin without chemical analysis or size distribution measurements that could distinguish larger bioparticles from other sources (e.g. mineral dust). Furthermore, I think direct comparison with results from the BEACHON-RoMBAS campaign is difficult, because this campaign was conducted in a different season and climate zone (as mentioned by the authors), which can result in different vegetation and therefore resulting in different pollen species.

Response: In regard to the first question, we believe that the burst is biological in origin (1) because the onset temperature of the IN burst on April 12 was ~ 12 °C, (2) because other sources such as mineral dust, black carbon, and volcanic ash become inefficient IN on a per surface area basis at T > -20°C; efficient IN that require minimal supercooling to induce freezing tend to be biologically-derived (e.g. Murray et al., 2012, Fig. 18), and (3) because Huffman et al. (2013) clearly demonstrated that a biological release mechanism can be responsible for the enhancement. We clearly state that the evidence is circumstantial in the text and will make that more clear in the abstract.

In regard to the second question, the comparison with BEACHON-RoMBAS is important since the BEACHON and our observations are very similar. It is interesting precisely because the vegetation and climate zone is different. Overall these observations may point to a ubiquitous process that is not necessarily tied to pollen alone but all bioaerosols. We hope that their and our work provide motivation for future studies on the rain-splash mechanism.

We revised the paragraph as follows to more clearly explain our reasoning:

Revisions: **The April 12 data show that ice nuclei concentrations increased ~30-fold at T ~ -20 ºC relative to background concentrations directly after the precipitation event. These findings are consistent with previous observations of the precipitation trigger (Huffman et al., 2013; Prenni et al., 2013). For example, Prenni et al. (2013) observed that IN concentrations were enhanced by an order of magnitude after rainfall with a concomitant increase in fluorescent particles. The increase in fluorescent particles combined with DNA analysis suggests that the enhancement was driven by biological particles (Huffmann et al., 2013). The rainfall-induced IN burst observed in our study has an onset freezing temperature of -12 ºC (Fig. 8) and concentrations are significantly higher than those observed by Prenni et al. (2013) at T = -15 ºC (cf. their Table 1). Similarly, Huffman et al. (2013) observed lower IN concentrations ~0.6 L^{-1} at -12 ºC for particles in the size range of ~2 to 5 µm.**

We argue that the observed onset freezing temperature of -12 ºC (Fig. 8) points to a biological origin of the IN. First, non-biological sources such as mineral dust, black carbon, and volcanic ash become inefficient IN on a per surface area basis at T > -20°C (Murray et al., 2012). Conversely, efficient IN that induce freezing at T > -15 ºC are a select species from the bacteria (Maki et al., 1974), lichen (Kieft, 1988), pollen (Diehl et al., 2002), and fungi (Richard et al., 1996; Huffmann et al., 2013) groups. Second, the humidity and wind-speed related mechanisms that lead to bioaerosol emission during rain storms (e.g. Webster et al., 1984; Pasanen et al., 1991; Paul et al., 2004) are likely applicable in any densely populated ecosystem. Thus, although we do not have direct measurements of IN composition we believe that the release of biologically-derived particles (presumably the sum over all sources and not just pollen) are a plausible explanation for the IN burst observed on April 12.

Technical corrections

R2 E#1: Droplet size is often given in volume. A droplet diameter would be helpful for comparison with previous publications on immersion and droplet freezing techniques (e.g. Pummer et al., 2012; Augustin et al., 2013; Diehl et al., 2002), which are referred to in the paper.

Response: We went through the manuscript and added droplet volumes where appropriate.

Revisions: Added volumes at the following locations:
Page 31676, Line 23
Page 31678, Line 4
Page 31683, Line 17
Page 31684, Line 12
Page 31684, Line 14
Page 31684, Line 16

Example Page 31676, Line 23: "Droplets smaller than 250 pL (*D* = 78.2 μm)…"

R2 E#2: Page 31675, Line 22: The phrase 'pollen particles' should be replaced by 'pollen grains' as it refers to the whole grain without bursting or multiplication processes taking into account.

Response: Done.

Revision: Pollen grains are released over a period of 2-4 weeks (Williams, 2010) and can be transported as far as 3,000 km from the emission source (Campbell et al., 1999).

R2 E#3: Page 31692, Line 25: Change 'been' to 'be'.

Response: Done.

Revision: … the IN activity of some of the nuclei can be irreversibly lost when …

R2 E#4: In general I would like to suggest color coding for the figures to help the eye of the reader. Additionally, the authors should check figures for consistency (e.g. x-axis labelling for 'Freezing temperature' and Temperature' as well as 'IN' and 'IN concentration').

Response: Revised figures can be viewed at the end of the document and in the revised manuscript.

Revisions: Figures 3, 4, 6, and 7 have had color added to them

Figure 5 updated horizontal axis label to 'Freezing temperature'

Updated legend information for Figures 3, 4, 5, 6, and 8

Figure 8 has the ordinal axis updated to 'IN concentration (#/L)'

Updated Figure 6 caption to reflect color change

R2 E#5: Figure 3: Due to the number of measurement points it would be helpful to color code the figure to support the reader in finding the main result of this figure (e.g. filtered vs. unfiltered experiments; nanodrops vs. picodrops). This statement applies to the following figures as well. The 'solid black line' mentioned in the caption in reality is 'grey'.

Response: Updated figure (See R2 E#4). Updated caption as follows.

Revisions: The dark grey line corresponds to…

R2 E#6: Figure 4: The top edge of the figure is cut and the label 'homogeneous freezing of pure water' is not completely visible.

Response: This is due to a type-setting error. We will ensure that this will not be an issue in the final manuscript.

Revisions: None.

R2 E#7: Figure 5: Font and symbol size should be magnified in both panels.

Response: Done.

Revisions: The font size will be identical to or larger than the caption font size.

R2 E#8: Figure 6: I suggest to color the different data points.

Response: We believe that using different colors and symbols creates more of a visual puzzle rather than providing more clarity. We therefore elect to keep the figure as is.

Revisions: None.

R2 E#9: Figure 7: Color coding the three y-axes would be much easier for the eye and would make labelling in the plot redundant.

Response: Thank you for the suggestion. We revised the figure.

Revisions: Please see end of this document and revised manuscript.

R2 E#10: Figure 8: The figure is too small and not readable. Please also indicate the unit (exponent) for IN number per sample in the graphs y-axis labels directly instead of in the caption only. Think of maybe combining all the daily data in one plot.

Response: The fonts are small because ACPD did not allow for landscape wide figures. Nonetheless, we decided to split the figure into two separate figures. Figure 8 will contain ambient measurements and Figure 9 will contain rain water data.

Revisions: Please see the end of this document or the revised manuscript for updated figures.

> **Updated Figure 8 caption to: Summary of ambient air ice nuclei data during the April pollen season. Number of IN per litre of air is obtained from the SAC. The label in the bottom left of each plot indicates the date and time the sample was collected. The "N" value in the top right corner denotes the average number of pollen grains per litre of air during the closest 24 h period that N.C. Department of Air Quality pollen counts coincided with sample collection. The symbols corresponding to picodrops and nanodrops are identical to those in Fig. 3. The horizontal dashed line corresponds to IN concentrations of 1 L^{-1} and is added to guide the eye.**
>
> **Created new Figure 9 caption: The primary y-axis gives the number of ice nuclei pL^{-1} of water measured in precipitation samples. The secondary y-axis roughly approximates IN concentrations per litre of air, assuming that 1 pL ~ 1 cloud droplet and a cloud droplet number concentration of 100 cm^{-3}. The label on the bottom left of the plot indicates the date and time during which the sample was collected. The horizontal dashed line corresponds to IN concentrations of 1 L^{-1} and is added to guide the eye.**

Anonymous Referee #3

This study addresses the hypothesis that macromolecules may become dispersed by rupturing of pollen sacs during wetting and drying cycles in the atmosphere and that this could be a significant source of ice nuclei in the atmosphere in the pollen season.

The manuscript present results from a field study carried out in Raleigh, North Carolina USA in 2013. Seven samples collected using a Swirling Aerosol Collector were analyzed for IN activity on a drop freezing assay. In addition rain water samples were analysed similarly. The hypothesis above was tested by comparing ice nuclei spectra with pollen concentrations.

The topic is and timely, the authors present an interesting data set. Sampling and analysis seem to be carefully done. I have some comments and suggestions for improvement as outlined below and I agree with the comments/concerns already provided by two reviewers and others. I can recommend publication after these have been addressed.

Response: We thank the referee for his/her support for publication of this manuscript.

General comments

R3 Issue #1: The authors attempt to extrapolate to make conclusions about pollen in general, the global scale and feed-back cycles in the Amazon. These brought conclusions do not seem justified based on the available dataset, and are not needed to merit publication of the data. I suggest the authors to shorten and tighten the discussion and conclusion sections.

Response: We removed this part from the manuscript.

Revisions: Removed statement from abstract and conclusions.

R3 Issue #2: The methods section could be organized better. It is unclear to the reader what the 15ul aliquot of water sample is and how it is sampled until having read quite a bit of the text. It would seem more natural to me to start with (2.1) the sampling procedure (p. 31679) and explain about the ice nuclei spectra afterwards in a new subsection (2.2). When explaining the nomenclature about the droplets (pico, nano) it would be helpful to mention what droplet diameters that correspond to right from the beginning.

Response: We agree that the methods section could be better organized. We rearranged the paragraphs in the methods sections and made minor adjustments to several paragraphs. As discussed R2 Issue #1 we added drop diameter references.

Revisions: Moved paragraphs starting with "Aerosol was sampled at North Carolina…" and "Rainfall totals were obtained…" to the beginning of the methods section.

Moved sentence "Rainfall totals were obtained from the on-site weather station that is operated and maintained by the North Carolina State Climate Office." to end of preceding paragraph.

Edited sentence Page 31676, Line 20 to: "A 15 µL aliquot of bulk sample water (water from SAC or rainwater) is mixed with squalene, emulsified using a vortex mixer, and poured onto a siliconized glass cover slide that is placed inside an aluminium dish."

R3 Issue #3: Pollen particles, pollen grain, granules particles per pollen grain, macromolecules, cellular debris – it seems that some of these words are used for the same thing - the notation should carefully explained and be consistent throughout.

Response: Thank you for this suggestion. The terminology is explained in the text in the paragraph that starts on Page 31675, Line 21. We have unified all references to pollen grain/sac/granule/etc. to follow this terminology.

Revisions:

Page 31674, Line 4 and 16: "pollen sac" to "pollen grain"
Page 31674, Line 15: "ambient pollen counts" to "ambient pollen grain counts"
Page 31675, Line 22: "pollen particles" to "pollen grains"
Page 31676, Line 2: "pollen sac" to "pollen grain"
Page 31676, Line 10: "pollen concentrations" to "pollen grain concentrations"
Page 31680, Line 4: "pollen concentrations" to "pollen grain concentrations"
Page 31680, Line 7: "pollen are" to "pollen grains are"
Page 31680, Line 14: "ambient pollen concentrations" to "ambient pollen grain concentrations"
Page 31680, Line 16: "Whole pollen cones" to "Whole male strobili"
Page 31680, Line 17: The cone was" to "The strobili were"
Page 31680, Line 21: "Pollen number concentrations" to "Pollen grain number concentrations"
Page 31688, Line 4: "pollen concentration" to "pollen particulate concentration"
Page 31688, Line 15: "pollen sac" to "pollen grain"
Page 31688, Line 16: "pine pollen cone" to "harvested male strobili"
Page 31688, Line 21: "pollen concentrations" to "pollen grain concentrations"
Page 31688, Line 22: "pollen counts" to "pollen grain counts"
Page 31688, Line 25: "pollen concentrations" to "pollen grain concentrations"
Page 31690, Line 26: "pollen concentrations" to "pollen grain concentrations"
Page 31691, Line 1 and 6: "pollen concentrations" to "pollen grain concentrations"
Page 31693, Line 18: "pollen concentrations" to "pollen grain concentrations"
Page 31693, Line 19: "pollen counts" to "pollen grain counts"
Page 31693, Line 24: "pollen" to "pollen and pollen-derived particles"
Page 31694, Line 2: "pollen sac" to "pollen grain"
Page 31694, Line 7: "pollen" to "pollen species"
Page 31694, Line 8: "pollen concentrations" to "pollen grain concentrations"
page 31695, Line 9: "grain bursting" to "pollen grain bursting"
Page 31697, Line 7 and 11: "pollen concentrations" to "pollen grain concentrations"
Page 31697, Line 11: "pollen grains" to "pollen derived IN"
Fig 7 caption: "pollen concentrations" to "pollen grain concentrations"

R3 Issue #4: Figures: Figures 5, 7 and 8 are too small. It should be checked that all symbols, lines etc. are explained in the figure captions – e.g red lines are not explained in caption for Fig 8.

Response: We have revised these Figures in response to referee #2

Revisions: Please see the end of this document and the revised manuscript for the updated figures and captions.

R3 Issue #5: Page 31680: In the introduction it is stated that pollen can be transported more than 3000 km from their emission source, but it is assumed that the pollen samples studied are only from local sources? Could the samples not be influenced by pollen from other than local sources?

Response: Yes, it is possible that some of the pollen we sampled was from sources other than those present in central North Carolina. However, the fraction of pollen grains that undergo long-range transport is generally less than 10% (Gregory, 1978) pending atmospheric conditions (Noh et al., 2013). The contribution of long-range transported pollen is probably less since the date of peak pollen emission slowly migrates Northward throughout the spring. We revised the paragraph to account for the possibility of long-range transport.

Revisions: The climate is temperate and humid sustaining a dense mixed hardwood forest composed primarily of oak, hickory, and pine species that surrounds the city (LeGrand, Jr. and Wiecek, 2003). Consequently, ambient pollen from local sources during this time period in the Raleigh area is dominated by tree pollen. Some unknown fraction of pollen may have originated from long-range transport into the region (Gregory, 1978; Noh et al., 2013).

Revision added the following citations to the reference list:
References: Gregory, P. H.: Distribution of airborne pollen and spores and their long distance transport, Pure Appl. Geophys. PAGEOPH, 116(2-3), 309–315, doi:10.1007/BF01636888, 1978.
Noh, Y. M., Lee, H., Mueller, D., Lee, K., Shin, D., Shin, S., Choi, T. J., Choi, Y. J. and Kim, K. R.: Investigation of the diurnal pattern of the vertical distribution of pollen in the lower troposphere using LIDAR, Atmos. Chem. Phys., 13(15), 7619–7629, doi:10.5194/acp-13-7619-2013, 2013.

R3 Issue #6: Page 31676: "rainwater samples were collected during rain events at the peak of the pollen season" ... it was only collected during some rain events. Why were these selected?

Response: We sought to sample rainwater near the peak of the pollen season. We did not have the staff/resources available to anticipate rainfall and be prepared to sample during night-time or during times the students involved in the project had to pursue coursework.

Revisions: None.

R3 Issue #7: Page 31679: Why was the rain water filtered and resuspended?

Page 31680: It is not clear how the reported recovered percentages (20% for rainwater, 100 % for ATD) were obtained.

Response: It was filtered/resuspended to lower the limit of detection and/or provide better sampling statistics for the warmer temperatures. Since this step is not crucial and since the resuspended fraction was difficult to estimate (see R2 Issue #10), we removed these data points.

Revisions: Please see our response to R2 Issue #10

R3 Issue #8: Page 31680: It should be explained how the representative pollen sample was used. What are the 4 samples? As I understand only one pollen sampled was collected?

Response: It is correct that only one pollen sample was collected. The pollen cones were processed in the way described on page 31680 and suspended in ultrapure water. We then conducted drop freezing analysis on this suspension in order to compare the ice nucleating ability of this pollen to others conducted in previous studies. The 4 separate pollen samples were replicate samples taken from the original, bulk pollen sample that were placed on filter papers and imaged in order to estimate the concentration of particles in the suspension. We revise this sentence in order to more clearly portray our process:

Revision: From these measurements we estimate a particle concentration of ~ 20 particles μL^{-1} of suspension, averaged over four repeated measurements of these 1 – 2.5 µL samples.

R3 Issue #9: Page 31685: the text says that Figure 3 shows that 10% of the particles froze heterogeneously and this corresponds to certain limits of detection – this should be explained.

Response: We clarified this statement.

Revision: Figure 1c shows that approximately 10% of the pure water droplets froze heterogeneously. This heterogeneous tail corresponds to the change in slope in the grey shaded region shown in Figure 3. Consequently, IN concentrations less than 10^{-7} pL^{-1} at T = -20 °C and 10^{-4} pL^{-1} at T ~ -36 °C cannot be detected with this particular setup and water purity.

R3 Issue #10: Page 31688: "The main conclusion from fig 6 is that as a first order approximation a generic pollen grain is expected to induce ice formation at T=-20C - this should be explained.

Response: Please see our detailed response to R2 Issue #13.

Revision: Please see our revisions in response to R2 Issue #13.

R3 Issue #11: Page 31689-31690: Comparison with Fletcher model: "Data are in reasonable agreement", most of the spectrum are in "excellent agreement" nonetheless the shape does not always follow... These are quite quantitative statements. The model results should be shown in the figure together with the data.

Response: We added the Fletcher line the right panel Figure 5.

Revision: Please see Figure 5 at the end of this document and in the revised manuscript. Caption revised to add: "Overlaid in blue is the Fletcher parameterization using Eq. 7."

R3 Issue #12: Page 31692: "Composition of rainwater solutions could lead to freezing point depression within cloud drops" This statement should be explained – what compounds in the rain water are the authors thinking about?

Response: We reworded this awkwardly stated idea.

Revision: However this solution may differ from the composition and concentrations within the solutions generated within clouds and in rain drops that transport the IN to the surface. Dissolved compounds found in rainwater solutions, e.g. nitrate and sulfate salts and/or various organic compounds could lead to freezing point depression. The magnitude of the freezing point depression is directly related to the water activity of the solution (e.g. Koehler et al., 2006). Water activity is approaching unity at the solute concentrations found during and after cloud droplet activation (Petters et al., 2009) and thus the total freezing point depression is expected to be small.

Revision added the following citation to the reference list:
Koehler, K. A., Kreidenweis, S. M., DeMott, P. J., Prenni, A. J., Carrico, C. M., Ervens, B. and Feingold, G.: Water activity and activation diameters from hygroscopicity data - Part II: Application to organic species, Atmos. Chem. Phys., 6(3), 795–809, doi:10.5194/acp-6-795-2006, 2006.

Minor comments:

R3 E#1: Page 31675: at temperatures as low as -9 – should be "as high as" ?.

Response: This change was incorporated into a sentence rewrite as part of Grothe Issue #4 below.

Revision: See Grothe Issue #4

R3 E#2: Page 31677, line 28: Exact droplet volumes are estimated… It does not sound right to estimate something exact. I suggest to delete exact and just write: Droplet volumes are estimated from...

Response: Done.

Revision: Droplet volumes for picodrops and nanodrops are estimated from…

Response to comment by Jaenicke:

The paper calculates nicely the collection efficiency of the SKC Incorporation Bioaerosol sampler for particles smaller then 1 um using the proper reference. But that is only part of the story, because particles have to enter the inlet tubing first. The collection efficiency of the inlet tubing has not been discussed (it could be calculated accordingly). Usually the collection efficiency of those inlet tubings drops rapidly for particles greater then 1 um. And pollen particles are larger then 1 um.

Response: We thank Dr. Jaenicke for his comment and defer to our direct reply on the ACPD discussion forum. In response to this comment we added our calculations of the collection efficiency in the form of an Appendix to the manuscript. We also added discussion regarding the washing of pollen along the 90 degree bend.

Revisions: Page 31679 starting line 13: Therefore the flow was stopped temporarily every hour and the collection well was refilled by spraying ultra-pure water through the collection inlet. This has the added benefit of washing any particles that impacted and remained on the collection inlet into the collection well (see Appendix A for details). It should be noted that this procedure was not performed after the last hour of measurement.

Added "Appendix A – Particle collection and sampling efficiencies". See revised manuscript for material.

Addition of Appendix added the following citations to the reference list:

Baron, P. A. and Willeke, K.: Aerosol Measurement, Principles, Techniques, and Applications, 2nd ed., Wiley-Interscience, New York., 2001.

Di-Giovanni, F., Kevan, P. G. and Nasr, M. E.: The variability in settling velocities of some pollen and spores, Grana, 34(1), 39–44, doi:10.1080/00173139509429031, 1995.

Juozaitis, a, Willeke, K., Grinshpun, S. A. and Donnelly, J.: Impaction onto a Glass Slide or Agar versus Impingement into a Liquid for the Collection and Recovery of Airborne Microorganisms., Appl. Environ. Microbiol., 60(3), 861–70

Kannosto, J., Yli-pirilä, P., Hao, L. and Leskinen, J.: Bounce characteristics of α -pinene-derived SOA particles with implications to physical phase, , 6095(June), 329–340, 2013.

Riediker, M., Koller, T. and Monn, C.: Differences in size selective aerosol sampling for pollen allergen detection using high-volume cascade impactors, Clin. Exp. Allergy, 30(6), 867–873, doi:10.1046/j.1365-2222.2000.00792.x, 2000.

Von der Weiden, S.-L., Drewnick, F. and Borrmann, S.: Particle Loss Calculator – a new software tool for the assessment of the performance of aerosol inlet systems, Atmos. Meas. Tech., 2(2), 479–494, doi:10.5194/amt-2-479-2009, 2009.

Added Fig. A1 and caption (see revised manuscript)

Response to comment by Grothe et al.

This paper describes field experiments regarding the contribution of pollen to atmospheric ice nuclei concentrations. We would like to emphasise that we highly appreciate that the authors have performed this field study and have tested our laboratory experiments and our hypothesis regarding pollen IN in the field (made in Pummer 2012 and Augustin 2013). However, we have detected some experimental details and some measurement strategies, which we think have to be described in more detail and there are some conclusions which we think are too strict.

Response: We thank Dr. Grothe and coworkers for their comments and suggestions. In response to the raised concerns we have narrowed our conclusions and clarified our measurement strategies where appropriate.

Grothe Issue #1: The region of investigation is Raleigh, North Carolina, USA situated 35° 49' N, 78° 39' W, which is several thousand kilometres away from the northern timberline, where we have assumed to find tree pollen with ice nucleation activity (Pummer 2012). Already then, we found different IN activities between pollen samples from the same genus: 4 K between 2 juniper and 3 K between 2 Thuja samples. However, the main trees of North Carolina are conifers, which in principle might be counted into the group of IN active trees. Nevertheless, we found in our experiments that the down-washable molecular IN from pine pollen is several orders of magnitude smaller in mass than from birch pollen. Therefore, we think that the conclusions might be restricted to the region and the trees of this region.

Response: We agree and it wasn't our intent to extrapolate our findings with respect to pollen globally. We have gone through the manuscript and made clear that this applies only to North Carolina.

Revisions: Please see the paragraph for our revisions to Vali Issue #3. Specifically we restrict the conclusions to a single ecosystem: "Our results, restricted to a single ecosystem with limited variation in meteorological conditions, are inconsistent with this hypothesis."

Grothe Issue #2: The authors have distinguished between dry particles and wet particles after rainfall. Obviously, among the dry particles IN are either inactive or low in numbers, but the rainfall is activating the particles and IN activity is 30 times increased afterwards (see fig. 7: 4/11 vs. 4/19). The contribution of these particles is most likely the sum over all kind of biological particles and this conclusion cannot be restricted to a particular sort. Therefore, filtration and analysis are extremely important issues. Here, we have not understood the filtration procedure described in the paper:

a) The rain water has been filtered but the dry particles have not been filtered (p.31679,19). Why?

b) Filtered and non-filtered rain water has been compared. The filtered one has been corrected by an efficiency factor of 0.2 (p.31694). Why?

c) Why has the filtrate not been analysed? This would have been of particular interest since it would include the molecular IN fraction.

Response: Please see our response to R2 Issue #10 and R3 Issue #7. The point is well taken and studies on the filtrate would have been desirable. The purpose was to lower the limit of detection and/or provide better sampling statistics for the warmer temperatures. Since this step is not crucial and since the resuspended fraction was difficult to estimate (see R2 Issue #10), we removed these data points. Filtration/resuspension on the SAC suspension was not possible due to insufficient sample amounts.

Revisions: Removed filtered/resuspended rainwater data points from Fig. 7. Removed discussion from the text.

Grothe Issue #3: In general, we stated the hypothesis that macromolecules can be released from pollen grains, which then can be distributed further independently from the mother grains, and thus, are not directly interconnects with pollen grain concentrations. In principle, the results of this paper do not contradict this idea. Therefore, the sentence "However, Pummer et al. (2012) suggest that the impact of pollen on atmospheric clouds might have been underestimated due to the ejection of IN active macromolecules from the pollen sac. Our results do not support this hypothesis" seems much too general.

Response: Please see our response to Vali Issue #3 and Grothe Issue #1.

Revisions: Please see our revisions in response to Vali Issue #3 and Grothe Issue #1.

Grothe Issue #4: Abstract, line 23-25: "Some pollen species contain some fraction of grains that induce freezing at temperatures as low as -9 C (Diehl et al., 2002)." It should be mentioned here that these high freezing tempeatures might also orignate in the fact that the particles examined in this study contained a large number of pollen, which, as shown in Augustin et al. (2013) and also Hartmann et al. (2013) increases the freezing temperature.

Response: We agree and we did explicitly mention in the original manuscript that only 1:1000 grains nucleated ice at this temperature. We revised the statement in the introduction to make this clear early on.

Revisions: Most species require supercooling to temperatures near -20 ºC or colder in order to induce freezing (Diehl et al., 2002; Pummer et al., 2012; Augustin et al., 2013). Select species contain low fractions of grains (1 in 1000) that induce freezing at temperatures as high as -9 ºC (Diehl et al., 2002).

Grothe Issue #5: Abstract, line 28: "associated with the grain" The ice nucleating entitiy of pollen grains does not have to be assiciated with a grain, as it can be washed off (Pummer et al. 2012 and Augustin et al. (2013)).

Response: We agree and used poor wording. We revised the sentence.

Revisions: Pummer et al. (2012) show that the ice nucleating activity of pollen is derived from non-proteinaceous macromolecules derived from the grain.

Grothe Issue #6: Ad Fig. 6: Please indicate which data set of Pummer et al. 2012 you have used. This is important, since high concentration (100% freezing) used in the equation of Vali et al. is underestimating the number of IN significantly. We may offer to support you with our latest data from pine and birch.

Response: The data for this figure was read from Murray et al., 2012. Correspondence with Dr. Sullivan identified the sample containing 50 mg/ml of water (5 wt%). The updated figure caption to reflect this information.

Revisions: Please see end of this document and revised manuscript for updated figure.

Grothe Issue #7: Ad Fig. 7: Were there no IN concentration measurements carried out during some rainfalls? Why are there some data holes? Since dry bioaerosols (including the pollen) are less likely to fragment, it is not so surprising that during the dry seasons IN concentrations are lower (and probably barely exceed whole-cell concentrations) than during rainfall. This is in good agreement with the paper by Huffman et al. 2013.

Response: In response to the first question. Indeed there are 'data-holes'. These can be attributed to the fact that our method is very labor intensive. Studies at much higher temporal resolution are a desirable and necessary next step to better understand this process.

In response to the second point that dry bioaerosols (including the pollen) are less likely to fragment: yes, this seems to be true. However, we note that (1) pollen in the SAC sample and rainwater were in contact with water and thus should have fragmented, (2) the atmospheric pollen are free to cycle in the boundary layer where they undergo the humidity cycling that in principle should be responsible to lead to the proposed fragmentation (see R2 Issue #1). We maintain our original argument that pollen are weak IN, low in number, and an obvious and ubiquitous fragmentation pathway was not observed in Raleigh. We are open to the idea that pollen are important contributors to atmospheric IN, either locally or globally, but this needs to be demonstrated with field measurements.

We are also open to the idea that the rainfall enhanced IN are due to either pollen fragmentation or due to another bioaerosol process. Given the warm onset temperature of -12C in both Huffman et al. (2012) and this study and that Birch pollen are not abundant in Raleigh seems unlikely that these are derived from pollen. However, we cannot say this for certainty and do not seek to make any claims regarding the specific biological source of the rain-triggered IN.

Revisions: We hope that the combined revisions clarified our views.

Revised figures and captions

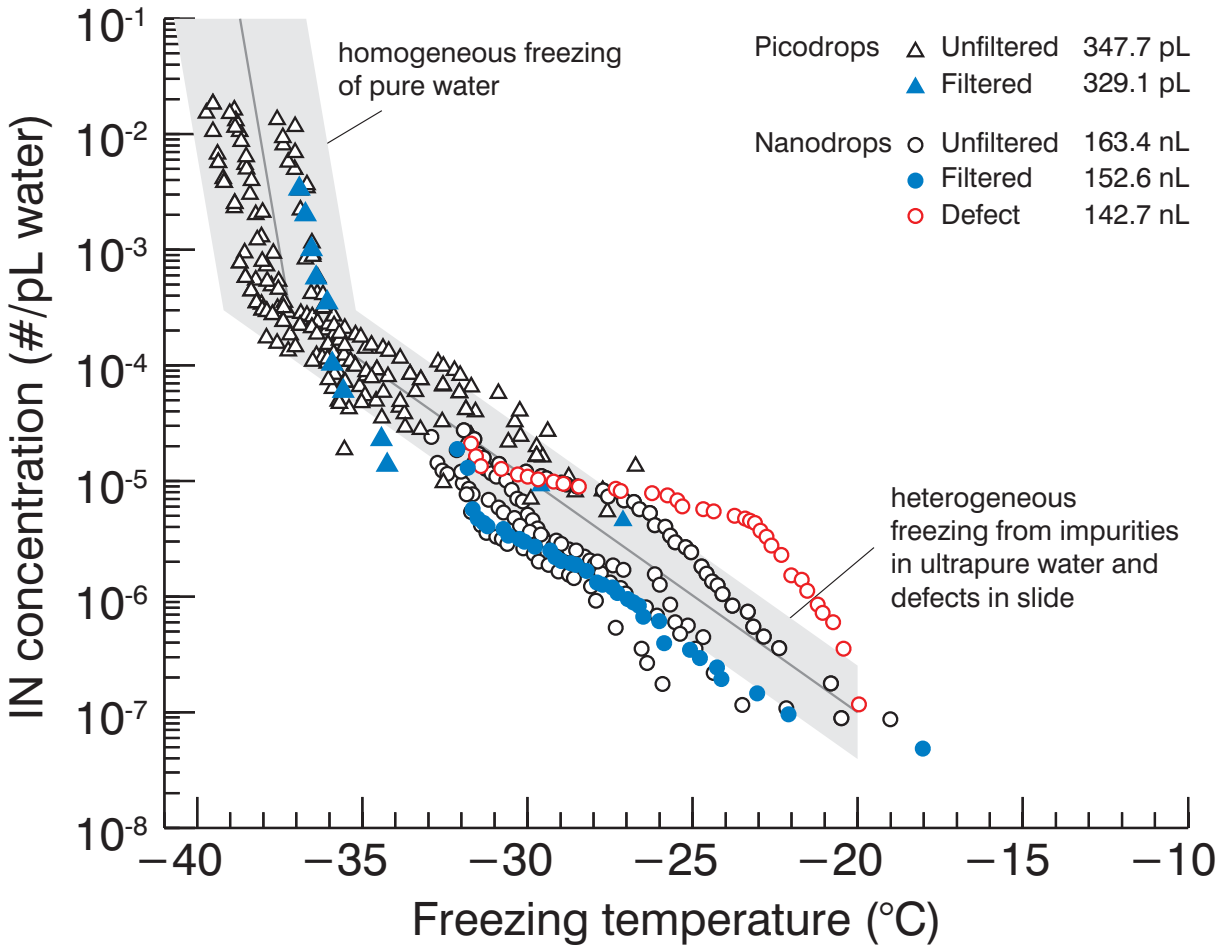


Figure 3. Summary of ice nucleation experiments with ultrapure water. Ice nuclei are expressed as the number of apparent ice nuclei per picolitre of water. Triangles represent picodrop experiments and circles represent nanodrop experiments. Filled symbols indicate filtered/resuspended data. Red circles demonstrate transient noise in the nanodrop experiments in the -20 to -30 °C range. Indicated in the top right corner is the average median drop volume for each class of droplets. The grey shaded area indicates an estimate of the experiment-to-experiment variability. The dark grey line corresponds to the average concentration of impurities present in the water.

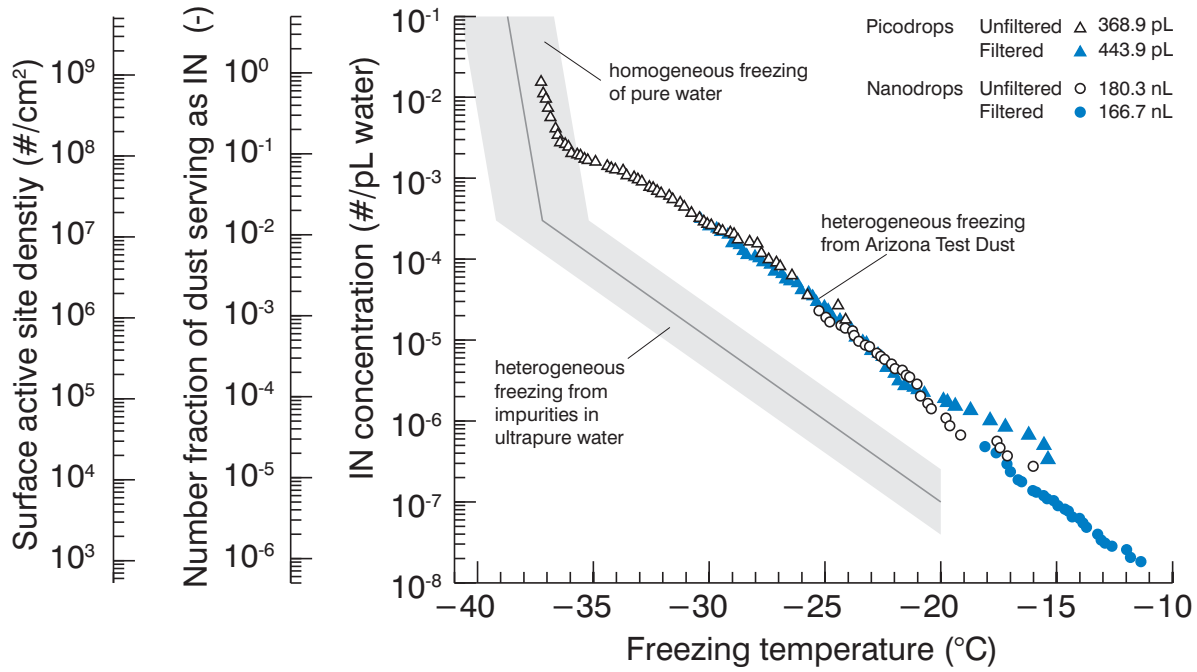


Figure 4. Cumulative ice nuclei spectrum for a suspension of 0.01 wt% of ATD in ultra pure water. Open and filled symbols correspond to unfiltered and filtered/resuspended experiments, and the numbers in the top right are similar to those in Fig. 3. The filtered/resupspended experiments correspond to a pre-concentration of ATD of 50:1. The second axis expresses the data as number fraction of dust serving as IN based on the dust number to mass ratio. The third axis expresses the data as IN active site (INAS) density based on the specific surface area and density of dust provided by the manufacturer.

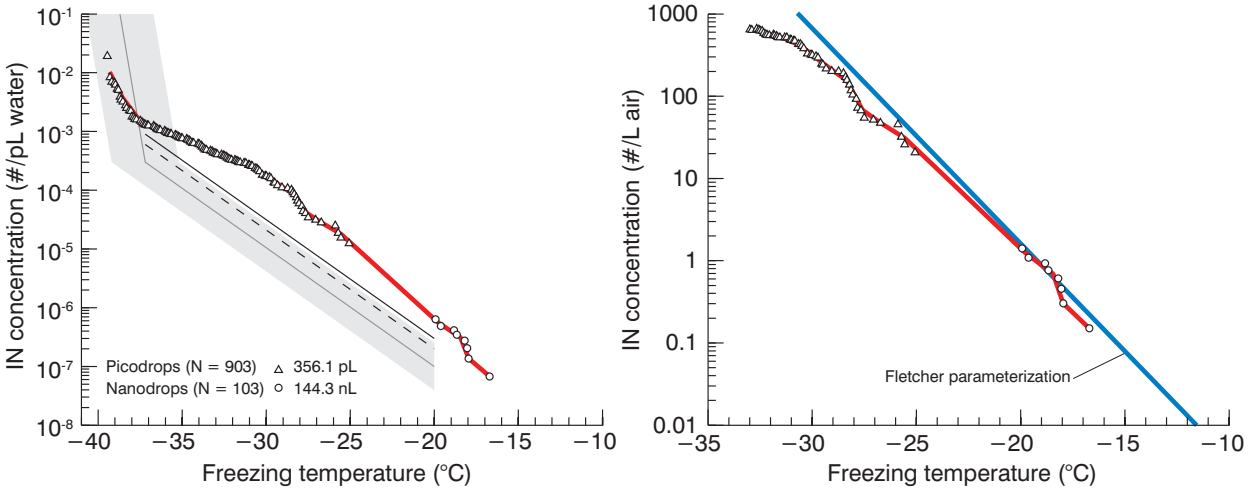


Figure 5. Example cumulative ice nuclei spectra from the SAC on April 8. Left: analysed in the same manner as shown in Fig. 4. The grey shaded area corresponds to the background concentration in the water samples shown in Fig. 3. Due to the addition of pure water to the SAC to maintain operation, the noise level was either multiplied by two (dashed line) or three (thick solid line) depending on the SAC run time. Right: same data as in the left plot but with background concentration (thick solid line in this case) of IN in the ultra-pure water subtracted and expressed as IN L^{-1} of air. The red line is the one degree average of the IN concentration. Overlaid in blue is the Fletcher parameterization using Eq. 7.

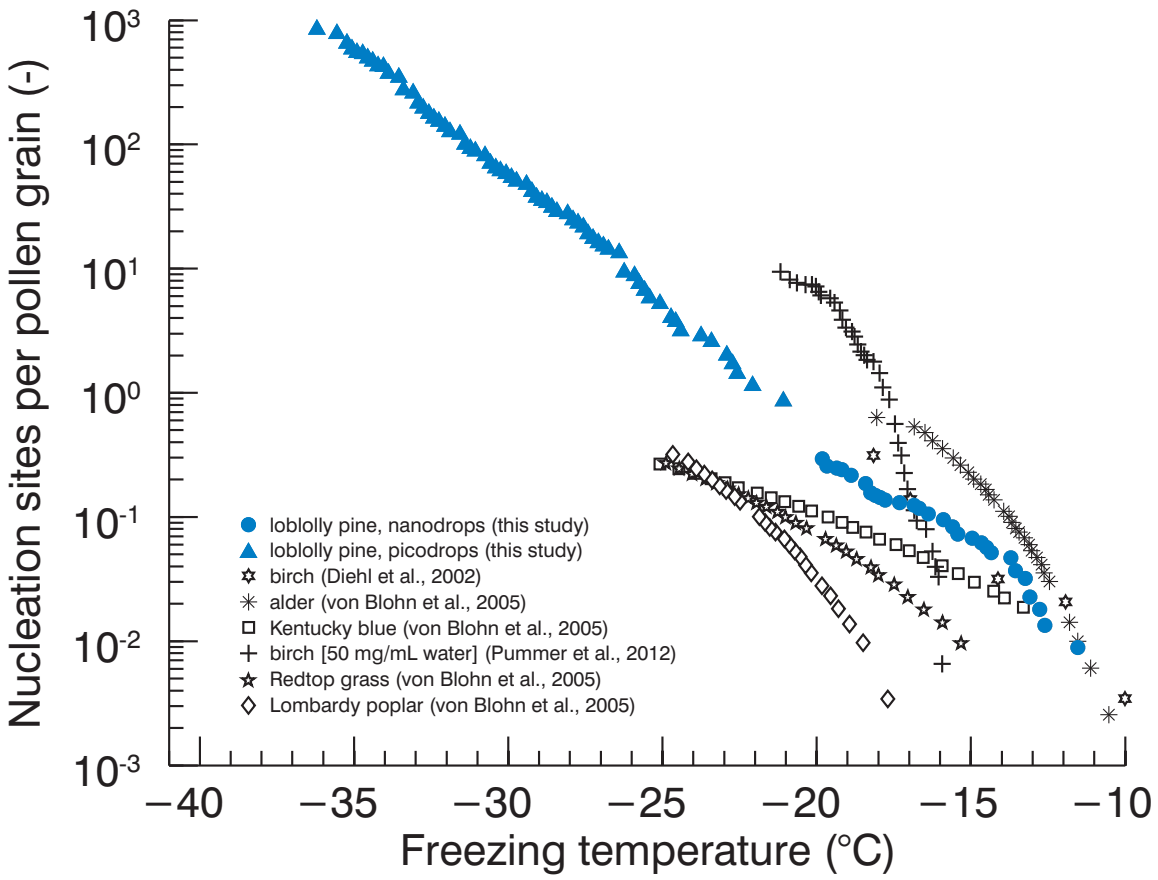


Figure 6. Number of nucleation sites per pollen grain as a function of temperature for various pollen types. Data from this study is shaded in blue. All other data were obtained from Fig. 15 of Murray et al. (2012). References to the original source are provided in the legend.

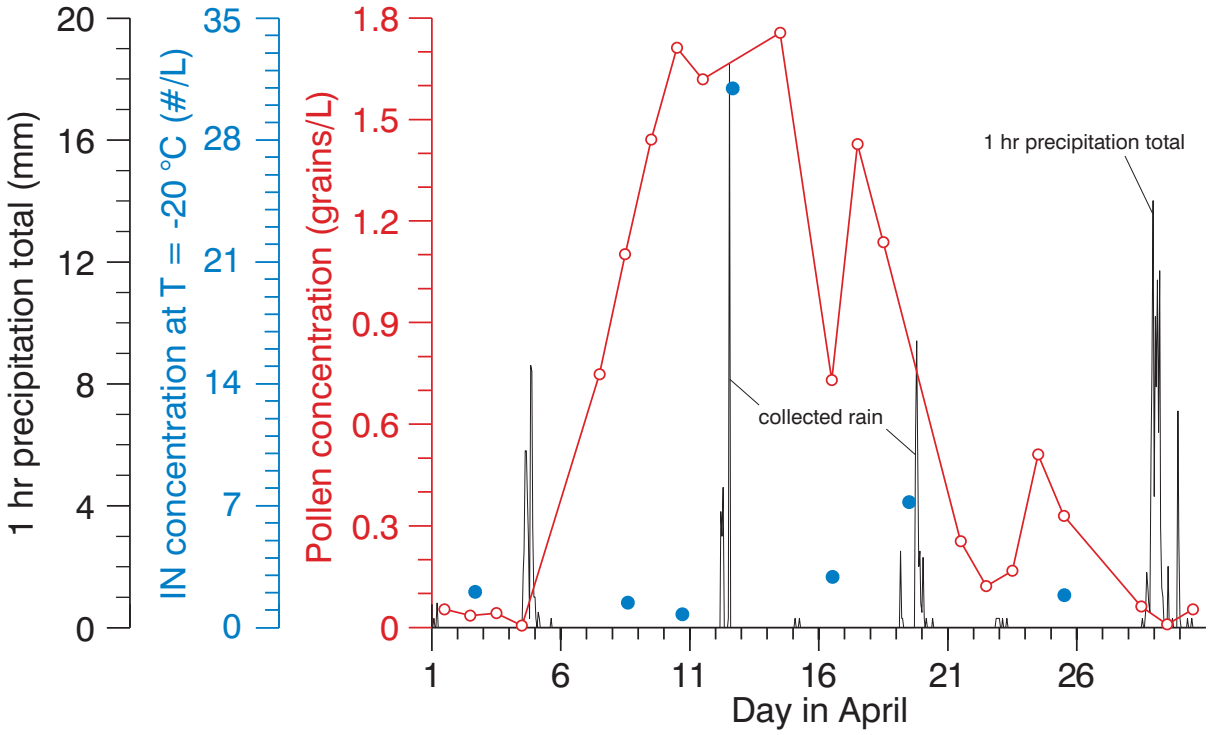


Figure 7. Hourly precipitation (black line), 24 hour pollen grain concentrations (open circles), and derived IN concentrations at T = -20 ºC (filled circles) for the month of April 2013.

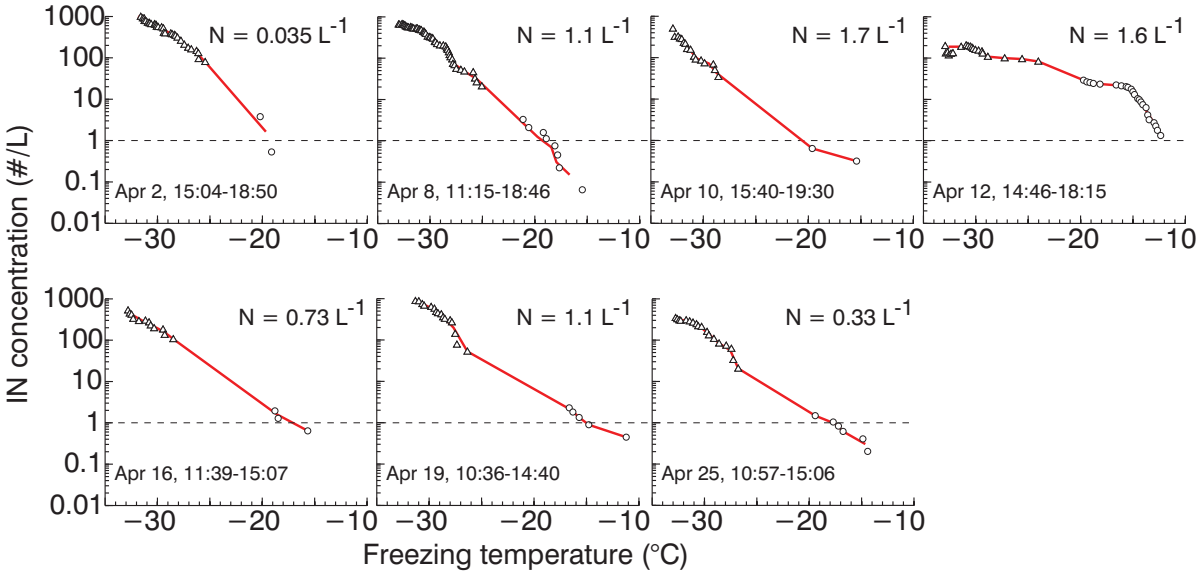


Figure 8. Summary of ambient air ice nuclei data during the April pollen season. Number of IN per litre of air is obtained from the SAC. The label in the bottom left of each plot indicates the date and time the sample was collected. The "N" value in the top right corner denotes the average number of pollen grains per litre of air during the closest 24 h period that N.C. Department of Air Quality pollen counts coincided with sample collection. The symbols corresponding to picodrops and nanodrops are identical to those in Fig. 3. As in Fig. 5, the red lines are the one degree averages of the IN concentrations. The horizontal dashed line corresponds to IN concentrations of 1 L^{-1} and is added to guide the eye.

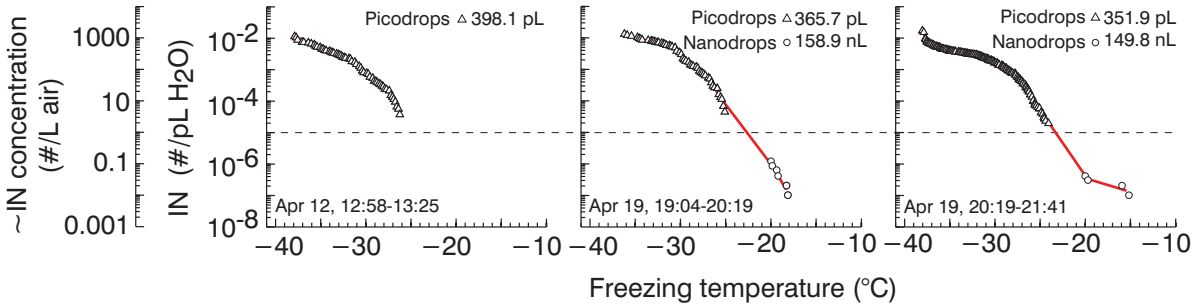


Figure 9. The primary y-axis gives the number of ice nuclei pL^{-1} of water measured in precipitation samples. The secondary y-axis roughly approximates IN concentrations per litre of air, assuming that 1 pL ~ 1 cloud droplet and a cloud droplet number concentration of 100 cm^{-3}. The label on the bottom left of the plot indicates the date and time during which the sample was collected. The horizontal dashed line corresponds to IN concentrations of 1 L^{-1} and is added to guide the eye.

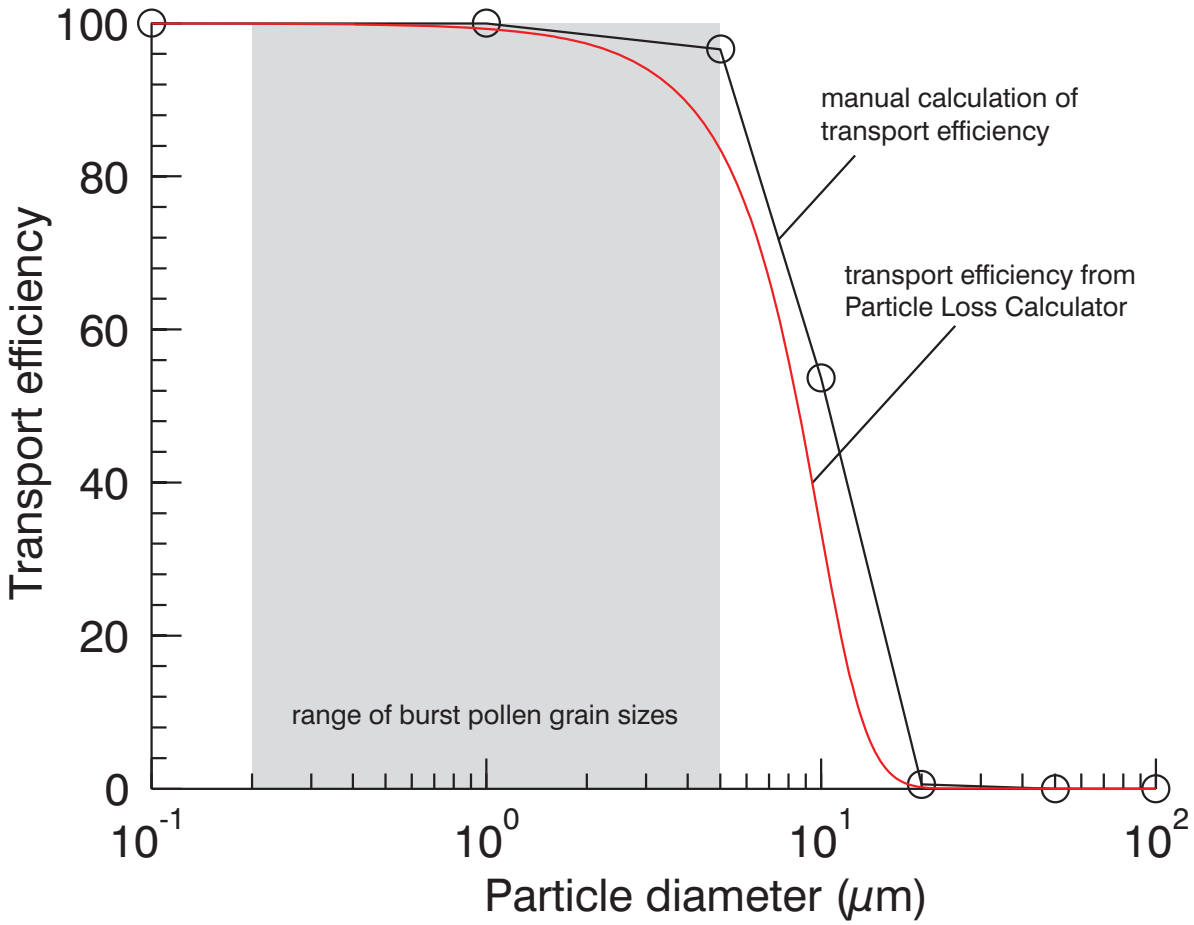


Figure A1. Particle transport efficiency as a function of particle size through the inlet of the SKC swirling aerosol collector. The black line is the calculated transport efficiency using Eq. 8-67 from Baron and Willeke (2001). The red line is the transport efficiency found using the Particle Loss Calculator (von der Weiden et al., 2009). The shaded grey area is range of particle diameters for burst pollen grains (Suphioglu et al., 1992; Taylor et al., 2002).