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Interactive comment on "Spores of most common airborne fungi reveal no ice nucleation activity" *by* B. G. Pummer et al.

Anonymous Referee #3

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In search of IN that may induce ice nucleation at low supercooling and explain glaciations in mixed-phase clouds, there has been revived interest in primary biological ice nuclei (IN). This study investigates a wide range of fungal spores and fits well into the current debate. It confirms older studies that most fungal spores showed no significant ice nucleation activities. Nevertheless a reconsideration and reinvestigation of this type of biological IN is worthwhile. The paper is well structured and the results are well placed in the context of related published work. The method applied in this study has been described in more detail in a previous publication. Nevertheless, in view of the negative result (i.e. no IN activity of airborne fungi), the experiment has to be discussed in more detail to exclude a misinterpretation of the absent IN activity. In "Results and Discussion", the authors mention that the IN inactivity could be the result of low concentration of fungal spores in the water phase. If this were indeed the case,

the statement made in the title of the manuscript (no ice nucleation activity of fungal spores) would not hold. When water-in-oil emulsions are prepared, hydrophobic IN may partition to the oil phase instead of the water phase. The authors describe the surface of the fungal spores as rather hydrophobic. It is therefore not obvious that they should partition to large extent to the water phase. If they were contained in the oil phase, this would be an alternative reason why they did not show any ice nucleation activity in the experiments. Since the fungal spores are rather large, it should be possible to spot them in a light microscope with large magnification. The authors should therefore add a figure to the manuscript with microscope images that show the location of the fungal spores within the emulsion.

In addition, whether droplets contain IN or not depends on the droplet size distribution and the IN size distribution. Therefore additional information should be provided for the emulsion preparation and data evaluation procedure covering the following questions: For how long were the samples emulsified with what stirring speed (RPM)? How stable were the emulsions? What was the size distribution of the droplets? Were only the larger droplets considered for evaluation (as stated in Pummer et al., 2012)? How many evaluated droplets were in the field of view of the microscope? How many times was a freezing experiment repeated? How was the reproducibility? What was the spread of freezing temperatures? E.g. add freezing temperature for 1 or 5 % activated fraction to table 2.

Figure 2 should be improved: It should be indicated which data point belongs to which sample. Why are some samples shown as frozen and others as non-frozen? This does not become clear.

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