

Interactive comment on “Twin-plate ice nucleation assay (TINA) with infrared detection for high-throughput droplet freezing experiments with biological ice nuclei in laboratory and field samples” by Anna T. Kunert et al.

Anonymous Referee #2

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Kunert et al. present a method, Twin-plate ice nucleation assay (TINA), for droplet freezing using infrared detection based on the heat release at freezing. Two types of biological INPs and an ambient sample collected on filter were used to test the method. The effect of O₃ and NO₂ exposure on IN activity of *P. syringae* is also tested. In the manuscript, the authors concluded that this is a high-throughput method which can be applied to lab and field studies. The authors showed that the exposure to high O₃ and NO₂ could decrease the ice nucleation efficiency of Snomax. From the field sample test, it is concluded that there are efficient biological IN that were smaller than 0.1 μm.

C1

This manuscript is suitable for publication in AMT. It is recommended to published in AMT after the following issues are addressed.

Comments:

1. Line 21, please be clear what does “deviations <0.5 K” mean and why it is high-precision temperature control.
2. Line 37, it is not clear why this method is suitable for high-throughput experiments and efficient analysis for field samples. I guess the authors don't mean in-situ field measurement? If I understood it right, as for the demonstration of the field sample in the manuscript, one should collect enough sample and extract them carefully to get different dilutions for nm(T) measurements.
3. Line 52-53, why the IR detector is better for droplet freezing detection? Please provide more details.
4. Line 79-80, the two biological INPs are very efficient IN, TINA could work very well with such efficient INPs. How about the situation when the unknown samples are less efficient especially when background freezing start contribute the freezing at e.g., -26C or higher.
5. Line 140, how the droplet temp. is calibrated? you only calibrate the sensors as described? Line 150, the sample temperature needs to be calibrated., not just the sensor. Where are the thermistors in the experimental setup? Depending the location, likely there will be thermal lag due to the plate thickness and different cooling rate. And is the temp. uniform cross such large sample plate? This could also contribute to the different freezing curves for different plates (or plate designs).
6. Line 188, when using cooling rate of 1K/min, does the Wegener-Bergeron-Findeisen process affect the measurement?
7. Line 256-257, please provide a brief description for the O₃ and NO₂ exposure experiment. Why such high concentration for both O₃ and NO₂ is used?

C2

8. Line 277, please provide the ice nucleation data for the field blank samples for comparison with ambient sample.
9. Line 298-299, it is not clear why the decrease in IN activity after heat treatment is indication of the presence of biological IN.
10. Line 307-314, it is suggested to carefully evaluate the discussion and conclusion in this section. As showing in Fig. 8, there is less than one order of magnitude different between the samples after 5 μm and 0.1 μm filtration. This could be just within measurement uncertainty, which is showing in Fig. S1 and S2, for each three independent samples that there is about one order of magnitude variation at certain temperature range.
11. Fig. 4, please provide the detail description for freezing determination using IR camera.

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