https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

Biogeosciences Discussions
Open Access
EGU

Highly active and stable fungal ice nuclei are widespread among *Fusarium* species

Anna T. Kunert [1], Mira L. Pöhlker [1], Carola S. Krevert [1], Carsten Wieder [1], Kai R. Speth [1], Linda E. Hanson [2], Cindy E. Morris [3], David G. Schmale III [4], Ulrich Pöschl [1], and Janine Fröhlich-Nowoisky [1]

[1]Multiphase Chemistry Department, Max Planck Institute for Chemistry, D-55128 Mainz, Germany
[2]USDA-ARS and Department of Plant, Soil and Microbial Science, Michigan State University, East Lansing, MI 48824, USA
[3]Plant Pathology Research Unit, INRA, 84143 Montfavet, France
[4]School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA 24061, USA

Correspondence: Janine Fröhlich-Nowoisky (j.frohlich@mpic.de)

Abstract. Some biological particles and macromolecules are particularly efficient ice nuclei (IN), triggering ice formation at temperatures close to 0 °C. The impact of biological particles on cloud glaciation and the formation of precipitation is still poorly understood and constitutes a large gap in the scientific understanding of the interactions and co-evolution of life and climate. To investigate the frequency and distribution of IN activity within the fungal genus *Fusarium*, more than 100 strains from 65 different *Fusarium* species were screened. In total, ∼11 % of all tested species included ice nucleation-active (IN-active) strains, and ∼16 % of all tested strains showed IN activity above -14 °C. Besides *Fusarium* species with known IN activity, *F. armeniacum*, *F. begoniae*, *F. concentricum*, and *F. langsethiae* were newly identified as IN-active. The cumulative number of IN per gram of mycelium for all tested *Fusarium* species was comparable to other biological IN like *Sarocladium implicatum*, *Mortierella alpina*, and Snomax®. Filtration experiments suggest that the single cell-free *Fusarium* IN is smaller than 100 kDa, and that aggregates can be formed in solution. Long-term storage and freeze-thaw cycle experiments revealed that the *Fusarium* IN remain active in solution for several months and after repeated freezing and thawing. Oxidation and nitration reactions, as occurring during atmospheric aging, did not affect the activity of the *Fusarium* IN. The high frequency of *Fusarium* and the wide distribution of IN activity within the genus, combined with the high stability of the IN, suggest a significant impact of fungal IN on the Earth's water cycle and climate.

1 Introduction

Ice particles in the atmosphere are formed either by homogeneous nucleation at temperatures below -38 °C or by heterogeneous nucleation catalyzed by particles or macromolecules serving as ice nuclei (IN) at warmer temperatures (Pruppacher and Klett, 1997). Biological particles in particular are expected to play an important role as IN in the temperature range from -15 °C to 0 °C, but the impact of biological particles on cloud glaciation and the formation of precipitation is still poorly understood (Coluzza et al., 2017). Several studies suggest a triggering effect of biological IN for cloud glaciation and formation of precipitation (Creamean et al., 2013; DeMott and Prenni, 2010; Pratt et al., 2009), and former studies have shown that

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

biological particles are more efficient than mineral IN (DeMott and Prenni, 2010; Després et al., 2012; Hill et al., 2014; Hoose and Möhler, 2012; Huffman et al., 2013; Möhler et al., 2007; Morris et al., 2014; Murray et al., 2012; Pratt et al., 2009).

The best characterized biological IN are common plant-associated bacteria of the genera *Pseudomonas*, *Pantoea*, and *Xanthomonas* (Garnham et al., 2011; Govindarajan and Lindow, 1988; Graether and Jia, 2001; Green and Warren, 1985; Hill et al., 2014; Kim et al., 1987; Ling et al., 2018; Schmid et al., 1997; Wolber et al., 1986). The first identified ice nucleation-active (IN-active) fungi were strains of the genus *Fusarium* (Hasegawa et al., 1994; Pouleur et al., 1992; Richard et al., 1996; Tsumuki et al., 1992). To date, a few more fungal genera with varying initial freezing temperatures such as *Isaria farinosa* ($\sim$ -4 °C), *Mortierella alpina* ($\sim$ -5 °C), *Puccinia* species (-4 °C to -8 °C), and *Sarocladium* (formerly named *Acremonium*) *implicatum* ($\sim$ -9 °C) have been identified as IN-active (Fröhlich-Nowoisky et al., 2015; Huffman et al., 2013; Morris et al., 2013; Richard et al., 1996).

The genus *Fusarium* is cosmopolitan and includes saprophytes and pathogens of plants and animals (Leslie and Summerell, 2006; Nelson et al., 1994). Although they are considered to be primarily soil-borne fungi, many species of *Fusarium* are airborne (Prussin et al., 2014; Schmale et al., 2012; Schmale and Ross, 2015), and they were found in atmospheric and cloud water samples (e.g., Amato et al., 2007; Fröhlich-Nowoisky et al., 2009; Fulton, 1966). Some species can cause wilts, blights, root rots, and cankers in agriculturally important crops worldwide (e.g., Schmale and Gordon, 2003; Wang and Jeffers, 2000). Other species can produce secondary metabolites known as mycotoxins that can cause a variety of acute and chronic health effects in humans and animals (e.g., Bush et al., 2004; Ichinoe et al., 1983).

Whereas the positive selective pressure for IN activity in *Fusarium* and other fungi has not been directly identified, an ecological advantage of initiating ice formation is easily conceivable. Indeed, most IN-active bacteria and fungi are isolated from regions with seasonal temperatures below 0 °C (Diehl et al., 2002; Schnell and Vali, 1972). Ice nucleation activity at temperatures close to 0 °C could be beneficial for pathogens or might provide an ecological advantage for saprophytic *Fusarium* species by facilitating in the acquisition of nutrients liberated during cell rupture of the host (Lindow et al., 1982). Furthermore, IN on the surface of the mycelium could avoid physical damage of the fungus by protective extracellular freezing (Fröhlich-Nowoisky et al., 2015; Zachariassen and Kristiansen, 2000) or to bind moisture as ice in cold and dry seasons (Pouleur et al., 1992). With increasing temperatures, the retained water can be of advantage in early vegetative periods and for bacterial movement on the mycelial water film known as fungal highway (Kohlmeier et al., 2005; Warmink et al., 2011). Moreover, IN activity might be beneficial for airborne *Fusarium* and for their return to the Earth's surface under advantageous conditions in a feedback cycle known as bioprecipitation (Després et al., 2012; Morris et al., 2013, 2014; Sands et al., 1982). In addition, once the IN are released into the environment, they can adsorb to clay and might also be available in the atmosphere associated with soil dust particles (Conen et al., 2011; Fröhlich-Nowoisky et al., 2015, 2016; Hill et al., 2016; O'Sullivan et al., 2014, 2015, 2016; Sing and Sing, 2010).

The sources, abundance, and identity of biological IN are not well characterized (Coluzza et al., 2017), and it has been proposed that systematic surveys will likely increase the number of IN-active fungal species discovered (Fröhlich-Nowoisky et al., 2015). *Fusarium* is the best-known IN-active fungus, but the frequency and distribution of IN activity within *Fusarium* is not well known. In this study, more than 100 strains from 65 different *Fusarium* species were tested for IN activity in three

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

laboratories with different freezing methods. A high-throughput droplet freezing assay was used to quantify the IN of selected *Fusarium* species, and filtration experiments were performed to estimate the size of the *Fusarium* IN. Furthermore, the stability of *Fusarium* IN upon exposure to ozone and nitrogen dioxide, during freeze-thaw cycles, and after short- and long-term storage under various conditions was investigated.

2 Materials and methods

2.1 Origin and growth conditions of fungal cultures

Thirty *Fusarium* strains from USDA-ARS/Michigan State University (L. Hanson, East Lansing, MI, USA), 13 strains from the Schmale Laboratory at Virginia Tech (D. Schmale, Blacksburg, VA, USA), and 69 strains from the Kansas State University Teaching Collection (J. Leslie, Manhattan, KS, USA) were screened for IN activity (Table S1).

The USDA-ARS/Michigan State University strains were cultivated on dextrose peptone yeast extract agar, containing 10 g L^{-1} dextrose (VWR, Radnor, PA, USA), 3 g L^{-1} peptone (Difco Proteose Peptone No. 3, Becton, Dickinson and Company, Franklin Lakes, NY, USA), and 0.3 g L^{-1} yeast extract (Merck, Kenilworth, NJ, USA), filtered through a 0.2 µm pore diameter filter (PES disposable filter units, Life Science Products, Frederick, CO, USA). After filtration, 12 g L^{-1} agarose (Certified Molecular Biology Agarose, Bio-Rad, Hercules, CA, USA) was added, and the medium was sterilized by autoclaving at 121 °C for 20 min. The colonies were grown at 22 °C to 24 °C for 7 to 19 days. The strains from the Schmale Laboratory at Virginia Tech and the Kansas State University Teaching Collection were maintained in cryogenic storage at -80 °C and were grown on quarter-strength potato dextrose agar (Difco Laboratories, Detroit, USA) on 100 mm Petri plates at ambient room temperature for four days prior to ice nucleation assays.

For quantitative analysis, exposure experiments, freeze-thaw cycles, as well as short- and long-term storage tests a selection of IN-active tested strains was grown on full-strength potato dextrose agar (VWR International GmbH, Darmstadt, Germany) first at room temperature for four to six days and then at 6 °C for about four weeks. For filtration experiments, the fungal cultures were grown at 6 °C for up to six months.

2.2 Preparation and treatments of aqueous extracts

For LED-based Ice Nucleation Detection Apparatus (LINDA) (Stopelli et al., 2014) experiments (see Sect. 2.3), 4 mL of sterile 0.9 % NaCl was added to each of eight petri plates, and the fungal cultures were scraped with the flat end of a sterile bamboo skewer. The resulting suspension of mycelium and spores was filtered through a 100 µm filter (Corning Life Sciences, Reims, France).

For Twin-plate Ice Nucleation Assay (TINA) (Kunert et al., 2018) experiments (see Sect. 2.3) the fungal mycelium was scraped off the agar plate and transferred into a 15 mL tube (Greiner Bio One, Kremsmünster, Austria). The fresh weight of the mycelium was determined gravimetrically. Pure water was prepared as described in Kunert et al. (2018). Aliquots of 10 mL pure water were added before vortexing three times at 2 700 rpm for 30 s (Vortex-Genie 2, Scientific Industries, Inc., Bohemia,

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019


Biogeosciences Discussions Open Access EGU

NY, USA) and centrifugation at 4 500 g for 10 min (Heraeus Megafuge 40, Thermo Scientific, Braunschweig, Germany). For all experiments the aqueous extract was filtered successively through a 5 µm and a 0.1 µm PES syringe filter (Acrodisc®, Sigma-Aldrich, Taufkirchen, Germany), and the aqueous extract contained IN from spores and mycelial surfaces.

For filtration experiments, the 0.1 µm filtrate was further filtered successively through 300 000 MWCO and 100 000 MWCO PES ultrafiltration units (Vivaspin®, Satorius AG, Göttingen, Germany). After each filtration step, the IN concentration was determined using TINA.

For exposure experiments, aqueous extracts of *F. acuminatum* 3-68 and *F. avenaceum* 2-106 were exposed to high concentrations of O_3 and NO_2 as described in Liu et al. (2017). Briefly, a mixture of 1 ppm O_3 and 1 ppm NO_2 was bubbled through 1 mL aliquots of aqueous extract for 4 h, which represents an exposure to an atmospherically relevant amount of approximately 200 ppb of each gas for about 20 h. Afterwards, the IN concentration was determined using TINA.

For freeze-thaw cycles, the IN activity of *F. acuminatum* 3-68 was determined shortly after preparation of the aqueous extract and after storage at 6 °C for 24 h using TINA. Then, the aqueous extract was stored at -20 °C for 24 h and thawed again. The IN activity was tested before storage at -20 °C for an additional 24 h. After thawing, the IN activity was determined again.

For long-term storage experiments, the aqueous extract of various *Fusarium* species was stored at 6 °C for about four months or at -20 °C for about eight months, and the IN activity was determined using TINA.

2.3 Ice nucleation assays

Two independent droplet freezing assays conducted in two laboratories were used to investigate the distribution of IN activity within *Fusarium* in an initial screening.

First, a thermal cycler (PTC200, MJ Research, Hercules, CA, USA) was used as described in Fröhlich-Nowoisky et al. (2015) to screen 30 *Fusarium* strains from seven species from USDA-ARS/Michigan State University in the temperature range from -2 °C to -9 °C. Mycelium was picked with sterile pipette tips (Eppendorf, Westbury, NY, USA) into 80 µL aliquots of 0.2 µm pore diameter filtered dextrose peptone yeast extract broth in sterile 96-well polypropylene PCR plates (VWR International, LLC, Radnor, PA, USA).

Second, the LED-based Ice Nucleation Detection Apparatus (LINDA) was used as described by Stopelli et al. (2014) to screen 13 strains from the Schmale Laboratory at Virginia Tech and 69 strains from the Kansas State University Teaching Collection. Aliquots of 200 µL of each aqueous extract were transferred to three separate 500 µL tubes and placed on ice for 1 h prior to the LINDA experiments. LINDA was run from -1 °C to -14 °C, and images of the samples were recorded every six seconds. As positive control, aqueous suspensions of *Pseudomonas syringae* CC94 from the collection of INRA (Avignon, France) (Berge et al., 2014) (with a final OD_{580} of 0.5 to 0.7, i.e. $\sim 10^9$ bacteria mL^{-1}) were used for each experiment. The bacteria were grown on King's medium B (King et al., 1954) at 22 °C to 25 °C for 48 h, and aqueous suspensions were incubated at 4 °C for 1 h to 4 h before LINDA experiments. The aqueous extract was prepared in 0.9 % NaCl solution, which reduced the freezing temperatures about 0.5 °C based on theoretical calculations.

Ice nuclei of selected *Fusarium* species were further analyzed using the high-throughput Twin-plate Ice Nucleation Assay (TINA) (Kunert et al., 2018). The aqueous extract was serially diluted 10-fold with pure water by a liquid handling station

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

(epMotion ep5073, Eppendorf, Hamburg, Germany), and 96 droplets (3 µL) were tested per dilution with a continuous cooling rate of 1 °C min^{-1} from 0 °C to -20 °C. The temperature was measured with an accuracy of 0.2 K (Kunert et al., 2018). The obtained fraction of frozen droplets (f_{ice}) and the counting error were used to calculate the cumulative number of IN (N_m) with the associated error using the Vali formula and the Gaussian error propagation (Kunert et al., 2018; Vali, 1971). For each experiment, the cumulative number of IN was averaged over all dilutions. If the experiment was repeated, the cumulative number of IN was averaged over all experiments, and the standard error was calculated. Three independent experiments with aqueous extract from three individual fungal culture plates of the same strain showed similar results with only slight variation. An example of results is presented for *F. armeniacum* 20970 (Fig. S1).

3 Results and discussion

3.1 IN-active *Fusarium* species

Although several IN-active *Fusarium* species are known, the frequency and distribution of IN activity within the fungal genus *Fusarium* is still not well studied (Hasegawa et al., 1994; Humphreys et al., 2001; Pouleur et al., 1992; Richard et al., 1996; Tsumuki and Konno, 1994). Two initial screenings in the temperature range from -1 °C to -14 °C were performed to better evaluate the frequency of IN activity within *Fusarium*.

In total, ∼ 16 % (18/112) of the tested strains showed IN activity with initial freezing temperatures of -3.5 °C to -11.2 °C (Table 1) in the typical range known for *Fusarium* (-1 °C and -9 °C) (Hasegawa et al., 1994; Humphreys et al., 2001; Pouleur et al., 1992; Richard et al., 1996; Tsumuki et al., 1992; Tsumuki and Konno, 1994). Most formerly reported initial freezing temperatures were obtained with different *Fusarium* strains, growth conditions, and freezing assays, which might explain differences compared to our results. The high proportion of IN-active strains within *F. acuminatum* is consistent with previous reports (Pouleur et al., 1992; Tsumuki et al., 1995). Overall, ∼ 11 % (7/65) of the tested species included IN-active strains. In addition to strains from *Fusarium* species with known IN activity, four *Fusarium* species were newly identified as IN-active: *F. armeniacum*, *F. begoniae*, *F. concentricum*, and *F. langsethiae*. In further experiments, the IN activity of *F. begoniae* and *F. concentricum* could not be verified.

The newly identified IN-active species are cosmopolitan. *Fusarium armeniacum* is a toxigenic saprophyte (Burgess et al., 1993) causing seed and root rot on soybeans (Ellis et al., 2012). The geographical distribution has been reported as tropical and subtropical (Leslie and Summerell, 2006), but it was also found in Minnesota, USA (Kommedahl et al., 1979) and Australia (Burgess et al., 1993). *Fusarium begoniae* is a plant pathogen of Begonia found in Germany with a potential wider distribution (Nirenberg and O'Donnell, 1998). *Fusarium concentricum* is a plant pathogen, frequently found in Central America and isolated from bananas (Aoki et al., 2001; Leslie and Summerell, 2006), and *F. langsethiae* is a broadly distributed cereal pathogen (Torp and Nirenberg, 2004). Some strains of these newly identified IN-active species are known to produce mycotoxins, which can threaten the health of humans and animals (Fotso et al., 2012; Kokkonen et al., 2012; Wing et al., 1993a, b).

The results suggest that the IN activity within *Fusarium* is more widespread than previously known. Not all *Fusarium* species include IN-active strains and not all strains within one species show IN activity. *Fusarium* IN are thought to be proteins or at

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

least to contain a proteinaceous compound (Hasegawa et al., 1994; Pouleur et al., 1992). Their production requires energy, and we might assume that this trait would not be expressed or maintained unless there was an ecological advantage. It is known that *Fusarium* can regulate the gene expression for IN production depending on environmental conditions such as nutrient availability (Richard et al., 1996), and some *Fusarium* species reduce or lose their IN activity after several subcultures (Pummer et al., 2013; Tsumuki et al., 1995). Thus, we cannot exclude that all *Fusarium* strains have the ability to produce IN. From the phylogenetic distribution of IN activity across the genus *Fusarium*, we can speculate that IN activity is a very old trait, but either the gene expression requires a trigger, which is not yet identified, or the trait might be in the process of being lost. It is unlikely, however, that the age of the genetic determinants of fungal IN activity is older than that in bacteria, since fungi diverged well after the age that has been attributed to the bacterial IN gene (Morris et al., 2014), and the genetic determinants are not the same as those in bacteria.

3.2 Quantification and size determination of IN from selected *Fusarium* species

A selection of IN-active *Fusarium* species was further investigated by extensive droplet freezing assay analysis using TINA. All tested *Fusarium* strains initiated ice nucleation between -3 °C and -4 °C (Fig. 1). Differences in the initial freezing temperature between the initial screening and the quantitative analysis can be due to different growth conditions and freezing assays. The cumulative number of IN (N_m) per gram of mycelium was in the range between 10^8 g^{-1} and 10^{13} g^{-1}. *Fusarium acuminatum* 3-68 showed the highest IN activity and *F. langsethiae* the lowest per gram of mycelium. The results are comparable to other IN-active microorganisms like *Sarocladium implicatum* (10^8 g^{-1}, Pummer et al., 2015, *Mortierella alpina* (10^9 g^{-1}, Fröhlich-Nowoisky et al., 2015; 10^{10} g^{-1}, Kunert et al., 2018), and the bacterial IN-active substance Snomax® containing *Pseudomonas syringae* (10^{12} g^{-1}, Budke and Koop, 2015; Kunert et al., 2018).

The size of the *Fusarium* IN was investigated by filtration experiments. Filtration through a 5 µm and a 0.1 µm filter did not affect the IN activity (Fig. 2), revealing that *Fusarium* IN are cell-free, easily removed from the fungus, and stay active in solution. This is consistent with previous studies (O'Sullivan et al., 2015; Pouleur et al., 1992; Tsumuki and Konno, 1994). Moreover, the IN are smaller than 100 nm for all tested *Fusarium* strains. Filtration through a 300 000 MWCO filter unit decreased the cumulative number of IN per gram of mycelium about 50 % to 75 % depending on the *Fusarium* species, but a tremendous number of IN (10^{10} - 10^{13} g^{-1}) still passed through the filter. The initial freezing temperature was slightly shifted towards lower temperatures. Further filtration through a 100 000 MWCO filter unit reduced the IN number to 10^8 - 10^{10} g^{-1}, which is less than 1 % of the initial IN concentration. Additionally, the initial freezing temperature was shifted about one degree towards lower temperatures.

As IN activity was found in all filtrates, the aqueous extract of *Fusarium* consists of a mixture of IN-active proteins with different sizes. We hypothesize that *Fusarium* IN are single proteins smaller than 100 kDa, which agglomerate to large protein complexes in solution. Some of these complexes fall apart upon filtration, so that the single IN proteins can pass through the filter. The small shift in the initial freezing temperature suggests that these proteins reassemble again to aggregates after filtration, as larger IN nucleate at warmer temperatures (Govindarajan and Lindow, 1988; Pummer et al., 2015). Assuming the protein as smooth spherical particle, the minimum diameter of the single IN protein would be smaller than 6.1 nm according

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

to Erickson (2009). Our results are in accordance with Lagzian et al. (2014), who cloned and expressed a 49 kDa IN-active protein from *F. acuminatum*.

As *Fusarium* IN are cell-free and can easily be washed off the fungal surface, they can be released in high numbers into the environment. If they are not degraded by microorganisms before, the IN can adsorb to soil dust and be aerosolized attached to these particles (Conen et al., 2011; Fröhlich-Nowoisky et al., 2015; Hill et al., 2016; O'Sullivan et al., 2014, 2015, 2016; Sing and Sing, 2010). This is in good agreement with Pruppacher and Klett (1997), who found a positive correlation between IN number concentration and particles in the coarse mode. Other releasing processes cannot be excluded, however, it is unlikely that the single proteins are present in the atmosphere as individual aerosol particles.

3.3 Stability of *Fusarium* IN

In the atmosphere, IN can interact with other aerosol particles or gases. They can be exposed to chemically modifying agents like ozone and nitrogen dioxide, and physical stressors like low temperatures and quickly changing temperatures. To investigate the stability of *Fusarium* IN, we performed exposure experiments, freeze-thaw cycles, and long-term storage tests.

The influence of chemical processing on the *Fusarium* IN, in particular oxidation and nitration reactions as occurring during atmospheric aging, was investigated by exposing aqueous extracts from *F. acuminatum* 3-68 and *F. avenaceum* 2-106 to high concentrations of ozone and nitrogen dioxide in liquid phase. Figure 3 shows that for both species neither the initial freezing temperature nor the cumulative number of IN per gram of mycelium was affected by exposure. These results demonstrate a high stability of *Fusarium* IN under oxidizing and nitrating conditions. This is in contrast to bacterial IN (Snomax®), which were reduced upon exposure (Kunert et al., 2018).

To study the effects of short-term storage and freeze-thaw cycles on the IN activity of *F. acuminatum* 3-68, IN measurements of the same aqueous extract were performed at different time points (Fig. 4). The results of freshly prepared aqueous extract revealed that the highest activity of fungal IN was already developed during preparation of the filtrate and no time for equilibration was required. Storage of aqueous extract at 6 °C for 24 h did not affect the IN activity. Also, further storage at -20 °C for another 24 h, and repeated freeze-thaw cycles had no impact on the IN activity. This means, that, once in the atmosphere, the IN can undergo several freeze-thaw cycles without losing their activity and are still able to influence cloud glaciation and the formation of precipitation. This could be an explanation why not all fungi are always IN-active as their IN are highly stable and quasi recyclable. Ice nuclei might influence the availability of moisture over long times periods, and if enough moisture is available in the environment, the necessity of IN production would be omitted and the fungus could safe energy.

In addition, the stability of *Fusarium* IN was studied in long-term storage tests, where aqueous extract of various *Fusarium* species was stored at different temperatures for a long period of time. Figure 5 shows that storage at 6 °C for four months and -20 °C for eight months, respectively, did not influence the IN activity of *F. armeniacum* 20970, *F. acuminatum* 1-4, *F. avenaceum* 2-106, and *F. acuminatum* 2-38. The results demonstrate the high stability of *Fusarium* IN in liquid and frozen solutions over long time periods, which makes *Fusarium* well applicable for laboratory IN studies. Moreover, the high stability is likely an advantage for these fungi to be linked to atmospheric processes.

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

4 Conclusions

The frequency and distribution of IN activity within the fungal genus *Fusarium* was investigated in a screening of more than 100 strains from 65 different *Fusarium* species. In total, ∼ 11 % (7/65) of all tested species included IN-active strains, and ∼ 16 % (18/112) of all tested strains showed IN activity, demonstrating the wide distribution of IN activity within *Fusarium*. Filtration experiments suggest that *Fusarium* IN form aggregates consisting of single IN smaller than 100 kDa (∼ 6 nm). Exposure experiments, freeze-thaw cycles, and long-term storage tests revealed a high stability of *Fusarium* IN, demonstrating the suitability of *Fusarium* in laboratory IN studies. The wide distribution of IN activity within the genus *Fusarium* together with the high stability of the *Fusarium* IN under atmospherically relevant conditions, suggest that the impact of these IN on the Earth's water cycle and climate might be more significant than previously assumed. Additional research is necessary to characterize the *Fusarium* IN and processes, which can result in their agglomeration to larger protein complexes. To evaluate the impact of these IN on the Earth's climate, additional work is required to study the abundance of *Fusarium* IN in environmental samples on a global scale.

Data availability. All data are available from the corresponding authors upon request.

Author contributions. C.E.M., J.F.-N., U.P. designed the experiments. D.G.S. III and L.E.H. provided fungal cultures. C.E.M., D.G.S. III, and J.F.-N. performed the initial screenings. A.T.K., C.S.K., C.W., and K.R.S. performed the experiments. A.T.K., J.F.-N., M.L.P., and U.P. discussed the results. A.T.K. and J.F.-N. wrote the manuscript with contributions of all co-authors.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements. We thank C. Bartoli, J.-D. Förster, T. Godwill, N.-M. Kropf, and E. Stopelli for technical assistance, G. D. Franc, T. C. J. Hill, B. Sánchez-Parra, J. F. Scheel, and M. G. Weller for helpful discussions, and the Max Planck Society (MPG), the Deutsche Forschungsgemeinschaft (DFG, FR3641/1-2, FOR 1525 INUIT) for financial support. This work is dedicated to the memory of Gary D. Franc, whose pioneering work in atmospheric microbiology has been an inspiration for this work.

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

References

Amato, P., Parazols, M., Sancelme, M., Laj, P., Mailhot, G., and Delort, A. M.: Microorganisms isolated from the water phase of tropospheric clouds at the Puy de Dôme: Major groups and growth abilities at low temperatures, FEMS Microbiology Ecology, 59, 242–254, 2007.

Aoki, T., O'Donnell, K., and Ichikawa, K.: *Fusarium fractiflexum* sp. nov. and two other species within the *Gibberella fujikuroi* species complex recently discovered in Japan that form aerial conidia in false heads, Mycoscience, 42, 461–478, 2001.

Berge, O., Monteil, C. L., Bartoli, C., Chandeysson, C., Guilbaud, C., Sands, D. C., and Morris, C. E.: A User's Guide to a Data Base of the Diversity of *Pseudomonas syringae* and Its Application to Classifying Strains in This Phylogenetic Complex, PLoS ONE, 9, e105 547, 2014.

Budke, C. and Koop, T.: BINARY: an optical freezing array for assessing temperature and time dependence of heterogeneous ice nucleation, Atmospheric Measurement Techniques, 8, 689–703, 2015.

Burgess, L. W., Forbes, G. A., Windels, C., Nelson, P. E., Marasas, W. F. O., and Gott, K. P.: Characterization and distribution of *Fusarium acuminatum* subsp. *armeniacum* subsp. nov., Mycologia, 1993.

Bush, B. J., Carson, M. L., Cubeta, M. A., Hagler, W. M., and Payne, G. A.: Infection and Fumonisin Production by *Fusarium verticillioides* in Developing Maize Kernels, Phytopathology, 94, 88–93, 2004.

Coluzza, I., Creamean, J., Rossi, M. J., Wex, H., Alpert, P. A., Bianco, V., Boose, Y., Dellago, C., Felgitsch, L., Fröhlich-Nowoisky, J., Herrmann, H., Jungblut, S., Kanji, Z. A., Menzl, G., Moffett, B., Moritz, C., Mutzel, A., Pöschl, U., Schauperl, M., Scheel, J., Stopelli, E., Stratmann, F., Grothe, H., and Schmale III, D. G.: Perspectives on the Future of Ice Nucleation Research: Research Needs and Unanswered Questions Identified from Two International Workshops, Atmosphere, 8, 138, 2017.

Conen, F., Morris, C. E., Leifeld, J., Yakutin, M. V., and Alewell, C.: Biological residues define the ice nucleation properties of soil dust, Atmospheric Chemistry and Physics, 11, 9643–9648, 2011.

Creamean, J. M., Suski, K. J., Rosenfeld, D., Cazorla, A., DeMott, P. J., Sullivan, R. C., White, A. B., Ralph, F. M., Minnis, P., Comstock, J. M., Tomlinson, J. M., and Prather, K. A.: Dust and Biological Aerosols from the Sahara and Asia Influence Precipitation in the Western U.S., Science, 339, 1572–1578, 2013.

DeMott, P. J. and Prenni, A. J.: New Directions: Need for defining the numbers and sources of biological aerosols acting as ice nuclei, Atmospheric Environment, 44, 1944–1945, 2010.

Després, V. R., Huffman, J. A., Burrows, S. M., Hoose, C., Safatov, A. S., Buryak, G., Fröhlich-Nowoisky, J., Elbert, W., Andreae, M. O., Pöschl, U., and Jaenicke, R.: Primary biological aerosol particles in the atmosphere: a review, Tellus B: Chemical and Physical Meteorology, 64, 15 598, 2012.

Diehl, K., Matthias-Maser, S., Jaenicke, R., and Mitra, S. K.: The ice nucleating ability of pollen: Part II. Laboratory studies in immersion and contact freezing modes, Atmospheric Research, 61, 125–133, 2002.

Ellis, M. L., Diaz Arias, M. M., Leandro, L. F., and Mungvold, G. P.: First report of Fusarium armeniacum causing seed rot and root rot on soybean (Glycine max) in the United States, Plant Disease, 2012.

Erickson, H. P.: Size and Shape of Protein Molecules at the Nanometer Level Determined by Sedimentation, Gel Filtration, and Electron Microscopy, Biological Procedures Online, 11, 32–51, 2009.

Fotso, J., Leslie, J. F., and Smith, J. S.: Production of Beauvericin, Moniliformin, Fusaproliferin, and Fumonisins B_1, B_2, and B_3 by Fifteen Ex-Type Strains of *Fusarium* Species, Applied and Environmental Microbiology, 68, 5195–5197, 2012.

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

Fröhlich-Nowoisky, J., Pickersgill, D. a., Després, V. R., and Pöschl, U.: High diversity of fungi in air particulate matter, Proceedings of the National Academy of Sciences of the United States of America, 106, 12 814–12 819, 2009.

Fröhlich-Nowoisky, J., Hill, T. C. J., Pummer, B. G., Yordanova, P., Franc, G. D., and Pöschl, U.: Ice nucleation activity in the widespread soil fungus *Mortierella alpina*, Biogeosciences, 12, 1057–1071, 2015.

Fröhlich-Nowoisky, J., Kampf, C. J., Weber, B., Huffman, J. A., Pöhlker, C., Andreae, M. O., Lang-Yona, N., Burrows, S. M., Gunthe, S. S., Elbert, W., Su, H., Hoor, P., Thines, E., Hoffmann, T., Després, V. R., and Pöschl, U.: Bioaerosols in the Earth system: Climate, health, and ecosystem interactions, Atmospheric Research, 182, 346–376, 2016.

Fulton, J. D.: Microorganisms of the Upper Atmosphere: IV. Microorganisms of a Land Air Mass as it Traverses an Ocean, Applied and Envrionmental Microbiology, 1966.

Garnham, C. P., Campbell, R. L., Walker, V. K., and Davies, P. L.: Novel dimeric β-helical model of an ice nucleation protein with bridged active sites, BMC structural biology, 11, 36, 2011.

Govindarajan, A. G. and Lindow, S. E.: Size of bacterial ice-nucleation sites measured *in situ* by radiation inactivation analysis, Proceedings of the National Academy of Sciences of the United States of America, 85, 1334–1338, 1988.

Graether, S. P. and Jia, Z.: Modeling *Pseudomonas syringae* Ice-Nucleation Protein as a β-Helical Protein, Biophysical journal, 80, 1169–1173, 2001.

Green, R. L. and Warren, G. J.: Physical and functional repetition in a bacterial ice nucleation gene, Nature, 317, 645–648, 1985.

Hasegawa, Y., Ishihara, Y., and Tokuyama, T.: Characteristics of Ice-nucleation Activity in *Fusarium avenaceum* IFO 7158, Bioscience, biotechnology, and biochemistry, 58, 2273–2274, 1994.

Hill, T. C. J., Moffett, B. F., DeMott, P. J., Georgakopoulos, D. G., Stump, W. L., and Franc, G. D.: Measurement of Ice Nucleation-Active Bacteria on Plants and in Precipitation by Quantitative PCR, Applied and Environmental Microbiology, 80, 1256–1267, 2014.

Hill, T. C. J., DeMott, P. J., Tobo, Y., Fröhlich-Nowoisky, J., Moffett, B. F., Franc, G. D., and Kreidenweis, S. M.: Sources of organic ice nucleating particles in soils, Atmospheric Chemistry and Physics, 16, 7195–7211, 2016.

Hoose, C. and Möhler, O.: Heterogeneous ice nucleation on atmospheric aerosols: a review of results from laboratory experiments, Atmospheric Chemistry and Physics, 12, 9817–9854, 2012.

Huffman, J. A., Prenni, A. J., DeMott, P. J., Pöhlker, C., Mason, R. H., Robinson, N. H., Fröhlich-Nowoisky, J., Tobo, Y., Després, V. R., Garcia, E., Gochis, D. J., Harris, E., Müller-Germann, I., Ruzene, C., Schmer, B., Sinha, B., Day, D. A., Andreae, M. O., Jimenez, J. L., Gallagher, M., Kreidenweis, S. M., Bertram, A. K., and Pöschl, U.: High concentrations of biological aerosol particles and ice nuclei during and after rain, Atmospheric Chemistry and Physics, 13, 6151–6164, 2013.

Humphreys, T. L., Castrillo, L. a., and Lee, M. R.: Sensitivity of Partially Purified Ice Nucleation Activity of *Fusarium acuminatum* SRSF 616, Current Microbiology, 42, 330–338, 2001.

Ichinoe, M., Kurata, H., Sugiura, Y., and Ueno, Y.: Chemotaxonomy of *Gibberella zeae* with Special Reference to Production of Trichothecenes and Zearalenone., Applied and Environmental Microbiology, 1983.

Kim, H. K., Orser, C., Lindow, S. E., and Sands, D. C.: *Xanthomonas campestris* pv. *translucens* Strains Active in Ice Nucleation, Plant Disease, 71, 994–996, 1987.

King, E. O., Ward, M. K., and Raney, D. E.: Two simple media for the demonstration of pyocyanin and fluorescin, Translational Research, 1954.

Kohlmeier, S., Smits, T. H. M., Ford, R. M., Keel, C., Harms, H., and Wick, L. Y.: Taking the Fungal Highway: Mobilization of Pollutant-Degrading Bacteria by Fungi, Environmental Science and Technology, 39, 4640–4646, 2005.

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

Kokkonen, M., Jestoi, M., and Laitila, A.: Mycotoxin production of *Fusarium langsethiae* and *Fusarium sporotrichioides* on cereal-based substrates, Mycotoxin Research, 28, 25–35, 2012.

Kommedahl, T., Windels, C. E., and Stucker, R. E.: Occurrence of *Fusarium* Species in Roots and Stalks of Symptomless Corn Plants During the Growing Season, Phytopathology, 1979.

Kunert, A. T., Lamneck, M., Helleis, F., Pöhlker, M. L., Pöschl, U., and Fröhlich-Nowoisky, J.: Twin-plate ice nucleation assay (TINA) with infrared detection for high-throughput droplet freezing experiments with biological ice nuclei in laboratory and field samples, Atmospheric Measurement Techniques, 11, 6327–6337, 2018.

Lagzian, M., Latifi, A. M., Bassami, M. R., and Mirzaei, M.: An ice nucleation protein from *Fusarium acuminatum*: cloning, expression, biochemical characterization and computational modeling, Biotechnology Letters, 36, 2043–2051, 2014.

Leslie, J. F. and Summerell, B. A.: The Fusarium Laboratory Manual, 2006.

Lindow, S. E., Hirano, S. S., Barchet, W. R., Arny, D. C., and Upper, C. D.: Relationship between Ice Nucleation Frequency of Bacteria and Frost Injury, Plant Physiology, 1982.

Ling, M. L., Wex, H., Grawe, S., Jakobsson, J., Löndahl, J., Hartmann, S., Finster, K., Boesen, T., and Šantl-Temkiv, T.: Effects of Ice Nucleation Protein Repeat Number and Oligomerization Level on Ice Nucleation Activity, Journal of Geophysical Research: Atmospheres, 123, 1802–1810, 2018.

Liu, F., Lakey, P. S. J., Berkemeier, T., Tong, H., Kunert, A. T., Meusel, H., Cheng, Y., Su, H., Fröhlich-Nowoisky, J., Lai, S., Weller, M. G., Shiraiwa, M., Pöschl, U., and Kampf, C. J.: Atmospheric protein chemistry influenced by anthropogenic air pollutants: nitration and oligomerization upon exposure to ozone and nitrogen dioxide, Faraday Discussions, 200, 413–427, 2017.

Möhler, O., DeMott, P. J., Vali, G., and Levin, Z.: Microbiology and atmospheric processes: the role of biological particles in cloud physics, Biogeosciences, 4, 2559–2591, 2007.

Morris, C. E., Sands, D. C., Glaux, C., Samsatly, J., Asaad, S., Moukahel, A. R., Gonçalves, F. L. T., and Bigg, E. K.: Urediospores of rust fungi are ice nucleation active at $>-10\,^{\circ}\mathrm{C}$ and harbor ice nucleation active bacteria, Atmospheric Chemistry and Physics, 13, 4223–4233, 2013.

Morris, C. E., Conen, F., Alex Huffman, J., Phillips, V., Pöschl, U., and Sands, D. C.: Bioprecipitation: a feedback cycle linking Earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere, Global Change Biology, 20, 341–351, 2014.

Murray, B. J., O'Sullivan, D., Atkinson, J. D., and Webb, M. E.: Ice nucleation by particles immersed in supercooled cloud droplets, Chemical Society Reviews, 41, 6519, 2012.

Nelson, P. E., Dignani, M. C., and Anaissie, E. J.: Taxonomy, biology, and clinical aspects of *Fusarium* species, Clinical Microbiology Reviews, 7, 479–504, 1994.

Nirenberg, H. I. and O'Donnell, K.: New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex, Mycologia, 1998.

O'Sullivan, D., Murray, B. J., Malkin, T. L., Whale, T. F., Umo, N. S., Atkinson, J. D., Price, H. C., Baustian, K. J., Browse, J., and Webb, M. E.: Ice nucleation by fertile soil dusts: relative importance of mineral and biogenic components, Atmospheric Chemistry and Physics, 14, 1853–1867, 2014.

O'Sullivan, D., Murray, B. J., Ross, J. F., Whale, T. F., Price, H. C., Atkinson, J. D., Umo, N. S., and Webb, M. E.: The relevance of nanoscale biological fragments for ice nucleation in clouds., Scientific Reports, 5, 8082, 2015.

O'Sullivan, D., Murray, B. J., Ross, J. F., and Webb, M. E.: The adsorption of fungal ice-nucleating proteins on mineral dusts: a terrestrial reservoir of atmospheric ice-nucleating particles, Atmospheric Chemistry and Physics, 16, 7879–7887, 2016.

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

Pouleur, S., Richard, C., Martin, J.-G., and Antoun, H.: Ice Nucleation Activity in *Fusarium acuminatum* and *Fusarium avenaceum*, Applied and Environmental Microbiology, 1992.

Pratt, K. A., DeMott, P. J., French, J. R., Wang, Z., Westphal, D. L., Heymsfield, A. J., Twohy, C. H., Prenni, A. J., and Prather, K. A.: *In situ* detection of biological particles in cloud ice-crystals, Nature Geoscience, 2, 398–401, 2009.

Pruppacher, H. R. and Klett, J. D.: Microphysics of Clouds and Precipitation, Springer Netherlands, Dordrecht, 2 edn., 1997.

Prussin, A. J., Li, Q., Malla, R., Ross, S. D., and Schmale, D. G.: Monitoring the Long-Distance Transport of *Fusarium graminearum* from Field-Scale Sources of Inoculum, Plant Disease, 98, 504–511, 2014.

Pummer, B. G., Atanasova, L., Bauer, H., Bernardi, J., Druzhinina, I. S., Fröhlich-Nowoisky, J., and Grothe, H.: Spores of many common airborne fungi reveal no ice nucleation activity in oil immersion freezing experiments, Biogeosciences, 10, 8083–8091, 2013.

Pummer, B. G., Budke, C., Niedermeier, D., Felgitsch, L., Kampf, C. J., Huber, R. G., Liedl, K. R., Loerting, T., Moschen, T., Schauperl, M., Tollinger, M., Morris, C. E., Wex, H., Grothe, H., Pöschl, U., Koop, T., and Fröhlich-Nowoisky, J.: Ice nucleation by water-soluble macromolecules, Atmospheric Chemistry and Physics, 15, 4077–4091, 2015.

Richard, C., Martin, J. G., and Pouleur, S.: Ice nucleation activity identified in some phytopathogenic Fusarium species, Phytoprotection, 77, 83–92, 1996.

Sands, D. C., Langhans, V. E., Scharen, A. L., and de Smet, G.: The association between bacteria and rain and possible resultant meteorological implications, J. Hungarian Meteorol. Service, 1982.

Schmale, D. G. and Gordon, T. R.: Variation in susceptibility to pitch canker disease, caused by *Fusarium circinatum*, in native stands of *Pinus muricata*, Plant Pathology, 52, 720–725, 2003.

Schmale, D. G. and Ross, S. D.: Highways in the Sky: Scales of Atmospheric Transport of Plant Pathogens, Annual Review of Phytopathology, 53, 591–611, 2015.

Schmale, D. G., Ross, S. D., Fetters, T. L., Tallapragada, P., Wood-Jones, A. K., and Dingus, B.: Isolates of *Fusarium graminearum* collected 40–320 meters above ground level cause Fusarium head blight in wheat and produce trichothecene mycotoxins, Aerobiologia, 28, 1–11, 2012.

Schmid, D., Pridmore, D., Capitani, G., Battistutta, R., Neeser, J.-R., and Jann, A.: Molecular organisation of the ice nucleation protein InaV from *Pseudomonas syringae*, FEBS Letters, 414, 590–594, 1997.

Schnell, R. C. and Vali, G.: Atmospheric Ice Nuclei from Decomposing Vegetation, Nature, 236, 163–165, 1972.

Sing, D. and Sing, C. F.: Impact of Direct Soil Exposures from Airborne Dust and Geophagy on Human Health, International Journal of Environmental Research and Public Health, 7, 1205–1223, 2010.

Stopelli, E., Conen, F., Zimmermann, L., Alewell, C., and Morris, C. E.: Freezing nucleation apparatus puts new slant on study of biological ice nucleators in precipitation, Atmospheric Chemistry and Physics, 7, 129–134, 2014.

Torp, M. and Nirenberg, H. I.: *Fusarium langsethiae* sp. nov. on cereals in Europe, International Journal of Food Microbiology, 95, 247–256, 2004.

Tsumuki, H. and Konno, H.: Ice Nuclei Produced by *Fusarium* sp. Isolated from the Gut of the Rice Stem Borer, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae), Bioscience, Biotechnology, Biochemistry, 1994.

Tsumuki, H., Konno, H., Maeda, T., and Okamoto, Y.: An ice-nucleating active fungus isolated from the gut of the rice stem borer, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae), Journal of Insect Physiology, 38, 119–125, 1992.

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

Tsumuki, H., Yanai, H., and Aoki, T.: Identification of Ice-nucleating Active Fungus Isolated from the Gut of the Rice Stem Borer, *Chilo suppressalis*Walker (Lepidoptera: Pyralidae) and a Search for Ice-nucleating Active *Fusarium* Species, Annals of the Phytopathological Society of Japan, 61, 334–339, 1995.

Vali, G.: Quantitative Evaluation of Experimental Results an the Heterogeneous Freezing Nucleation of Supercooled Liquids, Journal of the Atmospheric Sciences, 28, 402–409, 1971.

Wang, B. and Jeffers, S. N.: Fusarium Root and Crown Rot: A Disease of Container-Grown Hostas, Plant Disease, 84, 980–988, 2000.

Warmink, J., Nazir, R., Corten, B., and van Elsas, J.: Hitchhikers on the fungal highway: The helper effect for bacterial migration via fungal hyphae, Soil Biology and Biochemistry, 43, 760–765, 2011.

Wing, N., Bryden, W., Lauren, D., and Burgess, L.: Toxigenicity of *Fusarium* species and subspecies in section Gibbosum from different regions of Australia, Mycological Research, 97, 1441–1446, 1993a.

Wing, N., Lauren, D. R., Bryden, W. L., and Burgess, L. W.: Toxicity and Trichothecene Production by *Fusarium acuminatum* subsp. *acuminatum* and *Fusarium acuminatum* subsp. *armeniacum*, Natural Toxins, 1993b.

Wolber, P. K., Deininger, C. A., Southworth, M. W., Vandekerckhovet, J., Van Montagut, M., and Warren, G. J.: Identification and purification of a bacterial ice-nucleation protein, Proc. Natl. Acad. Sci., 1986.

Zachariassen, K. E. and Kristiansen, E.: Ice Nucleation and Antinucleation in Nature, Cryobiology, 41, 257–279, 2000.

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

Table 1. Ice nucleation-active *Fusarium* strains with corresponding initial freezing temperatures of the initial screening. The newly identified IN-active *Fusarium* species are marked with an asterisk (*).

Species	Strain	T_i (°C)
F. acuminatum	1-3	-5.6
F. acuminatum	1-4	-5.0
F. acuminatum	1-5	-5.6
F. acuminatum	1-24	-3.5
F. acuminatum	2-38	-5.0
F. acuminatum	2-48	-5.6
F. acuminatum	2-109	-5.6
F. acuminatum	3-48	-3.7
F. acuminatum	3-68	-3.5
F. acuminatum	20964	-6.2
*F. armeniacum**	20970	-5.3
F. avenaceum	2-106	-5.0
F. avenaceum	11440	-7.6
*F. begoniae**	10767	-11.2
*F. concentricum**	10765	-4.6
*F. langsethiae**	19084	-9.4
F. tricinictum	20990	-7.3

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

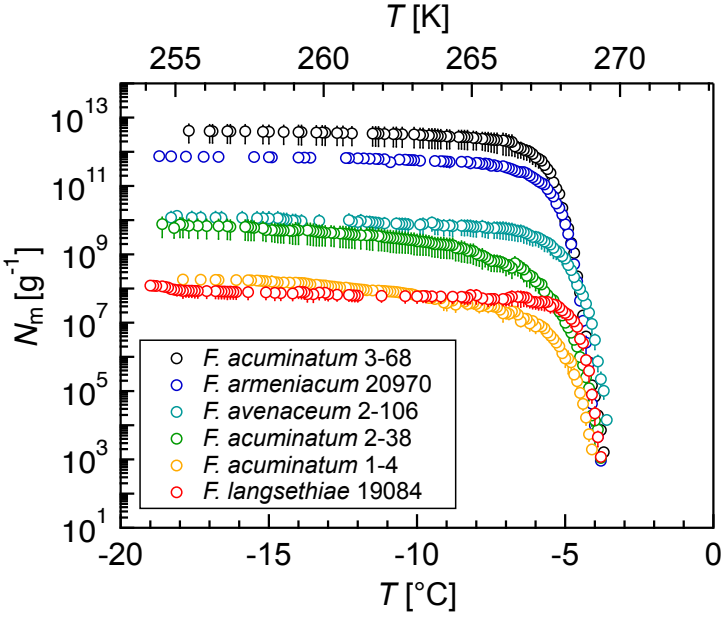

Figure 1. Overview of IN activity for selected *Fusarium* species and strains: cumulative number of IN (N_m) per gram of mycelium plotted against the temperature (T); arithmetic mean values and standard error of three independent experiments with aqueous extracts from different fungal culture plates.

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

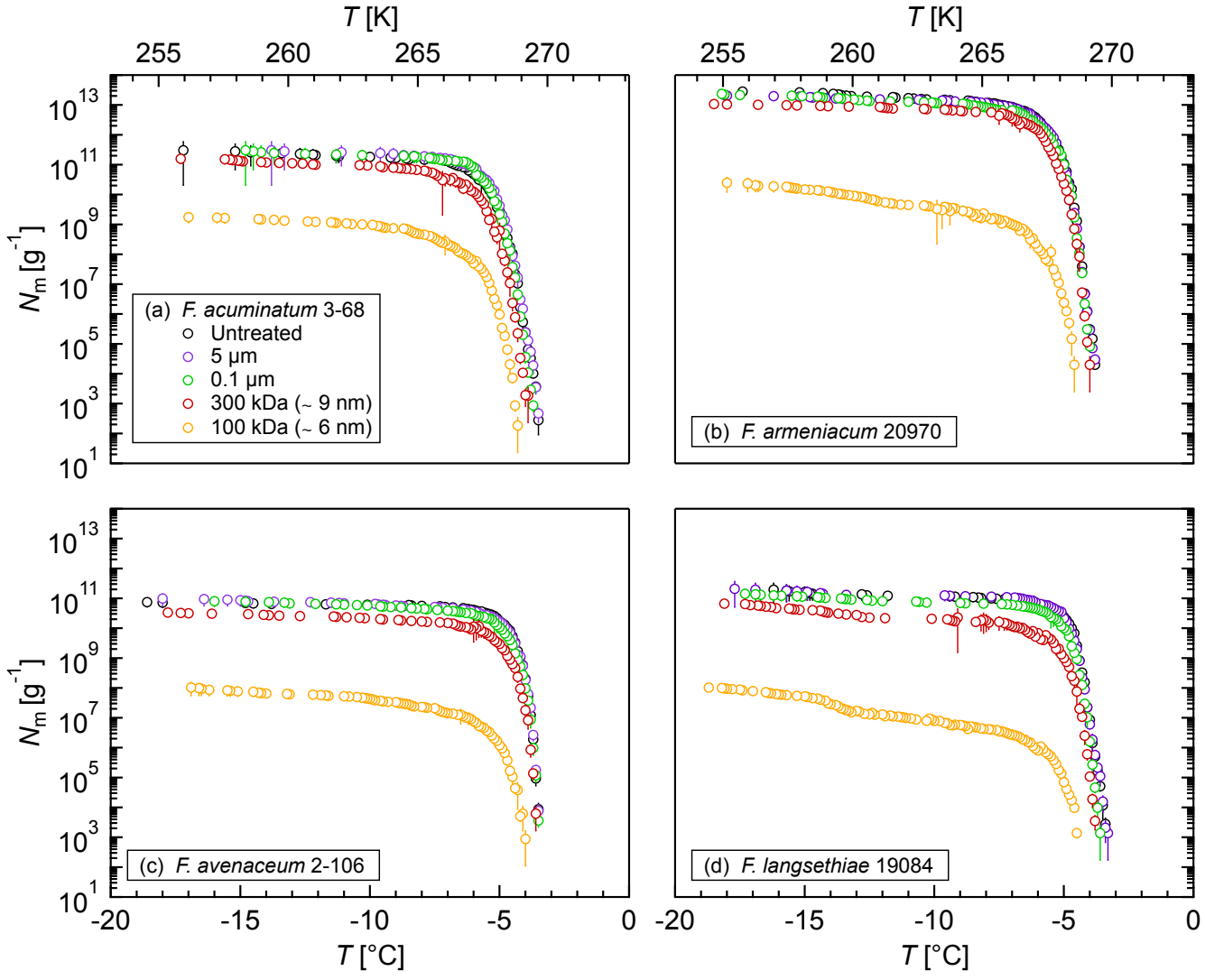

Figure 2. Size determination of the *Fusarium* IN upon filtration: cumulative number of IN (N_m) per gram of mycelium plotted against the temperature (T) for (a) *F. acuminatum* 3-68, (b) *F. armeniacum* 20970, (c) *F. avenaceum* 2-106, and (d) *F. langsethiae* 19084. The error bars were calculated using the counting error and the Gaussian error propagation.

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

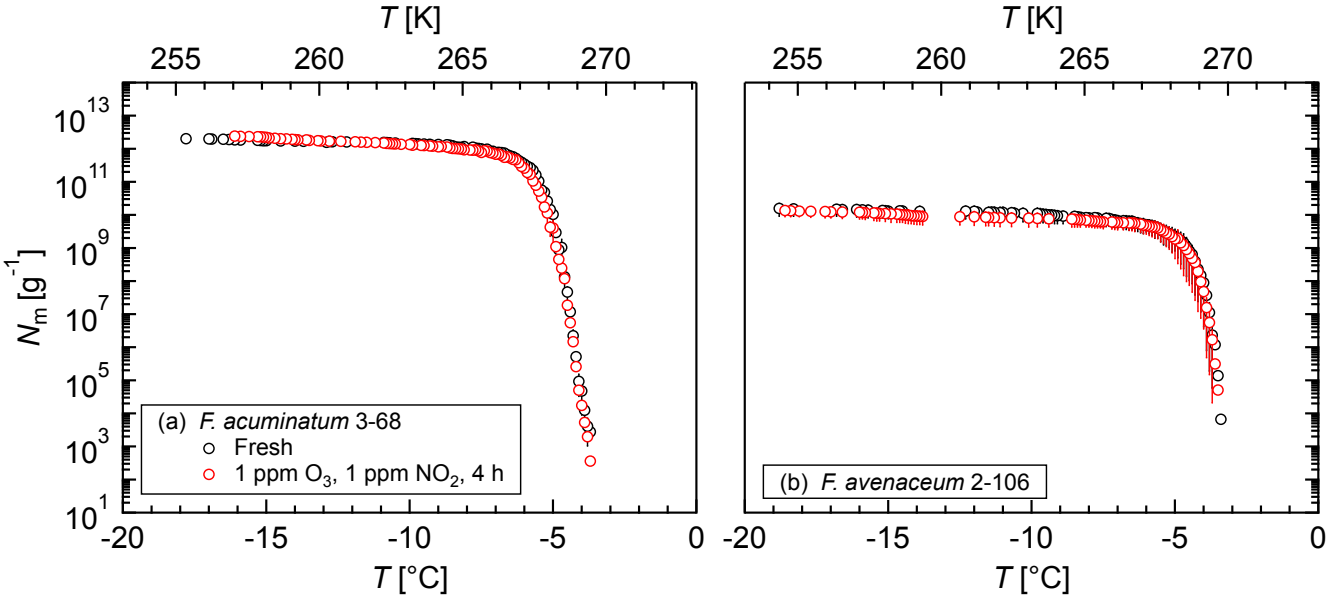

Figure 3. Exposure of aqueous extract from *Fusarium* to ozone and nitrogen dioxide: cumulative number of IN (N_m) per mass of mycelium plotted against the temperature (T) for (a) *F. acuminatum* 3-68 and (b) *F. avenaceum* 2-106; arithmetic mean values and standard error of two independent experiments with aqueous extracts from different fungal culture plates.

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

Biogeosciences
Discussions
Open Access
EGU

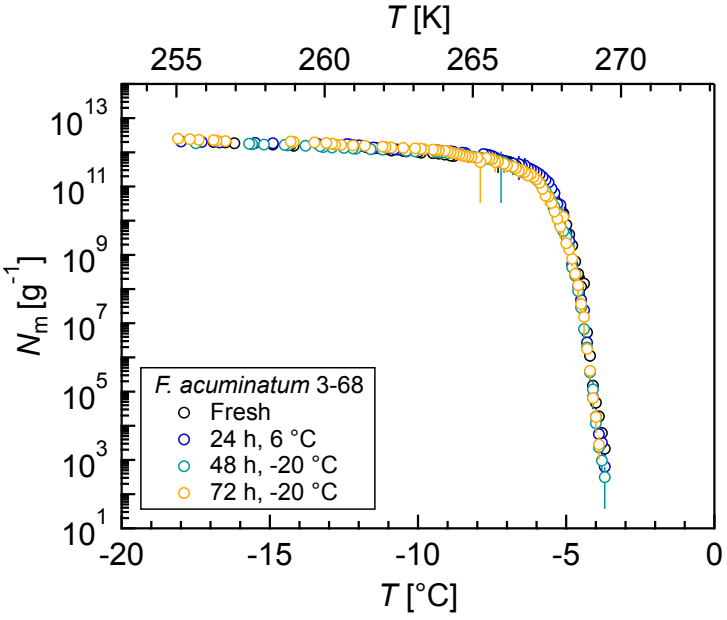

Figure 4. Effects of short-term storage and freeze-thaw cycles on the IN activity of *Fusarium acuminatum* 3-68: cumulative number of IN (N_m) per gram of mycelium plotted against the temperature (T). The same aqueous extract was measured immediately after preparation (black), after storage at 6 °C for 24 h (blue), after another 24 h stored at -20 °C (total 48 h; green), and after another 24 h stored at -20 °C (total 72 h; yellow). The error bars were calculated using the counting error and the Gaussian error propagation.

https://doi.org/10.5194/bg-2019-276
Preprint. Discussion started: 31 July 2019

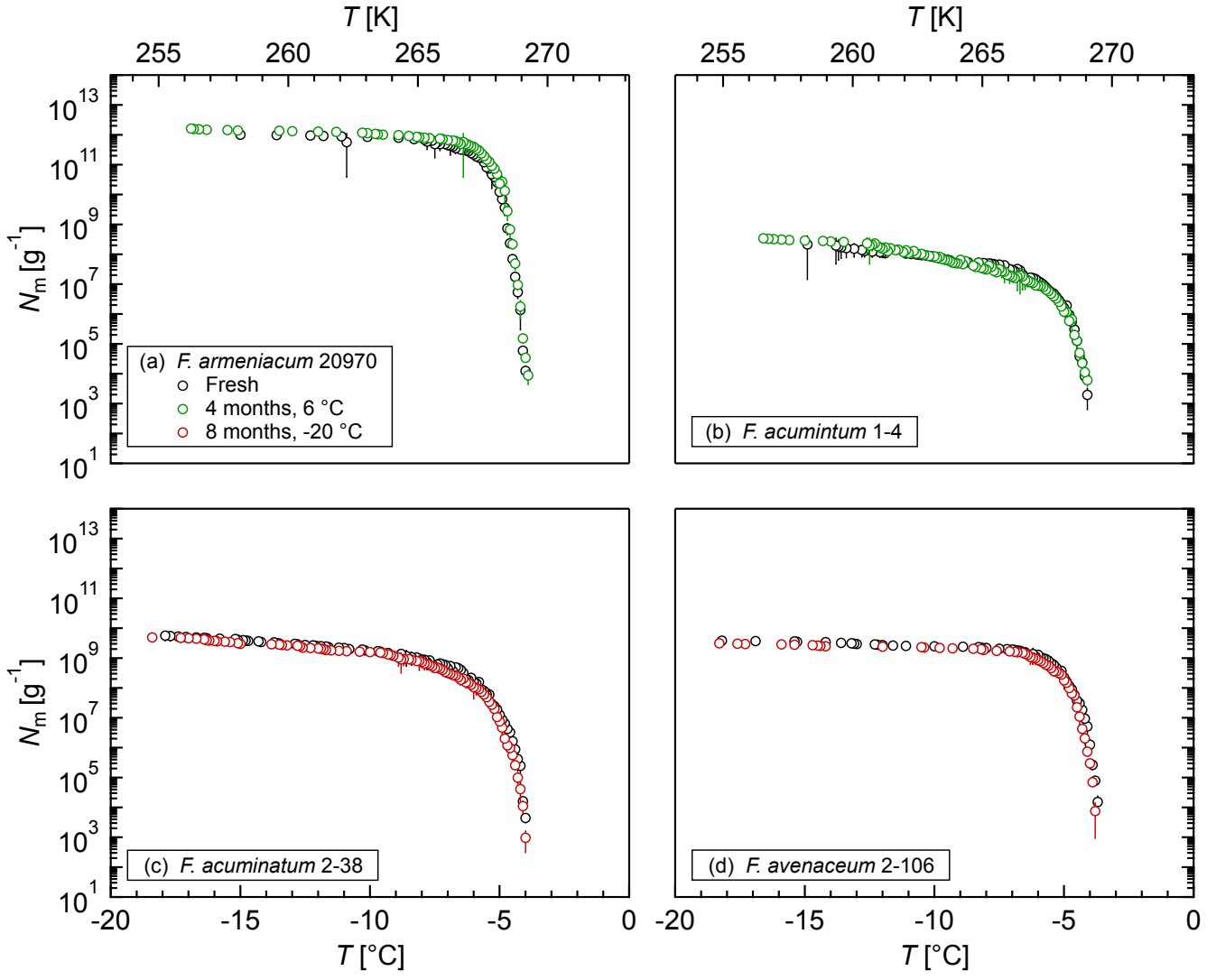

Figure 5. Effect of long-term storage on the IN activity of (a) *F. armeniacum* 20970, (b) *F. acuminatum* 1-4, (c) *F. acuminatum* 2-38, and (d) *F. avenaceum* 2-106: cumulative number of IN (N_m) per gram of mycelium against the temperature (T). The error bars were calculated using the counting error and the Gaussian error propagation.