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Ice nucleation by fungal spores from the classes *Agaricomycetes*, *Ustilaginomycetes*, and *Eurotiomycetes*, and the effect on the atmospheric transport of these spores

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Abstract. We studied the ice nucleation properties of 12 different species of fungal spores chosen from three classes: *Agaricomycetes*, *Ustilaginomycetes*, and *Eurotiomycetes*. *Agaricomycetes* include many types of mushroom species and are widely distributed over the globe. *Ustilaginomycetes* are agricultural pathogens and have caused widespread damage to crops. *Eurotiomycetes* are found on all types of decaying material and include important human allergens. We focused on these classes because they are thought to be abundant in the atmosphere and because there is very little information on the ice nucleation ability of these classes of spores in the literature. All of the fungal spores investigated contained some fraction of spores that serve as ice nuclei at temperatures warmer than homogeneous freezing. The cumulative number of ice nuclei per spore was 0.001 at temperatures between −19 °C and −29 °C, 0.01 between −25.5 °C and −31 °C, and 0.1 between −26 °C and −31.5 °C. On average, the order of ice nucleating ability for these spores is *Ustilaginomycetes* > *Agaricomycetes* ≃ *Eurotiomycetes*. The freezing data also suggests that, at temperatures ranging from −20 °C to −25 °C, all of the fungal spores studied here are less efficient ice nuclei compared to Asian mineral dust on a per surface area basis. We used our new freezing results together with data in the literature to compare the freezing temperatures of spores from the phyla *Basidiomycota* and *Ascomycota*, which together make up 98 % of known fungal species found on Earth. The data show that within both phyla (*Ascomycota* and *Basidiomycota*), there is a wide range of freezing properties, and also that the variation within a phylum is greater than the variation between the average freezing properties of the phyla. Using a global chemistry–climate transport model, we investigated whether ice nucleation on the studied spores, followed by precipitation, can influence the transport and global distributions of these spores in the atmosphere. Simulations suggest that inclusion of ice nucleation scavenging of these fungal spores in mixed-phase clouds can decrease the annual mean concentrations of fungal spores in near-surface air over the oceans and polar regions, and decrease annual mean concentrations in the upper troposphere.

1 Introduction

Fungal spores, which are the reproductive structures in the lifecycle of fungi, are abundant in the atmosphere (Després et al., 2012; Madelin, 1994; Bauer et al., 2008). Average fungal spore concentrations of 1 to 10 L^{-1} have been reported in the continental boundary layer (Elbert et al., 2007), and peak concentrations of 20 to 35 L^{-1} have been observed

previously (Oliveira et al., 2005; Ho et al., 2005; Goncalves et al., 2010; Quintero et al., 2010; Khattab and Levetin, 2008; Nayar and Jothish, 2013). These spores can be transported large distances and transported to high altitudes in the troposphere (Hirst et al., 1967; Gregory, 1978; Bowers et al., 2009; Amato et al., 2007; Ebner et al., 1989; Fulton, 1966; Kelly and Pady, 1953; Pady and Kelly, 1953; Pady and Kapica, 1955; Proctor and Parker, 1938), and even into the stratosphere (Smith et al., 2010; Imshenetsky et al., 1978; Griffin, 2004).

Ice nucleation on fungal spores may be important because this process could influence the frequency and properties of ice and mixed-phase clouds in the atmosphere. On a global annual scale, ice nucleation on fungal spores may be less important than ice nucleation on mineral dust (Hoose et al., 2010a; Sesartic et al., 2013), but ice nucleation on fungal spores could still influence the frequency and properties of ice and mixed-phase clouds regionally and seasonally (Delort et al., 2010; DeMott and Prenni, 2010; Möhler et al., 2007; Morris et al., 2004; Morris et al., 2011; Pöschl et al., 2010; Sands et al., 1982; Phillips et al., 2009; Creamean et al., 2013; Spracklen and Heald, 2013). As examples, numerous field studies have illustrated that at certain times, locations and conditions, a fraction of the total ice nuclei (IN) population may contain biological material (Pratt et al., 2009; Prenni et al., 2009; Garcia et al., 2012; Huffman et al., 2013; Tobo et al., 2013; Hader et al., 2013). Ice nucleation on fungal spores may also be important because ice nucleation on fungal spores, followed by precipitation, may be an important removal mechanism of these spores from the atmosphere (Morris et al., 2013a). The main mechanism for the spread of fungi is by wind dissemination of spores (Webster and Weber, 2007), and accurate models to predict the spread of fungal spores are needed. In the past, researchers have predicted the spread of fungal spores using various models (Andrade et al., 2009; Aylor, 1986, 2003; Fröhlich-Nowoisky et al., 2012; Isard et al., 2005; Kim and Beresford, 2008; Magarey et al., 2007; Pan et al., 2006; Pfender et al., 2006; Skelsey et al., 2008; Wang et al., 2010; Wilkinson et al., 2012), but ice nucleation followed by precipitation was not included in these models as a sink of the spores from the atmosphere.

Fungi can be designated into 35 different classes (Hibbett et al., 2007). So far, the ice nucleation properties of spores from the following classes have been investigated: *Dothideomycetes*, *Exobasidiomycetes*, *Eurotiomycetes*, *Pucciniomycetes*, and *Sordariomycetes* (Haga et al., 2013; Morris et al., 2013b; Iannone et al., 2011; Jayaweera and Flanagan, 1982; Pouleur et al., 1992; Pummer et al., 2013). There is some evidence that proteins are involved in ice nucleation by fungal spores, at least for spores from the genus Fusarium (Hasegawa et al., 1994). To add to the limited number of data on the ice nucleation properties of fungal spores, we studied the ice nucleation properties of 12 different species of fungal spores chosen from three classes: *Agaricomycetes*, *Ustilaginomycetes*, and *Eurotiomycetes*. The species and classes of the fungal spores investigated are listed in Table 1. We focused on these classes since they are thought to be abundant in the atmosphere (discussed below). Table S1 in the Supplement lists previous field studies that have identified these spores in the atmosphere. Recently, Pummer et al. (2013) studied a few of the same species as were studied here (*Agaricus bisporus* and *Aspergillus niger*). Our studies are complimentary to the studies by Pummer et al. (2013), since we measured the cumulative number of IN per spore as a function of temperature, as well as the ice nucleation active surface site density as a function of temperature, while Pummer et al. (2013) reported the temperature at which 50 % of all of the droplets froze using an average spore mass concentration of about $20\,\mathrm{mg\,mL^{-1}}$.

Agaricomycetes is a class of fungi that includes many types of mushroom species, and is widely distributed over the globe (Webster and Weber, 2007). This class includes *Agaricus bisporus*, the common or button mushroom. Spores from this class have been found to be important components of the air-borne fungal spore population near the surface (Fröhlich-Nowoisky et al., 2012; Mallo et al., 2011; Yamamoto et al., 2012; Oliveira et al., 2009a, b; Magyar et al., 2009; Fröhlich-Nowoisky et al., 2009; De Antoni Zoppas et al., 2006; Herrero et al., 2006; Morales et al., 2006) and also at high altitudes (Bowers et al., 2009; Amato et al., 2007). For example, over half of the fungal species identified from continental air by Fröhlich-Nowoisky et al. (2012) were from the class *Agaricomycetes*. The few studies that have quantified *Agaricomycetes* spore concentrations at the genus level have reported surface concentrations of roughly 10^{-3} to $1\,\mathrm{L^{-1}}$ (Magyar et al., 2009; Morales et al., 2006; Li, 2005).

Ustilaginomycetes spores have frequently been identified from surface air samples (Mallo et al., 2011; Pyrri and Kapsanaki-Gotsi, 2007), and have been shown to make up to a third of the total fungal spores in some regions (Herrero et al., 2006; Morales et al., 2006; Hasnain et al., 2005; Mitakakis and Guest, 2001). Boundary layer concentrations have been measured to be roughly 0.05 to $6\,\mathrm{L^{-1}}$ (Nayar and Jothish, 2013; Magyar et al., 2009; Khattab and Levetin, 2008; Pyrri and Kapsanaki-Gotsi, 2007; Morales et al., 2006; Hasnain et al., 2005; Wu et al., 2004; Troutt and Levetin, 2001; Sabariego et al., 2000; Calderon et al., 1995; Hirst, 1953; Gregory, 1952), and spores from this class have been identified at high altitudes in the troposphere by Pady and Kelly (1954). *Ustilagomycetes* are agricultural pathogens that belong to the group of smut fungi, causing widespread damage to cereal crops (Webster and Weber, 2007). Hence, understanding the transport of these spores in the atmosphere (which may involve ice nucleation) is of interest from an economic perspective.

Eurotiomycetes are found on all types of decaying material, and are one of the most ubiquitous types of fungi (Webster and Weber, 2007). *Eurotiomycetes* have also been identified as important human allergens (see, for example, Horner et al. (1995), and references therein). The specific

Table 1. Description of the fungal spores investigated in the current study.

Class	Species	Spore size[a] (µm)	Spore type[b]	Spores/Drop		
				Median	Q1[c]	Q3[c]
Agaricomycetes	*Agaricus bisporus*	8(±2) × 7(±2)	Basidiospore	3	2	5
	Amanita muscaria	10(±2) × 7(±2)	Basidiospore	28	8	65
	Boletus zelleri	13(±2) × 6(±2)	Basidiospore	4	2	8
	Lepista nuda	8(±2) × 5(±2)	Basidiospore	6	3	14
	Trichaptum abietinum	7(±2) × 4(±2)	Basidiospore	15	7	57
Ustilaginomycetes	*Ustilago nuda*	7(±2) × 6(±2)	Teliospore	9	5	18
	Ustilago nigra	9(±2) × 7(±2)	Teliospore	4	2	8
	Ustilago avenae	8(±2) × 6(±2)	Teliospore	15	7	31
Eurotiomycetes	*Aspergillus brasiliensis*	4(±1)	Conidiospore	1	0	2
	Aspergillus niger	4(±1)	Conidiospore	2	1	5
	Penicillium sp.[d]	4(±1) × 3(±1)	Conidiospore	15	7	29
	Penicillium brevicompactum	4(±1) × 3(±1)	Conidiospore	6	4	9

[a] The uncertainties in the size measurements correspond to the resolution limit of the Olympus IX70 and Zeiss Axiolab A microscopes. The 20 × objective was used for the *Agaricomycetes* and the *Ustilaginomycetes* spores, with an uncertainty of approximately 2 µm, and 30 ×, 40 × and 50 × objectives were used for the *Eurotiomycetes*, resulting in uncertainties of approximately 1 µm.

[b] Definitions for the spore types shown in Table 1:

- Basidiospore: a sexually reproductive spore produced by fungi of the phylum *Basidiomycota*; typically they are unicellular. Most basidiospores are ballistospores, which are violently dispersed from the apex of the basidium.
- Teliospore: produced by smut and rust fungi near the end of the growing season; consist of two cells with heavily thickened and darkly pigmented walls. Teliospores overwinter.
- Conidiospore: an asexually produced fungal spore for aerial dispersal, formed on the surface of modified hyphae called conidiophores.

[c] Q1: first quartile; 25 % of the data were less than or equal to this value. Q3: third quartile; 75 % of the data were less than or equal to this value.

[d] "sp." indicates an unidentified species.

types of spores from the class *Eurotiomycetes* that were studied here are from the *Penicillium* and *Aspergillus* genera. These spores are frequently present in surface air (Pyrri and Kapsanaki-Gotsi, 2012; Pyrri and Kapsanaki-Gotsi, 2007; Goncalves et al., 2010; Mallo et al., 2011; Shelton et al., 2002; Fröhlich-Nowoisky et al., 2009), and they often represent a large fraction of identified spores (up to 35 %), with typical surface concentrations of roughly 0.1 to 5 L^{-1} (Nayar and Jothish, 2013; Iglesias Fernández et al., 2012; Crawford et al., 2009; Pyrri and Kapsanaki-Gotsi, 2012; Quintero et al., 2010; Khattab and Levetin, 2008; Wu et al., 2004; Li and Kendrick, 1995). During periods of high spore productivity, concentrations of *Penicillium* spores as high as 10 L^{-1} have been observed (Pyrri and Kapsanaki-Gotsi, 2012). *Penicillium* spores have also been identified at high altitudes in the troposphere and stratosphere (Smith et al., 2010; Amato et al., 2007; Griffin, 2004; Jayaweera and Flanagan, 1982; Imshenetsky et al., 1978; Fulton, 1966; Pady and Kapica, 1955; Pady and Kelly, 1954; Kelly and Pady, 1953; Proctor and Parker, 1938), as have *Aspergillus* spores (Imshenetsky et al., 1978; Fulton, 1966; Pady and Kapica, 1955; Proctor and Parker, 1938; Amato et al., 2007).

Fungal spores from the phyla *Basidiomycota* and *Ascomycota* make up approximately 98 % of known fungal species found on Earth (James et al., 2006). Recent field measurements observed higher ratios of *Ascomycota* to *Basidiomycota* in remote marine regions compared to continental regions (Fröhlich-Nowoisky et al., 2012). One possible explanation for this was that *Basidiomycota* spores are more efficient ice nuclei compared to *Ascomycota* spores, resulting in *Basidiomycota* spores being more efficiently removed from the atmosphere by precipitation from ice and mixed-phase clouds (Fröhlich-Nowoisky et al., 2012). We also use our new freezing data, together with freezing data from the literature, to assess if fungal spores from the phylum *Basidiomycota* are better ice nuclei than fungal spores from the phylum *Ascomycota*.

In addition to studying the ice nucleation properties of spores from the classes *Agaricomycetes*, *Ustilaginomycetes*, and *Eurotiomycetes*, we also investigated whether ice nucleation on these spores, followed by precipitation, can influence the transport and removal of these spores in the atmosphere. We did this using a global chemistry–climate transport model and by comparing simulations with and without ice nucleation included in the model. These simulations suggest that ice nucleation on the spores studied may be important for the transport of these spores to remote regions (such as the marine boundary layer, polar regions, and the upper troposphere).

2 Methods

2.1 Spore samples and slide preparation

Agaricus bisporus, the common button mushroom, was purchased from a local grocery store in Vancouver, British Columbia (BC), Canada. The remaining *Agaricomycetes* fungi (*Amanita muscaria*, *Boletus zelleri*, *Lepista nuda*, and *Trichaptum abietinum*) were harvested from the Pacific Spirit Regional Park surrounding the University of British Columbia (UBC) campus in Vancouver, BC. To prepare slides containing *Agaricomycetes* spores, sections of the fruiting body were placed on a wire mesh in a sealed and humidified chamber. Hydrophobic glass slides (Hampton Research, HR3-215) were placed underneath the wire mesh exposed to the part of the fungus that releases spores under humidified conditions. Over time, spores were released naturally and deposited onto the glass slides.

Ustilaginomycetes spores (*Ustilago nuda*, *Ustilago nigra*, and *Ustilago avenae*) were acquired from the Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba, Canada. Slides containing *Ustilaginomycetes* spores were prepared analogously to the method used by Haga et al. (2013) for rust (*Pucciniomycetes*) spores. Briefly, a sealed glass vessel containing spores and a small fan were immersed in a sonicator bath. The combination of the fan and the vibrations from the sonicator bath generated a spore cloud within the vessel, and resulted in spores being deposited onto glass slides that were suspended on a wire mesh within the vessel.

The *Eurotiomycetes* species studied here were obtained from the UBC Department of Chemistry Bioservices Laboratory collection: *Aspergillus brasiliensis* (collection #56), *Aspergillus niger* (collection #161), *Penicillium* sp. (collection #58), and *Pencillium brevicompactum* (collection #54). *Aspergillus* spores were isolated using techniques similar to those described by Jones et al. (1992) and Allan and Prosser (1983). In short, the fungi were grown in petri dishes, and approximately 10^3 glass beads (0.5 mm in diameter) (BioSpec Products Inc.) were rolled over the surface of the fungal culture, which resulted in the spores being dislodged from the culture and attached to the beads. The beads and spores were then transferred to a sample vial containing ultrapure water. This resulted in a suspension of spores, while the glass beads settled on the bottom of the sample vial. The resulting spore suspension was sprayed onto hydrophobic glass slides using a Meinhard nebulizer (model TR-30-A1). *Pencillium* spores were aerosolized from fungal cultures and deposited onto a hydrophobic glass slide using a custom-built flow cell recently developed in our laboratory and previously used for depositing *Cladosporium* spores onto hydrophobic surfaces (Iannone et al., 2011).

2.2 Freezing experiments

The instrument used to study the immersion freezing properties of the fungal spores consisted of an optical microscope (Zeiss Axiolab A with a $10\times$ or $50\times$ objective) coupled to a flow cell that had temperature and relative humidity control. This apparatus or a similar apparatus has been used in many previous ice nucleation studies (Iannone et al., 2011; Dymarska et al., 2006; Koop et al., 1998; Chernoff and Bertram, 2010; Wheeler and Bertram, 2012), and will only be described briefly here. A hydrophobic glass cover slide is used to support the fungal spores inside the flow cell. First, water vapor is condensed on the spores to grow droplets approximately 30–150 µm in size by adjusting the dew point of the carrier gas to 2 °C and the temperature of the flow cell to 0 °C. Next, the flow cell was isolated from the humidified flow, and the temperature was lowered at a rate of 5–10 °C min^{-1} down to −40 °C. Finally, the temperature was raised to room temperature to evaporate the droplets, leaving only the fungal spores on the glass slide. Digital video and temperature were recorded during the freezing experiments. For each droplet, the freezing temperature and the 2-D surface area of the fungal spores contained within each droplet were determined.

2.3 Spore properties and number of spores per drop

Using microscope images, we calculated the average projected 2-D surface area per spore and the spore dimensions (length and width) for each species. From this information and the 2-D surface area of spores per drop (Sect. 2.2), we determined the number of spores per drop.

Scanning electron microscopy (SEM) images, acquired at the UBC Bioimaging Facility, were used to determine the surface features of the spores. Images for *A. muscaria*, *B. zelleri*, *L. nuda*, *T. abietinum*, *A. brasiliensis*, *A. niger*, *U. nuda*, *U. nigra*, and *U. avenae* were acquired using variable pressure SEM (Hitachi S2600 VP-SEM). *A. bisporus*, *Penicillium* sp., and *P. brevicompactum* spores were imaged by field emission SEM (Hitachi S4700 FESEM). Glass slides containing *Agaricomycetes* spores (*A. bisporus*, *A. muscaria*, *B. zelleri*, *L. nuda*, and *T. abietinum*) and *Aspergillus* spores (*A. brasiliensis* and *A. bisporus*) were affixed onto aluminum SEM stubs using double-sided glue tabs, sputter coated with 12 nm gold (Au) palladium (Pd) and mounted in the SEM instrument. Similarly, loose *Ustilaginomycetes* (*U. nuda*, *U. avenae*, and *U. nigra*) spores were deposited directly onto double-sided glue tabs, affixed onto aluminum SEM stubs, sputter coated with 8 nm Au, and mounted in the SEM instrument. To prepare *Penicillium* sp. and *P. brevicompactum* samples for imaging, cultures of each fungal species were first fixed using osmium vapor (4 % OsO_4 (aq)), and then a portion of the culture was excised and mounted onto an aluminum SEM stub using Aquadag (a graphite-based adhesive). Finally, the samples were allowed to air-dry prior to

imaging. For the SEM experiments, images were captured using secondary electrons, a working distance between 5 and 15 mm, and an acceleration voltage between 3 and 25 kV.

2.4 EMAC global chemistry–climate transport model

We conducted idealized simulations of fungal spore transport to investigate the sensitivity of simulated atmospheric concentrations to the activity of spores as ice nuclei. We simulated the transport and removal of atmospheric aerosol particles in a global chemistry–climate model, ECHAM–MESSy Atmospheric Chemistry (EMAC). The EMAC model is a numerical chemistry and climate simulation system that includes sub-models describing tropospheric and middle atmosphere processes and their interaction with oceans, land and human influences (Jöckel et al., 2006; Jöckel et al., 2005; Kerkweg et al., 2006; Tost et al., 2006; Lawrence and Rasch, 2005). It uses the Modular Earth Submodel System (MESSy) to link multi-institutional computer codes. The core atmospheric model is the fifth-generation European Centre Hamburg general circulation model (ECHAM5 GCM) (Roeckner et al., 2003). For the present study, we applied EMAC (ECHAM5 version 5.3.01, MESSy version 1.9) in the T63L31 resolution; i.e., with a spherical truncation of T63 (corresponding to a quadratic Gaussian grid of approximately $1.9 \times 1.9\,^{\circ}$ in latitude and longitude, or approximately 140×210 km at middle latitudes), with 31 vertical hybrid pressure levels up to 10 hPa. This model resolution provides a reasonable balance between accuracy and computational expense: tracer transport in the ECHAM5 GCM has been shown to be comparatively insensitive to further increases in model resolution (Aghedo et al., 2010).

Simulations were performed for five years and an additional year of spin-up time, with the model setup described in detail by Burrows et al. (2009), and with modifications to the scavenging scheme (Tost et al., 2010), including the slow downward transport of crystal-borne species due to the sedimentation of crystals, and the release and repartitioning of tracers associated with evaporation of droplets and melting of ice crystals. Briefly, aerosols are treated as monodisperse passive aerosol tracers emitted at the Earth's surface. As a simplification, we assumed constant emissions from all land surfaces (excluding land ice) at a rate of $200\,\mathrm{m^{-2}\,s^{-1}}$ (Elbert et al., 2007). All transport and removal processes in the model depend linearly on spore concentration, and spore concentrations do not affect clouds or radiation in the model. For these idealized simulations, we do not distinguish between different fungal spore species. Atmospheric transport is simulated (including advection and parameterized convective mass flux), and tracers are removed by dry and wet deposition to the surface. The EMAC model, used in similar configurations, has been shown to be capable of realistic simulations of aerosol transport and deposition for the transport of African dust to Europe (Gläser et al., 2012) and radioactive aerosol particles from the Chernobyl accident (Lelieveld et al., 2012).

Removal processes of particles simulated in the model included sedimentation, dry deposition, impaction scavenging, and nucleation scavenging by liquid, mixed-phase, and ice clouds. We use the methods outlined by Burrows et al. (2009) to describe sedimentation, dry deposition, and impaction scavenging. Nucleation scavenging was prescribed with a scavenging parameter, R, where $R_{\mathrm{nuc,\,liq}}$ and $R_{\mathrm{nuc,\,ice}}$ are the fractions of particles embedded in cloud droplets or ice particles within clouds, respectively. For warm liquid clouds (i.e., clouds at temperatures $> 0\,^{\circ}$C), we assume that $R_{\mathrm{nuc,\,liq}}$ is unity, as done previously (Heald and Spracklen, 2009). A value of unity assumes that the particles are efficient cloud condensation nuclei (CCN). Here, we use the immersion freezing results to assess implications for fungal spore global transport. In order for the spores to act as immersion-freezing nuclei, they would first have to be incorporated into liquid cloud droplets during the droplet nucleation and growth process, which is ensured by the assumption $R_{\mathrm{nuc,\,liq}} = 1$. For mixed-phase clouds (i.e., clouds at temperatures between $0\,^{\circ}$C and $-35\,^{\circ}$C), we carried out simulations with two different values of $R_{\mathrm{nuc,\,ice}}$. These two different simulations were carried out to test if ice nucleation can impact the long-distance transport of the spores in the atmosphere. In the first simulation (referred to as IN-Inactive), we used an $R_{\mathrm{nuc,\,ice}}$ value of zero at all temperatures relevant for mixed-phase clouds (below 0 to $-35\,^{\circ}$C), and in the second simulation (referred to as IN-Active), we assumed that $R_{\mathrm{nuc,\,ice}}$ is zero for mixed phase clouds at temperatures above $-25\,^{\circ}$C, but equal to unity for temperatures between -25 and $-35\,^{\circ}$C. A temperature of $-25\,^{\circ}$C for the onset of mixed-phase ice nucleation scavenging was chosen because it represents the approximate temperature where freezing occurs for the spores studied here (see Fig. 3). For ice clouds (i.e., clouds at temperatures $\leq -35\,^{\circ}$C, which is the homogeneous freezing temperature of water droplets), we assume that $R_{\mathrm{nuc,\,ice}}$ is 0.05 for both simulations mentioned above (IN-Inactive and IN-Active). 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 $\leq -35\,^{\circ}$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. A summary of the nucleation scavenging coefficients used in the different simulations is included in Table 2.

Table 2. Nucleation scavenging schemes used in EMAC.

Scavenging scheme	Liquid nucleation scavenging ($R_{\text{nuc, liq}}$)	Ice nucleation scavenging ($R_{\text{nuc, ice}}$)	
		Mixed-phase clouds ($0\,^{\circ}\text{C} > T > -35\,^{\circ}\text{C}$)	Ice clouds ($T \leq -35\,^{\circ}\text{C}$)
IN-Inactive	1	0 for entire temperature range	0.05
IN-Active	1	0 for $T > -25\,^{\circ}\text{C}$ 1 for $-25\,^{\circ}\text{C} \geq T > -35\,^{\circ}\text{C}$	0.05

The in-cloud local rate of change in the concentration of particles (i.e., the number of particles per number of air molecules X_i) due to nucleation scavenging and subsequent precipitation is

$$\frac{\Delta X_i}{\Delta t} = X_i f^{\text{cl}} \left(\frac{R_{\text{nuc, ice}} \cdot F_{\text{snow}}}{\text{iwc}} + \frac{R_{\text{nuc, liq}} \cdot F_{\text{rain}}}{\text{lwc}} \right), \quad (1)$$

where Δt is the length of the model time step, f^{cl} is the grid-box mean cloud fraction, F_{snow} and F_{rain} are the fluxes of snow and rain, respectively, *iwc* and *lwc* are the ice water content and liquid water content of the cloud, respectively, and $R_{\text{nuc, ice}}$ and $R_{\text{nuc, liq}}$ are the nucleation scavenging efficiencies of cloud ice and cloud liquid water, respectively (and as described in Table 2).

For nucleation scavenging, the same scavenging parameterizations were applied to both stratiform clouds and parameterized convective clouds. Simulations were run for 3 µm and 8 µm diameter spherical particles, which overlaps with the sizes (volume equivalent diameters) of the spores studied here (see Table 1).

3 Results and discussion

3.1 Spore properties

Listed in Table 1 are the average dimensions of the spores studied here, determined by optical microscopy. Values reported in Table 1 are consistent with the literature values: Bockus et al. (2010), Gilbertson and Ryvarden (1987), Lincoff (1981), Mathre (1982), Mitchell and Walter (1999), Pasanen et al. (1991), Raper et al. (1949), and Vanky (1987). SEM images of the fungal spores studied are shown in Fig. 1. In many of the images, the spores appear deflated (*A. bisporus*, *U. nuda*, *U. nigra*, *U. avenae*, *A. brasiliensis*, *A. niger*, *Penicillium* sp., and *P. brevicompactum*) or as partial spheroids (*T. abietinum*). These features are likely due to the sample processing prior to imaging (see Sect. 2.3), or to a loss in turgidity of the fungal spores in the vacuum of the SEM instrument.

3.2 Fraction of droplets frozen vs. temperature

Shown in Fig. 2 is the fraction of frozen droplets as a function of temperature, including all freezing events that were observed during these experiments. Results for droplets containing *Agaricomycetes* spores are shown in blue (open symbols). *Ustilaginomycetes* spores are shown in green (open symbols with a horizontal line) and *Eurotiomycetes* spores are in red (open symbols with a vertical line). The median number of spores per droplet is given for each spore species in Table 1, as well as the first and third quartiles. Also included in Fig. 2 (as *x*s) are previous results from Iannone et al. (2011) for homogeneous nucleation using the same experimental method as used here. Figure 2 shows that the fungal spores studied here contained some fraction of spores that initiated freezing at warmer freezing temperatures compared to the homogeneous results of Iannone et al. (2011). Since the number of spores per droplet varies for the different fungal species studied, conclusions about the relative freezing properties of these spores should not be formulated from Fig. 2.

3.3 Cumulative number of ice nuclei per spore as a function of temperature, *INperSpore(T)*

Using the method presented by Vali (1971), the cumulative number of ice nuclei per spore as a function of temperature, *INperSpore(T)*, was determined from the number of spores per drop and the fraction of droplets frozen as a function of temperature (Fig. 2). First, the droplets were binned by the number of spores per drop. Then, from the binned data, the cumulative number of ice nuclei per spore as a function of temperature was calculated with the following equation (note that only those bins containing at least 5 droplets were used in the calculation in order to have reasonable statistics in each bin):

$$INperSpore(T) = \frac{\sum_{j=1}^{n} \left[-\ln\left(\frac{N_{\text{unfrozen,j}}(T)}{N_{\text{total,j}}} \right) \times N_{\text{total,j}} \right]}{\sum_{j=1}^{n} \text{SPD}_j \times N_{\text{total,j}}}, \quad (2)$$

where j is the bin number, n is the total number of bins, $N_{\text{unfrozen},\,j}(T)$ is the number of unfrozen drops in bin j at

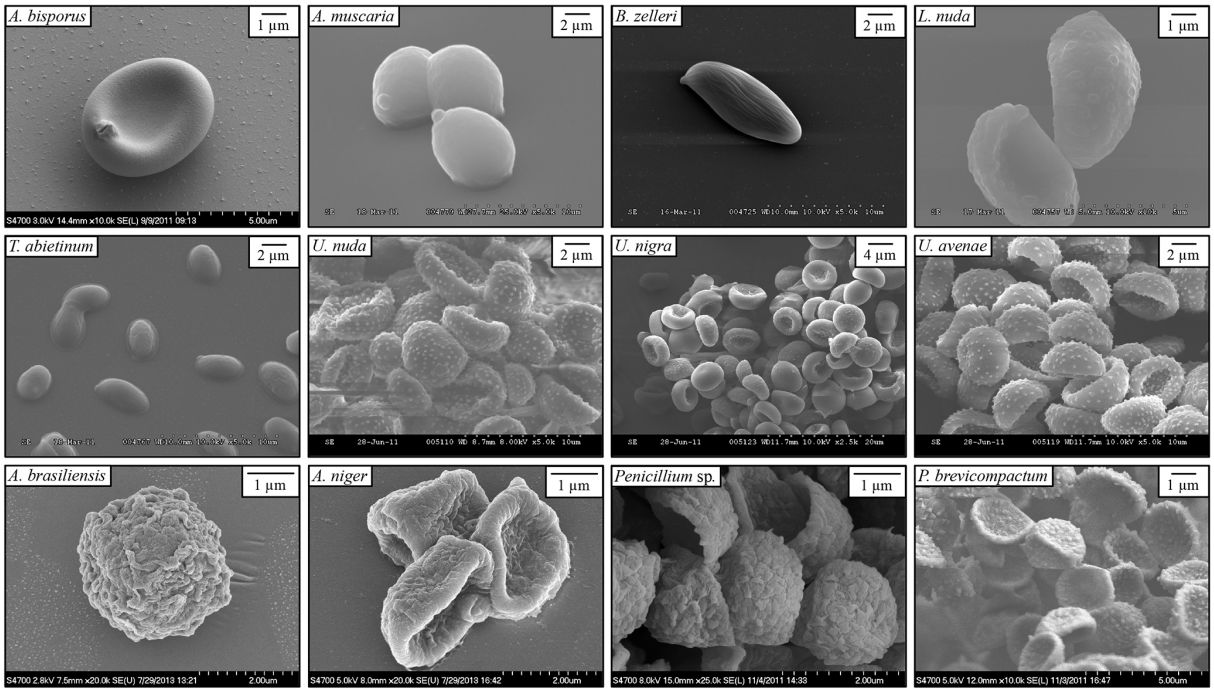

Figure 1. SEM images of spores from the classes *Agaricomycetes* (*A. bisporus*, *A. muscaria*, *B. zelleri*, *L. nuda*, and *T. abietinum*), *Ustilaginomycetes* (*U. nuda*, *U. nigra*, and *U. avenae*), and *Eurotiomycetes* (*A. brasiliensis*, *A. niger*, *Penicillium* sp., and *P. brevicompactum*).

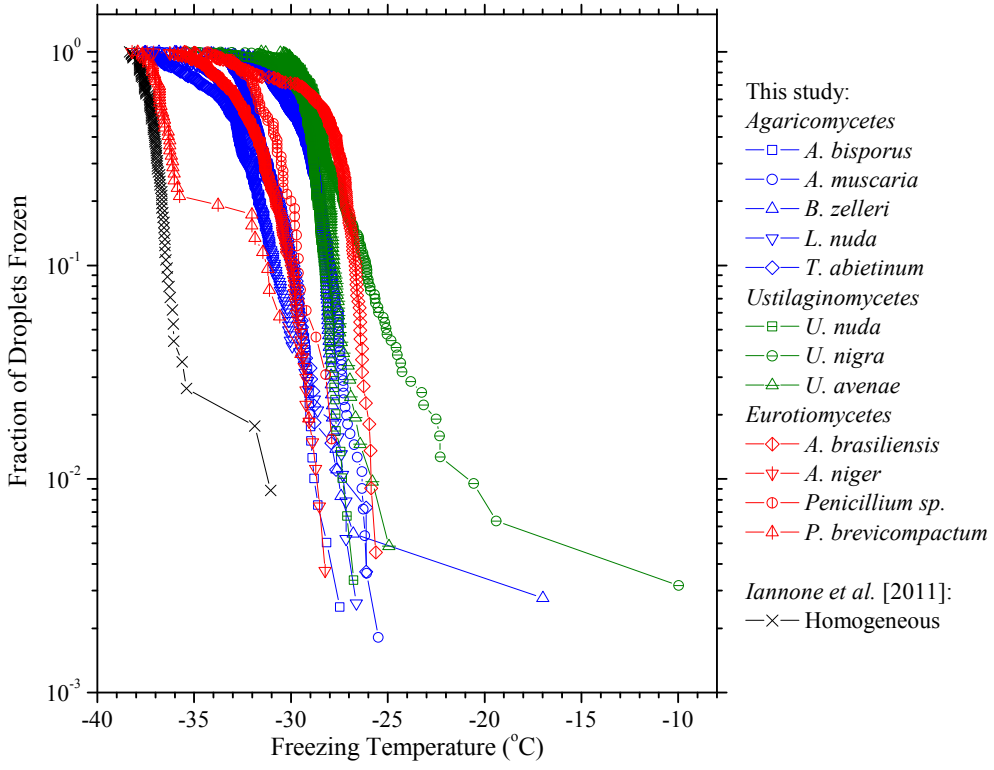

Figure 2. Fraction of droplets frozen as a function of temperature. *Agaricomycetes* spores (*A. bisporus*, *A. muscaria*, *B. zelleri*, *L. nuda*, and *T. abietinum*) are in blue (open symbols); *Ustilaginomycetes* spores (*U. nuda*, *U. nigra*, and *U. avenae*) are in green (open symbols with a horizontal line); *Eurotiomycetes* spores (*A. brasiliensis*, *A. niger*, *Penicillium* sp., and *P. brevicompactum*) are in red (open symbols with a vertical line). Homogeneous freezing results (×) (Iannone et al., 2011), obtained using the same apparatus as this study, are also included.

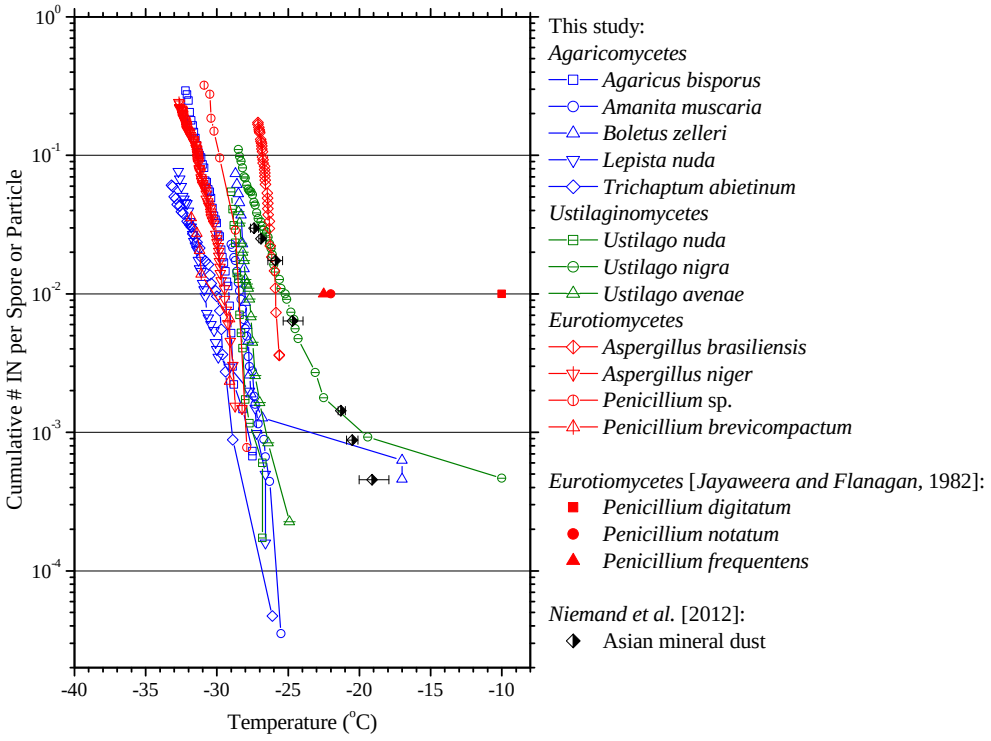

Figure 3. Cumulative number of ice nuclei per spore as a function of temperature, *INperSpore(T)*, as derived from the measurement data shown in Fig. 2. *Agaricomycetes* spores (*A. bisporus*, *A. muscaria*, *B. zelleri*, *L. nuda*, and *T. abietinum*) are in blue (open symbols); *Ustilaginomycetes* spores (*U. nuda*, *U. nigra*, and *U. avenae*) are in green (open symbols with a horizontal line); *Eurotiomycetes* spores (*A. brasiliensis*, *A. niger*, *Penicillium* sp., and *P. brevicompactum*) are in red (open symbols with a vertical line). Also shown is the *INperSpore(T)* for *Eurotiomycetes* spores studied by Jayaweera and Flanagan (1982) (filled symbols), and the cumulative number of ice nuclei per particle *INperParticle(T)* for submicron Asian dust studied by Niemand et al. (2012) (half-filled diamonds).

temperature T, $N_{\text{total},j}$ is the total number of drops in bin j, and SPD_j is the number of spores per drop in bin j. Note that the cumulative number of ice nuclei per spore is equivalent to the average number of ice nucleation sites per spore. Values of *INperSpore(T)* were not calculated for temperatures below –35 °C since at these temperatures homogeneous freezing of the droplets can dominate.

Shown in Fig. 3 are the *INperSpore(T)* values for the different fungal species studied. *Agaricomycetes* spores are shown in blue (open blue symbols), *Ustilaginomycetes* spores are shown in green (open green symbols with a horizontal line), and *Eurotiomycetes* spores are in red (open red symbols with a vertical line). From Fig. 3, the cumulative number of ice nuclei per spore was 0.001 between −19 °C and −29 °C, 0.01 between −25.5 °C and −31 °C, and 0.1 between −26 °C and −31.5 °C. Also, on average, the order of ice nucleating ability for these spores is *Ustilaginomycetes* > *Agaricomycetes* ≃ *Eurotiomycetes*. To illustrate this point, as an example, the cumulative number of ice nuclei per spore was 0.01 at approximately −27 °C for the *Ustilaginomycetes* spores, 0.01 at approximately −29 °C for the *Agaricomycetes* spores, and 0.01 at approximately −28.5 °C for the *Eurotiomycetes* spores.

Jayaweera and Flanagan (1982) and Pummer et al. (2013) are the only other studies to measure the ice nucleation ability of spores from the three classes investigated here. The results from Jayaweera and Flanagan (1982) are included in Fig. 3 for comparison (filled symbols). The results from Pummer et al. (2013) are not included in Fig. 3, since they did not report the cumulative number of IN per spore, and it was not possible to calculate this value from their experimental description.

From Fig. 3, when comparing the temperatures at which the cumulative number of ice nuclei per spore is equal to 0.01, all of the *Ustilaginomycetes*, *Agaricomycetes* and *Eurotiomycetes* spores studied here are poorer IN than those studied by Jayaweera and Flanagan (1982). Comparing just the *Penicillium* results from the current study to those from Jayaweera and Flanagan (1982) suggests that (1) *Penicillium* sp. and *P. brevicompactum* are poor IN compared to *P. digitatum*, *P. notatum*, and *P. frequentens*, and that (2) within the same genus, the *INperSpore* values can vary drastically (e.g., the freezing temperature at an *INperSpore* value of 0.01 varies by approximately 20 °C in the genus *Penicillium* as evident in Fig. 3).

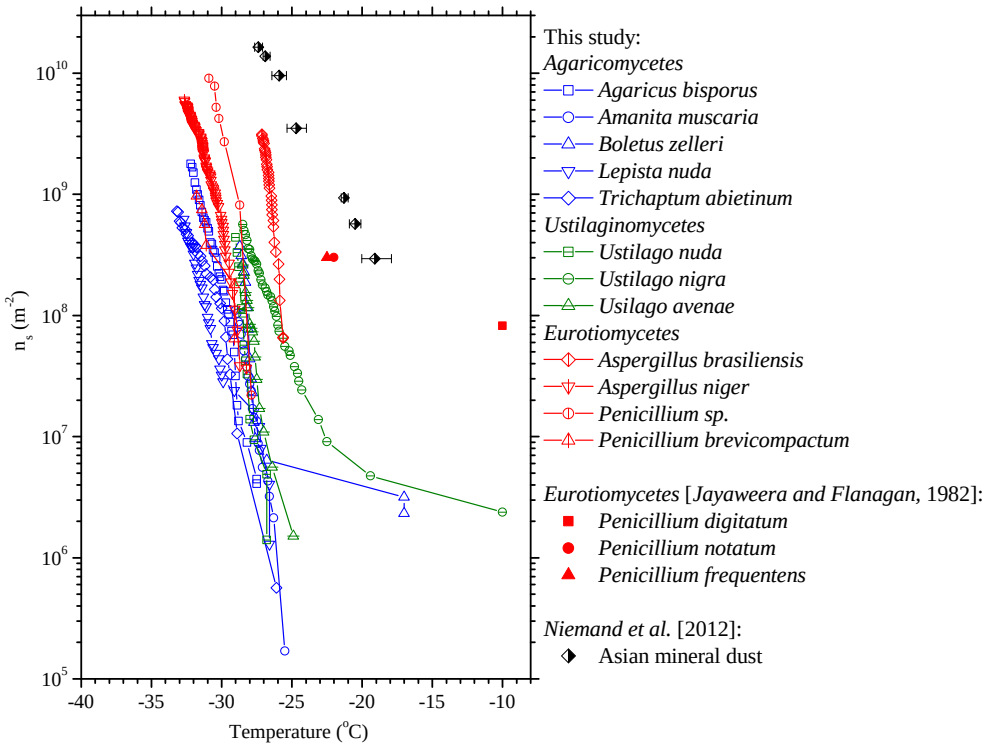

Figure 4. Ice nucleation active surface site density, n_S (m^{-2}), for fungal spores determined using the data in Fig. 3. *Agaricomycetes* spores (*A. bisporus*, *A. muscaria*, *B. zelleri*, *L. nuda*, and *T. abietinum*) are in blue (open symbols); *Ustilaginomycetes* spores (*U. nuda*, *U. nigra*, and *U. avenae*) are in green (open symbols with a horizontal line); and *Eurotiomycetes* spores (*A. brasiliensis*, *A. niger*, *Penicillium* sp., and *P. brevicompactum*) are in red (open symbols with a vertical line). Also shown are n_S values for *Eurotiomycetes* spores studied by Jayaweera and Flanagan (1982) (filled symbols), as well as for Asian mineral dust studied by Niemand et al. (2012) (half-filled diamonds).

It is important to consider the possibility that the fungal spores studied here may contain bacterial or fungal contaminants on their surface, and to discuss the likelihood that these contaminants will change the ice nucleating properties of the spores (Morris et al., 2013b). From the SEM images presented in Fig. 1, there is no indication of any bacterial or fungal surface contamination on the fungal spores (personal communication, D. Horne, UBC Bioimaging Facility, 2013). Since the *Eurotiomycetes* studied here (*A. brasiliensis*, *A. niger*, *Penicillium* sp., and *P. brevicompactum*) were grown in culture, any bacterial contamination of the spores would have been apparent in the culture stage of these experiments. The remaining spores (with the exception of *A. bisporus*) were qualitatively tested for surface contamination by bacteria and/or fungi using the same method from Haga et al. (2013) for rust and bunt spores: the spores to be tested were cultured in media (nutrient agar and tryptic soy agar) that would support contaminant growth, and the cultures were monitored for two weeks. The test indicated low levels of surface contamination for four of the fungal spores studied here. Specifically, *U. avenae*, *U. nigra*, and *U. nuda* cultures indicated surface contamination on the spores after 4 days, and colonies were observed in the *T. abietinum* culture after 13 days. For the remaining spores, *B. zelleri*, *L. nuda*, and *A. muscaria*, there was no indication of surface contamination. From the discussion above, it seems unlikely that the ice nucleation properties of the spores studied here are affected appreciably by surface contamination by fungi or bacteria. However, we cannot rule out this possibility, and so the *INperSpore* spectra presented in the current study should be taken as upper limits.

Mineral dust particles are considered to be important atmospheric ice nuclei. For comparison in Fig. 3 (half-filled diamonds), we have included recent immersion freezing results by Niemand et al. (2012) for submicron Asian mineral dust (experiments ACI04_16 and ACI04_19). *INperParticle(T)* for the Asian mineral dust was calculated from the ice nucleation active surface site density, n_S, values from Niemand et al. (2012), and as described by Haga et al. (2013). With the exception of *U. nigra* and *A. brasiliensis*, all of the spores studied here are poorer IN than the Asian mineral dust studied by Niemand et al. (2012) on a per particle basis. The *INperSpore(T)* values for *U. nigra* are similar to the *INperParticle(T)* values for Asian mineral dusts over the entire temperature range studied by Niemand et al. (2012); for *A. brasiliensis*, the *INperSpore(T)* values are comparable to the *INperParticle(T)* values for the dust only at temperatures below approximately −25°C.

3.4 Ice nucleation active surface site density, n_s

Since heterogeneous ice nucleation is a surface process, larger particles may be more efficient IN on a per particle basis because they have more surface area available for nucleation. To take into account the difference in surface area per particle, we calculated the ice nucleation active surface site density, n_s, for the fungal spores in the current study. The ice nucleation active surface site density corresponds to the number of ice nucleation sites per unit surface area of a particular type of IN (Connolly et al., 2009), and was calculated according to

$$n_s = \frac{INperSpore}{A_{aer}}. \quad (3)$$

To calculate the surface area for each fungal spore (A_{aer}), we approximated all spores other than the *Aspergillus* spores as prolate spheroids having the lengths and widths shown in Table 1. *Aspergillus* spores were approximated as being spheres with the diameters given in Table 1.

The temperature dependent n_s values for the fungal spores studied here are shown in Fig. 4. For comparison, included in Fig. 4 are n_s values for Asian mineral dust (Niemand et al., 2012) (experiments ACI04_16 and ACI04_19), and also results from Jayaweera and Flanagan (1982) for *Eurotiomycetes* spores. The n_s values for the *Eurotiomycetes* spores studied by Jayaweera and Flanagan (1982) were calculated using spore dimensions and morphologies from the literature (Raper et al., 1949). Specifically, *P. digitatum*, was approximated to be a prolate spheroid having dimensions 7.75×5.5 µm and both *P. notatum* and *P. frequentens* spores were assumed to be spheres with a diameter of 3.25 µm.

After accounting for surface area, Fig. 4 shows that the n_s spectra for all of the fungal species studied here are similar. Figure 4 also shows that a difference in surface area cannot explain the difference in freezing temperatures within the same genus observed in Fig. 3 for species of *Penicillium*. Finally, Fig. 4 suggests that, at temperatures ranging from $-20\,^{\circ}C$ to $-25\,^{\circ}C$, all of the fungal spores studied here are less efficient ice nuclei compared to Asian mineral dust on a per surface area basis.

Some of the spores studied have rough surfaces (see Fig. 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 upper limits to the true n_s values. A similar argument can be applied to the mineral dust n_s values shown in Fig. 4, since Niemand et al. (2012) used the volume-equivalent spherical diameter when determining n_s for mineral dust particles.

3.5 *Ascomycota* vs. *Basidiomycota*

The phyla *Basidiomycota* and *Ascomycota* together make up 98 % of known fungal species found on Earth (James et al., 2006). Recent field measurements found increased concentrations of *Ascomycota* spores relative to *Basidiomycota* spores in remote marine regions, and it was suggested (based on preliminary ice nucleation results) that these findings may be explained by *Basidiomycota* spores being better IN compared to *Ascomycota* spores (Fröhlich-Nowoisky et al., 2012). To investigate further whether there is a systematic difference in the ice nucleating ability of the *Ascomycota* and *Basidiomycota* fungi, we have compared the cumulative number of ice nuclei per spore and the ice nucleation active surface site density of *Basidiomycota* and *Ascomycota* fungal spores in Fig. 5 and Fig. 6, respectively. The data are taken from the current study as well as from the following studies: Haga et al. (2013), Iannone et al. (2011), Jayaweera and Flanagan (1982), and Morris et al. (2013b).

To calculate *INperSpore* for *Cladosporium* sp., studied by Iannone et al. (2011), we reanalysed the results from Iannone et al. (2011) using the same procedure as described above (i.e., procedures described in Sects. 2.2 and 2.3). All of the other *INperSpore* values shown in Fig. 5 were provided in the original studies (Haga et al., 2013; Morris et al., 2013b; Jayaweera and Flanagan, 1982). Based on Fig. 5, some of the *Ascomycota* spores (*Penicillium* sp. and *P. brevicompactum*) are the poorest IN, but, on the other hand, some of the spores from this phylum are also the best IN (*P. digitatum*, *P. notatum*, *P. frequentens*, *A. brasiliensis*, and *C. herbarum*). Within both phyla, *Ascomycota* and *Basidiomycota*, there is a wide range of freezing properties. Also, the variation within each phylum is greater than the variation between the average freezing properties of each phylum. Overall, we conclude that *Basidiomycota* spores are not all better IN compared to *Ascomycota* spores. Fig. 5 also shows that the ice nucleating ability of Asian mineral dust particles studied by Niemand et al. (2012) is approximately in the middle of the range observed for *Basidiomycota* and *Ascomycota* fungal spores.

Figure 6 shows previous results of n_s for fungal spores from the phyla *Basidiomycota* and *Ascomycota*. To calculate n_s for the spores studied by Morris et al. (2013b), we approximated the spores as prolate spheroids based on literature values for spore morphology and size ranges (Cummins and Husain, 1966; Bruckart et al., 2007; Anikster et al., 2005; Anikster et al., 2004; Laundon and Waterston, 1964). Specifically, we used dimensions of length and width as follows: 29.0×25.0 µm for *P. aristidae*, 14.6×12.5 µm for *P. lagenophorae*, 25.0×20.6 µm for *Puccinia* sp., 25.0×21.7 µm for *P. allii*, 24.4×19.7 µm for *P. striiformis*, 28.3×17.5 µm for *P. graminis*, 32.0×23.0 µm for *H. vastratrix*, and 23.3×20.4 µm for *P. triticina*. The calculation of n_s for the *Penicillium* spores studied by Jayaweera and Flanagan (1982) was discussed in Sect. 3.4. The other

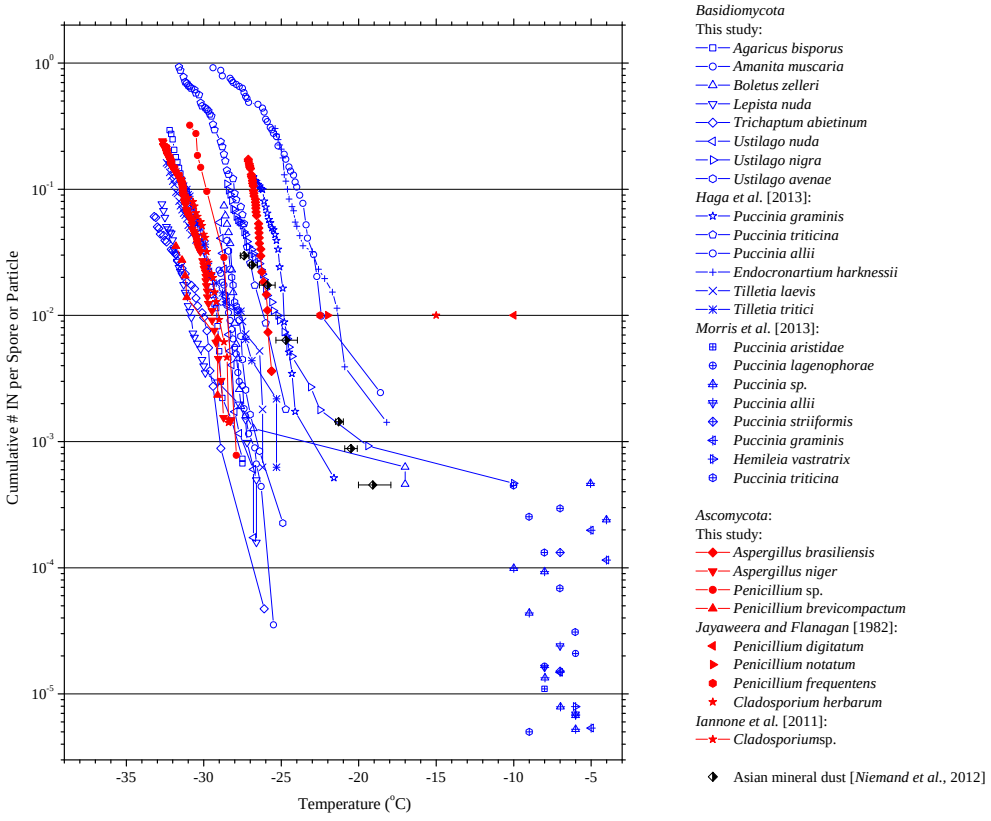

Figure 5. Comparison between the cumulative number of ice nuclei per spore as a function of temperature, *INperSpore(T)*, for *Ascomycota* and *Basidiomycota* spores investigated in this study and earlier studies, including Haga et al. (2013), Iannone et al. (2011), Jayaweera and Flanagan (1982), and Morris et al. (2013b). Also shown are *INperParticle(T)* values for submicron Asian mineral dust (Niemand et al., 2012).

Ascomycota spore studied by Jayaweera and Flanagan (1982) was *Cladosporium herbarum*, and to calculate n_s this spore was approximated as a prolate spheroid with dimensions of $19.5 \times 6.0\,\mu m$ (Schubert et al., 2007). To calculate n_s for *Cladosporium* sp. from Iannone et al. (2011), the spores were approximated as prolate spheroids with dimensions of $4.6 \times 3.2\,\mu m$ (based on microscope images of the spores).

Figure 6 shows that, once normalized to surface area, *Basidiomycota* spores are not all better IN compared to *Ascomycota* spores. Fig. 6 also shows that the variability in freezing properties within both phyla (*Basidiomycota* and *Ascomycota*) cannot be accounted for by a difference in surface area between different spore species. Finally, Fig. 6 suggests that, at temperatures ranging from $-20\,°C$ to $-25\,°C$, all fungal spores for which data are available are poorer immersion IN than Asian mineral dust on a per surface area basis.

3.6 Modeling results

We used the global chemistry–climate transport model, EMAC, to study the effects of ice nucleation on the transport and distribution of fungal spores in the atmosphere. Two scavenging scenarios were compared: in the first scenario (IN-Inactive), ice nucleation scavenging by fungal spores was set to zero for mixed-phase cloud temperatures (i.e., 0 to $-35\,°C$). In the second scenario (IN-Active), ice nucleation scavenging for fungal spores was set to zero for temperatures above $-25\,°C$, but unity for temperatures at or below $-25\,°C$ and above $-35\,°C$ (see Table 2). Results from the simulations are presented in terms of percentage difference in the fungal spore concentration between the two scavenging scenarios (i.e., (IN-Active – IN-Inactive)/IN-Inactive $\times$ 100 %). Positive non-zero values indicate increased concentrations due to scavenging from heterogeneous ice nucleation at temperatures at or below $-25\,°C$ and above $-35°C$, whereas negative non-zero values indicate decreased concentrations due to scavenging from heterogeneous ice nucleation over this temperature range. The results from these simulations are given in Figs. 7 and 8. In both figures, the top and bottom panels correspond to results for the 3 µm and 8 µm particles, respectively. Differences between simulations with 3 µm and 8 µm particles are minor. Figures 9 and 10 display, respectively, the simulated annual surface mean concentrations and the annual zonal mean concentration of spores in the global atmosphere, for all simulation cases (i.e., IN-Inactive and IN-Active). Because emissions are held constant, but removal

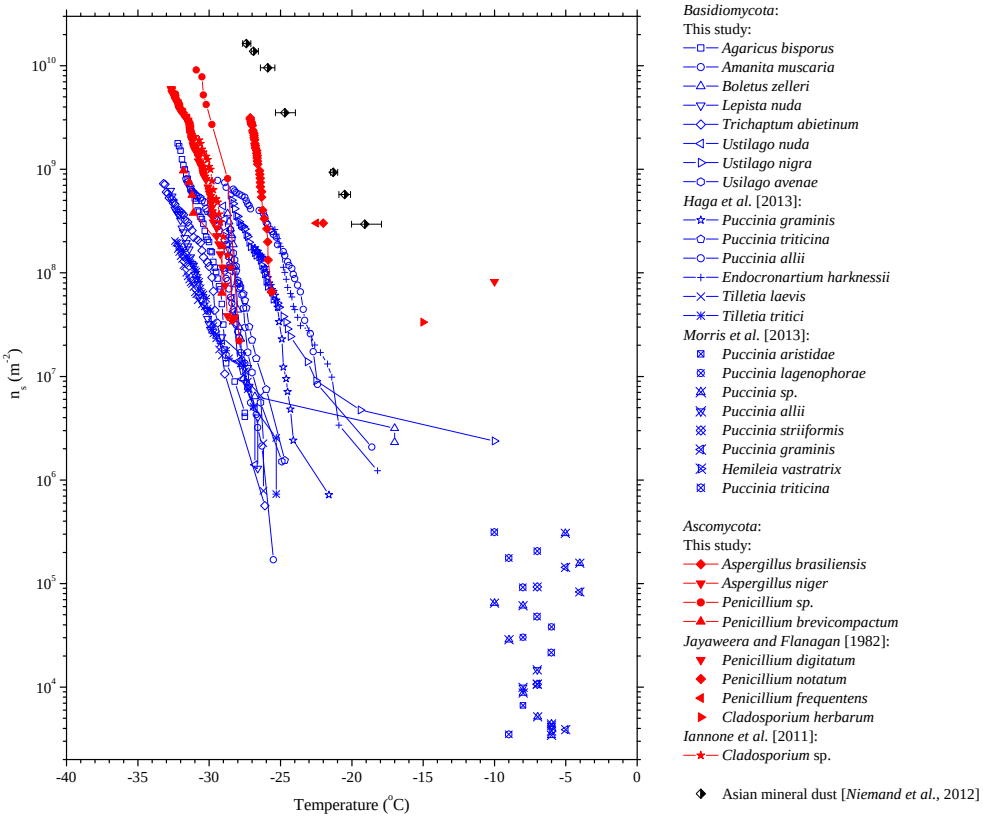

Figure 6. Comparison between ice nucleation active surface site density spectra (n_S vs. T) for *Ascomycota* and *Basidiomycota* fungal spores calculated using the data in Fig. 5 and using approximations for spore size and shape detailed in the text. Included are results from the current study as well as results from the following studies: Haga et al. (2013), Iannone et al. (2011), Jayaweera and Flanagan (1982), and Morris et al. (2013b). Also shown in the figure are previous results for submicron Asian mineral dust (Niemand et al., 2012).

processes are more efficient for 8 µm than for 3 µm particles, the absolute concentrations are higher for the 3 µm particles.

Figure 7 illustrates that the inclusion of ice nucleation scavenging of fungal spores in mixed-phase clouds can decrease the surface annual mean concentrations of fungal spores over the oceans and in polar regions. Since the source of fungal spores in the simulations was land surfaces, excluding land ice, the decrease in the surface concentrations of fungal spores over these regions can be explained by a decrease in the long-distance transport of fungal spores from land surfaces to these remote regions due to ice nucleation followed by precipitation. In stark contrast, scavenging by ice nucleation in mixed-phase clouds increased surface concentrations of fungal spores in the region between roughly 50 and 80° S. This can be explained by increased downward transport of ice-borne spores from mixed-phase clouds at higher altitudes to lower altitudes, where ice evaporates and releases spores in the region between 50 and 80° S. This explanation is supported by results shown in Fig. 8, which shows the percentage change in a zonally averaged vertical profile of the annual mean concentration. This figure illustrates that the inclusion of ice nucleation scavenging in mixed-phase clouds leads to a decrease in mean concentrations in the middle to upper troposphere, particularly at the middle latitudes, where precipitation scavenging in mixed-phase clouds exerts a strong control on aerosol transport to polar regions (Bourgeois and Bey, 2011; Zhang et al., 2012). Below 5 km and in the region between 50 and 80° S, there is an increase in concentrations of fungal spores when ice nucleation in mixed-phase clouds is included. An explanation for this observation is ice nucleation occurring at high altitudes, followed by evaporation of the ice crystals at heights between 0 and 5 km. Since the only change in the simulation 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 simulation. Large relative increases in concentrations are possible because the initial concentrations are extremely low in this remote region (Figs. 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

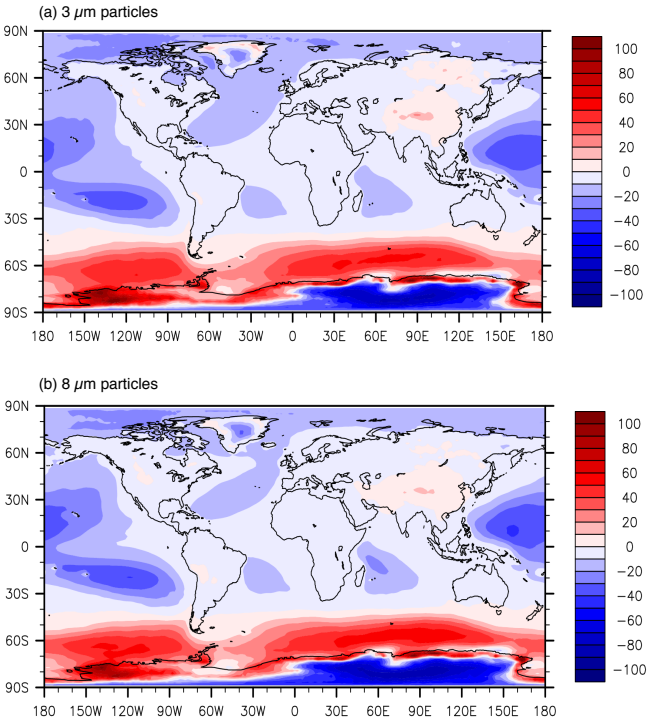

Figure 7. Global model results showing percentage change between IN-Active and IN-Inactive EMAC simulations in surface annual mean concentration, with percentage change being calculated as (IN-Active − IN-Inactive)/IN-Inactive × 100 %. **(a)** 3 µm particles; **(b)** 8 µm particles. In all simulations, spores are assumed to be CCN active. IN-Active assumes that particles are removed by ice-phase nucleation at temperatures at and below −25 °C and above −35 °C. Constant emissions were simulated from land surfaces, except for land covered with ice.

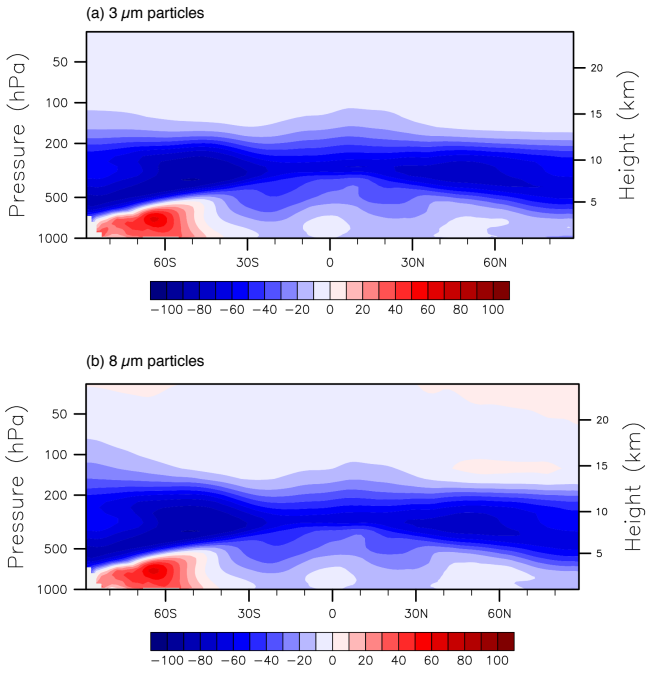

Figure 8. Global model results showing percentage change between IN-Active and IN-Inactive EMAC simulations in zonally averaged vertical profiles of annual mean concentrations, with percentage change being calculated as (IN-Active − IN-Inactive)/IN-Inactive × 100 %. **(a)** 3 µm particles; **(b)** 8 µm particles. In all simulations, spores are assumed to be CCN active. IN-Active assumes that particles are removed by ice-phase nucleation at temperatures at and below −25 °C and above −35 °C. Emissions were simulated from land surfaces, with the exception of land covered with ice.

frequent precipitation, which efficiently scavenges particles within and below precipitating clouds in these regions.

Our results represent an upper-bound estimate of the effect of ice nucleation on the transport of the spores studied, since the freezing efficiency of the studied spores is less than 1 at temperatures between −25 and −30 °C (Fig. 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.

4 Summary and conclusions

4.1 Ice nucleation on fungal spores

To add to the limited number of data on the ice nucleation properties of fungal spores, we studied the ice nucleation properties of 12 different species of fungal spores chosen from three classes: *Agaricomycetes*, *Ustilaginomycetes*, and *Eurotiomycetes*. We focused on these classes because they are thought to be abundant in the atmosphere, and also because there are currently few quantitative data on the ice nucleation ability of spores from these classes.

All of the fungal spores investigated contained some fraction of spores that serve as ice nuclei at temperatures warmer than homogeneous freezing. The cumulative number of ice nuclei per spore was 0.001 at temperatures between −19 °C and −29 °C, 0.01 between −25.5 °C and −31 °C, and 0.1 between −26 °C and −31.5 °C (Fig. 3). On average, the order of ice nucleating ability for these spores was *Ustilaginomycetes* > *Agaricomycetes* ≃ *Eurotiomycetes*. On a per surface area basis, all of the fungal spores studied had similar freezing properties (Fig. 4). In addition, the freezing data suggest that at temperatures ranging from −20 °C to −25 °C,

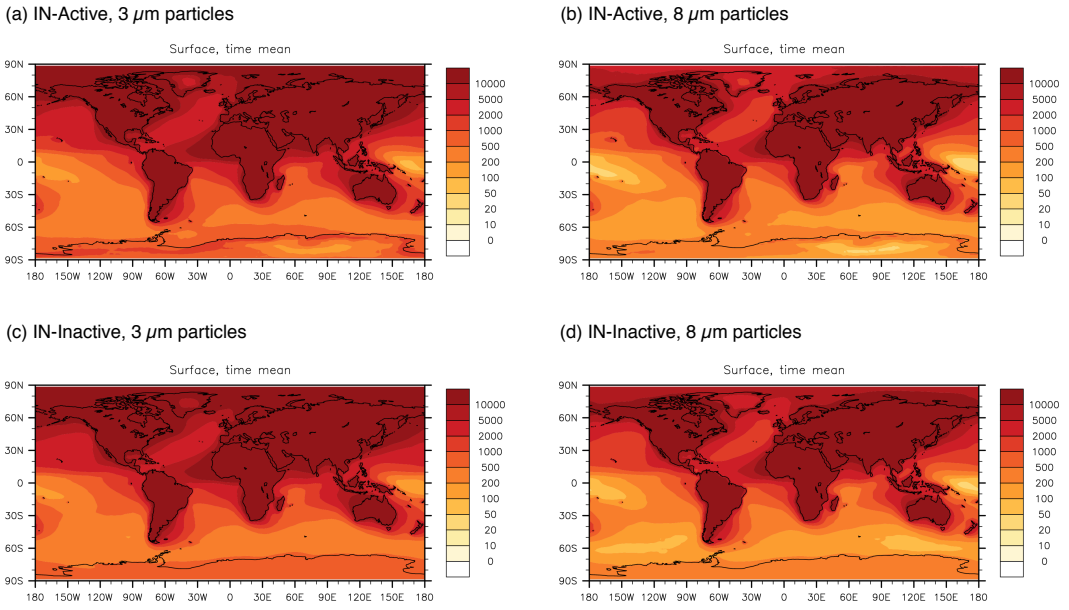

Figure 9. Annual mean spore concentrations (m^{-3}) at the surface in each global model simulation. **(a)** IN-Active, 3 µm particles; **(b)** IN-Active, 8 µm particles; **(c)** IN-Inactive, 3 µm particles; **(d)** IN-Inactive, 8 µm particles.

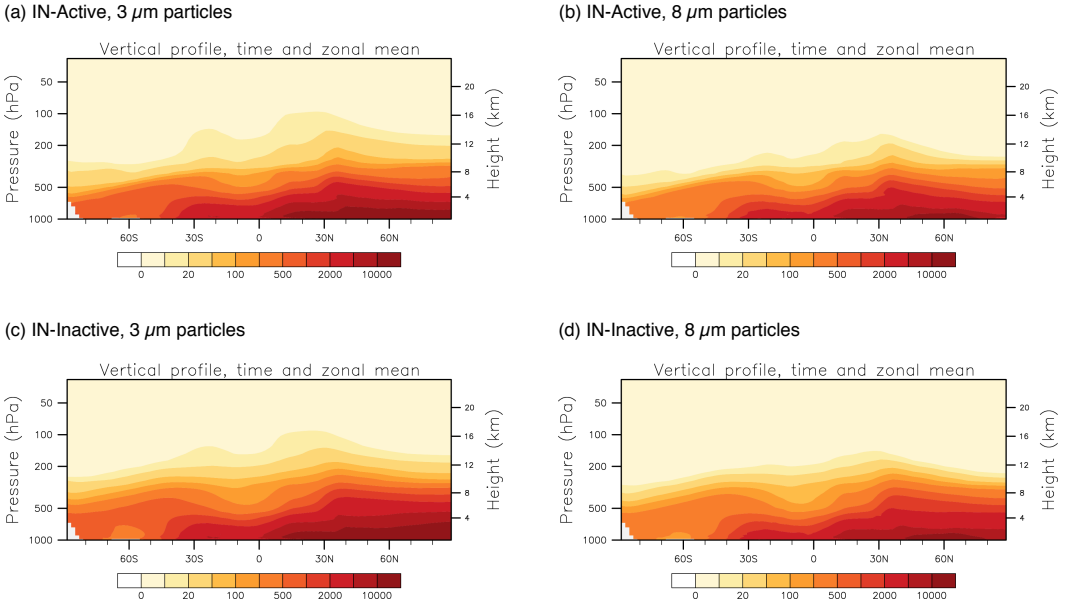

Figure 10. Annual zonal mean spore concentrations (m^{-3}) in each global model simulation. **(a)** IN-Active, 3 µm particles; **(b)** IN-Active, 8 µm particles; **(c)** IN-Inactive, 3 µm particles; and **(d)** IN-Active, 8 µm particles.

all of the fungal spores studied here were less efficient ice nuclei compared to Asian mineral dust on a per surface area basis.

Comparing our results to previous results we show that, within the same genus the freezing temperatures can vary drastically (e.g., the freezing temperature at an *INperSpore* value of 0.01 can vary by approximately 20 °C). We used our freezing results together with data from the literature to compare the freezing temperatures of spores from the phyla *Basidiomycota* and *Ascomycota*, which together make up 98 % of known fungal species found on Earth (James et al., 2006). The data show that within both phyla, *Ascomycota* and *Basidiomycota*, there is a wide range of freezing properties, and also that not all *Basidiomycota* spores are better IN than *Ascomycota* spores.

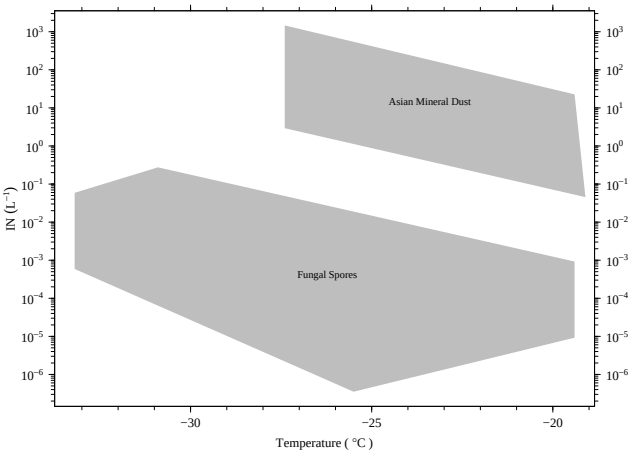

Figure 11. Global annual IN concentrations (L^{-1}) for the fungal spores studied here and for Asian mineral dust (Niemand et al., 2012), estimated using the method of Murray et al. (2012) and Eqs. (4) and (5). The IN concentrations for fungal spores should be considered as upper limits.

4.2 Global transport of fungal spores

Using a global chemistry–climate transport model, we show that ice nucleation on the fungal spores studied may modify their atmospheric transport and global distributions by providing an additional removal mechanism of the spores from the atmosphere. Specifically, we show that the inclusion of ice nucleation scavenging of fungal spores in mixed-phase clouds may (1) decrease the surface annual mean concentrations of fungal spores over the oceans and polar regions, and (2) decrease the annual mean concentrations in the upper troposphere.

To our knowledge, this study is the first to model globally the effect of IN activity on the distribution of fungal spores in the atmosphere and their long-distance transport. While the treatment of ice nucleation scavenging is simplified, this study gives an initial indication of the regions that are most sensitive to removal of spores due to scavenging by ice nucleation, as well as the potential magnitude of these effects. In the real atmosphere, scavenging of fungal spores through their activity as IN may be reduced by competition for freezing with other more effective or more abundant ice-nucleating particles.

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 sizes, 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 activities have interacting effects on aerosol burdens (Burrows et al., 2013). In addition, the freezing results shown here were for immersion freezing only. Experiments that explore ice nucleation by these fungal spores in other freezing modes would be useful, and they would complement the current results.

4.3 Atmospheric implications for the formation and properties of ice and mixed-phase clouds

In order for the fungal spores studied here to influence mixed-phase cloud formation and properties on a global scale, their IN concentrations must be comparable to other important atmospheric IN such as mineral dust. To investigate this further, the global annual mean IN concentration for the fungal spores studied here were calculated using the same method as Haga et al. (2013) for rust and bunt fungal spores, and based on the method of Murray et al. (2012). The formula used to perform this calculation is shown below (Hoose and Möhler, 2012; Vali, 1971):

$$[IN(T)] = \left[Spores\right] \times \{1 - \exp\left(-INperSpore(T)\right)\}, \quad (4)$$

where [*IN(T)*] is the IN concentration and [*Spores*] is the global annual mean concentration of fungal spores. For comparison, a similar calculation was done for the Asian mineral dust studied by Niemand et al. (2012) as follows:

$$[IN(T)] = [Dust] \times \{1 - \exp\left(-INperParticle(T)\right)\}, \quad (5)$$

where [*Dust*] is the global annual mean concentration of mineral dust.

Equations (4) and (5) were evaluated using the *INperSpore* and the *INperParticle* results from Section 3.3 (Fig. 3), and using particle concentrations from the global modeling study of Hoose et al. (2010b) at the 200 and 800 hPa levels. Specifically, [*Spores*] was set equal to 10^{-2} to $10^{0}\,L^{-1}$, and [*Dust*] was set equal to 10^{2} to $10^{4}\,L^{-1}$. The value of [*Spores*] should be considered as an upper limit, since the fungal spores studied here are a subset of all of the species of fungal spores that exist in the atmosphere.

The global annual mean IN concentrations calculated with the assumptions discussed above are shown in Fig. 11. Even using an upper limit for fungal spore concentrations, the global annual mean IN concentrations for the spores studied here are expected to be lower than for Asian mineral dust at temperatures less than −20 °C. This is because mineral dusts have higher atmospheric concentrations compared to fungal spores. These results suggest that the fungal spores studied here are not important IN on global and annual scales at temperatures less than −20 °C, a finding that is consistent with recent modeling studies (Hoose et al., 2010a; Sesartic et al., 2012, 2013).

Fungal spores may be important IN for mixed-phase cloud processes on a local and regional scale, especially during times of high spore emissions and when the concentration of mineral dust is low, and at warmer temperatures where mineral dust may be less important for ice nucleation (Spracklen and Heald, 2013). The results from this study should be

useful in future modeling studies examining the effects of fungal spore ice nucleation on regional mixed-phase cloud processes.

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