Biogeosciences Discussions

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Reviewer comment on Burkart et al. 2021

Anonymous Referee #1

Referee comment on "Isolation of subpollen particles (SPPs) of birch: SPPs are potential carriers of ice nucleating macromolecules" by Julia Burkart et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-8-RC1, 2021

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:

- 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?

- 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; Šantl-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.

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"?

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)

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.

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

Introduction:

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

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.

Methods:

Lines 116-118: add the date collection information.

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

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

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?

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

Results:

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

Line 210: "volume equivalent diameters" can be defined here.

Lines 237-240: good control experiment.

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

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

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?

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

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

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.

Figure 5: Cool data!

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.

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))

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)

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

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., Emmerson, 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.

Hiranuma, N., Möhler, O., Yamashita, K., Tajiri, T., Saito, A., Kiselev, A., Hoffmann, N., Hoose, C., Jantsch, E., Koop, T., and Murakami, M.: Ice nucleation by cellulose and its potential contribution to ice formation in clouds, Nat. Geosci., 8, 273–277, https://doi.org/10.1038/ngeo2374, 2015.

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.

Kunert, A. T., Pöhlker, M. L., Tang, K., Krevert, C. S., Wieder, C., Speth, K. R., Hanson, L. E., Morris, C. E., Schmale III, D. G., Pöschl, U., and Fröhlich-Nowoisky, J.: Macromolecular fungal ice nuclei in *Fusarium*: effects of physical and chemical processing, Biogeosciences, 16, 4647–4659, https://doi.org/10.5194/bg-16-4647-2019, 2019.

Ling, M. L., Wex, H., Grawe, S., Jakobsson, J., Löndahl, J., Hartmann, S., Finster, K., Boesen, T., and Šantlâ□□Temkiv, 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.

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.

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.

Tong, H.-J., Ouyang, B., Nikolovski, N., Lienhard, D. M., Pope, F. D., and Kalberer, M.: A new electrodynamic balance (EDB) design for low-temperature studies: application to immersion freezing of pollen extract bioaerosols, Atmospheric Meas. Tech., 8, 1183–1195, https://doi.org/10.5194/amt-8-1183-2015, 2015.

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.