Authors Response to Anonymous Referee #2

The authors wish to thank Referee #2 for his/her thoughtful comments and useful discussions. Below are our point-by-point responses (in blue texts) to the reviewer's comments. Corresponding modifications are reflected in the manuscript and figures.

Referee Comment: My main concern regarding this paper is that only three types of cellulose have been investigated. However, cellulose is the most common organic compound on Earth and it is the most common polysaccharide. Of course, there are many, many cellulose types and MCC, FC and NCC are only a very few representatives. It comes not clear from the manuscript how and why these three have been chosen.

Authors Response: The reason we choose three cellulose types was because these have diverse surface structures (**Table 1**), and their INP properties differ by orders of magnitude. Further, such a family of cellulose types allows us to probe the sensitivity of ice nucleation experimental techniques towards detecting non-proteinaceous biological materials. This point is now clarified in the end of **Sect. 1.3.**: "The motivation of using multiple types of cellulose was to (1) examine the immersion freezing abilities of both predominantly supermicron (MCC and FC) and submicron (NCC) cellulose particles to assess a wide size range of chemically uniform biological particles and (2) look into diverse surface structure (**Table 1**)".

Referee Comment: In general, I miss a more elaborated introduction (1.1 background) where the sources of cellulose in the biosphere and finally in the atmosphere are discussed.

Authors Response: We added the following introductory sentences regarding general cellulose source in **Sect. 1.1.**: "Cellulose is a linear polymer of 1–4 linked β-d-anhydroglucopyranose molecules, derived from plant fragments, leaf litter, wood fiber, non-wood fiber and/or even microbes (*Quiroz-Castañeda & Folch-Mallol*, 2013; *Thakur and Thakur*, 2014; *Chawla et al*., 2009). The composition and structure of cellulose-containing bio fiber depends on the source and several different factors, summarized in *Khalil et al*. (2012) and *Dittenber and GangaRao* (2012).".

Reference:

- Quiroz-Castañeda, R. E. and Folch-Mallol, J. L.: Hydrolysis of Biomass Mediated by Cellulases for the Production of Sugars. In: Sustainable Degradation of Lignocellulosic Biomass - Techniques, Applications and Commercialization, edited by: Chandel. A., ISBN: 978-953-51-1119-1, InTech, doi: 10.5772/53719, 2013.
- Chawla, P. R., Bajaj, I. B., Survase, S. A., Singhal, R.S.: Microbial cellulose: Fermentative production and applications, Food Technology and Biotechnology, 47, 107–124, 2009.
- Dittenber, D. B., and GangaRao, H. V. S. Critical review of recent publications on use of natural composites in infrastructure, Composites Part A: Applied Science and Manufacturing, 43, 1419–1429, doi: https://doi.org/10.1016/j.compositesa.2011.11.019, 2012.
- Khalil, H. P. S. A., Bhat, A. H., and Yusra, A. F. I.: Green composites from sustainable cellulose nanofibrils: A review, Carbohydrate Polymers, 87, 963–979, doi: https://doi.org/10.1016/j.carbpol.2011.08.078, 2012.
- Thakur, V. K., and Thakur, M. K.: Processing and characterization of natural cellulose fibers/thermoset polymer composites, Carbohydr. Polym., 109, 102–117, doi: https://doi.org/10.1016/j.carbpol.2014.03.039, 2014.

Referee Comment: Also relevant literature should be discussed (regarding marine aerosols, bio-aerosols (fungi, pollen, bacteria, plant fragments, leaf litter etc.)), e.g. the fact that water extractable INPs consist of polysaccharides should be mentioned (Dreischmeier 2017,Pummer 2012).

Authors Response: These polysaccharides are not discussed since these pollen release INM polysaccharides are fundamentally different from cellulose. The authors note that cellulose is a specific allomorph of polysaccharides (i.e., polymer containing D-glucose residues linked by β-1,4-glycosidic bonds). *Dreischemeier et al*. (2017) addresses boreal pollen INM saccharide vs. "*other polysaccharides such as cellulose*.". For given specific reason, the authors would like to omit any extensive discussion of INM in general in the current manuscript. Nevertheless, the authors now address the importance of more comprehensive study of plant constituents, including INM polysaccharides, with suggested citations in our conclusion section: "…it is important to further conduct comprehensive study on ice nucleation activities

of other important plant structural materials, such as cellulose polymorphs, lignin materials, lipids, carbohydrates and other macromolecule saccharides (e.g., *Pummer et al*., 2012; *Dreischmeier et al*., 2017), as well as natural plant debris in simulated super-cooled clouds of the lower and middle troposphere.".

Reference:

- Dreischmeier, K., Budke, C., Wiehemeier, L., Kottke, T., and Koop, T.: Boreal pollen contain ice-nucleating as well as ice-binding 'anti-freeze' polysaccharides, Sci. Rep., 7, 41890.
- Pummer, B. G., Bauer, H., Bernardi, J., Bleicher, S., and Grothe, H.: Suspendable macromolecules are responsible for ice nucleation activity of birch and conifer pollen, Atmos. Chem. Phys., 12, 2541-2550, https://doi.org/10.5194/acp-12-2541-2012, 2012.

Referee Comment: In principle, the physical and chemical properties of cellulose depend a lot on the history of the respective sample: water uptake, swelling, drying, shrinking, are inherently important for the INA.

1. Even a freeze-thawing cycle of the same cellulose-water system could change the INA from one experiment to the other. These are just some points which should be discussed in more detail and might also help to understand the results of the paper.
2. From my point of view, cellulose is not the ideal candidate for an intercomparison program due to its unstable INA.
3. On the other hand, this study gives good proof that it is not so much the influence of the different instruments which are responsible for the differing results, but much more the cellulose sample, since it properties are not sufficiently constant.
4. Another important point is the specific surface area of cellulose, since the calculation of the ice active site number inherently depends on it. However, the specific surface area of dry cellulose is not the same as the surface area in aqueous solution after swelling. Much more area becomes available and also the surface chemistry exhibited to the water interface might be changed. The authors should explain how they include this into their parametrization.

Authors Response: The reviewer makes good points. See below our four comments:

1. For clarity, all of our analyses were done only when cellulose materials were dry or newly wet-generated by purpose to minimize the bias from these potential artifacts and not to refrain from comparing wet and dry. Nonetheless, it is possible that "water uptake, swelling, drying, shrinking" processes may affect (therefore, cellulose may not be stable). The impact of freeze-thawing cycle as well as pre-activation (*Wagner et al*., 2016) should be carefully looked into. The authors clarify this point in the **Supplemental Sect. S.10.** as follows: "Though looking into the stability of the samples is beyond the scope of the current study, it is necessary in the future to carry out a more detailed study in characterizing the saturation level and temperature dependence of specific adsorption-desorption processes at atmospherically relevant heterogeneous freezing temperature range of cellulose at <-4 °C (*this study*) by applying a modern surface physisorption characterization tool. It is possible that the freeze-thawing processes affect stability of cellulose materials due to water uptake, swelling, drying and/or shrinking. It is also desired to carefully look into pre-activation (e.g., *Wagner et al*., 2016).".

Reference:

- Wagner, R., Kiselev, A., Möhler, O., Saathoff, H., and Steinke, I.: Pre-activation of ice-nucleating particles by the pore condensation and freezing mechanism, Atmos. Chem. Phys., 16, 2025-2042, https://doi.org/10.5194/acp-16-2025-2016, 2016.

2. Our thought is now clearly addressed in the conclusion section as well as in the **Supplemental Sect. S.10.** as follows:

- "…These diversities suggest the complex surface structure and compositional heterogeneity may play a substantial role to explain the diversity. This also implies that the cellulose system might not be suitable as a calibrant at this stage unless we completely understand the complex properties of cellulose materials."

- "…The observed discrepancy may be due to non-uniform active site density for different sizes and/or the alteration in physico-chemical properties of cellulose by liquid-suspending it. Unless otherwise defined, the cellulose system may not be an ideal calibrant at this moment."

3. The authors agree. As addressed above, stability of the sample is different issue.

4. The reviewer is right – swelling may alter the specific surface area of cellulose. To rigorously address this point, it is necessary to carry out a more detailed study in characterizing and quantifying surface properties of cellulose materials by applying a modern surface physisorption characterization tool in the future (currently not available). Here we outline the necessary two steps of potential future studies:

- Step 1: Quantitatively determine the BET-SSA, the pore size distribution and the void volume density of cellulose samples via the standard isothermal physisorption method (at STP) using Krypton as low saturation pressure gasses. Krypton allows us to assess porous but low SSA materials like cellulose (personal communication with the manufacturer). Additionally, CO_2 will be used for nanoscopic pore (≈3.5 to 15 Å) distribution analysis, if necessary, due to its lower molecular quadrupole moment (*Rouquerol et al*., 1989). Nitrogen can be used for BET surface area, micro-pore characterization, and meso-pore characterization (≈20 to 3000 Å). Finally, we will relate the measured quantities to the number of ice-nucleating surface active sites (i.e., $n_s(T)$) to develop the morphology-resolved parameterization, which can be formulated in the atmospheric models.
- Step 2: Assess the saturation level and temperature dependence of specific adsorption-desorption processes at atmospherically relevant heterogeneous freezing temperature range of cellulose at <-4 °C. An advanced physisorption characterization tool (e.g., Micromeritics, 3Flex) enables the cryogenic-physisorption of H_2O and N_2 for temperature above -28 °C and pressure below 100 mmHg (personal communication with the manufacturer). These ranges are relevant to atmospheric mixed-phase clouds, where immersion freezing dominates the ice nucleation process (*Hande and Hoose*, 2017). Assessing the effect of variabilities in thermodynamic conditions will allow us to define the relationship between material porosity and reactivity, separately and in conjunction with Step 1.

Reference:

- Rouquerol, J.; Rouquerol, F.; Grillet, Y., ENERGETICAL ASPECTS OF N2 AND AR ADSORPTION - SPECIFIC ADSORPTION, TWO-DIMENSIONAL PHASE-CHANGES AND ADSORPTION IN MICROPORES. *Pure and Applied Chemistry* **1989,** *61*, (11), 1933-1936.
- Hande, L. B.; Hoose, C., Partitioning the primary ice formation modes in large eddy simulations of mixed-phase clouds. *Atmospheric Chemistry and Physics* **2017,** *17*, (22), 14105-14118.

Referee Comment: So there are many sources of cellulose but most cellulose is not ice nucleation active. Then it is important to understand what makes the difference in terms of INA. Why are some cellulose samples so much more ice nucleation active than others? The authors might at least try to find an answer on this question in order to enhance the scientific value of the manuscript.

Authors Response: The statement of "most cellulose is not ice nucleation active" seems speculative. As described above, the authors offer logical steps and a potential approach to find an ultimate answer for the question raised (i.e., why are some cellulose samples so much more ice nucleation active than others). This point is addressed in the **Supplemental Sect. S.10.** ("…it is necessary in the future to carry out a more detailed study in characterizing the saturation level and temperature dependence of specific adsorption-desorption processes at atmospherically relevant heterogeneous freezing temperature range of cellulose at <-4 °C (*this study*) by applying a modern surface physisorption characterization tool."). Indeed, these points warrant some follow up studies.

The authors note that our knowledge of whether the laboratory results of a few cellulose materials can be representatively scaled up to the total plant fiber content in the atmosphere to assess the overall role of non-proteinaceous bio-INPs in clouds and the climate system is still limited. Luckily, there has been

another on-going AIDA study to investigate if other important plant constituents, such as cellulose polymorphs, lignin materials, lipids and carbohydrates, as well as natural plant debris can act as bio-INPs in simulated super-cooled clouds of the lower and middle troposphere. Preliminary scientific results have been presented in three conferences as of 2015 (*Hiranuma et al*., 2015; *Steinke et al*., 2017 and 2018; Note both corresponding authors participate in all of these plant fiber INP studies). Overall, our findings support the view that MCC may be a good proxy for inferring ice nucleating properties of natural plant debris. Our detailed outcomes will be presented in another paper (currently in preparation).

To clarify this important point, the authors added the following sentence in the end of the conclusion section: "Our knowledge of non-proteinaceous biological INPs is still limited. Thus, it is important to further conduct comprehensive studies on the ice nucleation activity of other important plant structural materials, such as cellulose polymorphs, lignin materials, lipids, carbohydrates and other macromolecule saccharides (e.g., *Pummer et al*., 2012; *Dreischmeier et al*., 2017; *Suski et al*., 2018), as well as natural plant debris in simulated supercooled clouds of the lower and middle troposphere. Such additional studies are especially important for assessing the overall role of non-proteinaceous bio-INPs in clouds and the climate system.".

Reference:

- Hiranuma, N., Hoose, C., Järvinen, E., Kiselev, A., Möhler, O., Schnaiter, M., Ulrich, R., Cziczo, D.J., Zawadowicz, M., Felgitsch, L., Grothe, H., Kulkarni, G., Reicher, N., Rudich, Y., and Tobo, Y: Ice nucleation by plant structural materials and its potential contribution to glaciation in clouds, AGU Fall Meeting, San Francisco, CA, USA, Dec., 2015.
- Steinke, I., Funk, R., Hiranuma, N., Möhler, O., and Zhang, K.: Immersion freezing properties of complex biological aerosols derived from plants, INUIT Final Conference and 2nd Atmospheric Ice Nucleation Conference, Grasellenbach, Germany, Feb.-Mar., 2018.
- Steinke, I., Funk, R., Hiranuma, N., Möhler, O., Shen, X.: From macromolecules to plant related aerosols – investigating the ice nucleation properties of complex biological particles, 1st Atmospheric IN Conference, Leeds, UK., Jan., 2017.
- Pummer, B. G., Bauer, H., Bernardi, J., Bleicher, S., and Grothe, H.: Suspendable macromolecules are responsible for ice nucleation activity of birch and conifer pollen, Atmos. Chem. Phys., 12, 2541–2550, https://doi.org/10.5194/acp-12-2541-2012, 2012.
- Dreischmeier, K., Budke, C., Wiehemeier, L., Kottke, T., and Koop, T.: Boreal pollen contain ice-nucleating as well as ice-binding 'anti-freeze' polysaccharides, Sci. Rep., 7, 41890, 2017.
- Suski, K. J., Hill, T. C. J., Levin, E. J. T., Miller, A., DeMott, P. J., and Kreidenweis, S. M.: Agricultural harvesting emissions of ice-nucleating particles, Atmos. Chem. Phys., 18, 13755-13771, https://doi.org/10.5194/acp-18-13755-2018, 2018.

Referee Comment: Minor comment Fig. 3, y-axis: "relative intensity (a.u.)"

Authors Response: We omit Fig. 3 concerning the comment provided by Referee #1 (and upon the agreement with the relevant data providers).

Note: Dr. Romy Ullrich has been added as an author for her extensive contribution to the database work.