Alexander Laskin,
Editor of Atmospheric Chemistry and Physics
Pacific Northwest National Laboratory

Dear Alex,
Listed below are our responses to the comments from reviewers 1-3. The reviewer's comments are in bold type and our responses are in normal text. We thank the referees for carefully reading our manuscript and for excellent comments.

Sincerely,

Allan Bertram
Professor of Chemistry
University of British Columbia

Anonymous Referee #1

This is nice study using experimental and model tools to investigate the importance of fungal spores in the atmosphere. I have some minor comments, and I recommend publication after these comments are addressed. Classes of fungal spores are examined for their ice nucleation properties. Atmospheric relevance of these spores needs to be discussed. Why these spores are important; any ice residues or rain water samples show the evidence of these spores? Atmospheric relevance needs to be discussed?

To address the referee's comment, a new table (Supplemental Table S1) will be added to the manuscript, which lists all of the fungal spores studied here as well as the previous studies that have identified these spores in the atmosphere.

Figure 2: Show mineral dust immersion freezing data points from literature. Authors have compared Ns densities in Figure 4 to Asian mineral dust, but surface area approximation used in this study (for spores) would affect the comparison. Looking at Figure 1, it is clear that spores are not spherical particles. Actual surface area would be higher than assumed. If substituted in the Ns calculation, the actual Ns densities would be even lower than shown. Please add these error bars. Please also revisit the conclusions stated in section 4.1 (line 11 to 25 on page 5033 and line 1 to 2 on page 5034).

The referee raises a good point. To address the referee's comment, we will add a paragraph where we will discuss the uncertainties in n_s values due to the uncertainties in surface areas. The paragraph that will be added to the document is included below:

"Some of the spores studied have rough surfaces (see Figure 1). Based on the SEM images we estimate that the surface areas of some of the spores may be up to a factor of 10 higher than the geometric surface areas. As a result the n_s values reported here, which were calculated assuming a geometric surface area, should be considered as an upper limit to the true n_s values. A similar argument can be applied to the mineral dust n_s values shown in Figure 4 since Niemand et al. [2012] used volume-equivalent spherical diameters when determining n_s values for mineral dust particles."

In addition to adding the information above, we will restrict our conclusions on the difference in ns values to the temperature range of -20 to -25 °C. This will ensure that our conclusions are not sensitive to the uncertainties in surface areas.

Figure 8 showing comparison with previous studies should be revisited. I suggest show the raw data, for e.g., Figure 2. Surface area approximations would affect the comparison, as mentioned above. If authors like to compare, I suggest CNT derived nucleation rates per unit area should be showed.

The referee suggests that we should compare raw data without taking into account surface area differences between experiments. We would prefer not to do this since previous work has shown that surface areas should be taken into account when comparing freezing results [Hoose and Mohler, ACP, 2012]. The referee also suggests using CNT derived nucleation rates per unit

area. Previous work has shown that CNT with a single contact angle does not reproduce freezing data in most cases (see for example Hoose and Mohler [ACP, 2012] and Wheeler and Bertram [ACP, 2012]). We are currently working on a manuscript where we apply CNT theory together with active sites to the current data, but we feel this is beyond the scope of the current manuscript.

For EMAC studies it is assumed that spores are efficient CCN. I'm not sure how CCN and IN activities are related. Efficient CCN does not mean efficient IN. According to Figure 2, this frozen fraction plot shows that spores are not efficient IN at mixed-phase cloud (just because their frozen efficiency is less than 1). How this assumption affects the cloud model results should be discussed, at least briefly. Also, I'm not sure about the emission rates. It is fixed at a single rate from all land surfaces. Line 12 to 15, page 5025. Discuss how this assumption will affect the results. This will give an idea about sensitivity of this parameter. Is it right to assume a single rate from all land surfaces.

We agree with the referee that efficient CCN activity does not mean efficient IN activity. We mentioned the CCN activity because both CCN activity and IN activity affect the transport of particles in the atmosphere. We have shown elsewhere that CCN activity and IN activity (as represented by mixed phase cloud scavenging) have interactive effects on the simulated atmospheric transport of aerosols in our model [Burrows et al., 2013]. Furthermore, the model requires some information about CCN activity to be prescribed for transported particles. Because of their large size, we feel it is a good approximation to assume that fungal spores are very efficient CCN and essentially always active in liquid cloud droplet nucleation.

Since the concentrations of fungal spores are quite low compared to those of other particles, we assume that fungal spores do not significantly influence liquid cloud formation. Therefore, no effect of fungal spore concentrations on liquid cloud properties is implemented in the model simulations.

We will add text to clarify these points in the manuscript, extending the text on p. 5034, lines 16-19 (new text in italics):

In the simulations we assumed that the fungal spores were efficient cloud condensation nuclei, as done previously [Heald and Spracklen, 2009]. Experiments to verify this assumption are needed, *however, because of their large size, it is a reasonable first assumption that fungal spores will nucleate cloud droplets. The CCN activity of particles significantly affects their removal by precipitation in the atmosphere, and CCN and IN activity have interacting effects on aerosol burdens [Burrows et al., 2013].*

Page 5034: line 21 to 28. Why acid coating studies are important. Which acid, sulfuric or nitric?. Why organic coating studies are not important? You may want to generalize the statement otherwise. I was not sure why so many papers were cited for coating work. Do all these papers studied acid coating affect. Is there any evidence (field, laboratory) that shows the spores can be coated with acids?

Since this section raised more questions than it answered and since this section is not an integral part of the paper (i.e. no conclusions are based on this section), we will delete this section from the document.

Anonymous Referee #2

Haga et al. present a survey study of the ice nucleation properties of different classes of fungal spores. Experiments were performed using the well-established drop freezing method with an apparatus that has been successfully employed in previous studies. ECHAM5 model simulations were used to test the implications of the fungal spore IN activity on the transport and distribution of spores on a global scale. The main findings of the manuscript are (1) that after normalizing per unit surface area the IN activity for different classes and species are relatively similar and (2) that the inclusion of IN scavenging in model simulations alters the concentration and distribution in the atmosphere.

This a well-referenced manuscript that incrementally improves upon a previous manuscript of Haga et al. [2013, JGR]. Aside from a few aspects of the manuscript that require clarification I recommend publication in ACP.

Comments: The authors have done a nice job in researching the different classes of fungal spores in the atmosphere. In the process several terms of the biological classification scheme ranging from phylum to species are being used without clear demarcation. Unlike IN active bacteria that can be characterized via 16s rRNA sequences and the presence of an IN active protein, the molecular mechanism responsible of fungal spore IN activity is unknown. It is difficult to argue a priori why different phyla, classes, genera, families, or species would differ in their IN activity. It would help if the authors better define what the expected similarities and differences are at the class and species level and how those might effect the IN activity. I don't know much about fungal spores but an example borrowed from algae would be different contact angles and materials of the surface as with cocolithophores and diatoms. Even if the root cause of the IN activity of the fungi is beyond the scope of this study that information is needed to begin exploring similarities and differences in IN activity between the different experiments. Also, some terms are never explained and/or used such as teliospore and conidiospore only appear in Table 1.

To address the referee's comments, first we will add definitions for the terms in Table 1. Second, we will point out in the manuscript that there is some evidence that proteins are involved in ice nucleation by fungal spores, at least for the spores from the genus Fusarium [Hasegawa et al. Biosci Biotechnol Biochem, 1994].

Throughout the manuscript the authors use "Number of nuclei per spore". I generally think of 1 nucleus = 1 particle. It may be better to say "fraction of spores that serve as IN at temperature T" or "average number of nucleation sites per spore"

This is a good point. We have followed the nomenclature used by us (Haga et al. [ACP, 2013]) and others (see Morris et al. [ACP, 2013]) and think it would be better to be consistent with what has been used previously in the literature. To address the referee's comments we will add a comment to the document to indicate that "number of nuclei per spore" is equivalent to "average number of nucleation sites per spore".

Figure 2 shows that the experiment with P. brevicompactum has a signficant number of drops that freeze homogeneously, either because no spore was present in the drop or because the spore present did not contain an active site. Effectively those drops did not contain an active ice nucleus. Should those data then be presented as IN per spore in Figure 3?

This is a great point raised by the referee. To address the referee's comments we will remove the data in Figure 3-6 for P. brevicompactum that correspond to homogeneous freezing temperatures (i.e. we will removed all data at temperature ≤ -35 °C).

A related question is with respect to the average number of spores per drop. What is the variability of that average when considering individual drops? Using the Vali method assumes that the distribution of nuclei in the drop is fairly uniform. This is ensured when working with liquid suspensions. But I wonder if this is the case in the two-step procedure used here. First spores are deposited on the slide, then drops form on that slide by water nucleation. At minimum the authors should show (maybe they have in past work and then need to reference it) that the spores were distributed spatially uniformly over the slide and or that droplets formed on the slide are also formed spatially uniformly. Placing a virtual grid over the wet and dry images, computing the distribution of number of drops/spores per grid square and showing that it follows a Poisson distribution will do the trick. If the uniform condition is not satisfied it will be necessary to quantify how badly the maldistribution of spores per drop would interfere with the construction of Figure 4.

Before calculating the cumulative number of IN per spore we first binned the freezing data so that we have the same number of particles per drop in each bin. This avoids the issues raised by the referee above. To address the referee's comment we will add a description on how we determined the cumulative number of ice nuclei per spore and add the formula used to calculate this value. In addition in Table 1 we will add information on variability in the number of spores/drop.

It is claimed that "all of the fungal spores investigated were found to cause freezing". It seems more appropriate claim that all of the species/classes studied contained some fraction of spores that serve as IN at temperatures warmer than homogeneous freezing.

This is an excellent point raised by the referee. In the Abstract and Section 4.1, line 11 we will change "All of the fungal spores investigated were found to cause freezing of water droplets at temperatures warmer than homogeneous freezing" to "all of the fungal spores investigated contained some fraction of spores that serve as ice nuclei at temperature warmer than homogeneous freezing".

The comparison to active site densities to dust are interesting but ultimately unhelpful. The much more relevant comparison would be the number of IN from dust and fungal spores, which requires scaling INAS with the surface area of dust and fungal spores in various environments.

To address the referee's comments, we will add a new figure to the document that shows estimated numbers of IN from dust and fungal spores. Thank you for the suggestion.

pg. 5017 "cosmopolitan" is a strange word to use here.

To address the referee's comments "cosmopolitan" will be changed to "widely distributed over the globe" at this location and also in the abstract.

Anonymous Referee #3

This study combined laboratory measurements and modeling effort to investigate: 1) immersion freezing on 12 types of fungal spores and 2) possible effects of ice nucleation on the atmospheric transport and distributions of these species. The results show that ice nucleation ability of Ustilaginomycetes is higher than the other two classes. These fungal spores are less efficient than Asian mineral dust when comparing on surface area basis. Using a global chemistry-climate transport model, the authors conclude that the ice nucleation of these spores can influence their distribution in the atmosphere. Because of the limit ice nucleation data in the literature, this study provided substantial new data for fungal spores and should be archived. The paper is well written and contains interesting and original work. Therefore the manuscript is suitable for publication in ACP once the following comments/issues are addressed.

Major comments:

1), the manuscript gave a detail summary on the identification and abundant of fungal spores in the atmosphere and showed possible importance of ice nucleation on these spores. As shown in the Introduction section, the reported concentrations of these spores are often less than 100 L-1 in the boundary layers. These are relatively low concentrations compared to other types of atmospheric particles. In addition, due to the large sizes of the spores and long term transport, what could be the possible fractions of these spores that would be transported to remote regions and higher altitude? The manuscript provided insufficient discussion on the concentration and distribution of investigated species in the model which may affect the modeling results. What emission rate was used in the model (page 5025, line 13)? It could be very informative to show the global and vertical distribution of the investigated spores, although using a single emission rate for all the land surface is oversimplified.

Fungal spores are implemented as passively transported tracers in the model, i.e. they do not feed back on to model transport. As the referee points out, spore concentrations are quite low compared to other types of particles in the atmosphere, so we expect their impacts on clouds or radiative transfer to be minor in most of the global atmosphere [Hoose et al., 2010].

We agree with the referee that the distribution of the emissions within the continents will change the model simulations, and the use of a single emission rate is a simplification. It was not our intention to provide fully realistic simulations of fungal spores, but simply to illustrate the potential for higher removal rates in mixed-phase clouds to significantly impact spore transport. The simulations are therefore somewhat idealized.

To address the referee's comments, we will include the emission rate used in the simulations. In addition we will add figures displaying the mean surface concentrations and the mean vertical distribution of spores from our simulations.

It is not clear that whether model simulation included all the spores at once or separately. In Table 2, the simulation use Rnuc,ice =1 in IN-Acitve case for T between -25 and -35C, this assumption clearly will result in overestimation of percentage change between IN-

Active and IN-Inactive in mixing ratios due to ice nucleation (the results shown in Figures 5 and 6). Rnuc,ice is temperature and spore type dependent which can be seen from the results shown in Figures 2 and 3.

The model simulations did not differentiate between different types of spores, so in that sense the simulations included all the spores at once. We performed idealized simulations to give an initial estimate of the effect of ice nucleation on transport of the studied fungal spores. This effect should be taken as an upper bound.

We agree with the referee that the use of Rnuc,ice =1 will result in a high estimate of the percentage change between IN-active and IN-inactive in mixing ratios due to ice nucleation. This estimate should be interpreted as an upper bound, also in light of the fact that competition with other ice-nucleating particles is not implemented in the simulations, as pointed out in the next referee comment. We will add some text to clarify this, as noted in our response to the next comment.

2), could you comment on the possible effects of the ice nucleation competition between fungal spores and other atmospheric particles (for example, dust and marine diatoms for open ocean) on the redistribution of fungal spores? When co-exiting with more efficient ice nucleating atmospheric particles and when ice crystals form more efficient IN, the fraction of fungal spores forming ice will be significantly lower than the current IN-Active calculations or assumptions. Thus the results shown in this study could be overestimated if consider the realistic atmospheric implications.

In short, caution is needed to interpret the results from these model simulations with current assumptions. The conclusions from the modeling section need to be reworded or additional discussion is needed to clarify the potential bias.

We agree with the referee that competition with more efficient or more abundant ice nucleating particles would potentially reduce the effects discussed here, particularly in clouds with low updraft velocities. This is an important point, which it would be interesting to address in more detail in future studies. Current global models do not yet have the capability to simulate the differences in ice nucleating ability of different particle types, or mixed-phase cloud microphysical processes, in sufficient detail to address this question as fully as we would like. Such capabilities are the subject of significant ongoing research and model development efforts, and beyond the scope of this study. Given the inherent limitations of current model capabilities, we agree that the results in the modelling section of this paper should be taken as an upper bound for the effects of scavenging on the transport of spores (or of other particles with an idealized continental source).

We will add the following text to the discussion of the modelling results to point out these limitations and help clarify the appropriate interpretation of the modelling results for the reader: "Our results represent an upper-bound estimate for the effect of ice nucleation on the transport of the spores studied since the freezing efficiency of the spores studied is less than one between -25 C and -30° C (Figure 3). Furthermore, these simulations do not treat competition between multiple ice nucleating species for freezing and water vapor uptake; to our knowledge this

capability is not yet present in any global models, and is a topic of ongoing research and development. Competition with other ice nucleating species would tend to decrease the scavenging of fungal spores, since fewer fungal spores would nucleate ice, and therefore more spores would be returned to the interstitial phase by evaporation during the Bergeron-Findeisen process."

We will also add a sentence at the beginning of Section 2.4 stating the goal of the simulations and pointing out that the simulations are idealized, and therefore not necessarily realistic in all aspects:
"We conducted idealized simulations of fungal spore transport to investigate the sensitivity of the atmospheric mixing ratios to the activity of spores as ice nuclei."

Specific comments:

1, Since the modeling results (section 3.5) don't directly related to the discussion in section 3.6, it is suggested to move section 3.6 right after section 3.4. The paper may flow better. If the authors choose to do so, the order of corresponding figures and last two paragraphs in page 5019 also need to be changed.

We will move section 3.6 right after section 3.4. Thank you for the suggestion.

2, Page 5024, Line 19-26, please provide a brief discussion or justification for using 0.05 for ice cloud in the simulation (Table 2). Due to the lack of data in lower temperatures (<-35C), ice cloud is not necessary needed in the simulations and the modeling investigation should only focus on mixed phase clouds.

The value 0.05 is used in the simulations because it was a default value in the EMAC scavenging code, which was chosen because it was in agreements with the very low fractions of aerosols that are contained in cloud hydrometeors at these low temperatures [Henning et al., 2004; Verheggen et al., 2007]. We include this value in the table solely for completeness, since we did not alter it from the default value in this study. However, in other work [Burrows et al., 2013], we have shown that the effect of changing the ice cloud scavenging parameter on the simulation of aerosol transport is negligible (at least for this model).

To improve clarity and address the referee comment, we will modify that text as follows:
"...This is the default value used in this EMAC version, chosen because it is consistent with measurements of cloud scavenging at low temperatures [Henning et al., 2004; Verheggen et al., 2007]. We also note that the value of this parameter has a negligible effect on modeled aerosol transport compared to other model parameters, as we have shown elsewhere [Burrows et al., 2013], and that measurements are not available for fungal spore IN activity at temperatures $\leq$ -35 °C. Therefore, although we acknowledge that scavenging in ice clouds may also differ between IN-Active and IN-Inactive particles, in this study we focus on the sensitivity to scavenging in mixed-phase clouds and do not vary the ice-phase cloud scavenging parameter."

3, Page 5026, Line9, since surface area data are critical, please provide the range of Spores/Drop in Table 1 or other percentiles. Also, it could be useful to include the surface

area data in figure 4 as a second panel. Line 13-15, did the authors consider plotting the surface area vs. freezing temperature including each nucleation event? The freezing temperature vs. surface area plot could provide additional information on surface dependence.

To address the referee's comment, the range of spores/droplets will be added to Table 1. Regarding surface area data, in a previous paper we investigated the effect of surface area on freezing temperatures of fungal spores [Iannone et al., ACP, 2011]. We did not include such an analysis in the current manuscript since the current manuscript is already quite long and this analysis is not crucial for the conclusions reached in the current manuscript.

4, Page 5026, Line 21, It is not very clear what is cumulative number of ice nuclei "per spore", why not using surface area for each nucleation event? The definition or the formula used here and in the paper by Haga et al, 2013 are slightly different compared to the formula used by Vali (1971). Please provide a brief description on the determination of cumulative number of ice nuclei per spore and give the formula. This could be placed in supporting document. This is easier for readers to follow the paper.

To address the referee's comment we will add to the manuscript a description on how the cumulative number of ice nuclei per spore was determined and the formula used to calculate this value.

5, Page 5030, Line 25-28, as described in the Section 2.4 (page 5023,line 9), the dry deposition is considered, so if consider the size of 3 and 8 micrometer of spores and dry deposition, what are the concentrations/mixing ratios at about, for example, 300 hPa and 900 hPa? Please see the comments above regarding the vertical distribution of fungal spores before any ice nucleation simulation. The impression is that the mixing ratios at 300 hPa level could be significant lower than those at 900 hPa, especially for 8 micrometer case. Even if all spores nucleated ice and are transported to lower altitudes, they won't account for all the percentage increases below 5 km (as for now, from Figs. 5 and 6, the absolute percent changes for high (negative) and low (positive) altitudes are similar). A back-of-the-envelope calculation may help to understand the contribution of downward transport (due to ice nucleation) to the changes in low altitudes. I could be completely wrong on this speculation, but current manuscript doesn't provide sufficient data or evidences to support the explanations and conclusions.

The meteorology (winds, precipitation, etc.) is identical in all simulations, so all differences are due to changes in scavenging rates related to particle size and Rnuc,ice. Since ice nucleation scavenging was the only parameter changed in the simulations, and its only effect is to increase downward transport and removal of IN-active particles, this is the only possible explanation for the simulated increases in concentrations in the atmospheric boundary layer over the Southern Ocean and Antarctica, as well as the Himalayas.

To understand why the relative changes at low altitudes are so large, it is important to recognize that the simulated concentrations of spores at low altitudes over the Southern Ocean and Antarctica are extremely low. This is due to the high amount of precipitation in this region and

its extremely effective removal of particulate matter. As a result, in this region, simulated concentrations are higher in the free troposphere, where long-range transport from continents provides a source and wet removal processes are less efficient. Even a relatively small amount of downward transport in this region can have a proportionally large impact on the boundary-layer concentrations.

In response to this comment and the referee's major comment #1, we will add figures displaying the vertical and horizontal distribution of fungal spores in the different simulation cases. We will also add the following text to the discussion of the model results:

"Since the only change in simulations is the ice nucleation scavenging efficiency, and meteorological fields are identical between simulations, downward transport within frozen hydrometeors is the only possible explanation for the changes in the simulations. Large relative increases in concentration are possible because the initial concentrations are extremely low in this remote region (Figures 9 and 10), so even a small source of particles from above can have a large relative impact on boundary-layer concentrations. The very low concentrations in the boundary layer over the Southern Ocean and Antarctica are the result of the large distance from continental sources, as well as strong and frequent precipitation, which efficiently scavenges particles within and below precipitating clouds in this region."

6, Table 2, missing degree signs in the table.

This correction will be made in the revised manuscript.

7, Figure 1, the scale bars are not very clear.

New scale bars will be added to the figure.