**Anonymous Referee #2**

We would like to thank you for your comments and remarks to our manuscript. In the following your comments will be in regular letters and our answers in blue italic letters.

**General comments**

This paper presents a study of the generation, ice nucleation ability, and modeling of an internally mixed-aerosol composed of mineral dust and water soluble biological material. The mineral dust chosen in this case (NX-illite) has been used extensively in the past as a surrogate for atmospheric mineral dusts. Many biological materials such as the Birch pollen washing water (BPWW) presented here have been previously shown to exhibit significant ice nucleation activity though few studies have examined a combination of the two. The authors use of the recently developed modified Soccer Ball Model (SBM) for parameterizing internally mixed aerosols based on parameters derived from the pure components is novel. However, there are several issues which need to be addressed before publication.

A significant amount of the manuscript deals with the characterization of the mixed aerosols used in the freezing study. The authors utilize multiple techniques to characterize the mixing state of the aerosols though only two of the techniques (humidity and volatility growth factors) provide any conclusive evidence as to the mixing state. The other techniques while not contradicting the growth factor results do not provide much additional information on the mixing state. I would suggest reworking these sections to remove unnecessary details which make the paper more confusing while not adding any additional information. The SEM and EDX sections, for example, could be removed or shortened as they provides little additional information.

*We decided to revise the manuscript to put more emphasis on the characterization methods for external/internal particle mixtures, as these were an important part of our work. This implies that abstract, introduction and conclusion will be extended respectively, and the focus of the new version of the manuscript will be more on the characterization of the mixed particles. Thus also the title changes to:*

*"Laboratory-generated mixtures of mineral dust particles with biological substances: Characterization of the particle mixing state and immersion freezing behavior"*

*As part of the changes, we decided to delete Table 2, as it gives only less information and maybe leads to confusion.*

*Concerning the modeling part of the manuscript, we will add an additional theory section after the introduction, where the Soccerball model will be explained.*

Secondly, the reader is left confused when going through the paper as to whether the pure particle freezing results (illite-NX and BPWW) were performed for this study or are simply reproduced from previous papers. For example, Table 3, clearly states that the SBM parameters used were taken from the literature while page 29655, lines 6-9 indicate that a different sample of BPWW was used for this study. Clarification throughout the manuscript as to which of the measurements were made for this study is necessary before publication.

*As we need to use a new Swedish birch pollen batch (the other one was used up), we performed new measurements with the washing water of this batch. We observed slightly different lambda values but the values for the parameters of the contact angle distribution were identical to those presented in Augustin et al., 2013. The data of pure BPWW particles which is shown in Figure 4 belongs to the* *new batch. This new birch pollen batch was also used for the mixtures. We mentioned that on page 29655, lines 6-11 in the first version, and now changed that passage slightly:*

*"These values differ a little from the respective values given in Augustin et al. (2013). The reason for that is that for the measurements done for the here presented study (meaning both, those of pure BPWW and those for mixed illite–BPWW particles) a new birch pollen batch was used. It is not surprising that due to natural variability the number of INMs produced per pollen grain or per mass of pollen varies. As a result, the number of INMs per particle also differs from batch to batch. But the ice nucleation properties (μ and σ) which were determined for the old pollen batch can be used to model the ice nucleation behavior of particles produced from the new batch, as seen by the good agreement between measured and modeled data for BPWW shown in Fig. 5. This is a strong indication for the fact that the two types of INMs in the new batch as such are the same than those in the formerly used batch.."*

*Additionally, we add the following sentence to section 4.2 (Immersion freezing experiments) of the new manuscript: "Concerning the BPWW measurements shown here, we should mention that we had to use another birch pollen batch than in Augustinet al., 2013 as this one was used up. Similarities and differences between these two batches are addressed further down in the manuscript."*

*With respect to the illite-NX measurements, please refer to your comment on page 29643, lines 2-3 further down on this review.*

Finally, the results presented here only use a single particle size (500nm) and a single coating thickness. While it is useful to show that the modified SBM is capable of predicting the freezing results of a monodisperse sample of internally mixed particles such as those presented here, additional measurements showing the model's capability with different particle sizes or relative amounts of illite and BPWW would significantly enhance the conclusion that the SBM can be used to predict the freezing behaviour of mixed aerosols as presented in the manuscript. While not essential to the publication of this manuscript, I strongly suggest expanding the laboratory results presented here.

*The measurements to that topic, immersion freezing measurements as well as characterization measurements, were not trivial and required several months. To increase the dataset would take too long thus we decided to leave it with this one dataset for the time being. As the main massage of our work would not change it would make no sense to put that much effort and time in new measurements.*

page 29641, lines 8-9 – The references here refer only to P. syringae. Additional references showing the ice nucleation ability of biological particles should be added.

*The sentence you refer to here explicitly deals with ice activity of P. Syringae and observations of that at particularly high temperatures. We added a sentence following that one:*

*"Already Szyrmer and Zawadzki (1997) and later Murray et al. (2012) give detailed overviews over different types of INP and denominate biological materials as those being ice active at higher temperatures above about -15°C."*

page 29642, line 4 – Provide a reference for the size of INM (10nm).

*We will add the following reference: Pummer et al., 2012.*

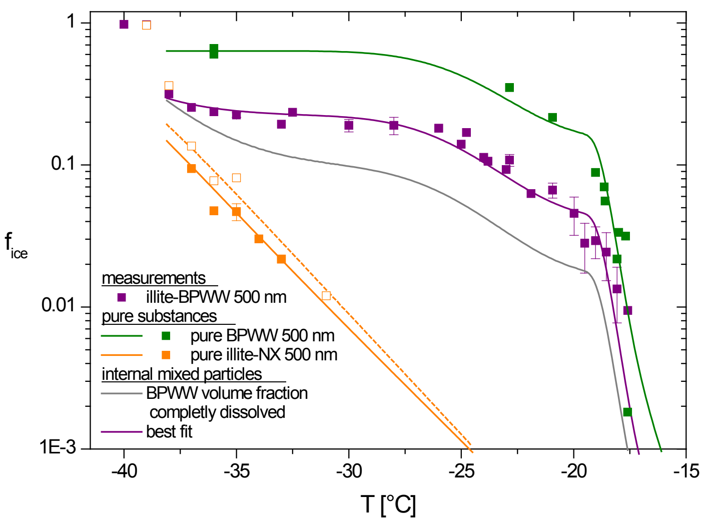
page 29642-29643, lines 26-1 – How was the concentration of illite-NX determined from the suspension? Please add detail regarding how these measurements were performed.

*To determine the concentration of the illite-NX suspension, 10 ml of the pipetted suspension were dried in a petri dish. A precision balance was used to first determine the weight of the empty petri dish. After drying the suspension the petri dish with the residues was weighted again. This procedure was repeated several times. The mean concentration of the illite-NX suspension is about 0.01 g/mL.*

*This information is given in the text now, previous to the sentence you mention above.*

page 29643, lines 2-3 – A comparison of the freezing ability of suspended illite-NX particles with those of dry generated particles is discussed though no results are presented. Are these measurements performed using size-selected particles or the full spectrum of generated aerosols? I recommend adding a figure showing the freezing comparison in the supplemental information so that the reader can see for themselves that the results are the same.

*Thank you for this suggestion. We will add the data points to Figure 4 (new version see here) and replace "not shown here" with "filled and open orange squares in Fig. 4, respectively". Also, the sentence you cite here was slightly modified, being now "… compared to the freezing ability of dry generated illite-NX particles of the same size (500 nm), ". We also now show both parameterizations, for wet generated illite-NX particles (straight orange line) and dry generated illite-NX particles (dashed orange line). The whole discussion to that topic was moved from the materials section to section 4.2 (Immersion freezing experiments).*




page 29643, lines 11-12 – The authors should indicate the pore size of the filters used to remove the pollen grains and comment on whether or not they expect any solid material to pass through the filters.

*The pore sizes of our filters were 4-7µm.We add this to the new manuscript. Whole pollen grains are in a size range of 20 µm and it thus is not possible that we have whole pollen grains in our washing water. Besides the soluble material of the pollen grains which goes into solution it is possible that fragments are formed due to the bursting of pollen grains and that they are present in the washing water. But as size selected particles were used, fragments of the size of 500 nm or smaller have had to be present. We do not know if such small fragments are there, but even if there were, they would not change our general results. From the investigations of Pummer et al., 2012 we know that only the single ice active macromolecules (INM) are the freezing catalysts and that the grains themselves do not induce ice nucleation, and these INM can be incorporated in any particle that is generated from the suspension, no matter if it also includes some pollen grain fragment or not.*

page 29643, lines 12-13 – Similar to above, detail should be added indicating how the Swedish birch pollen concentration was determined.

*The procedure was the same as that for the illite-NX suspension (see above), and we revised the respective sentence: "After filtration the concentration of the Swedish birch pollen material in the suspension was determined with the same procedure that was described for the illite-NX suspension above, and it was determined to be about 0.004 gmL$^{-1}$."*

page 29644, line 13 – Since the point is made regarding doubly charged particles, the size cut ($D_{50}$) of the cyclone used should be provided to indicate that these particles are unlikely to be present.

*The cut off diameter of the cyclone was 500 nm. We will add this in the manuscript.*

page 29646, lines 11-12 – While it is obvious why the C/Si ratio would provide some measure of the relative amounts of biological material and mineral dust in each particle, no explanation is given as to why a factor of 10 was chosen as the cutoff (ie. why a C signal greater by a factor of 10 indicates a purely biological particle and similarly why a Si signal greater by a factor of 10 indicates a purely mineral dust particle). The authors should add an explanation as to why these values were chosen. Would this technique work using different chemical tracers (eg. C/Al ratio)?

*We agree. The SEM/EDX section was rewritten and an explanation for the use of the C/Si ratio was added.*

*"The typical routine for the EDX based identification of particles as internal/external mixtures is the use of elemental ratios of main elements of the pure components (within the individual particles in the mixed sample) as classification criteria. In this study the classification of the analyzed particles is based on the determined Carbon/Silicon C/Si ratio. The choice of the boundary values of the elemental ratios for the classification as internal respectively external mixture depends on the detection limit and uncertainty of the EDX measurement. Marginal carbon and silicon signals (close to detection limit) are often observed in SEM-EDX measurements. In this way only particles with a C/Si ratio (based on net count rate) between 0.1 and 10 can be classified as internal illite/BPWW mixtures. As EDX analysis is limited to major and minor elements, internal mixtures cannot be identified when one component is only present in traces."*

page 29647, lines 1-13 – Similar to the above comment, the authors should provide a reference or a rationale for using the intensity ratio (Na/(Na + SiO)) as a metric for biological versus mineral dust particles. Additionally, results presented in the supplemental information suggest that many pure BPWW particles have intensity ratios between the chosen cutoffs of 0.1–0.65. Justification should be provided for the chosen cutoffs.

*We copy here the reply to reviewer #1 who made the same comment:*

*The thresholds have been chosen in a very conservative way based on the measurements of the pure substances: No illite-NX particles have been observed that had a ratio Na/(Na+SiO) greater than 0.65. This means that particles showing a ratio larger than 0.65 are considered to be pure BPWW particles. No BPWW particles have been observed having a ratio smaller than 0.10, meaning that particles with ratios smaller than 0.10 are assumed to be pure illite-NX particles.*
*In the mixed particle experiment, only 59 particles (14%) showed a ratio < 0.1 (thereby being pure illite-NX) and only 16 (4%) showed a ratio > 0.65 (pure BPWW). Thus, the remaining particles (82%) are likely mixed particles.*
*However, there is an uncertainty introduced by the finding that many pure BPWW and some illite-NX particles have intensities between 0.1 and 0.65. This was considered in our estimates: these particles represent 44% of the pure BPWW particles and 37% of the pure illite-NX particles. This increases the possible fraction of pure BPWW particle in the mixture to 24% pure illite-NX (14% * 1.37) and to 7% pure BPWW (4% * 1.44).*
*With this "worst case scenario", still 69% of the particles are internally mixed.*
*This is explained separated in sections 2.2 and 3.1. We will summarize these two separate sections in one paragraph in the revised version to make it clearer.*

page 29649, lines 1-4 – No mention is given of the range of expected coating thicknesses. While the growth factors for pure illite paritcles are quite narrow in distribution, the growth factors for the pure BPWW and the hygroscopic growth factor for the mixed particles is broader. This would result in a range of κ values as well as a range of possible coating thicknesses. The authors should provide an estimate of the spread in coating thicknesses used.

*For the calculations referred to here, average values were used (i.e., average k values were determined based on the average growth factors), and therefore also the derived coating thickness is an average value. We added this information to the text*

*Adding a more thorough estimation of the possible width of the coating (which, based on a very first quick estimate, would range from no coating to double the amount of the volume fraction) would put way too much emphasis on the here derived coating thickness, as the uncertainty of this value is rather large, given e.g, uncertainties in hygroscopic growth measurements and also the assumption of spherical particles. Hence we prefer to not give a range, here.*

page 29649, lines 11-13 – An indication of the number of individual particles observed for the determination should be given to indicate the statistical validity of the statement made here.

*To answer your question, we counted the particles on the images and found that from 145 particles only one shows a shape similar to the pure BPWW particles. The paragraph dealing with the SEM results was completely rewritten and we add the following in the new manuscript:*

*"Furthermore, when looking at the illite–BPWW SEM images, nearly none of the viscous droplets, which would indicate the presence of pure BPWW particles could be observed in the SEM images of the illite-BPWW particles ( 145 particles were counted from which only 1 shows a shape similar to the pure BPWW particles)."*

page 29651, lines 6-8 – All of the methods used to characterize the particles do not indicate that the particles contain both biological material and mineral dust. The SEM and EDX results could not identify any biological material on the mixed particles. This statement should be rewritten to make this clear.

*You are right in mentioning that for some of the methods we used rather logic reasoning to argue that there has to be biological material included in the particles. For some methods (as we found out also only during our measurements), the (low) amounts of biological material were problematic. And the VHTDMA measurements gave clear indication that the particles are internally mixed. We did modify the statement as follows:*

*"Nevertheless, all methods showed (albeit some only indirectly) that a significant fraction …"*

*Furthermore, the chapter in which the findings of the SEM/EDX measurement (no biological material in the mixtures could be determined with this method, because of the EDX limitations) and the implications of this finding are presented, was completely rewritten.*

page 29654, lines 3-4 – The results presented here and in Figure 4 indicate that the pure illite-NX and pure BPWW particles do not reach an $f_{ice} = 1$ at the lowest temperatures measured. Results for the illite-BPWW mixed particles attain $f_{ice} = 1$ below -38°C which the authors indicate is the onset of homogeneous freezing. Why was the homogeneous onset not observed for the pure particles? Were measurements not made at these temperatures or are the homogeneous results removed from the dataset presented? This should be mentioned in the manuscript.

*We typically measure down to -40°C, particularly for substances we didn't examine before or when checking that LACIS is still functioning well. This was done for the data shown in Fig. 4 for the mixed particles. But as we had examined particles from pollen before, where a frozen fraction of 1 (within measurement uncertainty) was observed below -38°C, we did not repeat many measurements at the low temperatures for the here presented dataset, as we were overall aiming at determining the frozen fraction in the plateau region and the slope of the curve. The same holds for illite-NX, where the measurements done for the present study were aiming at examining if there was a difference between dry and wet dispersed particles. Hence, measurements for pure particles were not done for the here presented datasets at the homogenous freezing range. We now do, however, include data obtained for the dry illite-NX in Fig. 4. To explain why we didn't measure the full freezing curves for the two pure substances, please understand that LACIS measurements are always quite time consuming, and that we had so much data on pollen and illite-NX overall that we decided*

page 29656, lines 2-4 – It is unclear here if measurements were made with polydisperse illite-NX particles and the value of $\lambda_{illite}(D_p)$ was determined in the present study or if this is taken from the literature.

*As we explained in the original manuscript on page 29644 lines 9-12, all our measurements were performed with monodisperse particles. The λillite(Dp) was determined in this study as this was not done in Augustin-Bauditz et al., 2014. We will mention this in the new version of the manuscript.*

page 29656, line 4 – The word "resulting" suggests that the values of $\mu\theta$ and $\sigma\theta$ are determined from the value of $\lambda_{illite}$ while the caption to Table 3 indicates that the values of $\mu\theta$ and $\sigma\theta$ are taken from Augustin-Bauditz et al. 2014. Please clarify which measurements are made in the present study and which are taken from the literature.

*The values of μθ and σθ for illite-NX particles were calculated within this study as it was not done in Augustin-Bauditz et al., 2014.We now show both parameterizations, the one for the dry generated particles which were measured in Augustin-Bauditz et al., 2014 and the one for wet generated particles which were measured within this study (see the new Table 3). For the calculations of the freezing behavior of the mixed particles the parameters of the wet generated particles were used. We also changed the caption of Table 3.*

page 29658, line 4 – The authors present the values of λ used for the illite-BPWW mixed particles. The specific values of λ used for both the illite-NX and BPWW pure particles fits as well as the completely dissolved BPWW case should be included as a comparison for the reader.

*We will add these values to the new manuscript. They can be calculated with the given relations on page 29655 lines 3 and 5 for BPWW particles and on page 29656 line 4 for illite-NX particles. We will give all lambda-values for the different samples in a new table:*

| 500 nm particle | λillite | λα | λβ |
|---|---|---|---|
| pure illite | 0.8125 | - | - |
| pure pollen (surface dependance) | - | 0.825 | 0.166 |
| Case b (Fig. 5) | 0.759 | 0.082 | 0.016 |
| Case c (Fig. 5) | 0.3293 | 0.2062 | 0.0412 |

*Thank you for your technical comments. We changed the manuscript accordingly.*

**Technical corrections**

page 29640, line 11 – "INUIT" should be defined in the manuscript.
page 29641, line 12 – Remove "e.g.,"
page 29641, line 25 – Define "INP".
page 29646, line 15 – Replace "illit" with "illite".
page 29649, line 27 – This was presented as C/Si on page 29646.
page 29651, line 8 – Remove comma after "both".
page 29651, line 20 – Replace "straight" with "solid".
page 29651, line 21 – Replace "In the next section, it will be described" with "The next section will describe".
page 29652, line 18 – Delete second "a".
page 29652, line 20 – Integration limits in equation (2) should be $\int_0^{\pi}$ for the first term and $\int_{\pi}^{\infty}$ for the third term.
page 29655, lines 3 & 5 – The dash before $\lambda_\alpha$ makes it appear as a negative symbol. This should be removed.
page 29655, line 7-9 – This sentence should be rewritten for clarity.
page 29657, line 3 – No comma after "both".
page 29657, line 13-25 – Multiple instances of "case a" or "panel c" the a, b, and c should be identified in brackets as done in Figure 5 to make it easier to read.
page 29658, line 4 – Remove dash before λα.
page 29658, line 14 – Remove commas after "both" and "material".
page 29658, line 24 – This sentence should be rewritten for clarity.
page 29664, Table 1 – The authors should indicate in the caption or the text that the fits for the grown factors determined are log-normal distributions. Additionally, the spread should be defined as either the standard deviation or the variance of the log-normal

distribution.
page 29668, Figure 2 – “Left part” and “Right part” should be replaced with “Panel a” and “Panel b”.
page 29670, Figure 4 – Replace “an mobility diameter” with “a mobility diameter”.

Supplemental information – Provide detailed captions for the figure and table. The histograms should have a labelled vertical axis. Table headers should be presented in English.
Interactive comment on Atmos. Chem. Phys. Discuss., 15, 29639, 2015.

Literature:

Murray, B. J., D. O'Sullivan, J. D. Atkinson, and M. E. Webb (2012), Ice nucleation by particles immersed in supercooled cloud droplets, *Chem. Soc. Rev.*, *41*, 6519-6554.

Szyrmer, W., and I. Zawadzki (1997), Biogenic and anthropogenic sources of ice-forming nuclei: A review, *BAMS*, *78*(2), 209-228.