

Interactive comment on “Ice Nucleation Activity in the Widespread Soil Fungus *Mortierella alpina*” by J. Fröhlich-Nowoisky et al.

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We thank the anonymous referee #1 for constructive comments and suggestions, which are highly appreciated and have been taken into account upon revision of our manuscript. Detailed responses are given below.

Referee #1

Referee #1: The phrase ice nucleation active (INA) is used at numerous points throughout paper, beginning at the abstract (page 12698, line 6). However, the successful experimental observation of heterogeneous ice nucleation in the immersion mode is dependent on the concentrations of the nucleating material employed, and the temperature range which can be probed, as subject to instrumental limitations. While I would

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prefer the use of more concise language (e.g. “efficient ice nucleators” rather than “ice nucleation active”), as this phrase appears so widely throughout the paper, a concise definition of what is meant by INA from its first occurrence would be easier than having to reword throughout.

Response: As suggested we added a definition of what is meant by INA in the text: “ice nucleation active (INA) = inducing ice formation in the probed range of temperature and concentration”

Referee #1: One of the key results highlighted by the authors is that the ice nucleating particles produced by the fungus seem to be < 300 kDa in size. However, there is very little discussion on the centrifuge ultrafilters used in the study (e.g. section 2.6). For instance, can the authors provide information on how wide the pore size distributions on these filters are? A discussion of this, perhaps as part of the experimental section, would be useful to give an idea as to how constrained this estimate on the protein size is.

Response: We used Viviaspin filter tubes with a molecular weight cut-off of 300 kDa and 100kDa (see section 2.6.). As the ice nucleating particles could be filtered through a 300 kDa device but with a few exceptions not through a 100 kDa filter tube we concluded that the ice nucleating particles are smaller than 300kDa (table 4, page 12708 lines 3-8).

Referee #1: Page 12702 line 6: For those not familiar with the experimental setup, can you describe what is meant by the “head” in this sentence? At what point in the temperature ramp was the temperature variation measured? Does the value of $\pm 0.2^\circ\text{C}$ for the temperature variation across the “head” translate into a droplet-to-droplet temperature uncertainty of $\pm 0.2^\circ\text{C}$?

Response: For clarification we changed this sentence into “ Temperature variation across the cooling block...” The temperature variation was measured using a Thermistor verification probe, supplied by the manufacturer to verify functioning of thermal cy-

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clers. The verification measurement was performed at each temperature of the ramp. The variation of $\pm 0.2^\circ\text{C}$ translates to a droplet-to-droplet temperature uncertainty of $\pm 0.2^\circ\text{C}$, comparable to the uncertainties of similar immersion freezing measurement instruments as summarized in Hiranuma et al., 2014.

Hiranuma, N., Augustin-Bauditz, S., Bingemer, H., Budke, C., Curtius, J., Danielczok, A., Diehl, K., Dreischmeier, K., Ebert, M., Frank, F., Hoffmann, N., Kandler, K., Kiselev, A., Koop, T., Leisner, T., Möhler, O., Nillius, B., Peckhaus, A., Rose, D., Weinbruch, S., Wex, H., Boose, Y., DeMott, P. J., Hader, J. D., Hill, T. C. J., Kanji, Z. A., Kulkarni, G., Levin, E. J. T., McCluskey, C. S., Murakami, M., Murray, B. J., Niedermeier, D., Petters, M. D., O'Sullivan, D., Saito, A., Schill, G. P., Tajiri, T., Tolbert, M. A., Welti, A., Whale, T. F., Wright, T. P., and Yamashita, K.: A comprehensive laboratory study on the immersion freezing behavior of illite NX particles: a comparison of seventeen ice nucleation measurement techniques, *Atmos. Chem. Phys. Discuss.*, 14, 22045-22116, doi:10.5194/acpd-14-22045-2014, 2014.

Referee #1: Figure 2 and Figure 3: Error bars in both temperature and the concentration of active ice nuclei should be shown. A discussion of the main uncertainties in the analysis used to produce this graph would also be useful in the main text.

Response: For the concentration of IN we added binomial confidence intervals (95%) derived by the formula 2 from Agresti and Coull (1998). We added this information in the text and figure captions. For the temperature we do not think that error bars would be beneficial (indeed, they may even be misleading) as the cooling was done in $0.5\text{--}1^\circ\text{C}$ steps which took 12 min for each step and included 5 min dwelling time at each T. We recorded the number of frozen droplets at these defined temperatures (like in other studies) but the freezing of the droplets already starts during the cooling to the next T due to the nature of the IN.

Referee #1: Page 12710 line 21: I'm unclear on exactly how the authors reach the conclusion that *M. alpina* seem to form only a "single activity class"? Could the authors

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elaborate?

Response: As written in the manuscript the statement that the *M. alpina* IN seem to form only a single activity class comes from the comparison to bacterial IN which are known to contain different classes of IN active at different temperatures due to different-sized aggregates. This can be seen by several strong increases of the number of IN at different temperatures as the IN active at lower temperatures are typically more abundant than the IN active at higher temperatures. We see no increases in the number of IN, over and above the initial onset of activity, within the tested temperature range and thus our samples might have contained IN proteins of only one single class.

Other comments/typos

Referee #1: Page 12708 line 14: Typo in the word significantly here.

Response: The typo was corrected.

Referee #1: Page 12704, line 19: I suggest it is worth spelling out for the reader why a $0.1\ \mu\text{m}$ filter was used here.

Response: Pore sizes of 0.2 or $0.1\ \mu\text{m}$ are used for sterile filtration to remove possible contamination with bacteria or other particles. To make it more clear we changed the text into "... sterile filtered deionized water".

Referee #1: Page 12709 line 9: Can the authors explain for readers interested in the study, but not necessarily possessing a background in biology, what "arbuscular mycorrhizal fungi" is?

Response: Arbuscular mycorrhizal fungi are fungi that form a symbiotic association (called mycorrhiza) with the roots of plants. They are found in more than 80% of the plant families. For clarification we changed the text into: "...but based on their ability to solubilize phosphorus, they can also form interactions with arbuscular mycorrhizal fungi, which are plant root symbionts."

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