

Anonymous Referee #1

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.

The submission by Augustin-Bauditz et al. describes a method for the generation of aerosolized insoluble solid particles mixed with soluble/water miscible organic substances, followed by a short laboratory study of the ice nucleating ability of such particles and a lengthy modelling study. Small ice nucleating particles and/or molecules (referred to as INM here and in the manuscript) of biological origin have previously been shown to in some way detach from their parent particle while in suspension. The finding that the artificial addition of these INM to mineral dust can significantly alter the ice nucleating ability of these particles is a strong support of previous studies that concluded the biological components of soil dusts enhance their ice nucleating behavior (e.g. Conen et al., 2011; O'Sullivan et al., 2014). However the amount of space dedicated to the separate parts in comparison with the abstract, introduction and conclusions results in the paper's emphasis being confusing, which I think should be redressed before publication.

Beyond the confusing paper emphasis, I have a number of minor comments/queries and technical issues, which are listed below.

Paper Emphasis:

The paper has a rather vague title and its main contents are split into three general parts – aerosol characterization, laboratory ice nucleation, and ice nucleation modelling, with characterization getting approx. eight pages, lab work approx. two pages and modelling work approx. six pages. However, there is no mention of modelling work in the abstract, no mention of characterization in the conclusions and no mention of either in the introduction. I would suggest two steps which would largely fix this:

- 1) Rework the abstract, introduction and conclusions. Give more emphasis towards the generation/characterisation of mixed aerosol, as this forms the largest part of the paper. Also cover the modelling work in the abstract, and perhaps move the introductory modelling material to the introduction. It should also be more clearly explained what the purpose of the modelling section is, as its conclusions are already fairly clear just from the three experimental datasets in figure 4.

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. The Reviewer is right when she/he says that our conclusion that the freezing behavior of the mixed particles can be explained by the freezing behaviors of the pure substances is already fairly clear just from the three experimental datasets in figure 4. Nevertheless we think that an adequate proof of this

conclusion is necessary. For this proof, modeling of the measured data is the best method. We will make this clearer in the new manuscript.

2) Expand the breadth of the laboratory data included. Not only will this address the paper emphasis, but providing only one experimental dataset weakens the conclusions. For example, experiments with particles generated from a number different illite-BPWW mixing ratios or sizes would result in much stronger conclusions.

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. As the main message of our work would not change it would make no sense to put that much effort and time in new measurements.

Minor Comments:

Throughout the paper there is a general assumption/presentation that the ice nucleating ability of INMs is superior to that of mineral dust. It is probably worth adding a clarifying statement somewhere that this is not always the case.

To our knowledge, there is no biological INM which shows a worse freezing behavior than mineral dust, especially in the particle size ranges relevant for atmospheric conditions (several hundred nanometers to some micrometers in diameter). We do not think that a clarifying statement is necessary here.

Page 29640, line 20. I think this statement is too strong. I recommend inserting the words an and could: It can be concluded that *an* INM located on a mineral dust particle *could* determine the freezing behavior of that particle.

We do not agree with the Reviewer here. The freezing behavior of the particles from the Dust-Bio mixture is clearly higher than that of the pure dust particles and the only explanation for this are the INMs in the Dust-Bio-mixture so we would prefer to leave the text as it is.

Page 29641, lines 1-4. This sentence implies that the publication by Murray et al. did not study particles of 'atmospherically relevant sizes'. Not only did this review include data from atmospherically relevant particles, but until the discussion as to whether multiple-particles-per-droplet techniques are comparable to single particle techniques in terms of available surface area is resolved, I would recommend avoiding such an implication. The individual particles used in such techniques are usually of relevant sizes.

Indeed, as ice nucleation activity scales with total particle surface area, our former formulation might have been misleading. However, droplets containing a larger number of particles (and hence a larger total particle surface area), as often is the case for measurements performed with cold stages, will freeze at higher temperatures, compared to a droplet that contains only one of these particles. We have shown this in the paper mentioned here, i.e., Augustin-Bauditz et al., 2014 (see supplement), and also in Niedermeier et al., 2015. In these two papers, the basic freezing behavior of the examined K-feldspar sample (reported a surface site density or contact angle distribution, respectively) was found to agree with that reported in Atkinson et al., 2013 for a similar sample, although the measured frozen fractions deviated by several °C (best seen in Fig. 1 (right panel) in Niedermeier et al., 2015, deviation up to more than 10°C, depending on particle size), due to the high number of particles per droplets in the latter.

Avoiding misinterpretation, the sentence in question was changed to: "Laboratory measurements indicate that mineral dust particles are efficient ice nucleating particles in a temperature range below -15°C (Murray et al., 2012) or probably only below -20°C (Augustin-Bauditz et al., 2014), where the latter examined droplets which each contained a single particle of atmospherically relevant sizes."

Page 29641, lines 9-19. This section could do with more references. For example, but not limited to, Murray et al., 2012; Hoose and Möhler, 2012; O'Sullivan et al., 2014; Conen et al., 2011; Wilson et al., 2015.

In these lines we exclusively talk about single macromolecules of biological particles like bacteria, pollen or fungi, and that these macromolecules keep their ice activity even if they are detached from their original carriers. We add Wilson et al., 2015 here, for material originating from algae contained in the sea surface microlayer.

The studies of Murray et al., 2012 and Hoose and Möhler 2012 do not contain any information about the ice nucleation ability of single macromolecules. Thus these references are not suitable at that point. Also the O'Sullivan et al., 2014 and Conen et al., 2011 studies do not address that topic. We mention these latter two studies further down in the manuscript where we talk about soil dust.

Page 29641, lines 24-26. I think this statement is too general. At which temperature ranges? Dust from what sources? Surely these things have an influence. There already seems to be some consensus that desert dust and soil dust are different things.

We are somewhat astounded, as also text prior to and after the sentence you mention here discusses this matter, starting in line 18 and extending to the next page, where soil dusts are explicitly mentioned. We did, however, add some details, first prior to the sentence you mention above:

"In that context, Kleber et al. (2007) describe that soil organic matter sorbs on mineral surfaces, preserving and maybe even accumulating INM when being connected to mineral surfaces."

And we also added details of the studies by Conen et al., 2011, O'Sullivan et al., 2014 and Tobo et al., 2014, following the sentence that originally ended on page 29642:

"...material (Conen et al., 2011; O'Sullivan et al., 2014; Tobo et al., 2014). However, the temperature ranges in which the organic fraction of the examined soil dusts was reported to be responsible for the ice activity differed between the studies, extending down to only -15°C for O'Sullivan et al., 2014 (where ice activation observed at lower temperatures was ascribed to mineral dust), but down to even below -30°C for Tobo et al., 2014."

Page 29642, lines 6-8. This needs many more references. Hiranuma et al is not the only NX-illite paper.

The work of Hiranuma et al., 2014 gives a nice overview over several different illite-NX studies, thus we thought that this is a sufficient reference at that point. We change the reference to: "Hiranuma et al., 2014 and references therein".

Page 29643, line 13. This works out to be 1/3 of the original mass of pollen. If such an amount makes it through the filtering, is it really realistic that it's just some released macromolecules and not fragments of pollen grains? In Pummer et al 2012, it's 2.4 % mass released into the water.

In the paper of Pummer et al., 2012 it is said that the residue content in the water is about 2.4 wt% (i.e., mass of residue related to the mass of water). In that study, originally 50 mg of pollen grains were put in 1 ml (i.e., 1 g) of water (5 wt%). Thus after filtering they still had nearly half of the original mass of pollen in the washing water. This is the same order of magnitude that was also observed by us. We mentioned that special point in the new manuscript.

There indeed is a lot of material on the pollen (waxes, proteins, sugars and others), having their function in the pollens role of carrying the genetic material to its destination. Material can also be released from the interior of the pollen, which, however, will not change the results presented here, as already also discussed in Pummer et al., 2012 and Augustin et al., 2013.

Page 29643, line 14. Please clarify the measure of mixing – volume of suspension or mass of suspended/dissolved material?

We mixed the same volume from both suspensions. 10 ml of the pollen washing water with 10 ml of the illite-NX suspension. We made this clear in the new text. The sentence now reads as follows: “The illite-NX suspension was mixed with the BPWW using 10 mL of each of the suspensions”

Additionally, the previous two paragraphs now have a better description of how the concentrations of illite-NX and birch pollen material in the respective suspensions were determined.

We hope that this resolves your request, here.

Other than that, the amount of suspended/dissolved material plays a different role in measurements for which dry particles are selected, compared to e.g., cold stage measurements where the suspensions are used directly. For our measurements, after spraying, suspensions were dried, prior to selecting a dry particle size. The total amount of material which is then included in a droplet (which in the next step is activated on each particle) then depends on the selected particle size. The fraction of illite-NX to that of birch pollen material in these dry particles, however, does not follow easily from how the suspension was made, which was one of the reasons why we started a rather extensive particle characterization.

Page 29644, line ~12. Do you have any information about whether the mineralogy of the size selected particles matches that of the bulk?

We assume that the mineralogy does not change over a wide range of particle sizes (including bulk), where the reason is as follows: Hiranuma et al., 2015 presents results for immersion freezing of illite-NX particles (same batch) from a number of different instruments. Results for those instruments which used dry particles initially, which were then activated to droplets in the instruments, agreed well, no matter if the instruments were fed with particles with sizes from 200nm to 1000nm as well as with polydisperse aerosol. This indicates that at least those properties responsible for the immersion freezing did not change.

Page 29649-29650 and elsewhere. I really don't think that the SEM section provides any useful results to the paper and I would strongly suggest removing it.

As we extended the paper with respect to the characterization methods, the SEM section is an important part of the paper even if the results are not that strong.

Page 29650, lines 1-3. If the BPWW from the mixed particles evaporated in vacuum, surely the pure BPWW particles should have evaporated as well?

As described in the SEM chapter the Darmstadt group has used boron substrates for the analysis of the mixed illite-BPWW particles. This substrate was chosen after we found out that morphologically the BPWW component was not identifiable in the mixed samples. Therefore, boron was chosen, in order to have the possibility to use the carbon signal as a fingerprint for the detection of the BPWW residuals.

If "liquid/organic/oily" particles like the residuals from the BPWW are deposited on boron, they form large and thin films, which are often hard to analyze/identify. In this way the pure BPWW residuals were also hardly analyzable on the boron substrates.

In contrast the residuals from the pure BPWW form droplets on the filter substrates (see Figure 3a), which were used for the SEM analysis of the pure BPWW particles.

Page 29650, lines 20-27. The thresholds used are not unambiguous, especially when the SPLAT data for the pure BPWW is considered. Please provide some additional comments/justification for the chosen thresholds.

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 was 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. It can be found in section 4.1.3 of the new manuscript.

Pages 29650-29651. Similarly to the SEM data, I don't really see how the addition of the SPLAT data improves the paper. The conclusion is just to ignore the SPLAT data and use the VH-TDMA data, which is a rather weak conclusion. Is there anything of any real importance to be said from the SPLAT data?

As already mentioned, we decided to rewrite the manuscript to put more emphasis on the characterization methods for external/internal particle mixtures. In this context, the methods will be compared and the advantages and disadvantages of each method will be discussed.

Page 29654, lines 1-3 and lines 18-20. I'm confused. Either these statements need to be clarified or they directly contradict each other. Is there one INM or two INM in BPWW? Also lines 18-20 feel very strong (especially considering that page 29655 lines 6-9 talk about how the current sample is different to previous). Is this fact, or just a conclusion that was consistent with the data?

The statement in line two is a general statement which says that ice nucleating macromolecules are responsible for the freezing ability of pollen grains. The “single” refers to the fact that not the whole pollen grain is needed but only a single macromolecule.

In Augustin et al., 2013 it was observed that the pollen grains from the Swedish birch pollen contain two distinctly different types of these ice nucleating macromolecules. That is what was referred to in lines 18-20, which now reads “contains two different types of INM”.

We additionally assume that the abbreviation “INM” was confusing to you, as it was defined as the plural “ice nucleating macromolecules”. We will change that so that “INM” is ice nucleating macromolecule throughout the text, and the plural form will be INMs.

We had to use a new Swedish birch pollen batch for this study simply because the old one was used up. Due to natural variability, it is possible that the composition of the birch pollen differs slightly from batch to batch. This is what we observed for the number of available ice nucleating macromolecules. The batch used for Augustin et al., 2013 contained slightly more INM per pollen mass than the batch used for this study. This manifests itself in different lambda values. But the slope of the freezing curve is the same for both batches, indicating that we investigated the same kind of INMs.

Technical issues

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

Page 29640, line 6. Delete “e.g.,”.

Page 29641, line 15. Please delete the double brackets }{.

Page 29642, lines 1-4. Please provide a reference.

Page 29645, line 12. Delete comma after RH.

Page 29646, line 12. Change netto to net.

Page 29648, line 16. Is VGF 0.57 as here or 0.56 as in Table 1?

Page 29648, line 20-22. Suggest deleting comma’s after suggests and both, and changing “material or, in other words” to “material. In other words”

Page 29649, line 8. Illite not illit

Page 29649, line 14-15. Suggest starting the sentence with As, deleting “On the other hand,”

Page 29649, line 26. Please replace the word results with conclusions.

Page 29650, lines 18-20. Delete comments about unusable data, it’s not important.

Page 29650, line 25. “Mentioned above”. Please specify.

Page 29651, line 8. Suggest deleting comma after both, and also replacing “or, in other words” with “and”

Page 29651, line 21. Suggest deleting both commas.

Page 29652, line 3. Delete comma and als “in the” -> “For this the SBM version...”

Page 29652, line 18. A a.

Page 29654, lines 2-3. Please add a reference to the single INM statement.

Page 29657, line 3. Delete comma after both.

Page 29657, line 22. Change depended to dependent.

Page 29658, lines 8-9. Suggest deleting “we can confirm that”. This phrase does not work with “possible”.

Page 29658, line 14. Delete commas

Page 29658, line 16. Change a to an.

Page 29658, line 17. The section in parentheses is not easy to read. Better to replace the symbols with “of the contact angle”. E.g. “mean and standard deviation of the contact angle”

Page 29658, line 23, Delete already.

Page 29658, line 24-25. Delete from already to conditions. i.e. “However, it was difficult to determine...”

Page 29659, line 1. Delete even.

Page 29659, line 4. Advice, not advices.

Figure 4. Consider reformatting the lines/symbols to make the chart clearer when printed in black and white.

Supplement: Please add y-axis labels to the figure and translate the table into English, specify in the table caption what data it refers to, and add a column for the bin sizes in the table.

Literature:

Atkinson, J. D., B. J. Murray, M. T. Woodhouse, T. F. Whale, K. J. Baustian, K. S. Carslaw, S. Dobbie, D. O'Sullivan, and T. L. Malkin (2013), The importance of feldspar for ice nucleation by mineral dust in mixed-phase clouds, *Nature*, 498(7454), 355-358, doi:10.1038/nature12278.

Augustin-Bauditz, S., H. Wex, S. Kanter, M. Ebert, F. Stolz, A. Prager, D. Niedermeier, and F. Stratmann (2014), The immersion mode ice nucleation behavior of mineral dusts: A comparison of different pure and surface modified dusts *Geophys. Res. Lett.*, 41, doi:10.1002/2014GL061317.

Conen, F., Morris, C. E., Leifeld, J., Yakutin, M. V., and Alewell, C.: Biological residues define the ice nucleation properties of soil dust, *Atmos. Chem. Phys.*, 11, 9643-9648, 10.5194/acp-11-9643-2011, 2011.

Hiranuma, N., O. Moehler, K. Yamashita, T. Tajiri, A. Saito, A. Kiselev, N. Hoffmann, C. Hoose, E. Jantsch, T. Koop, and M. Murakami (2015), Ice nucleation by cellulose and its potential contribution to ice formation in clouds, *Nat. Geosci.*, 8(4), 273-277, doi:10.1038/ngeo2374.

Hoose, C., and Möhler, O.: Heterogeneous ice nucleation on atmospheric aerosols: a review of results from laboratory experiments, *Atmos. Chem. Phys.*, 12, 9817-9854, 10.5194/acp-12-9817-2012, 2012.

Kleber, M., P. Sollins, and R. Sutton (2007), A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces, *Biogeochemistry*, 85, 9-24.

Murray, B. J., O'Sullivan, D., Atkinson, J. D., and Webb, M. E.: Ice nucleation by particles immersed in supercooled cloud droplets, *Chem. Soc. Rev.*, 41, 6519-6554, 10.1039/C2CS35200A, 2012.

Niedermeier, D., S. Augustin-Bauditz, S. Hartmann, H. Wex, K. Ignatius, and F. Stratmann (2015), Can we define an asymptotic value for the ice active surface site density for heterogeneous ice nucleation?, *J. Geophys. Res.*, 120, doi:10.1002/2014JD022814.

O'Sullivan, D., Murray, B. J., Malkin, T. L., Whale, T. F., Umo, N. S., Atkinson, J. D., Price, H. C., Baustian, K. J., Browse, J., and Webb, M. E.: Ice nucleation by fertile soil dusts: relative importance of mineral and biogenic components, *Atmos. Chem. Phys.*, 14, 1853-1867, 10.5194/acp-14-1853-2014, 2014.

Wilson, T. W., Ladino, L. A., Alpert, P. A., Breckels, M. N., Brooks, I. M., Browse, J., Burrows, S. M., Carslaw, K. S., Huffman, J. A., Judd, C., Kilthau, W. P., Mason, R. H., McFiggans, G., Miller, L. A., Najera, J. J., Polishchuk, E., Rae, S., Schiller, C. L., Si, M., Temprado, J. V., Whale, T. F., Wong, J. P. S., Wurl, O., Yakobi-Hancock, J. D., Abbatt, J. P. D., Aller, J. Y., Bertram, A. K., Knopf, D. A., and Murray, B. J.: A marine biogenic source of atmospheric ice-nucleating particles, *Nature*, 525, 234-238, 10.1038/nature14986, 2015.

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 message 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.

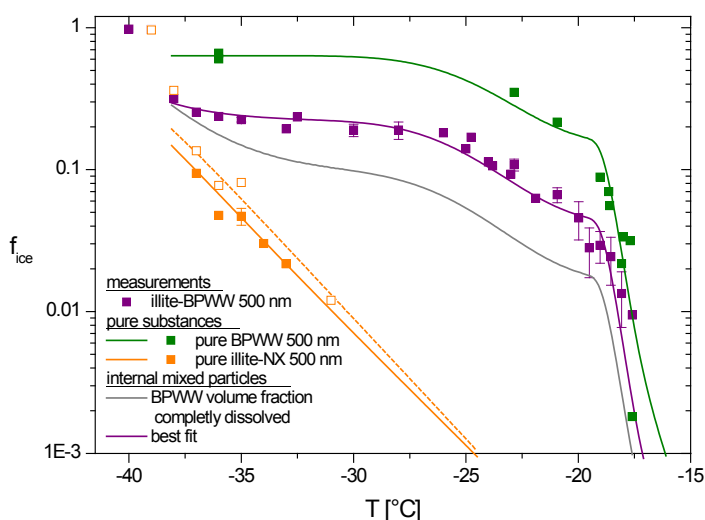
page 29642-29643, lines 26-1 – How was the concentration of illite-NX determined from the suspension? Please add detail regarding how these measurements were performed.

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 (D₅₀) 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 $\text{Na}/(\text{Na}+\text{SiO})$ 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 particles 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 k 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 $\lambda_{illite}(D_p)$ 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 μ_{θ} and σ_{θ} are determined from the value of λ_{illite} while the caption to Table 3 indicates that the values of μ_{θ} and σ_{θ} 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 $\mu\theta$ and $\sigma\theta$ 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	λ_{illite}	λ_{α}	λ_{β}
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^{π} for the first term and \int_{π}^{∞} for the third term.

page 29655, lines 3 & 5 – The dash before λ_{α} 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 λ_{α} .

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.

Summary of the main changes in the manuscript

- title changes to: “Laboratory-generated mixtures of mineral dust particles with biological substances: Characterization of the particle mixing state and immersion freezing behavior”
- the new manuscript provides 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 were extended respectively.
- section 5.1 (Basics of the Soccerball model) has been moved and is now section 2
- sections concerning the SPLAT and SEM/EDX measurements were rewritten
- the former table 2 was deleted
- a new table showing a summary of the λ values was added (the new table 2)
- table 3 was extended and includes now the SBM parameters of both wet generated illite-NX particles and dry generated illite-NX particles
- a new figure showing the Splat results was added (figure 4)
- the former figure 4 which is now figure 5 was extended. Now also the results of the measurements of illite-NX particles generated from suspension are shown. The SBM parameterization of both wet generated illite-NX particles (straight orange line) and dry generated illite-NX particles (dashed orange line) are shown.
- several smaller changes concerning technical issues

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

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Abstract

Biological particles such as bacteria, fungal spores or pollen are known to be efficient ice nucleating particles. Their ability to nucleate ice is due to ice nucleation active macromolecules (INM-INMs). It has been suggested that these INM-INMs maintain their nucleating ability even when they are separated from their original carriers. This opens the possibility of an accumulation of such INM-in-e.g., INMs in soils, resulting in an internal mixture of mineral dust and INM-INMs. If particles from such soils which contain biological INM-INMs are then dispersed into the atmosphere due to wind erosion or agricultural processes, they could induce ice nucleation at temperatures typical for biological substances, i.e., above -20 up to almost 0°C . ~~To explore this hypothesis, we performed a measurement campaign, while they might be characterized as mineral dust particles due to a possibly low content of biological material.~~

~~We conducted a study~~ within the research unit INUIT (Ice Nucleation research Unit), where we investigated the ice nucleation behavior of mineral dust particles internally mixed with INM. Specifically, we mixed a pure mineral dust sample (illite-NX) with ice active biological material (birch pollen washing water) and quantified the immersion freezing behavior of the resulting particles utilizing the Leipzig Aerosol Cloud Interaction Simulator (LACIS). ~~To characterize the~~ A very important topic concerning the investigations presented here as well as for atmospheric application is the characterization of the mixing state of the generated aerosol aerosol particles. In the present study we used different methods ~~which will also be discussed.~~ like single-particle aerosol mass spectrometry, scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX), and a volatility-hygroscopicity tandem differential mobility analyser (VH-TDMA) to investigate the mixing state of our generated aerosol. Not all applied methods performed similarly well in detecting small amounts of biological material on the mineral dust particles. Measuring the hygroscopicity/volatility of the mixed particles with the VH-TDMA was the most sensitive method. We found that internally mixed particles, containing ice active biological material, follow the ice nucleation behavior observed for the ~~purely biological particles, i. e. freezing occurs at temperatures at~~

~~which mineral dusts themselves are not yet ice active~~ pure biological particles. We verified this by modeling the freezing behavior of the mixed particles with the Soccerball model (SBM). It can be concluded that a single INM located on a mineral dust particle determine the freezing behavior of that particle with the result that freezing occurs at temperatures at which pure mineral dust particles are not yet ice active.

1 Introduction

In the last years a lot of effort has been made to characterize the freezing ability of different aerosol particles, which were thought to be ice nucleating active. Especially mineral dust particles were investigated quite intensively, as they were found in ice crystal residues most frequently (Kumai, 1961; DeMott et al., 2003; Pratt et al., 2009). Laboratory measurements indicate that mineral dust particles are efficient ice nucleating particles in a temperature range below -15°C (Murray et al., 2012) or probably only below -20°C (Augustin-Bauditz et al., 2014), where the latter examined ~~particles with droplets which each contained a single particle of~~ atmospherically relevant sizes. In contrast, atmospheric observations with lidar and radar showed that ice particles also formed at higher temperatures (Seifert et al., 2010; Kanitz et al., 2011; Bühl et al., 2013). It has been assumed in the past, that the presence of biological particles like bacteria, fungal spores or pollen is necessary to explain ice nucleation at higher temperatures, as these particles show ice nucleation ability up to temperatures of -2°C (Maki et al., 1974; Morris et al., 2008). ~~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 .~~ Only comparatively recent it became known that biological particles carry small ice nucleating macromolecules (~~INM~~INMs) which are responsible for their freezing ability. These ~~INM~~INMs could be e.g., proteins in case of bacteria (Wolber et al., 1986) ~~and~~, fungal spores (Fröhlich-Nowoisky et al., 2015) ~~and material originating from algae contained in the sea surface microlayer (Wilson et al., 2015)~~ or polysaccharides in case of pollen (Pummer et al., 2012). Furthermore several studies showed that ~~INM are~~

an INM is still ice active when the original carrier is detached or **non-viable** (e.g., in the case of bacteria **non viable** (Kleber et al., 2007; Pummer et al., 2012, 2015; Hartmann et al., 2013; Augustin et al., 2013; Fröhlich-Nowoisky et al., 2015)). So it is very likely that these **INM-INMs** are accumulated in the ground, where they come in contact with mineral dust particles. Already Schnell and Vali (1976) suggested that mineral dust particles may act as inert carriers for biological particles. Pratt et al. (2009) investigated 46 atmospheric cloud ice crystal residues with ATOFMS (Aerosol Time Of Flight Mass Spectrometer) and found that 60 % of the dust particles were likely a mixture of biological material and mineral dust. Also Michaud et al. (2014), investigating the residual particles in hail stones, found biological material to be attached to the surface of mineral dust particles. In that context, Kleber et al. (2007) describe that soil organic matter sorbs on mineral surfaces, preserving and maybe even accumulating INMs when being connected to mineral surfaces. Therefore, there is some indication that the ascription of mineral dust to the atmospheric **INP-ice nucleating particles (INP)** is, at least to a certain extent, due to unnoticed attached ice nucleating biological material (Conen et al., 2011). The question arises how the freezing ability of a mineral dust particle changes when there is some biological material attached to its surface. There are already some laboratory studies which confirm the enhanced freezing ability of soil dust due to the presence of biological material (Conen et al., 2011; O'Sullivan et al., 2014; Tobo et al., 2014). But soil dust However, the temperature ranges in which the organic fraction of the examined soil dusts was reported to be responsible for the ice activity differed between the studies, extending down to only -15 °C for O'Sullivan et al. (2014) (where ice activation observed at lower temperatures was ascribed to mineral dust), but down to even below -30 °C for Tobo et al. (2014) .

Soil dust is a very inhomogeneous substance and it is very difficult to characterize which of its constituents is responsible for the freezing initiation, particularly as **INM-INMs** were found to be on the size of a few 10 nm only (Pummer et al., 2012; Fröhlich-Nowoisky et al., 2015). Also, to quantify the freezing ability of an internal mixture of mineral dust and biological material it is advantageous to know the freezing ability of the individual materials. For

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this reason in the present study we mixed a well characterized mineral dust (~~illite-NX, Hiranuma et al., 2015~~) (~~illite-NX, Hiranuma et al., 2015; Augustin-Bauditz et al., 2014~~), well characterized biological material (birch pollen washing water, Pummer et al., 2012; Augustin et al., 2013) and investigated the immersion freezing ability of the resulting mixed particles, utilizing the Leipzig Aerosol Cloud Interaction Simulator (LACIS). The knowledge of the mixing state of the produced particles is essential for the understanding of the observed freezing abilities. Thus, we applied several methods for characterizing the mixing state of the generated aerosol: Single-particle aerosol mass spectrometry, scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX), and a volatility-hygroscopicity tandem differential mobility analyser (VH-TDMA). These methods each use a different approach to characterize the particle mixing state and can therefore be regarded as complementary. One aspect of this study is to compare these characterization methods and to assess their ability to identify the mixing state of a laboratory generated aerosol. Another important issue is the adequate interpretation of the freezing behavior of the mixed particles. For this purpose the Soccerball model (SBM, Niedermeier et al., 2015) is used. In the following ~~chapter the chapters~~ the basics of the SBM, the preparation of the mixture ~~is described~~ as well as the particle generation ~~-. We applied several will be explained. The applied~~ methods for characterizing the mixing state of the generated aerosol ~~-, which~~ are also described ~~in the next chapter~~, together with the measurement set-up used for the ice nucleation measurements. Afterwards the results are presented and discussed.

2 Basics of the Soccerball model

With the help of the SBM it is possible to describe and parameterize the freezing behavior of different materials. In general it is assumed that freezing is induced by single ice nucleating entities. These entities can be e.g., special sites on the surface of a particle (as it is assumed for mineral dust particles) or, in the case of biological material, single INMs. In any case, the ice nucleating entity has a defined two dimensional surface area s_{site} . A specific contact

angle θ is assigned to each ice nucleating entity which determines the ice nucleation ability of this particular entity in terms of a nucleation rate coefficient j_{het} based on classical nucleation theory (Zobrist et al., 2007). The overall contact angle distribution is described by a Gaussian probability density function with a mean value μ_θ and a standard deviation σ_θ , with each contact angle θ occurring with a probability of $p(\theta)$.

$$p(\theta) = \frac{1}{\sqrt{2\pi}\sigma_\theta} \exp\left(-\frac{(\theta - \mu_\theta)^2}{2\sigma_\theta^2}\right) \quad (1)$$

The probability P_{unfr} of a droplet (which contains an ice nucleating entity) to be unfrozen at a certain temperature T and a certain time t is defined as:

$$\begin{aligned} P_{\text{unfr}}(T, \mu_\theta, \sigma_\theta, t) = & \int_0^\pi p_\theta \exp(-j_{\text{het}}(T, \theta) s_{\text{site}} t) d\theta \\ & + \int_{-\infty}^0 p_\theta \exp(-j_{\text{het}}(T, \theta = 0) s_{\text{site}} t) d\theta \\ & + \int_\pi^\infty p_\theta \exp(-j_{\text{het}}(T, \theta = \pi) s_{\text{site}} t) d\theta \end{aligned} \quad (2)$$

As a next step, we consider a population of droplets, with each droplet containing a single particle and all particles having the same size. Naturally, the ice nucleating entities are Poisson distributed over the particle population. This means that each particle contains one, multiple or even none ice nucleating entities. Thus the average number of ice nucleating entities per particle is defined by the expected value of the Poisson distribution λ (Hartmann et al., 2013). The ice nucleating probability of each entity is determined by the

contact angle distribution. With this, the probability $P_{\text{unfr},\lambda}$ for droplets to remain unfrozen is given by:

$$\underline{P_{\text{unfr},\lambda}(T, \mu_\theta, \sigma_\theta, \lambda, t) = \exp\left(-\lambda(1 - P_{\text{unfr}}(T, \mu_\theta, \sigma_\theta, t))\right)} \quad (3)$$

Note that $P_{\text{unfr},\lambda} \neq P_{\text{unfr}}$. While P_{unfr} is only valid for droplets which contain an ice nucleating entity, $P_{\text{unfr},\lambda}$ is valid for the whole droplet population i.e. also for those droplets which do not contain an ice nucleating entity. The ice fraction, which is identical to the freezing probability $P_{\text{fr},\lambda}$ follows with:

$$\underline{f_{\text{ice}}(T, \mu_\theta, \sigma_\theta, \lambda, t) = 1 - P_{\text{unfr},\lambda}} \quad (4)$$

Equation (5) represents the combination of the original SBM from Niedermeier et al. (2014) and the CHES model from Hartmann et al. (2013) and was derived in detail in Niedermeier et al. (2015). The average number of ice nucleating entities λ is a material and size depending parameter and its determination is dependent on the freezing behavior of the investigated material.

3 Methods

3.1 Materials

To produce particles consisting of both dust and biological material, first a suspension containing both materials was prepared. For the experiments presented here illite-NX was chosen as the dust component, as this product has been used as a proxy for the natural dust composition found in the atmosphere ([Hiranuma et al., 2015](#)) ([Hiranuma et al., 2015, and references therein](#)). Furthermore, the freezing ability of pure illite-NX particles was already investigated with LACIS in a previous study (Augustin-Bauditz et al., 2014). For the illite-NX suspensions 510 g of illite-NX powder

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were suspended in ~~100~~200 mL of MilliQ water. After shaking the sample it was stored in the refrigerator for about 24 h. During that time large and heavy particles sedimented to the ground. An Eppendorf pipette was used to sample 2550 mL from the top part of the suspension. ~~This resulted in a suspension with an illite-NX concentration of about~~ 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 three times. The mean concentration of the illite-NX suspension is about 0.01 g mL⁻¹. ~~The freezing ability of illite-NX particles generated from this suspension was measured with LACIS and compared to the freezing ability of dry generated illite-NX particles as presented in Augustin-Bauditz et al. (2014) (not shown here). No significant difference was observed.~~

As biological component, washing water from Swedish birch pollen (in the following BPWW) was used. The INM responsible for the freezing ability of BPWW ~~are most likely polysaccharides~~ is most likely a polysaccharide (Pummer et al., 2012). The immersion freezing behavior of these ~~INM-INMs~~ was already investigated with LACIS and parameterizations are given in Augustin et al. (2013). The production of the BPWW suspension was done similar to that described in Augustin et al. (2013) but with a lower concentration of 1 g of pollen grains in 80 mL of MilliQ water. The birch pollen were mixed with the water and the pollen grains were then removed by filtration (pore size 4-7 μm) of the resulting suspension. 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 g mL⁻¹. Thus, after filtration we have about one third of the original mass of pollen in the washing water. This is the same order of magnitude than what was observed by Pummer et al. (2012).

The illite-NX suspension was mixed with the BPWW using ~~the same amount of both~~ 10 mL of each of the suspensions, which resulted in a dust-bio-mixture to which we refer to in the following as illite-BPWW suspension. Concerning this illite-BPWW suspension, one should be aware of the following facts: A high percentage of the BPWW consists of soluble material

which is released when the pollen grains are suspended in water. Thus the BPWW can be viewed as a suspension of INM-INMs (and other larger molecules) in a solution, rather than just a suspension in water. In contrast to that, the dust contains only a small amount of soluble material so the-its suspension consists mainly of solid particles and dilute water. These facts are important for the resulting mixing state of the internally mixed illite–BPWW particles and will be referred to again in the next section.

3.2 Particle generation and characterization

In Fig. 1 the generation pathway, starting from a suspension and ending with dry particles, is shown schematically. First an atomizer (following the design of TSI 3075) was used to generate droplets from the suspension illite–BPWW suspension as well as from the pure illite-NX and BPWW suspensions. As mentioned above, the BPWW contains a soluble fraction which was distributed in the whole suspension, so it is reasonable to assume that every droplet generated from the illite–BPWW suspension contained at least some soluble biological material, although not necessarily any INM-INMs. Most but maybe not all of these droplets also contained a mineral dust particle. After the atomization the droplets were dried by passing them through a silica gel diffusion dryer. ~~Considering the above mentioned composition of the generated droplets, the resulting dry particles were most likely an internal mixture of illite-NX and BPWW material. There might have been also some particles consisting only of BPWW material but we expect no pure illite-NX particles in the generated aerosol.~~ The dry particles were passed through a diffusion charger (85Kr) to achieve a bipolar charge distribution and afterwards size-selected using a differential mobility analyzer (DMA; Knutson and Whitby (1975); type “Vienna medium”). In the measurements presented here a mobility diameter of 500 nm was selected. Due to the use of a cyclone (cut off diameter: 500 nm) upstream of the DMA, the fraction of doubly charged particles contained in the aerosol is negligible: This was verified through measurements of the optical particle size distribution done downstream of the DMA using an UHSAS (Ultra-High Sensitivity Aerosol Spectrometer, Droplet Measurement Technologies). After size selection the aerosol stream was split and one part was always fed into a Condensation

Particle Counter (CPC, TSI 3010) to measure the total particle number concentration. The remaining aerosol was then available for particle characterization (e.g., sampling on filters) and for the Leipzig Aerosol Cloud Interaction Simulator (LACIS).

~~To analyze the~~ To understand the observed freezing abilities, the knowledge about the mixing state of the produced aerosol, generated particles is essential. Considering the above mentioned composition of the generated droplets, we assume that the resulting dry particles were most likely an internal mixture of illite-NX and BPWW material. There might have been also some particles consisting only of BPWW material but we expect no pure illite-NX particles in the generated aerosol. To proof this hypothesis several aerosol characterization methods were applied which will be ~~briefly~~ introduced in the following:

Volatility-Hygroscopicity Tandem Differential Mobility Analyser (VH-TDMA)

The volatility and hygroscopicity of ~~the BPWW and illite-NX materials~~ particles from all three suspensions, the two pure ones and the mixed one were used to infer the particle mixing state. In contrast to mineral dust, biological material is much more volatile and hygroscopic. Therefore, treatment with heat or humidity will change the size of particles consisting of biological material but likely will have a smaller effect on the mineral dust components. Measurements were performed with a custom-built VH-TDMA, which was composed of two DMAs and a CPC (TSI 3010) separated by a volatility (V-mode) and a humidity (H-mode) conditioning device. A mobility diameter of 500 nm, similar to that used for LACIS measurements, was selected with the first DMA. Monodisperse particles were then conditioned either in the thermodenuder section at 300 °C under dry conditions (10 % RH) or in the humidity section at 90 % RH at room temperature (20 °C). The residence times in the conditioning devices were approximately 2 s and 4 s for the V-mode and H-mode, respectively. The resulting particle size distributions obtained after conditioning were measured by the second DMA coupled to the CPC. The second DMA and the humidity section were confined in a temperature-controlled box at 20 °C. For the H-mode the sheath air of the second DMA was humidified to 90 % RH, ~~too~~. The volatile “growth” factor VGF obtained from the V-mode is defined as: $VGF = D_{p(300^{\circ}\text{C}, 10\% \text{RH})} / D_{p(20^{\circ}\text{C}, 10\% \text{RH})}$ where $D_{p(300^{\circ}\text{C}, 10\% \text{RH})}$ is

the measured diameter after heating at 300 °C and $D_{p(20^{\circ}\text{C},10\% \text{RH})}$ the selected dry mobility diameter at ambient temperature. The hygroscopic growth factor HGF using the H-mode is defined in a similar way: $\text{HGF} = D_{p(20^{\circ}\text{C},90\% \text{RH})} / D_{p(20^{\circ}\text{C},10\% \text{RH})}$ where $D_{p(20^{\circ}\text{C},90\% \text{RH})}$ is the measured diameter at 90 % RH. In this work, the distribution of growth factors refers to the growth factor probability density function (GF-PDF) and was fitted as a superposition of distinct Gaussian modes using the TDMAinv algorithm developed by Gysel et al. (2009). From comparing VGF and HGF distributions obtained for the pure and mixed particles, the fraction of internally mixed particles in the aerosol generated from the illite–BPWW suspensions can be derived.

Scanning Electron Microscope (SEM) and Energy Dispersive X-ray analysis (EDX)

In cooperation with the Leibnitz institute of surface modification in Leipzig (IOM) ~~filter samples of particles and the Technical University Darmstadt filter samples~~ generated from the ~~illite–BPWW–illite–BPWW~~ suspension as well as from the pure illite-NX and BPWW suspensions were imaged ~~with a SEM. With this technique by scanning electron microscopy (SEM). Analysis at the IOM was performed in a ULTRA 55, Carl Zeiss SMT, (Oberkochen, Germany) and in Darmstadt in a FEI Quanta 200 FEG Environmental Scanning Electron Microscope (Eindhoven, the Netherlands).~~

~~By SEM we intended to examine visual differences between particles generated from the two pure substances and from the illit–BPWW suspensions to see whether there are pure particles in the illite–BPWW aerosol.~~

Energy Dispersive X-ray analysis (EDX)

~~The Technical University of Darmstadt analyzed the generated particles using EDX. Particles were sampled on Bore-substrates and then sent to Darmstadt for the analysis. Analysis was performed in a FEI (Eindhoven, the Netherlands) Quanta 200 FEG Environmental Scanning Electron Microscope (ESEM) equipped with an energy-dispersive illite–BPWW suspensions to get more information about the exact mixing-state of the~~

illite-BPWW aerosol, i.e., if the sample can be described as an external mixture of the two components, as an agglomeration of the two components or as an internal mixture. Additionally, at the Technical University Darmstadt the pure samples and the particles from the illite-BPWW suspension were analyzed by energy dispersive X-ray microanalysis system analysis (EDX, Phoenix EDAX, Tilburg, the Netherlands). With this technique the elemental composition of individual particles is determined. The classification of the analyzed particles in pure illite particles, pure BPWW particles or internal mixture of both components was performed on base of the determined C/Si ratio. For particles with a C/Si ratio (based on netto count rate) larger than 10 or smaller than 0.1 external mixtures were assumed, all other particles are classified as internal illite/BPWW mixtures. Again, particles generated from the two pure suspensions were analyzed as well as particles generated from the illite-BPWW suspension. In order to prevent an influence of the sampling substrate on the chemical results (carbon signal from the filters) for these analysis the particles were sampled on Boron substrates.

Single Particle Laser Ablation Time-of-flight mass spectrometer (SPLAT)

Finally we also investigated the mixing state of the ~~illite-BPWW~~ illite-BPWW aerosol via single particle mass spectrometry. Here, the instrument SPLAT (~~Kamphus et al., 2008~~) (Kamphus et al., 2008) of the Max Planck Institute for Chemistry, Mainz, was used. For these experiments, particles were generated with the set-up described above and then examined in the SPLAT immediately after generation. This was done at first separately for an illite suspension and for BPWW, and then for the mixed particles generated from the ~~illite-BPWW~~ illite-BPWW suspension. In the SPLAT, single particles are hit by a laser pulse which vaporizes and ionizes the compounds of the particle. A bipolar time-of-flight mass spectrometer is used to detect the ions. The composition and thereby the mixing state of the individual particles can then be inferred from their mass spectra. ~~To decide whether a particle is an internal mixture or a pure particle, the peak intensity ratio of selected marker peaks ($\text{Na}/(\text{Na} + \text{SiO})$) was used (histograms of this ratio for the pure as well as for the mixed particles are shown in the Supplement).~~

The experiments with the pure particles showed that values of this ratio between 0 and 0.1 occurred only for pure illite, while ratios larger than 0.65 were only observed for pure BPWW particles. Thus, particles with ratios between these threshold values are likely mixtures of both components. The uncertainties of this method can be estimated as follows: 25 uncertainty originates from the amount of useful mass spectra per measurement (about 25 of the mass spectra recorded in each experiment had to be discarded out due to insufficient ion count rate or wrong mass calibration). Additionally, 37 of the pure illite values as well as 44 of the pure BPWW values have intensity ratios in the intermediate range from 0.1 to 0.65. These uncertainties result in a possible underestimation of externally mixed particles of up to about a factor of 1.6 for illite and 1.8 for BPWW.

3.3 Freezing experiments

For the freezing experiments the laminar flow tube LACIS was utilized. In the inlet section of LACIS, the aerosol flow is combined isokinetically with a humidified sheath air flow such that the aerosol forms a beam of approximately 2 mm in diameter along the center line of the flow tube. Supersaturated conditions are achieved by cooling the tube walls, and result in activation of the particles to droplets, with each droplet containing one size segregated particle. Further downstream of the flow tube, these droplets then may freeze due to further cooling. For detailed information on the operation mode of LACIS see Stratmann et al. (2004) and Hartmann et al. (2011).

At the outlet of LACIS, TOPS-Ice (thermally-stabilized optical particle spectrometer, Clauss et al., 2013) is used to discriminate between frozen and unfrozen droplets, and to quantify the fraction of frozen droplet (f_{ice} , number of frozen droplets divided by the total number of frozen and unfrozen droplets). In the investigations presented here, f_{ice} was determined in the temperature range between ~ -17 and -40 °C.

4 Measurement results

In the following we will first describe the results ~~of the methods that were used for obtaining information on the~~ concerning the determination of the mixing state of the aerosol ~~(3.1 generated aerosol (4.1))~~. Afterwards, ~~we will describe~~ the results of the immersion freezing experiments ~~(3.2 will be discussed (4.2))~~.

4.1 ~~Mixing state~~ Characterization of the produced aerosol particle mixing state using different methods

Several methods were applied to characterize the mixing state of the generated illite-BPWW aerosol. These were already introduced in Sect. 3.2 and their results will be discussed in the following.

4.1.1 VH-TDMA

The HGF and VGF distributions of the different particle types which were obtained from the VH-TDMA measurements are shown in Fig. 2. The values for the mean and spread standard deviation of the different curves are given in Table 1. For the pure illite-NX particles (orange line) almost no change in size was observed for both treatments, which results in mean growth factors of approximately 1. This confirms that the illite-NX contained no or only very little amounts of volatile or soluble material. In contrast to that, particles generated from the BPWW suspension (green line) showed a significant change in size for both treatments which lead to HGF and VGF of 1.38 and 0.570.56, respectively. The results for the particles generated from the illite-BPWW suspension (purple line) showed HGF and VGF values which are between those for the pure substances. It is also obvious that only one mode is present and that this mode shows no (for the VGF) or only little (for the HGF) overlap with the pure BPWW material. This suggests ~~;~~ that nearly all particles from the illite-BPWW suspension consisted of both, illite-NX and BPWW material ~~or, in~~ In other words: all particles were internally mixed. Furthermore, the HGF values can be used to determine the hygroscopicity parameters κ of the different particle types (Petters and Kreidenweis, 2007).

These κ values are 0, 0.176 and 0.017 for pure illite-NX, pure BPWW and internally mixed particles, respectively, determined from average HGF. For the κ of the internally mixed particles the simple mixing rule $\kappa_{\text{mix}} = \sum_i \epsilon_i \kappa_i$ can be applied (Petters and Kreidenweis, 2007). Here, ϵ_i depicts the amount of material i which has a κ value of κ_i . With this it is possible to calculate the average volume fraction of BPWW material on the internally mixed particles, which was found to be 9.7%. Assuming spherical illite-NX particles surrounded by a smooth layer of BPWW material, the BPWW layer thickness would be 8 nm.

Examples of SEM images of

4.1.2 SEM and EDX

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.

Following this scheme all particles from the illite–BPWW suspension would have been classified as pure illite samples. Including the morphological data from the SEM measurements it becomes obvious that the particles from the investigated particles are shown in Fig. 3. Particles generated from a BPWW suspension looked like viscous droplets (panel a) . In contrast to that the illite-NX particles show a clear, nearly spherical shape (panel b) . On a closer look, one recognizes that the illit-NX particles consists of small agglomerated plates. This structure was already described earlier by Broadley et al. (2012) . The image of the particles generated from the

illite-BPWW suspension is shown in the lower part of illite-BPWW suspension are neither morphologically (secondary electron images, see Fig. 3. In this example as well as in all other images that were taken, which are not all shown here, b and c) nor chemically (EDX) discriminable from the pure illite particles by SEM analysis. Furthermore, nearly none of the viscous droplets (Fig. 3a), which would indicate the presence of pure BPWW particles, could be observed in the illite-BPWW images. From the visual appearance, all particles resembled pure illite-NX particles. On the other hand, as described above, the generation method makes it very unlikely that pure illite-NX particles can be generated, as some material from the BPWW is always contained in the droplets generated from the atomizer. So we suggest, based on these images, that nearly all particles generated from illite-BPWW suspensions are internal mixtures, even if the BPWW material on the particles can not be identified visually. The VH-TDMA measurements already showed similar results and they indicated that the volume fraction of BPWW material is rather small compared to the volume fraction of illite-NX. As already assumed above, it might be that the illite-NX particles are covered with a thin SEM images of the illite-BPWW particles (145 particles were counted from which only 1 showed a shape similar to the pure BPWW particles).

With respect to the limitations of EDX analysis it can be concluded that the relative abundance of the BPWW residuals within the mixed particles is less than (maximum) 2 wt%. As the morphological surface features of the illite particles are still visible in the illite-BPWW suspension, a surface coating, which is thicker than a few tens of nanometer, can be excluded. This is consistent with the results from the VH-TDMA measurements, where a layer thickness of 8 BPWW film, which is not optically visible in the microscope. It is also possible that due to the very low pressure (near vacuum) which is needed for the microscope imaging some of the more volatile BPWW compounds are evaporated.

The Darmstadt EDX analysis showed similar results as the SEM images from the IOM. Following the Si/C ratio 100 of the analyzed particles generated from the illite-BPWW suspension were pure illite particles. An unambiguous identification of any BPWW material on the particles generated from this suspension was not possible by EDX. Again, it can

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be argued that thin films of organic material were evaporated due to the vacuum in the microscope or that these films are too thin to be detectable by EDX.

The results of the SEM/EDX measurements for the mixing state of the particles generated from the illite-BPWW suspension can be summarized in the following way: no externally mixed nonvolatile carbonaceous particles were found. The particles were rather determined to be pure illite particles, showing neither morphologically (by visualization) nor chemically (by EDX) any fingerprints for carbonaceous material. However, as the presence of the BPWW component was shown before by the VH-TDMA technique, these findings hint towards the biological material being present only nm was estimated. As the residual carbonaceous particles (which will contain the INMs) from the BPWW will be sited on the surface of the illite grains, it is highly likely that they are present either as very thin carbonaceous films on the illite particles or by evaporation in the high vacuum SEM environment. In principle it is not possible to make a clear statement about the mixing state of the illite-BPWW particles from the SEM/EDX analysis. Nevertheless, as no pure BPWW particles were observed and as we do not expect pure illite-NX particles in the illite-BPWW aerosol, the assumption could be made that nearly all particles are internally mixed and that the BPWW material on the particles is rather small, which was already suggested by the VH-TDMA measurements. film or as small "isles" at the surface of the illite particles.

For the illite-BPWW

4.1.3 Single Particle Mass Spectrometry

For the illite-BPWW suspension 549 mass spectra were measured with the SPLAT, 150 of which had to be discarded due to insufficient ion count rate or wrong mass calibration. The remaining 399 mass spectra were used for the further analysis (the Na/(Na + SiO) histograms and data can be found in the Supplement). Of these spectra, around

To decide whether a particle is an internal mixture or a pure particle, the peak intensity ratio of selected marker peaks (Na/(Na + SiO)) was used (histograms of this ratio as well as examples of mass spectra for the pure as well as for the mixed particles are shown in Fig. 4).

The experiments with the pure particles showed that values of this ratio between 0 and 0.1 occurred only for pure illite, while ratios larger than 0.65 were only observed for pure BPWW particles. Thus, particles with ratios between these threshold values are likely mixtures of both components.

328 (around 82) showed a ratio $\text{Na}/(\text{Na} + \text{SiO})$ % out of the 399 spectra showed a ratio $\text{Na}/(\text{Na} + \text{SiO})$ between 0.1 and 0.65, indicating internal mixtures of illite-NX and BPWW material. Only 59 particles (14 %) appeared to be pure illite, and 16 (4 %) appeared to be pure BPWW in the SPLAT. However, the possible underestimation-

The uncertainties of this method can be estimated as follows: 25% uncertainty originates from the amount of useful mass spectra per measurement (about 25% of the mass spectra recorded in each experiment had to be discarded out due to insufficient ion count rate or wrong mass calibration). Additionally, 37% of the pure illite values as well as 44% of the pure particles as mentioned above allows also BPWW values have intensity ratios in the intermediate range from 0.1 to 0.65. These uncertainties result in a possible underestimation of externally mixed particles of up to about a factor of 1.6 for pure illite and 1.8 for pure BPWW. This "worst case scenario" would lead to values of 24 pure illite-% pure illite particles and 7 pure BPWW-% pure BPWW particles, which still means that 69% of the particles are internally mixed).

On the other hand, as discussed above, it is rather unlikely to find pure illite-NX particles in the aerosol generated from the illite-BPWW-illite-BPWW suspension. But as the VH-TDMA measurements as well as the SEM images and the EDX analysis suggest that the amount of BPWW material on the particles might be rather small, it can not be ruled out that the SPLAT can not detect such small amounts of biological material and thus the amount of internally mixed particles is underestimated.

In Table 2 the results of all characterizations methods are summarized.-

It is obvious that the analysis of the mixing state of the generated illite-BPWW-illite-BPWW aerosol is not trivial and that it is bound to the limitations of the applied instruments. What we learned from these investigations is, that especially for particles with only small

amounts of biological material, the detection of this biological material is difficult or even not possible. Nevertheless, all methods showed ~~that a~~ (albeit some only indirectly) that a significant fraction, if not all, of the generated particles consisted of both ~~-,~~ biological material and dust ~~or, in other words,~~ and are internally mixed particles.

4.2 Immersion freezing experiments

In Fig. 5 the results of the immersion freezing experiments of the particles generated from the illite–BPWW suspension are shown (purple symbols). ~~A first increase of the ice fraction with decreasing temperature can be observed at a temperature of about -17C . After this first steep increase the ice fraction curve becomes shallower and finally reaches a saturation level where no more droplets freeze with decreasing temperature. At about -36C , ice fraction starts to slightly rise again until the homogeneous freezing sets in (at about -38C).~~ For a better understanding of the freezing behavior of the ~~particles generated from the illite–BPWW suspension~~ mixed particles it is necessary to first understand the freezing behavior of the pure materials. Therefore, results of measurements from the pure 500 nm illite-NX and BPWW particles are shown in Fig. 5, ~~too,~~ as orange and green symbols, respectively. The straight freezing ability of illite-NX particles generated from suspension was compared to the freezing ability of dry generated illite-NX particles of the same size (500 nm) as presented in Augustin-Bauditz et al. (2014) (filled and open orange squares in Fig. 5, respectively). The ice nucleation ability of the wet generated particles was only slightly below that of the dry generated ones. Concerning the BPWW measurements shown here, we should mention that we had to use another birch pollen batch than in Augustin et al. (2013) as the one used in the former study was used up. Similarities and differences between these two batches are addressed further down in the manuscript.

The comparison between the freezing behavior of the particles generated from the illite–BPWW suspension and the pure substances indicates that it is possible to explain the immersion freezing behavior of the mixture by the freezing abilities of the pure substances. Down to roughly -30C , the temperature range at which the first step increase is observed, and also the subsequent course of the ice fraction, are identical to those of pure

BPWW particles. The second increase in the frozen fraction below -30°C , from roughly 0.2 to above 0.3, occurs in a temperature range for which the ice nucleation is observed for pure illite-NX particles. At about -38°C the homogeneous freezing sets in. The solid lines in Fig. 5 represent modeled ice fractions for the different particle types. In the next section, it will be described. The next section will describe how these curves were obtained for the pure particles and how they can be combined to describe the ice nucleation behavior of the internally mixed particles.

5 Theoretical description and discussion

In the following, we will describe the modeling of the ice nucleation measurements and will show what can be learned from that. For this, the SBM in the version introduced in Niedermeier et al. (2015) will be used. First we will explain the basics of this model, then we will. On the first view it seems to be fairly clear just from the three experimental datasets in Fig. 5 that the freezing behavior of the mixed particles can be explained by the freezing behaviors of the pure substances. Nevertheless, this conclusion has to be checked by adequate modeling. For this purpose we decided to use the Soccerball model (SBM, Niedermeier et al., 2015, see section 2). We will first introduce the parameterizations for the pure substances, and finally afterwards the ice nucleation of the mixed illite-BPWW-illite-BPWW particles will be modeled.

5.1 Basics of the Soccerball model

In general it is assumed that freezing is induced by single ice nucleating entities. These entities can be e.g. special sites on the surface of a particle (as it is assumed for mineral dust particles) or, in the case of biological material, single INM. In any case, the ice nucleating entity has a defined two dimensional surface area s_{SITE} . A specific contact angle θ is assigned to each of the ice nucleating entities which determines the ice nucleation ability of this particular entity in terms of a nucleation rate coefficient j_{NET} based on classical nucleation theory (Zobrist et al., 2007). The contact angle θ of each surface site is

determined by a Gaussian probability density function with a mean value μ_θ and a standard deviation σ_θ :

$$p(\theta) = \frac{1}{\sqrt{2\pi}\sigma_\theta} \exp\left(-\frac{(\theta - \mu_\theta)^2}{2\sigma_\theta^2}\right)$$

The probability P_{unfr} of a single entity to not initiate ice nucleation at a certain temperature T and a certain time t is defined as:

$$\begin{aligned} P_{\text{unfr}}(T, \mu_\theta, \sigma_\theta, t) &= \int_0^x p_\theta \exp(-j_{\text{het}}(T, \theta) s_{\text{site}} t) d\theta \\ &+ \int_{-\infty}^0 p_\theta \exp(-j_{\text{het}}(T, \theta = 0) s_{\text{site}} t) d\theta \\ &+ \int_x^\infty p_\theta \exp(-j_{\text{het}}(T, \theta = \pi) s_{\text{site}} t) d\theta \end{aligned}$$

As a next step, we consider a population of droplets, with each droplet containing a single particle and all particles having the same size. It is assumed that the ice nucleating entities are Poisson distributed over the particle population. Thus the average number of ice nucleating entities per particle is defined by the expected value of the Poisson distribution λ (Hartmann et al., 2013). Depending on λ , each particle contains none, one or even multiple ice nucleating entities and all entities have the same freezing probability. With this, the probability $P_{\text{unfr},\lambda}$ for droplets to remain unfrozen is given by:

$$P_{\text{unfr},\lambda}(T, \mu_\theta, \sigma_\theta, \lambda, t) = \exp\left(-\lambda(1 - P_{\text{unfr}}(T, \mu_\theta, \sigma_\theta, t))\right)$$

The ice fraction, which is identical to the freezing probability $P_{\text{fr},\lambda}$ follows with:

$$f_{\text{ice}}(T, \mu_\theta, \sigma_\theta, \lambda, t) = 1 - P_{\text{unfr},\lambda}$$

Equation (5) represents the combination of the original SBM from Niedermeier et al. (2014) and the CHESS model from Hartmann et al. (2013) and was derived in detail in Niedermeier et al. (2015). λ is a material and size depending parameter and its determination is dependent on the freezing behavior of the investigated material.

5.1 Immersion freezing properties of BPWW particles

For the freezing behavior of BPWW (green line in Fig. 5) it was observed that the ice fraction reaches a saturation range below one (Augustin et al., 2013), which means that at a certain temperature no further increase in ice fraction with decreasing temperature is observed. It is known that single INM-INMs are responsible for the freezing behavior of the BPWW material (Pummer et al., 2012). The occurrence of a saturation range below one implies that not all of the particles generated from a BPWW suspension contain an INM (a similar behavior was observed for Snomax in Hartmann et al., 2013). In those cases, the average number of INM per droplet (λ) can be directly calculated from the ice fraction f_{ice}^* observed in the saturation range, with: $\lambda = -\ln(1 - f_{ice}^*)$.

In Augustin et al. (2013) it was possible to describe the λ value of the BPWW by both, a linear particle volume dependence and a linear particle surface area dependence, where a surface area dependence yielded a slightly better regression coefficient (surface area: $r = 0.9918$, and volume: $r = 0.9275$). This was somehow surprising as it was expected that the BPWW particles are fully soluble and thus should show a clear linear volume dependence (as for example in the case of Snomax; Hartmann et al., 2013). In Augustin et al. (2013) it was speculated that the BPWW contains some slowly dissolving material and that the generated particles may need more time than the few seconds they have in LACIS to dissolve completely, which would explain the surface dependence of λ .

It should be mentioned here that the Swedish birch pollen washing water which was used in this study contains two different types of INM (called INM- α and INM- β), which are internally mixed (Augustin et al., 2013). So for the BPWW used in this study Eq. (5) changes to:

$$P_{unfr,\lambda}^{BPWW}(T, \mu_\theta, \sigma_\theta, \lambda, t) = \exp\left(-\lambda_\alpha(1 - P_{unfr,\alpha}) - \lambda_\beta(1 - P_{unfr,\beta})\right) \quad (5)$$

The two different types INM are the reason for the change in the slope-shoulder of the BPWW ice fraction curve at around -20°C , where the increase at temperatures above and below this shoulder is caused by the more and the less ice active type of INM being active,

respectively. The values for μ_θ and σ_θ for both INM types contained in the BPWW were taken from Augustin et al. (2013) and are given in Table 3.

The size of a single INM was estimated to be 10 nm (Pummer et al., 2012). Assuming spherical ~~INM-INMs~~ this leads to a s_{site} of $3.14 \times 10^{-16} \text{ m}^2$. As explained above, the λ values of INM- α and INM- β can be described by both a linear volume dependence and a linear surface area dependence. The relations between λ and particle volume for INM- α and INM- β can be described as follows:

$$\underline{\lambda}_\alpha = 6.76 \times 10^{18} \text{ m}^{-3} \times D_p^3 \text{ and } \lambda_\beta = 1.31 \times 10^{18} \text{ m}^{-3} \times D_p^3.$$

When assuming a surface area dependence the relations are:

$$\underline{\lambda}_\alpha = 3.30 \times 10^{12} \text{ m}^{-2} \times D_p^2 \text{ and } \lambda_\beta = 6.65 \times 10^{11} \text{ m}^{-2} \times D_p^2.$$

The respective values for a 500 nm particle are given in Tab. 2. 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 a new birch pollen batch had to be used for the here presented measurements. This includes both, measurements of pure BPWW and those for mixed illite-BPWW particles) a new birch pollen batch was used~~ illite-BPWW particles. It is not surprising that due to natural variability the number of ~~INM-per-particle-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_θ and σ_θ) ~~are similar, as the same INM are present~~ 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.

5.2 Immersion freezing properties of illite-NX particles

In contrast to the BPWW particles we did not observe a saturation range for the frozen fraction for the illite-NX particles (Augustin-Bauditz et al., 2014). But, due to the lower ice nucleating ability, it ~~could be is~~ plausible that homogeneous ice nucleation, which is dominant for $T < -38$ °C, perhaps masks a potential leveling off of the frozen fraction curves. Therefore, we also used the presented procedure for representing the ice nucleating ability of the pure illite-NX particles (orange ~~line lines~~ in Fig. 5). To do so, the following assumptions were made. First, the λ value of illite-NX is assumed to be smaller than the determined λ value for feldspar given in Niedermeier et al. (2015). This is a reasonable assumption as K-feldspar was observed to be the most ice active mineral dust found so far (Atkinson et al., 2013; Augustin-Bauditz et al., 2014). Second, we assume that λ is directly correlated to the particle surface area, as for mineral dust it is assumed that the ice nucleating entities are special sites on the surface of the particle. This correlation between λ and particle surface area was already observed for feldspar particles (Niedermeier et al., 2015). Due to these assumptions we could distinctly narrow the range of the possible λ parameter. For a s_{site} of 10^{-14} m² as used in Niedermeier et al. (2015), the best fit between measured and modeled data was obtained for $\lambda_{\text{illite}} = 3.25 \times 10^{12} \text{ m}^{-2} \times D_{\text{p}}^2$. For a 500 nm particle the λ_{illite} would be 0.8125 (see Tab. 2). The resulting μ_{θ} and σ_{θ} for both wet and dry generated particles are given in Table 3. For the calculations in the next section, the parameters of the wet generated particles were used, as the illite-BPWW particles were also generated from a suspension.

5.3 Immersion freezing properties of illite-BPWW particles

~~The comparison between the freezing behavior of the particles generated from the illite-BPWW suspension and the pure substances indicates that it is possible to explain the immersion freezing behavior of the mixture by the freezing abilities of the pure substances. Down to roughly -30 °C, the temperature range at which the first steep increase is observed, and also the subsequent course of the ice fraction, are identical to those of pure BPWW~~

particles. The second increase in the frozen fraction below -30°C , from roughly 0.2 to above 0.3, occurs in a temperature range for which the ice nucleation is observed for pure illite-NX particles. In other words As already indicated, the freezing behavior of the mixed particles appears as superposition of the single substances' freezing behaviors, and the most ice active ice nucleation entity within the particle will dominate the freezing process. For the 500 nm illite-BPWW particles considered here the INM-INMs of the BPWW material are the dominant ice nucleation entity/entities in the temperature range between -17 and -30°C . At this point, it is worth mentioning that although we assume that every particle contains some material from the BPWW, not every particle will contain an INM, as already pure 500 nm BPWW particles did not all contain an INM. Hence in some illite-BPWW particles, it will be the illite which induces droplet freezing.

In the following, we model the ice nucleation behavior of the illite-BPWW particles based on the already-existing-SBM parameters (μ_{θ} and σ_{θ} from Table 3) for the pure substances. Therefore, we first For the illite-NX component the parameters of the wet generated particles were used.

First we consider that all particles are internally mixed (see discussion in Sect. 3.14.1). As independent probabilities are multiplicative, the $P_{\text{unfr, mix}}$ is calculated as follows:

$$P_{\text{unfr, mix}}(T, \mu_{\theta}, \sigma_{\theta}, \lambda, t) = P_{\text{unfr, } \lambda}^{\text{illite}} \times P_{\text{unfr, } \lambda}^{\text{BPWW}} \quad (6)$$

With that the ice fraction can be determined as follows:

$$f_{\text{ice}}(T, \mu_{\theta}, \sigma_{\theta}, \lambda, t) = 1 - P_{\text{unfr, mix}} \quad (7)$$

Equation (7) now represents the freezing behavior of particles, which consist of both illite-NX and BPWW material, assuming that the freezing behavior of the pure substances remains unchanged, even when they are mixed. As already mentioned, the μ_{θ} and σ_{θ} values of the pure substances are independent of the particle size. In contrast to that the parameters λ_{α} , λ_{β} and λ_{illite} change when the particle size changes.

From the VH-TDMA measurements we know that the soluble volume fraction of the BPWW material in the internally mixed particle is approximately 9.7%. As mentioned above

it might be that the BPWW material on the illite-NX material does not fully dissolve during the few seconds in LACIS, where the particle is immersed in a droplet. In the following we want to discuss three different behaviors, which the internally mixed particles may show during the freezing experiments (Fig. 6). Case a (a) depicts an extreme case, where the illite-NX particle is fully covered with BPWW material and the BPWW material does not dissolve at all. Only the BPWW material is exposed to the water and can trigger the freezing process. In this case the internally mixed particles would behave exactly like pure 500 nm BPWW particle (green line in Fig. 5). Obviously this case overestimates the measured ice fractions of the illite–BPWW particles. Case b (b) shows another extreme case. Here the whole BPWW material is dissolved in the droplet. Assuming the volume fraction of BPWW material on the illite–BPWW particles to be 9.7%, we can calculate the λ values of the BPWW material (assuming λ to be volume depended dependent) as well as λ_{illite} for the remaining spherical illite-NX particle. The values are given in Tab. 2. The resulting ice fraction curve is shown in Fig. 5 as a grey line. It is obvious that in this case the ice fraction of the illite–BPWW particles is underestimated. We can conclude from this that the real case is an intermediate case of a and b (a) and (b) (panel c (c) in Fig. 6). The BPWW material dissolves partly, as it was already suggested in Augustin et al. (2013). For this case the size parameters can not be calculated directly as we have no information of how much of the BPWW material will dissolve and how much will still cover the illite-NX core. So we fit Eq. (7) to the measured data with λ_α , λ_β and λ_{illite} being being the fit parameters, where the relation between λ_α and λ_β remains the same as derived from the measurements of the pure BPWW. This leads to the following results:

$$\underline{-}\lambda_\alpha = 0.2062, \lambda_\beta = 0.0412, \lambda_{\text{illite}} = 0.3293$$

This is depicted as purple line in Fig. 5, and fits the course of the measured data well over the whole temperature range. It should be noted at this point that in the model the ice nucleation properties (μ_θ and σ_θ) of both materials have not been changed and still are the same as for the pure materials. So with the modeled ice fraction curves we can confirm that it is possible to explain could show that the course of the immersion freezing behavior of

the mixture can be explained by the freezing abilities of the pure substances. This can be interpreted as a confirmation of our assumption that it is the ice nucleating active biological fraction which determines the INM containing mixed particles' ice nucleation ability.

6 Conclusions

Several studies showed that mineral dust particles can act as carriers for biological material. Up to now it was not shown clearly how a single particle which consists of both τ -mineral dust and biological material τ -behaves in terms of ice nucleation.

In this study we showed that it is possible to quantitatively describe the freezing behavior of particles generated from aan illite-BPWW suspension, based on parameters (mean values μ_{θ} and standard deviations σ_{θ} and standard deviation of the contact angle distribution) of the pure substances. In other words, the freezing behavior of the mixed particles appears as superposition of the single substances' freezing behaviors and for a droplet containing such a mixed particle, the most active freezing entity in the droplet will control the freezing process at a certain temperature. For the internally mixed particles presented here, this means that if there is an INM located on the surface of an illite-NX particle, this INM will initiate the freezing of the droplet already at much higher temperatures than the pure illite-NX particle.

~~However, already for the here examined particles generated in the laboratory under well defined conditions, it was~~ This study also indicates, that it is fairly difficult to determine the mixing state ~~of the particles, and it was seen that particularly the~~ even already of a laboratory generated aerosol. The VH-TDMA measurements strongly suggest that the generated particles are internally mixed. However, the amount of biological material on these particles was estimated to be less than 10 % of the whole particle mass. The microscope techniques (SEM and EDX) apparently did not detect this small biological fraction of a mixed bio-dust particle ~~might be overlooked. Atmospheric measurements are usually even more challenging, and therefore~~ because of their limited analytical possibilities. Also the single particle mass spectrometry (SPLAT) did not detect such small amounts

of biological material on all particles. As a result the amount of internally mixed particles appears to be underestimated. This may also be the case for atmospheric measurements. Therefore it can not be ruled out that ice nucleation attributed to mineral dust in the past occasionally might have been due to an undetected biological component.

Based on our results the following ~~advices~~ advice can be given for the modeling of atmospheric ice nucleation: On the one hand it is not necessary to define new parameterizations for dust-bio-mixtures as these particles, depending on actual composition, induce freezing similar to the pure substances. On the other hand, this implies that for proper modeling, the knowledge concerning the number of mineral and biological ~~IN~~ INP and their mixing state is highly desirable. ~~To find~~ Finding the respective atmospherically relevant values is still a big challenge as the particle characterization methods are limited in their ability to detect ~~rarely occurring materials~~ small amounts of certain substances.

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Table 1. Mean value and ~~spread~~ standard deviation of the HGF and VGF Gaussian distribution for the different heated (V) and humidified (H) particle types. The selected size prior to the treatment was 500 nm.

	suspension	mean	spread <u>standard deviation</u>
HGF	illite-NX	0.99	0.01
	BPWW	1.38	0.07
	illite-BPWW	1.05	0.05
VGF	illite-NX	0.99	0.01
	BPWW	0.56	0.02
	illite-BPWW	0.87	0.01

Table 2. Summary of the results of all characterization methods. The amounts of pure and internally mixed particles are given in %. For SEM and EDX no clear statement could be given, so the assumed values are given in parentheses. The values in squared brackets depict the possible SPLAT results if the underestimations of pure particles are implemented. Summary of the λ values for 500 nm particles of different composition. They were calculated with the given relations in section 5.1 for BPWW particles and section 5.2 for illite-NX particles. For the calculation of the values of case (b) it was assumed that the soluble volume fraction of the BPWW material in the internally mixed particle is approximately 9.7%. The values for case (c) were estimated by fitting Eq. (7) to the measured data with λ_α , λ_β and λ_{illite} being the fit parameters, where the relation between λ_α and λ_β remains the same as derived from the measurements of the pure BPWW. The λ values shown here, are only valid for the respective particles generated for the measurements in this study.

500 nm particle	λ_{illite}	λ_α	λ_β
pure illite	0.8125	-	-
pure BPWW (surface dependent)	-	0.825	0.166
Case (b) (Fig.6)	0.759	0.082	0.016
Case (c) (Fig.6)	0.5114	0.2056	0.0411

Table 3. Soccerball model parameters used for the calculations shown in Fig. 5. The values for μ_θ and σ_θ are determined from ~~former~~ measurements with the pure substances (~~Augustin et al., 2013 for the~~ The BPWW parameters and Augustin-Bauditz et al., 2014 were directly taken from Augustin et al. (2013). The parameters for the illite-NX particles (wet and dry generation) were calculated within this study.

	μ_θ [rad]	σ_θ [rad]
pure illite <u>illite-NX (dry generation)</u>	1.903 <u>1.903</u>	0.2743 <u>0.274</u>
pure <u>illite-NX (wet generation)</u>	<u>2.022</u>	<u>0.315</u>
pure BPWW (INM- α)	1.016	0.0803
pure BPWW (INM- β)	0.834	0.0005

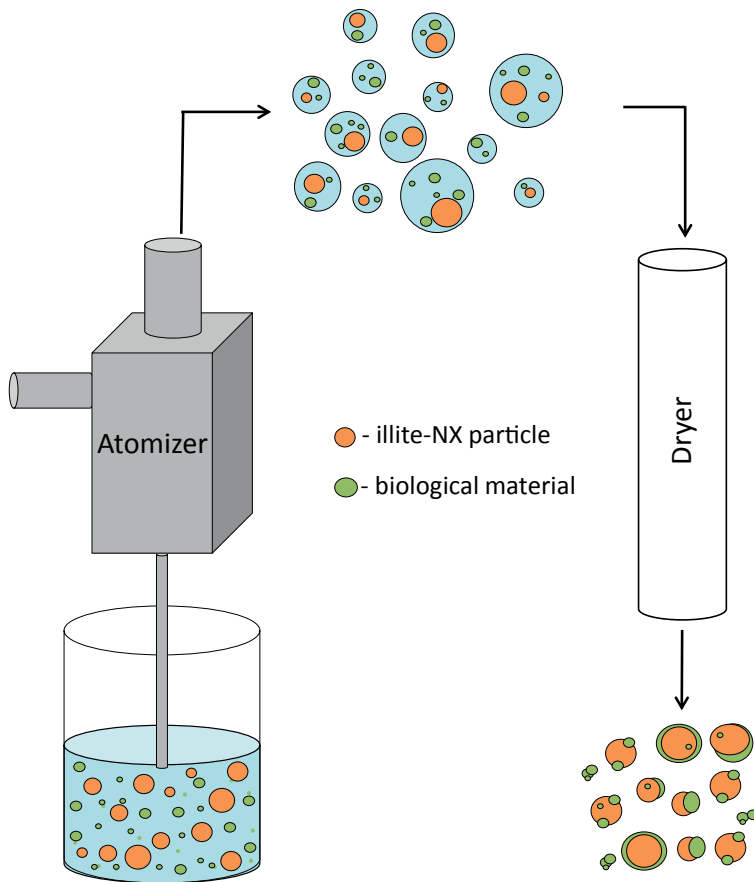


Figure 1. Schematic of the particle generation method used in this study.

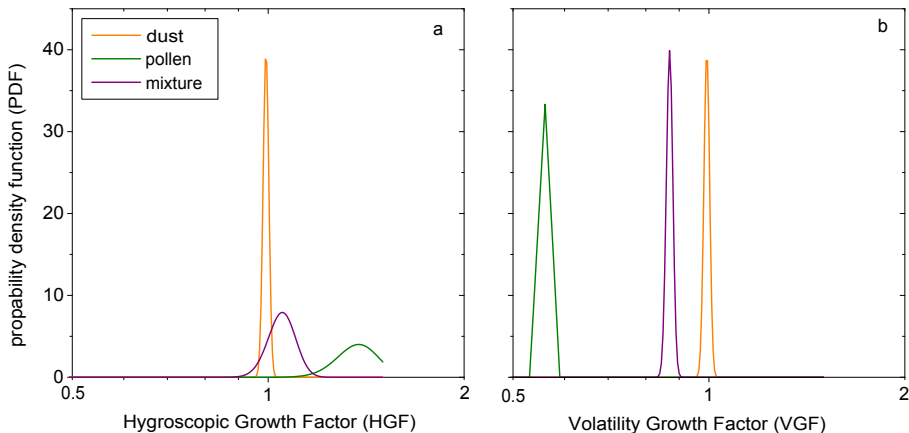


Figure 2. **Left part Panel a:** probability density function (PDF) of the hygroscopic growth factor (HGF) determined from the VH-TDMA measurements at 90 % relative humidity. **Right part Panel b:** PDF of the volatile “growth” factor (VGF) determined from the VH-TDMA measurements at 300 °C.

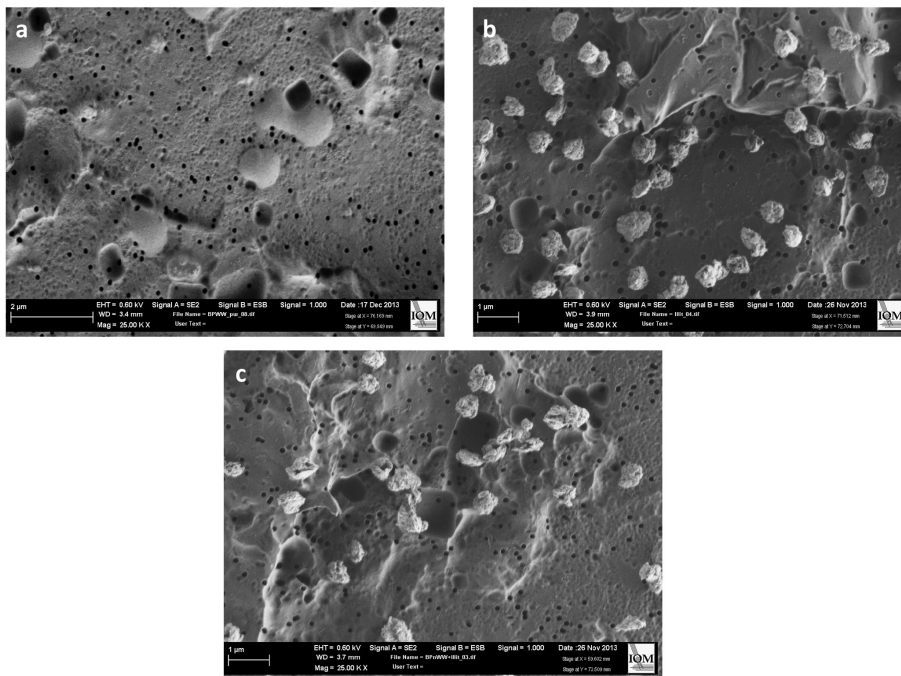


Figure 3. Examples of SEM images of the different suspensions. **(a)** BPWW, **(b)** illite-NX, **(c)** illite-BPWW.

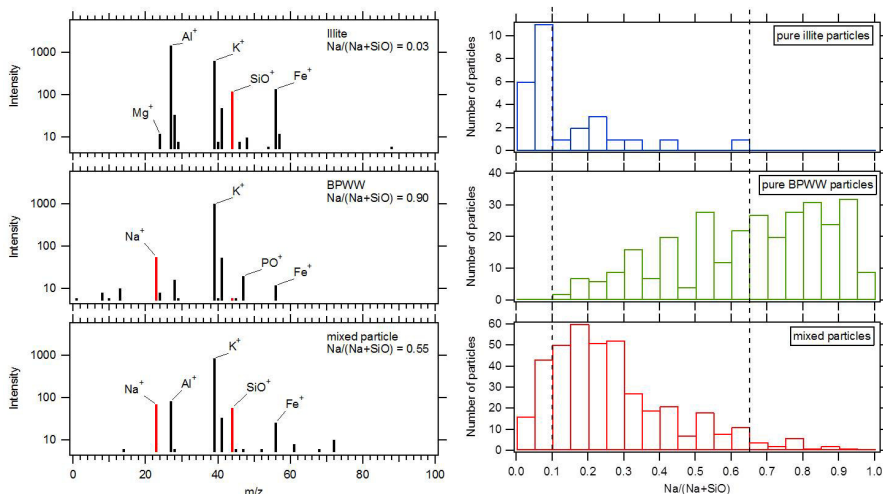


Figure 4. Left: Examples of single particle mass spectra of a pure illite particle, a pure BPWW particle, and a mixed particle. The marker peaks Na^+ (m/z 23) and SiO^+ (m/z 44) are highlighted. The values for $\text{Na}/(\text{Na} + \text{SiO})$ are given for each particle. Right: histograms of the ratio $\text{Na}/(\text{Na} + \text{SiO})$ for all particles. The dashed lines indicate the boundaries for pure illite (< 0.1) and for pure BPWW (> 0.65).

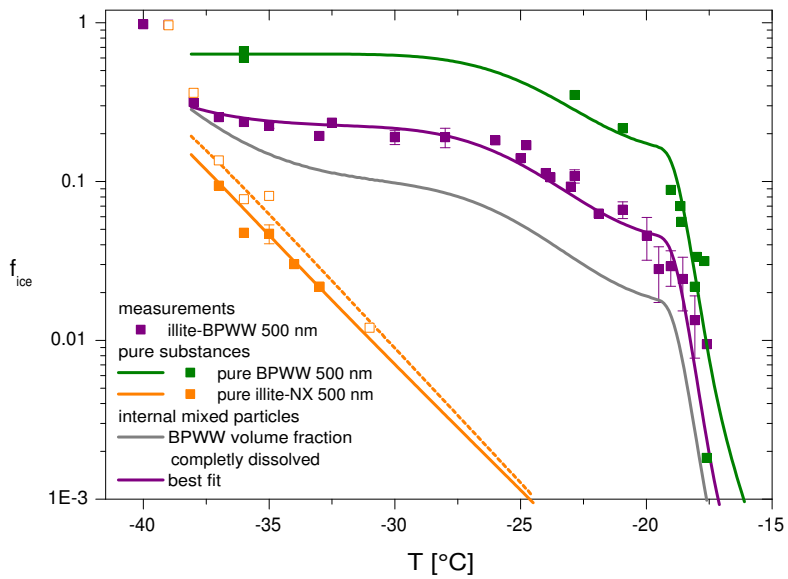


Figure 5. Results of the immersion freezing experiments of particles with a mobility diameter of 500 nm generated from the illite–BPWW-suspension as well as from the pure illite-NX and BPWW particles (purple, orange and green symbols, respectively). filled orange squares: illite-NX particles from suspension, open orange squares: illite-NX particles from dry generation. Error bars are the standard deviation of the experiments and were obtained for temperatures with at least three measurements. The green, the dashed orange and the straight orange line represent the freezing ability of pure BPWW, dry generated illite-NX and wet generated illite-NX particles, respectively (500 nm mobility diameter) based on the SBM parameters determined from measurements with the pure substances. The grey line represents an extreme case where all the available BPWW material (9.7% volume fraction) on the mixed particles has been dissolved in the droplet. The purple line represents the freezing behavior of internally mixed particles which was fitted to the measured data.

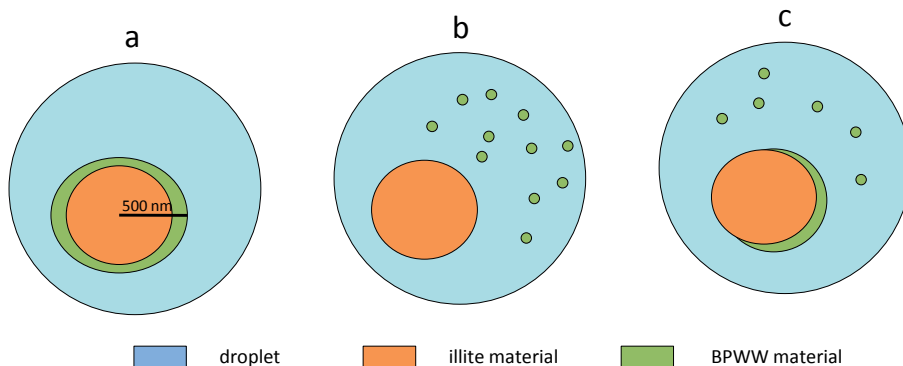


Figure 6. Theoretical forms of mixing of a spherical internally mixed illite–BPWW particle. **(a)** The illite–NX particle is completely covered with BPWW material which does not dissolve in the droplet. **(b)** The BPWW material is completely dissolved in the droplet. **(c)** The BPWW material is partly dissolved and partly still on the illite–NX particle.