

We thank referee #1 very much for the extensive review of our manuscript and the positive feedback. We also much appreciate the critical feedback and the suggestion to add a broader discussion of related literature. Addressing comments and suggestions clearly improved the quality of our manuscript.

The comments and our answers are listed below. The referee's comments are written in black, our responses in green.

Overview of the work:

The authors present a three-part study where they first developed a method to extract SPP from pollen grains, then measured the ice nucleating ability of SSP and its washing water, and finally, quantified the protein content of the INMs from the washing water. The authors investigated the ice nucleating ability of different extraction steps of the pollen grain, and found that the SPP themselves were not ice active. However, the authors determined that the washing of the SPP resulted in the isolation of ice nucleating macromolecules. This procedure is well explained and well-illustrated and the investigated work is clearly described. Furthermore, the authors used two methods, fluorescence spectroscopy and quantitative protein analysis assay, to determine the protein content of the ice nucleating macromolecules. This last chemical step is of particular value to the research field of biological ice nuclei. The work presented merits publication in Biogeosciences after minor revisions; my suggested revisions are focused on additional experimental details as well as on adding important literature context and comparisons to the results and discussion sections.

General feedback:

1. Additional details on the replicates and on the uncertainty of the ice nucleation results are necessary. Can the authors comment on the number of replicates necessary to generate Figures 4. What are the uncertainties associated with the immersion freezing technique and can the authors add appropriate error bars to their freezing data in Figure 4?

We thank the referee for this comment. Indeed, it is important to clarify the uncertainties of our ice nucleation assay. The temperature uncertainty of VODCA is 0.5 °C which was previously determined by Zolles et al., 2015. Furthermore, we determined the measurement uncertainty by calculating the counting error and performed a Gauß error propagation as described in Kunert et al., 2018. Errors larger than 100% were excluded in the graphs.

References:

Kunert, A. T., Lamneck, M., Helleis, F., Pöschl, U., Pöhlker, M. L., & Fröhlich-Nowoisky, J. (2018). 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(11), 6327-6337.

Zolles, T., Burkart, J., Häusler, T., Pummer, B., Hitzemberger, R., & Grothe, H. (2015). Identification of ice nucleation active sites on feldspar dust particles. *The Journal of Physical Chemistry A*, 119(11), 2692-2700.

2. The work is well presented but lacks discussions on the comparison of the results with previously published work in the results sections and conclusion. For example, do the results presented corroborate (or not) the work by (Dreischmeier et al., 2017)? In the results section, can the authors elaborate on the comparison of their findings with work on fungal spores (Haga et al., 2014, 2013; Kunert et al., 2019)? Can the authors discuss their findings in terms of structural studies of INMs such as (Ling et al., 2018; Šanti-Temkiv et al., 2015)? The authors mention how the intine is composed of cellulose, yet there is interesting literature in ice nucleation on the relevance of cellulose which can be mentioned (see (Hiranuma et al., 2015, 2018)). Is there any connection with SPP and lignin (see (Bogler and Borduas-Dedekind, 2020))? In an atmospheric implication section, can the authors discuss if airborne ice nuclei of unknown origin could be attributed to SPPs (like in (Lloyd et al., 2020))? Are the INMs in this work forming nanogels (see (Xi et al., 2021))? In the opinion of the authors, is the field converging towards proteinaceous material is the most important INMs? If not, why? If so, why? Discussing these details will place the presented work in the greater context of the current literature on INMs and will allow future work to more effectively build upon the work presented.

We thank the referee for the feedback and agree that a deeper discussion regarding the chemical analysis of INMs will improve the manuscript. We extended chapter 3.3 Chemical Analysis not only with a deeper discussion but also with an additional experiment where proteins of sample B were unfolded using urea as a reagent and subtilisin as an enzymatic digestion tool (please see the details about the experiment in answer 1 to referee 2). Furthermore, we decided to remove the sub-headlines 3.3.1 and 3.3.2.

We added the following discussion to chapter 3.3 starting in line 268:

“However, in literature only Tong et al., 2015 deal with proteins as INMs in birch pollen. Still, the exact chemical composition of the INM from primary biological aerosol particles (PBAPs) is a matter of controversial discussion. When Pummer et al., 2012 discovered that ice nuclei are water soluble and can be extracted from pollen, they proposed that polysaccharides are the responsible moieties. This was supported by infrared and raman spectra and by size filtration experiments pointing to INMs larger than 100 kDa bearing carboxylate groups (Pummer et al., 2013, Dreischmeier et al., 2017). INMs with similar physical and chemical properties were also extracted from other PBAPs such as fungi (Haga et al. 2013, 2014, Kunert et al. 2019). This led Hiranuma et al., 2015, 2018 to the conclusion that cellulose, which is a polysaccharide and part of PBAPs cell walls, might

be responsible for the INA. But also for other polymers such as lignin and nanogels, which can take up water in their structures as well, a similar ice nucleation activity as for cellulose was found (Bogler and Borduas-Dedekind 2020, Xi et al., 2021). Structure and size are also crucial for bacterial ice nucleation proteins, where repeat numbers and oligomerization contribute in a seemingly independent manner to the nucleation mechanism (Ling et al., 2018, Šanti-Temkiv et al., 2015). In general, INMs in the atmosphere are manifold and found from different sources such as forests, deserts and marine environments (Lloyd et al., 2020).

In summary, our experiments come to a similar conclusion as Tong et al., 2015 pointing in the direction of proteins but are not a priori in contradiction with the other recent literature. The piece of puzzle could be a glycoprotein, which exhibits carboxylate functionalities, is larger 100kDa, can bind water in tertiary structures and displays degeneration and unfolding of its secondary structure due to heat treatment or reaction with enzymes. Also glycoproteins are known to be part of the metabolism of cells regulating freezing stress tolerance (Zachariassen and Kristiansen, 2000).”

References:

Tong, H. J., Ouyang, B., Nikolovski, N., Lienhard, D. M., Pope, F. D., & Kalberer, M. (2015). A new electrodynamic balance (EDB) design for low-temperature studies: application to immersion freezing of pollen extract bioaerosols. *Atmospheric Measurement Techniques*, 8(3), 1183-1195.

Pummer, B. G., Bauer, H., Bernardi, J., Bleicher, S., & Grothe, H. (2012). Suspendable macromolecules are responsible for ice nucleation activity of birch and conifer pollen. *Atmospheric Chemistry and Physics*, 12(5), 2541-2550.

Pummer, B. G., Bauer, H., Bernardi, J., Chazallon, B., Facq, S., Lendl, B., ... & Grothe, H. (2013). Chemistry and morphology of dried-up pollen suspension residues. *Journal of Raman Spectroscopy*, 44(12), 1654-1658.

Dreischmeier, K., Budke, C., Wiehemeier, L., Kottke, T., & Koop, T. (2017). Boreal pollen contain ice-nucleating as well as ice-binding 'antifreeze' polysaccharides. *Scientific reports*, 7(1), 1-13.

Haga, D. I., Iannone, R., Wheeler, M. J., Mason, R., Polishchuk, E. A., Fetch Jr, T., ... & Bertram, A. K. (2013). Ice nucleation properties of rust and bunt fungal spores and their transport to high altitudes, where they can cause heterogeneous freezing. *Journal of Geophysical Research: Atmospheres*, 118(13), 7260-7272.

Haga, D. I., Burrows, S. M., Iannone, R., Wheeler, M. J., Mason, R. H., Chen, J., ... & Bertram, A. K. (2014). Ice nucleation by fungal spores from the classes Agaricomycetes, Ustilaginomycetes, and Eurotiomycetes, and the effect on the atmospheric transport of these spores. *Atmospheric Chemistry and Physics*, 14(16), 8611-8630.

Kunert, A. T., Pöhlker, M. L., Tang, K., Krevert, C. S., Wieder, C., Speth, K. R., ... & Fröhlich-Nowoisky, J. (2019). Macromolecular fungal ice nuclei in *Fusarium*: effects of physical and chemical processing. *Biogeosciences*, 16(23), 4647-4659.

Hiranuma, N., Möhler, O., Yamashita, K., Tajiri, T., Saito, A., Kiselev, A., ... & Murakami, M. (2015). Ice nucleation by cellulose and its potential contribution to ice formation in clouds. *Nature Geoscience*, 8(4), 273-277.

Hiranuma, N., Adachi, K., Bell, D., Belosi, F., Beydoun, H., Bhaduri, B., ... & Möhler, O. (2018). A comprehensive characterization of ice nucleation by three different types of cellulose particles immersed in water: lessons learned and future research directions.

Bogler, S., & Borduas-Dedekind, N. (2020). Lignin's ability to nucleate ice via immersion freezing and its stability towards physicochemical treatments and atmospheric processing. *Atmospheric Chemistry and Physics*, 20(23), 14509-14522.

Xi, Y., Mercier, A., Kuang, C., Yun, J., Christy, A., Melo, L., Maldonado, M. T., Raymond, J. A., and Bertram, A. K.: Concentrations and properties of ice nucleating substances in exudates from Antarctic sea-ice diatoms, *Environ. Sci. Process. Impacts*, 23, 323–334, <https://doi.org/10.1039/D0EM00398K>, 2021.

Ling, M. L., Wex, H., Grawe, S., Jakobsson, J., Löndahl, J., Hartmann, S., Finster, K., Boesen, T., and ŠantlâTemkiv, T.: Effects of Ice Nucleation Protein Repeat Number and Oligomerization Level on Ice Nucleation Activity, *J. Geophys. Res. Atmospheres*, 123, 1802–1810, <https://doi.org/10.1002/2017JD027307>, 2018.

Šantl-Temkiv, T., Sahyoun, M., Finster, K., Hartmann, S., Augustin-Bauditz, S., Stratmann, F., Wex, H., Clauss, T., Nielsen, N. W., Sørensen, J. H., Korsholm, U. S., Wick, L. Y., and Karlson, U. G.: Characterization of airborne ice-nucleation-active bacteria and bacterial fragments, *Atmos. Environ.*, 109, 105–117, <https://doi.org/10.1016/j.atmosenv.2015.02.060>, 2015.

Lloyd, G., Choularton, T., Bower, K., Crosier, J., Gallagher, M., Flynn, M., Dorsey, J., Liu, D., Taylor, J. W., Schlenczek, O., Fugal, J., Borrmann, S., Cotton, R., Field, P., and Blyth, A.: Small ice particles at slightly supercooled temperatures in tropical maritime convection, *Atmospheric Chem. Phys.*, 20, 3895–3904, <https://doi.org/10.5194/acp-20-3895-2020>, 2020.

Zachariassen, K. E., & Kristiansen, E. (2000). Ice nucleation and antinucleation in nature. *Cryobiology*, 41(4), 257-279.

Specific comments:

Title: I'm wondering if a title highlighting all three parts of the study (SPP extraction and INM isolation) may be more representative of the work. In addition, the major finding of the work is the chemical identification of proteinaceous material, which should also be mentioned in the title.

Would the authors consider revising their title along the lines of “Isolation of subpollen particles (SPP) and their ice nucleating ability: SPP are carriers of proteinaceous ice nucleating macromolecules”?

We thank the referee for the suggestion and decided to change the title. The new title now is: “Isolation of subpollen particles (SPP) of birch: SPP are potential carriers of ice nucleating macromolecules.”

Abstract:

Are the authors interested in citing the thunderstorm asthma literature to *motivate* their research? As it currently stands, the mention of thunderstorm asthma appears to be an afterthought in the introduction. I would recommend mentioning this context already in the abstract as well as move the introduction paragraph (lines 87-99) earlier. It's an arguably important motivation for the presented research. Recent reference (including refs therein): (Bannister et al., 2021)

We decided to mention thunderstorm asthma only as an afterthought, as the phenomenon of thunderstorm asthma is predominantly related to grass pollen. To our knowledge no case of thunderstorm asthma in relation to birch pollen has been reported.

Line 13: the authors state a gap in knowledge, “explanations on how these materials could distribute in the atmosphere are missing” but do not address this gap in their study. Perhaps this sentence can be reworded to address the gap indeed addressed here.

We reworded the sentence accordingly: “However, INM and SPP are not clearly distinguished. This has motivated the present study which focuses on birch pollen and investigates the relationship between pollen grains, INM and SPP.”

Line 16: what is meant by “loosely attached”? Van der Waals forces? Covalent bonds?

We could separate the INM from the SPP simply by rinsing the SPP with water. Due to the removability without reagents we believe that INMs are not covalently bonded. We decided to clarify the statement and reworded the sentence: “We show that INM are not bonded (i.e. can be washed off with water) to SPP.”

Introduction:

Lines 69-70: I would be interested to read a (Tong et al., 2015) discussion in the results section in more details.

We agree with the referee and added a discussion about the chemical analysis of INMs including a discussion about proteins (Tong et al., 2015) in chapter 3.3. starting in line 268 (please see the text in the comment above; point 2).

Lines 105-109: I would suggest also placing the emphasis of this work on the identification of proteinaceous material as the ice active INMs. The authors show clear chemical evidence of these types of molecules, and this finding is interesting and important.

We agree with the referee that the finding of proteinaceous material as the ice active INMs is important. To prove the findings, we conducted an additional enzymatic digestion experiment. At the end of the introduction in line 109 we also added a sentence: “To clarify the role of proteins in heterogeneous freezing we conducted a specific enzymatic digestion/ protein unfolding experiment.”

Methods:

Lines 116-118: add the date collection information.

We added the date of collection and the exact location of the birch tree. The sampling location has changed as we now refer to the pollen we used for the additional experiments (based on the suggestions of referee 2): “Freshly harvested pollen samples were collected from a birch tree at the Donaukanal (location birch tree: 48.237480, 16.362990) in Vienna. Collection took place on April 9, 2021.”

Lines 145-150: I wondered whether a figure with the described shapes could be helpful for the reader to visualize this calculation.

We think this is a good idea and decided to add a figure to visualize the calculation. In the text we also added the equation used to calculate the volume equivalent diameter.

“Based on the approximated shape the volume equivalent diameter d_v was calculated by equation:

$$d_v = s \sqrt[3]{\frac{1}{2} \left(\frac{3l}{s} - 1 \right)} \quad (1)''$$

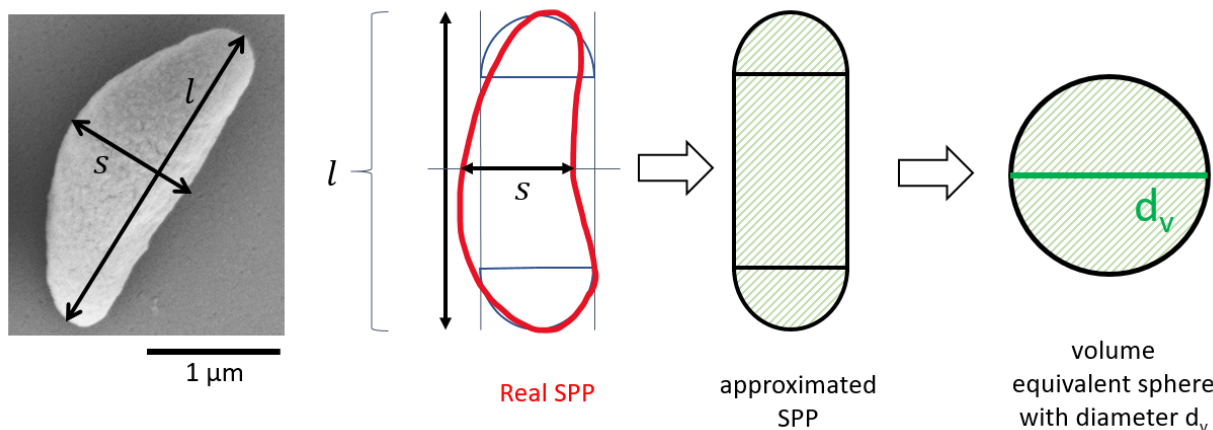


Figure 2: Approximation of the shape of an SPP and determination of the respective volume equivalent diameter d_v .

Line 155: can the formation of the emulsion be described in more detail?

The experimental procedure was published already in 2012 by Pummer et al., 2015 by Zolles et al., and 2018 by Felgitsch et al. To highlight the linkage, we added in line 155: “[...] a detailed description of the experiment is given in Felgitsch et al., 2018, Zolles et al., 2015 and Pummer et al., 2012.”

Furthermore, we described the preparation of the emulsion more clearly. We added in line 156 the mass percentage and the manufacturer/distributor: “(10 wt% lanolin, anhydrous, VWR Int., Radnor, PA, USA; 90 wt% paraffin, light, pure grade, AppliChem GmbH, Darmstadt GER)” and the information “by mixing with a pipette tip” to the sentence.

The improved description is stated as follows:

“INM content from birch pollen was quantified in immersion freezing mode by using the Vienna Optical Droplets Crystallization Analyzer (VODCA) setup (a detailed description of the experiment is given in Felgitsch et al., 2018, Zolles et al., 2015 and Pummer et al., 2012): An emulsion of 2 μl sample solution and 4 μl inert oil-mixture (10 wt% lanolin, anhydrous, VWR Int., Radnor, PA, USA; 90 wt% paraffin, light, pure grade, AppliChem GmbH, Darmstadt GER) is prepared by mixing with a pipette tip on a microscopic glass slide and transferred into a cryo-cell. Sample emulsions are cooled with a rate of $10\text{ }^\circ\text{C min}^{-1}$. The freezing process was monitored by videos at four different spots of each sample glass slide via a microscope camera (MDC320, Hengtech, GER).”

References:

Pummer, B. G., Bauer, H., Bernardi, J., Bleicher, S., & Grothe, H. (2012). Suspendable macromolecules are responsible for ice nucleation activity of birch and conifer pollen. *Atmospheric Chemistry and Physics*, 12(5), 2541-2550.

Zolles, T., Burkart, J., Häusler, T., Pummer, B., Hitzemberger, R., & Grothe, H. (2015). Identification of ice nucleation active sites on feldspar dust particles. *The Journal of Physical Chemistry A*, 119(11), 2692-2700.

Felgitsch, L., Baloh, P., Burkart, J., Mayr, M., Momken, M. E., Seifried, T. M., ... & Grothe, H. (2018). Birch leaves and branches as a source of ice-nucleating macromolecules. *Atmospheric Chemistry and Physics*, 18(21), 16063-16079.

Lines 166-168: Can't dilution have an effect on the protein structure and thus its ice nucleating ability? How did the authors control for this issue?

We confirm that aggregation can influence the ice nucleation behavior as shown in Qiu et al., 2019 for example. However, investigating the aggregation of birch INMs is out of focus for this paper. To avoid this issue, we just state the cumulative nuclei concentration (CNC) values in the paper (instead of e.g. number concentration of proteins). CNC refers to the number of ice nucleating particles or macromolecules independent of their structural composition (e.g. composed of one single protein or an aggregate). To clarify, we added the following sentence to the text (line 164): "Note that one ice nuclei can also be an aggregate of more than one single molecule (Qiu et al., 2019)."

We like the idea of the referee to further investigate the ice nucleation of single molecules and aggregates from birch pollen. This will be part of a follow-up paper.

References:

Qiu, Y., Hudait, A., & Molinero, V. (2019). How size and aggregation of ice-binding proteins control their ice nucleation efficiency. *Journal of the American Chemical Society*, 141(18), 7439-7452.

Linea 167-168: Why was this diameter range chosen? Can a brief explanation be given?

This diameter range is relevant for atmospheric cloud droplets as described by Pruppacher and Klett, 2010. Therefore, the freezing behavior is similar to atmospheric ice nucleation. To clarify we added in line 169: "The selected size range is relevant for atmospheric cloud droplets." to the experimental section.

References:

Pruppacher, H. R., & Klett, J. D. (2010). Microstructure of atmospheric clouds and precipitation. In *Microphysics of Clouds and Precipitation* (pp. 10-73). Springer, Dordrecht.

Results:

Lines 199-200: How does this process affect the atmospheric relevance of the results in this manuscript? Can the authors elaborate?

In our experiments with fresh pollen grains we observed that particulate material is expelled from pollen grains in water. This process has also been documented in literature (e.g. Grote et al., 2003; already referenced in the manuscript). We have now added a video (<https://ucloud.univie.ac.at/index.php/s/FuF5SVBfqayb0ta>) and additional electron microscope images to illustrate the process of pollen rupture as it naturally occurs with fresh pollen grains. Insoluble SPP are clearly visible. In contrast, we do not find any insoluble SPP with commercial pollen, as commercial pollen has lost the ability to rupture and germinate. To mimic the natural process and to access the insoluble SPP we cracked the grains in the mixing mill. We again emphasize that such insoluble SPP are not obtained when commercial pollen grains are just mixed with water. In nature the process of pollen rupture is believed to occur (Schäppi et al., 1999, Grote et al., 2003; Taylor et al 2004, Hughes et al., 2020).

Line 210: “volume equivalent diameters” can be defined here.

We added a figure to visualize the calculation and defined the volume equivalent diameter in the figure (see comment 145-150).

Lines 237-240: good control experiment.

We thank the referee for this comment.

Lines 242-244: this sentence seems out of place? Move to the introduction?

We moved the sentences “Purified SPP are composed of starch and are contained in the pollen grains as energy storage units. Starch is a polysaccharide made of amylose and amylopectin (Hancock & Bryon 2000; Buléon et al., 1998) inside the amyloplast membrane (Matsushima et al., 2016)” to the introduction (starting in line 104).

Line 262: What is the mass percentage of the sample that is thought to contain these concentrations of proteins?

Unfortunately, we were not able to determine the dry mass of our samples due to low concentration and limited sample volume.

Figure 1: it's not clear in the caption where images of 1a and 1b are from? Could additional details be added? Which part of the figure is copyrighted?

We realized that the phrase ‘information for the drawing is taken from....’ is confusing and overcautious. We deleted the phrase. All three images were created by the authors.

Figure 2: very clear! Well done! ï Small addition: could the instrument be specified in the caption as well?

We thank the referee for this feedback. We added the name and details of the instrument in the caption: “Extraction process of SPP. Step 1: Entire pollen grains mixed with ultrapure water are crushed in a mixer mill (MM400, Retsch GmbH, Haan, GER). [...]”

Figure 3: additional details in the caption of how these distributions were generated would be useful.

We added details to the caption of Figure 3. The caption now reads:

Figure 3: a) Size distribution of SPP. The volume equivalent diameter d_v was calculated by equation 1 that is based on the approximated shape of an SPP. The d_v of 326 SPP was binned into 23 equidistant intervals between 0.2-2.5 μm . b) Aspect ratio of SPP.

Figure 4: Compelling data. Good job. Related to the general feedback, a discussion of replicates and uncertainties could be reported here in the caption and displayed on the graphs.

We thank the referee for these remarks. As mentioned above we added a statement regarding the uncertainty of the ice nucleation assay in chapter 2.4 and added error bars to the graphs.

Figure 5: Cool data!

We thank the referee for this positive feedback.

SI: the SI for this manuscript is quite short, and I wondered whether the authors might consider including the SI into the text (easier for the reader). For example, Figure S1 could be merged within Figure 2 of the main text.

We leave the control experiment in the SI but added the additional experiment to the main text.

Conclusion:

Line 274: can the authors make connections between the water-soluble component to dissolved organic matter? (see (Borduas-Dedekind et al., 2019; Knackstedt et al., 2018))

We thank the referee for this remark and added the following to line 273:

“The soluble INMs are easily extracted with water. In nature, INMs from the surface of birches are washed down by rain (Seifried et al., 2020) certainly ending up in soil and/or rivers (Borduas-Dedekind et al., 2019; Knackstedt et al., 2018).”

References:

Seifried, T. M., Bieber, P., Felgitsch, L., Vlasich, J., Reyzek, F., Schmale III, D. G., & Grothe, H. (2020). Surfaces of silver birch (*Betula pendula*) are sources of biological ice nuclei: in vivo and in situ investigations. *Biogeosciences*, 17(22), 5655-5667.

Borduas-Dedekind, N., Ossola, R., David, R. O., Boynton, L. S., Weichlinger, V., Kanji, Z. A., and McNeill, K.: Photomineralization mechanism changes the ability of dissolved organic matter to activate cloud droplets and to nucleate ice crystals, *Atmospheric Chem. Phys.*, 19, 12397–12412, <https://doi.org/10.5194/acp-19-12397-2019>, 2019.

Knackstedt, K., Moffett, B. F., Hartmann, S., Wex, H., Hill, T. C. J., Glasgo, E., Reitz, L., Augustin-Bauditz, S., Beall, B., Bullerjahn, G. S., Fröhlich-Nowoisky, J., Grawe, S., Lubitz, J., Stratmann, F., and McKay, R. M.: A terrestrial origin for abundant riverine nanoscale ice-nucleating particles, *Environ. Sci. Technol.*, <https://doi.org/10.1021/acs.est.8b03881>, 2018.

Technical comments:

In many instances throughout the text, a comma is missing to separate a clause from the sentence. Ex: lines 13, 15, 18, 19, 31 and so on. (English vs German syntax and comma usage)

We have carefully gone through the manuscript and added missing commas.

Line 48: “The solution is” should be “was”

Corrected.

References:

Bannister, T., Ebert, E. E., Williams, T., Douglas, P., Wain, A., Carroll, M., Silver, J., Newbigin, E., Lampugnani, E. R., Hughes, N., Looker, C., Mulvenna, V., Csutoros, D., Jones, P. J., Davies, J. M., Suphioglu, C., Beggs, P. J., Emerson, K. M., Huete, A., and Nguyen, H.: A Pilot Forecasting System for Epidemic Thunderstorm Asthma in Southeastern Australia, *Bull. Am. Meteorol. Soc.*, 102, E399–E420, <https://doi.org/10.1175/BAMS-D-19-0140.1>, 2021.

Bogler, S. and Borduas-Dedekind, N.: Lignin’s ability to nucleate ice via immersion freezing and its stability towards physicochemical treatments and atmospheric processing, *Atmospheric Chem. Phys.*, 20, 14509–14522, <https://doi.org/10.5194/acp-20-14509-2020>, 2020.

Borduas-Dedekind, N., Ossola, R., David, R. O., Boynton, L. S., Weichlinger, V., Kanji, Z. A., and McNeill, K.: Photomineralization mechanism changes the ability of dissolved organic matter to activate cloud droplets and to nucleate ice crystals, *Atmospheric Chem. Phys.*, 19, 12397–12412, <https://doi.org/10.5194/acp-19-12397-2019>, 2019.

Dreischmeier, K., Budke, C., Wiehemeier, L., Kottke, T., and Koop, T.: Boreal pollen contain ice-nucleating as well as ice-binding “antifreeze” polysaccharides, *Sci. Rep.*, 7, <https://doi.org/10.1038/srep41890>, 2017.

Haga, D. I., Iannone, R., Wheeler, M. J., Mason, R., Polishchuk, E. A., Fetch, T., Kamp, B. J. van der, McKendry, I. G., and Bertram, A. K.: Ice nucleation properties of rust and bunt fungal spores and their transport to high altitudes, where they can cause heterogeneous freezing, *J. Geophys. Res. Atmospheres*, 118, 7260–7272, <https://doi.org/10.1002/jgrd.50556>, 2013.

Haga, D. I., Burrows, S. M., Iannone, R., Wheeler, M. J., Mason, R. H., Chen, J., Polishchuk, E. A., Pöschl, U., and Bertram, A. K.: Ice nucleation by fungal spores from the classes Agaricomycetes, Ustilaginomycetes, and Eurotiomycetes, and the effect on the atmospheric transport of these spores, *Atmospheric Chem. Phys.*, 14, 8611–8630, <https://doi.org/10.5194/acp-14-8611-2014>, 2014.

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Hiranuma, N., Adachi, K., Bell, D., Belosi, F., Beydoun, H., Bhaduri, B., Bingemer, H., Budke, C., Clemen, H.-C., Conen, F., Cory, K., Curtius, J., DeMott, P., Eppers, O., Grawe, S., Hartmann, S., Hoffmann, N., Höhler, K., Jantsch, E., Kiselev, A., Koop, T., Kulkarni, G., Mayer, A., Murakami, M., Murray, B., Nicosia, A., Petters, M., Piazza, M., Polen, M., Reicher, N., Rudich, Y., Saito, A., Santachiara, G., Schiebel, T., Schill, G., Schneider, J., Segev, L., Stopelli, E., Sullivan, R., Suski, K., Szakáll, M., Tajiri, T., Taylor, H., Tobo, Y., Weber, D., Wex, H., Whale, T., Whiteside, C., Yamashita, K., Zelenyuk, A., and Möhler, O.: A comprehensive characterization of ice nucleation by three different types of cellulose particles immersed in water: lessons learned and future research directions, *Atmospheric Chem. Phys. Discuss.*, 1–82, <https://doi.org/10.5194/acp-2018-933>, 2018.

Knackstedt, K., Moffett, B. F., Hartmann, S., Wex, H., Hill, T. C. J., Glasgo, E., Reitz, L., Augustin-Bauditz, S., Beall, B., Bullerjahn, G. S., Fröhlich-Nowoisky, J., Grawe, S., Lubitz, J., Stratmann, F., and McKay, R. M.: A terrestrial origin for abundant riverine nanoscale ice-nucleating particles, *Environ. Sci. Technol.*, <https://doi.org/10.1021/acs.est.8b03881>, 2018.

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