Responses to referee #1

We thank Referee #1 for acknowledging the improvement of our manuscript.

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

The authors did a good job addressing the reviewer comments. I have small additional suggestions at this stage:

1. The discussion in added to chapter 3.3 starting in line 268 is well done. I would also encourage the authors to state their key conclusions: "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." could also be added to the abstract.

We thank the referee for this suggestion and added the sentence to the abstract in line 27.

"The missing piece of the puzzle could be a glycoprotein, which exhibits carboxylate functionalities, can bind water in tertiary structures and displays degeneration and unfolding of its secondary structure due to heat treatment or reaction with enzymes."

2. Perhaps I can also further probe the authors on the idea of glycoproteins. Would they also explain the activity seen by dissolved organic matter in these papers (Borduas-Dedekind et al., 2019; Knackstedt et al., 2018; Moffett et al., 2018; Moffett, 2016)?

Theoretically, proteins might play an important role in the ice nucleation activity of dissolved organic matter. However, we have no specific information if this was the case in the here mentioned studies. We would propose to investigate those samples using advanced analytical methods such as fluorescence spectroscopy or mass spectrometry in future studies.

3. On the point of the comment on Line 16: what is meant by "loosely attached"? Van der Waals forces? Covalent bonds? The authors comment: "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." However I would argue that "washing with water" could also cause hydrolysis of ester bonds and therefore is not proof of the absence of covalently-bonded moieties. pH-dependent water washing could help shed light on this mechanism.

This is a good point. We agree that covalent bonds cannot be excluded entirely, since hydrolysis might have cleaved bonds during the rinsing process. We now stated clearly in line 301 that a definite statement cannot be made:

"However, based on our experiments we cannot make a definite statement on the type of bonding."

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.

Moffett, B., Hill, T., DeMott, P., Moffett, B. F., Hill, T. C. J., and DeMott, P. J.: Abundance of biological ice nucleating particles in the Mississippi and its major tributaries, Atmosphere, 9, 307, https://doi.org/10.3390/atmos9080307, 2018.

Moffett, B. F.: Fresh water ice nuclei, Fundam. Appl. Limnol., 188, 19–23, https://doi.org/10.1127/fal/2016/0851, 2016.

Responses to referee #2

We thank referee #2 for his/her additional comments.

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

(1) In response to my comment 4.) the authors wrote the following text:
"We observe that fresh pollen grains directly released from catkins germinate and rupture when immersed in water or exposed to high relative humidity (>95 %). To illustrate the process, we now also made a video of freshly collected birch pollen grains immersed in ultrapure water. It can be seen clearly that particulate material is expelled from the pollen grains. Additionally, we exposed fresh pollen grains to relative humidities above 90 % for several hours and also find ruptured pollen grains."
I cannot find this information on observations at high humidity, neither in the text nor in the supplement. Apparently, the authors have provided the video for review purposes only, but I recommend that it is made available to the readers directly as a supplement/asset.

Actually, we added the information in section 3.1, where we also provide a link to the video. We intend to provide the video also to the readers.

(2) Line 132/133: "With commercially purchased pollen this ability is almost lost."
Can you provide any reasonable explanation for why this is the case? What is the difference to fresh pollen material?

Pollen rupture and germination are closely related to the viability of pollen grains. Viability can be lost due to desiccation (e.g. during storing conditions or by exposure to sunlight) and aging of the pollen grain (e.g. Stanley and Linskens 1974; Siriwattanakul et al. 2019).

We added a brief explanation and changed the sentence in line 134 to:

"With commercially purchased pollen this ability is almost lost due to aging and desiccation of pollen and the loss of viability."

Siriwattanakul, U., Piboonpocanun, S., & Songnuan, W. (2019). Rapid pollen rupture and release of pollen cytoplasmic granules upon hydration of allergenic grass and weed species commonly found in subtropical regions. *Aerobiologia*, *35*(4), 719-730.

Stanley, R. G., Linskens, H. F. (1974). *Pollen: biology biochemistry management*. Berlin, Heidelberg (Germany): Springer Science & Business Media

(3) Figure 2a: Please indicate in Figure 2a which part is defined as sample B, C etc. Currently, it is mentioned that Step 3 yields sample B and Step4 yields sample C, although in the first case the sample is in the filter and in the other case it is the filtrate. Please indicate this directly in each figure panel so that it is directly obvious to the reader without having to read the detailed text in lines 141-151.

We realized that the reference to sample B in the text is confusing. Actually all samples refer to the filtrate. We have now explicitly described sample B in the text and also clarified the labelling in figure 2a.

(4) Line 181: “Note that one ice nuclei can also be…” Use singular for nuclei, i.e. nucleus.

Done.

(5) Line 189: “Errors larger than 100% were excluded in the graphs.” I do not understand this procedure, why is it done?

Indeed, the sentence is misleading. We excluded **data points** (not errors) with errors larger than 100% in the graphs. This approach is described in Kunert et al. (2018). We changed the sentence in line 189 to:

“Furthermore, we calculated the counting error and performed a Gauß error propagation as described in Kunert et al. (2018). Accordingly, data points with errors larger than 100% were excluded in the graphs.”

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.