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 massage 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 *cloud* 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 all., 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 relased 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.