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Interactive comment on "Birch leaves and branches as a source of ice-nucleating macromolecules" by Laura Felgitsch et al.

Anonymous Referee #2

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The manuscript submitted by Felgitsch et al. discusses work analyzing samples of leaves and wood from a number of birch trees in Austria. The authors subjected the samples to ice nucleation tests as well as to infrared and fluorescence spectroscopy. The main conclusions of the work were to report the concentration range of ice nuclei and the mean freezing temperature per sample. They also reported the spectral properties of a few selected samples to suggest that the chemical components in the samples were similar. The work showing data about ice nuclei in the birch material is interesting and could be useful to compare against other material types. The discussion was very thin and didn't make strong connections between the ice nuclei and spectroscopic analyses. The sections about optical spectral were not convincing. It wasn't obvious why infrared and fluorescence spectroscopy was used as the primary

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"chemical" test for comparison here. These shown broad similarity between the few samples discussed, but it was unclear that the spectral features shown would have been expected to be different between these different portions of the same plant. To make this portion more convincing, I think it would be useful to bring additional comparison to spectra of the same components of trees in order to show either comparability or contrast.

It was also not clear what the atmospheric implications of this work would be. I understand that atmospheric relevance of small pollen particles, and to a lesser extent leaves that could fragment and disperse as aerosols in the atmosphere. The relevance of ground wood as ice nuclei was less clear. The authors attempted to make a case that the chemical components of the birch material was relatively similar across the portions of the plant and that aspects of the macromolecules could play a role in atmospheric ice nucleation. I didn't quite see the link, and I think the manuscript could be improved by more clearly discussing a direct link between the observations reported and some atmospheric implications.

Overall, the manuscript does have some interesting and novel data, and so those are worth publishing in some form. I suggest that the discussion be improved to make some of the statements a bit clearer, more defensible, and more obviously linked to ambient atmospheric processes. Additional, specific statements are listed below.

Pg 3, line20 – The authors removed "visible" contamination such as lichen. How might leaving "sub-visible" contamination affect the outcomes? I would think that removing only the obvious layer could include still significant amounts of nuclei that could still influence results. Alternatively, by taking the same sample and stripping the outer bark so that there was no possible contamination between external molecules (whether lichen, deposited pollutants, etc.) could isolate this issue. Pg 3, line27 – The drying process was continued until the weight was constant. How did the authors define "constant?" Pg5, related to Fig. 2 – Since the authors draw conclusions about the types of birch material (leaves, primary wood, etc.), it would be good to show averages + std dev

of each type on either the left or right within this figure. Pg 6, line33 - "pointing to the importance of polysaccharides in our extracts" This is an example of an overstatement, in my opinion. While the polysaccharides may include these specific infrared bands, fundamentally these are vibrational features of individual chemical bonds that can exist in many types of molecules. Pg 6, line 34 - "can be assigned to other biomolecules" ... similar to the comment above. I think it would be better stated as "are consistent with" in place of "can be assigned to" Pg 7, Section 3.4 – Subtle differences in intensity of fluorescence peaks here could easily be a function of analyte concentration. How did the authors control for concentration? If the authors are suggesting that the $\sim 10\%$ differences in the peak heights (e.g. of the 260 nm Ex) are due to chemical or biological differences in the sample, they should discuss how they are confident it is not just subtle dilution effects. Pg7, line 24 - "Most of our samples froze at temperatures close to the freezing temperature of birch pollen washing water." This line is a bit vague. What do the authors mean by "close to" here and "most?" Pg 8. line33 – "show strong similarities .. shown by Chen et al." Can the authors expand the discussion on this point? After looking up the spectra shown by Chen et al., I was a bit confused. I see that the Chen spectra seem to be somewhat higher in resolution, but otherwise I wasn't sure what specific points the authors were trying to extract from the comparison. Pg 8, first paragraph - How would these spectra look if you did the same with material from other tree species? Fluorescence spectra are always broad (i.e. compared to IR spectra), and then when grinding large volumes of material to be mixed into a sample for a spectrum - the analysis is obviously very homogeneous and mixed with huge numbers of types of molecules. It does not surprise me that these four sets of spectra look similar - it would surprise me if they looked very different. In contrast, I would expect the same spectra from another tree species to look very similar, so it is hard to know what this fluorescence spectra adds to the overall analysis in the manuscript. Can the authors provide comparisons to fluorescence spectra published elsewhere? Surely this has been done and is otherwise reported. Page 9, line 24 - "suggest that birch tissues tested contained chemical substances similar to birch pollen." I disagree

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with the weight of this statement. I think that the results suggest that the samples may have exhibited broadly similar IR and fluorescence spectral features, but to extend the statement to say that the "chemical substances" were similar was never tested directly here. Also, the data shown in the paper suggest that spectra from different types of material from the same plant are relatively similar, but differences across plant samples are not directly shown.

Figures – In general, I would suggest using color for figures 2-4. For Fig 2, I would also put the circle/triangle/star detail into the figure legend, and not just in the caption. This would make the complex figure easier to read.

Figure 3 – How do these data compare to other atmospheric measurements using similar techniques?

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