Authors' response to reviewers' comments for "Microfluidic Immersion Freezing of Binary Mineral Mixtures Containing Microcline, Montmorillonite, or Quartz" by Nadia Shardt, Florin N. Isenrich, Julia Nette, Christopher Dreimol, Ning Ma, Zamin A. Kanji, Andrew J. deMello, Claudia Marcolli

We thank the reviewers for their time and their suggestions that have significantly improved our manuscript. Below, we provide our responses and summarise the changes that we have made with page and line numbers referring to the uploaded document with tracked changes. Other minor revisions were also made to improve the manuscript, and we updated the TOC graphic.

Reviewer 1

The manuscript describes the heterogeneous freezing of water microdroplets containing pure microcline (mc), montmorillonite (mm), or quartz (qu) particles, as well as binary mixtures thereof, as observed and analyzed in a microfluidic droplet assay device.

As natural mineral dust is typically a mixture of various minerals, one key assumption underlying many experiments on the ice nucleation ability of mineral dust is that individual dust particles do not interact in their ice nucleation ability; i.e., their joint effect can be described as the surface-weighted sum of the constituents.

This manuscript presents a counterexample to this assumption by showing that the addition of montmorillonite to a microcline suspension can even decrease the ice nucleation ability of the microcline particles. The authors discuss this in the light of chemical interactions between the two minerals. This is an important observation that will probably stimulate additional work, and therefore, the manuscript should be published in ACP.

The manuscript is written very well, and the findings are presented clearly and concisely. I have several minor questions and suggestions, which are listed below.

In total, the manuscript contains four parts:

1. Description of the apparatus, especially the addition of a semi-automated freezing detection scheme using a neural network for image analysis (Figs 1, 2)
2. Presentation and discussion of the results obtained for the individual minerals mc, qu, mm. (Figs. 3, 4)
3. Presentation and discussion of the results obtained for mixtures of the minerals at different mixing rations (Figs. 5, 6).
4. Presentation of the results obtained for a sample of Arizona Test Dust (ATD)

General remarks:

Part 4 of the manuscript seems somewhat unrelated to the main part, could the authors comment in the introduction why it was included?

> **Authors' response**
>
> We agree that the link between the binary mixture results and the ATD suspensions was not explicitly stated, as also noted by Reviewer 2. To strengthen the link between these sections, we have now added fitting of the experimental n_s vs. T values for each pure mineral and predictions of frozen fraction for ATD suspensions assuming additive surface areas of the constituent minerals.
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> **Changes to manuscript**
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> - Abstract: added "Predictions are also made for Arizona Test Dust from the obtained pure mineral fits, and general agreement with experiments is observed."
> - Page 4 starting on line 31: added "A comparison between the ice nucleation activity of the controlled mixtures and that of ATD is drawn to investigate whether samples mixed

from the pure components can explain the ice nucleation activity of samples that were collected as mixtures from the air or ground."

- Page 23, Fig. 8: added predictions of frozen fraction for ATD at three concentrations using the pure minerals' n_s fits from Fig. 3. The caption has the following additional text: "The dash-dotted lines are predictions (not fits) of frozen fractions calculated using Eq. (4) with inputs of each pure mineral's nucleation site density vs. temperature shown in Fig. 3 assuming an ATD composition of 29 wt.% microcline, 23 wt.% quartz, and 25 wt.% montmorillonite."
- Page 23 starting on line 17: added a new paragraph stating: "The median freezing temperatures at each concentration are in the same range as those that would be expected for the fraction of microcline present. If we assume the composition of ATD to be that reported by Kaufmann et al. (2016) (i.e., 29 wt.% microcline, 23 wt.% quartz, and 25 wt.% montmorillonite, we obtain the predictions of frozen fraction shown by the dash-dotted lines for each concentration of ATD in Fig. 8 using Eq. (4) with the fits obtained from each pure mineral's nucleation site density (shown in Fig. 3). There is general agreement between the experimental frozen fractions for ATD and the predicted frozen fractions obtained from the activity of the pure components. This agreement suggests that samples mixed from the pure components can explain the ice nucleation activity of samples that were collected as mixtures from the air or ground, such as ATD. Such an observation can inform future work in: (*i*) the design of experimental campaigns with controlled bottom-up mineral mixtures acting as surrogates of real field samples without their variability and complexity, and (*ii*) the development of composition-aware parameterizations for ice nucleation in the atmosphere."

Remarks on Abstract:
Line 3: Please delete the word "Unfortunately". Previous lab experiments have focused on individual constituents for a good reason, and for most of the results shown in this manuscript, the above-stated assumption on the additivity of ice nucleation efficiency holds. C.f. also to my remark 3 on part 3.

Authors' response and change to manuscript
Upon the reviewer's suggestion, we have deleted the word "Unfortunately" in the abstract.

Remarks on part 1:

1. Please give some references to the CNN setup and/or software packages used. In the central panel of Figure 1, there are abbreviations in the legend, that are not explained.

   Authors' response
   We thank the reviewer for finding this omission. We have made the modifications below.

   Changes to manuscript
   - Page 8 on lines 8 and 10–12: added "implemented with the TensorFlow and Keras packages in Python" and "The last layer used rectified linear unit (ReLU) and softmax activation functions to determine the final classification."

2. In Fig. 2, it appears that the number of droplet freezing events detected varies strongly between the 5 mixtures. Please explain. What was the total no. of droplets within the field of view for each experiment in Fig. 2? Please give this number in the description or in the Figure caption.

   Authors' response
   The number of droplet freezing events changes because the number of droplets in the field of view varies between experiments. We have now clarified this in the text and added information to the caption of Fig. 2.

Changes to manuscript

- Page 6 starting on line 15: added "Based on these recommendations around 100–200 droplets and three repeats were used for experiments in the current study. There were inherent variations in the number of droplets observed in each experiment because of variability in the process of droplet generation between experiments. The primary goal of droplet generation was to obtain a stable, monodisperse population of droplets with sufficient spacing to avoid droplet coalescence. The spacing proved to be slightly variable depending on the exact fluid flow rates required to achieve a stable droplet population, which influenced the number of droplets in the field of view."
- Figure 2 caption: added "The total number of droplets present in the field of view is assumed to be equal to the total number of freezing events detected, with variations between experiments arising from differences in droplet spacing during droplet generation."

Remarks on part 2:

1. In all freezing curves (Figs 3, 5, 6) a frozen fraction curve for pure water is reproduced from Isenrich et al. 2022. Shouldn´t blank reference measurements with pure water be repeated regularly to exclude contaminations in the tubing? Please comment on this in the manuscript.

   Authors' response

   We report the pure water data from Isenrich et al. (2022) as our background data, because it was performed contemporaneously as part of the same experimental campaign, and it is representative of the freezing behavior for pure water in the setup. To prevent contamination in the tubing between runs, the same tubing was only reused for experiments with the same mineral, and the lower concentration experiments were completed first. The tubing was also flushed with isopropanol after each day of experiments to avoid contamination.

   Changes to manuscript

   - Page 5 starting on line 24: added "To remove the possibility for contamination in the inlet PTFE tubing to the microfluidic device, the same tubing was only reused for experiments with the same mineral. The tubing was also flushed with isopropanol after each day of experiments. If multiple mineral concentrations were investigated on the same day, the lower concentration of each mineral suspension was investigated first to avoid potential contamination of subsequent experiments."

2. In Fig. 3, the n_s values collapse to a single curve for quartz, as they do in Fig. 7 for ATD. For mc and mm however, they are disjunct between the concentrations used. Please comment on this surprising finding in the manuscript.

   Authors' response

   We agree that this discrepancy between different concentrations for microcline and montmorillonite should be investigated further. Potential causes of this may be a real change in the nucleation site density for the concentrations and particles sizes we investigated or simply variability in the actual suspension concentration compared to the nominal concentration. This variability may also play a role in the interpretation of binary mixture frozen fractions (i.e., the microcline–montmorillonite mixture), which we now discuss in more detail in the manuscript.

   Changes to manuscript

   - Page 13 starting on line 4, added "For microcline and montmorillonite, the derived n_s values do not agree between different mineral concentrations, which suggests that there are differences in the number of nucleation sites per unit surface area as a function of concentration. However, we cannot exclude the possibility that these apparent discrepancies may be due to deviations in the actual suspension concentrations from the nominal ones in the process of preparing the samples from the stock suspension. To obtain a representative model of the site density, we therefore consider all n_s data over all mineral concentrations of a single mineral. The dashed lines in the plots of n_s in Fig. 3 are fits obtained using Eq. (2) for microcline, quartz, and montmorillonite with standard deviations (SD) for $\log_{10} n_s$ of 0.47, 0.19, and 0.22, respectively. The highest standard deviation is obtained for microcline due to the spread of the experimentally-derived values of n_s between different nominal suspension concentrations. The obtained fitting parameters for each pure mineral were used to calculate frozen fractions for each concentration using Eq. (1), as illustrated by dashed lines in Fig. 3, showing close agreement with experimental observations for quartz and montmorillonite. Some of the predicted frozen fractions for microcline, however, deviate from those obtained experimentally, with a steeper slope in frozen fraction predicted for the highest concentration of 0.1 wt.% and a shallower slope for the concentration of 0.001 wt.%. As in our analysis of n_s for microcline, we attribute these discrepancies to potential variation in the actual concentration of the suspensions. The three replicates shown at each concentration were prepared from the same starting suspension and show high reproducibility, while different concentrations were prepared independently, and variability may be greater."

- Page 19 starting on line 20: added "Yet we cannot exclude the possibility that experimental variability in the determination of microcline's ice nucleation activity explains the observed tail of the microcline–montmorillonite mixture compared to the microcline–quartz mixture. As shown in Fig. 3, the trend of n_s vs. T for microcline derived from experimental frozen fractions does not collapse onto a single line over all studied concentrations, and in Fig. 6, the obtained fit for n_s to this data thus yields a frozen fraction at lower temperatures (dashed blue line) than those observed experimentally at a nominal microcline concentration of 0.001 wt.% (open diamonds). If the true activity of 0.001 wt.% microcline is represented by the dashed blue line, then the frozen fraction observed in Fig. 6a for microcline–quartz is explained by the inherent activity of a 0.1 wt.% quartz suspension, and the frozen fraction observed in Fig. 6b for microcline–montmorillonite is explained by the additive activity of each constituent, as depicted by the solid purple line calculated using Eq. (4)."

3. It would be helpful to discuss the expected error margins in the data as given e.g. in Fig. 3. If possible, give error bars for one exemplary sample.

   Authors' response
   We have added the uncertainty in temperature to the caption of each relevant figure (being 0.2 K). Some indication of the variability between runs is shown through the three replicates, but this variability may not be classified fully as experimental error, since nucleation is a stochastic process and observations of nucleation must inherently show variability. We designed our experiments to align with the results of Monte Carlo simulations performed by Alpert & Knopf (2016), where a minimum number of droplets and minimum number of experiments were recommended to ensure adequate accuracy and interpretability from the results obtained.

   Changes to manuscript
   - Captions of Figs. 3, 5, 6, and 8: added "(temperature uncertainty is 0.2 K (Isenrich et al., 2022))"

Remarks on part 3:

1. In all freezing curves, but particularly in Figs. 5 and 6, the individual symbol shapes are hardly discernible. I do not have an easy solution for this, but plotting symbols open instead of closed and/or at a smaller size might help the reader.

Authors' response and changes to manuscript
We thank the reviewer for the suggestions to improve the clarity of the figure. We have now used open symbols for the pure mineral frozen fractions and reduced the opacity of the filled symbols for the mixture data. We also note that the raw data that is shown in the figure will be made available online for interested readers to better see the trends of specific datasets.

2. It would be helpful if expected "fraction frozen" curves could be constructed for the various mixtures from the results of part 2 using the individual n_s values and surface areas (the expected additive outcome) to compare to the measurement results. This would probably also avoid plotting too many individual data points in Figs. 5 and 6.

Authors' response
We thank the reviewer for this suggestion, and we have now added these predicted frozen fraction curves based on the pure mineral site densities. In each relevant figure, we plot the predictions in solid lines.

Changes to manuscript

- Page 10: added "Section 2.4 Governing Equations for Fitting and Prediction" to describe the methods used for fitting of the pure mineral n_s vs. T data and for predicting the frozen fractions for both binary mixtures and ATD suspensions.
- Page 12, Fig. 3: added dashed lines to indicate the fits of n_s and the corresponding frozen fractions obtained from these fits
- Page 12, Fig. 3 caption: added "The dashed lines are model fits for n_s as a function of temperature, from which frozen fraction curves are calculated using Eq. (1). The equations are given by: $n_{s,mc} = 10^{2.3344}\,[-1.6598(T + 23.054)]^{3.2291}$, $n_{s,qu} = 10^{0.8924}\,[-1.2756(T + 21.865)]^{3.7223}$, and $n_{s,mm} = 10^{-0.2189}\,[-0.5956(T + 20.744)]^{4.8178}$."
- Figs. 5, 6, and 8: added dashed lines for pure component frozen fractions calculated using the n_s fits, solid lines for predicted frozen fractions based on additive surface areas of the pure minerals, and dash-dotted lines for frozen fraction predictions for ATD suspensions.

3. Discussion section: Even though the non-additive behavior in the ice nucleating efficiency of mc and mm is surprising and interesting, it should be noted that it is only apparent if mm is present in a 100x higher concentration than mc, a situation that is not very likely to be encountered in nature in an individual droplet.

Authors' response
Due to the possibility of variability in our measured pure microcline n_s values explaining the tail end of the microcline–montmorillonite mixture, we have tempered our discussion and conclusions relating to interactions.

Changes to manuscript

- Page 25 starting on line 32: deleted "Interestingly, the median freezing temperature of the microcline (0.001 wt.%)–montmorillonite (0.1 wt.%) mixture was lower than that of the microcline (0.001 wt.%)–quartz (0.1 wt.%) mixture, likely due to ion exchange at the microcline surface."
- Page 26, starting on line 8: deleted "It highlights the dominance of microcline as an ice-nucleating particle in the investigated bottom-up mixtures, and it identifies a potential for interactions in an internal mixture of montmorillonite and microcline (depending on their relative weight fraction)."

No specific remarks on part 4 or the appendices.