The authors would like to thank the referee for the time and effort in reviewing our manuscript „Birch leaves and branches as a source of ice nucleating macromolecules"

We would like to discuss two general points brought up by the reviewer:

1) The usage of fluorescence and IR spectroscopy as methods of characterization

Fluorescence and IR spectroscopy have various upsides, including the fast measurements and very little requirements on samples and preparation and are therefore often applied, especially on complex systems as biological samples. Especially IR spectroscopy is widely used in literature since it gathers detailed molecular information related to the chemical reactivity and biological activity of the samples. This is especially true for literature on the ice nucleation activity of birches and birch pollen. We included a paragraph on the usage of IR spectroscopy and fluorescence spectroscopy in the introduction section (p3, l3-10)

"Spectroscopic methods are a key instrument in characterizing complex biological systems. One of the methods typically applied on biological materials is infrared spectroscopy (Baker et al., 2015). Infrared spectroscopy can be applied for the characterization and discrimination of plants (Kim et al., 2004; Gorgulu et al., 2007; Anilkumar et al., 2012; Carballo-Meilan et al., 2014). Further infrared spectroscopy has already shown to respond well on the biochemical features of pollen of different species, allows differentiation of such (Gottardini et al., 2007; Pummer et al., 2013; Zimmermann and Kohler, 2014; Bağcioğu et al., 2015) and can even be used to gain information on the environmental conditions (Zimmermann and Kohler, 2014). While fluorescence spectroscopy is currently not used to discriminate different species, Pöhlker et al. (2013) showed that discrimination of pollen is possible with this technique on a family level."

2) The atmospheric impact of our work

We included a paragraph about the possible atmospheric impact concerning plant debris and number concentrations of the INP of birch trees in the discussion (p9, l31-41).

"Some investigations on birch stands showed a dry weight of 2 to 25 t per ha for twigs and 1 to 8 t per ha for leaves (Johansson, 1999; Uri *et al.*, 2007). This leads to estimated INP concentrations on the order of 10^{16} to 10^{19} per ha for twigs and 10^{14} to 10^{18} per ha for leaves. Plant debris can be an important constituent of ambient particulate matter (Matthias-Maser and Jaenicke, 1995; Andreae, 2007; Winiwarter *et al.*, 2009). However, the underlying processes of the release of plant debris in the atmosphere is not fully understood, making predictions of their atmospheric impact hard (Andreae, 2007; Winiwarter *et al.*, 2009). Sánchez-Ochoa and colleagues analysed atmospheric aerosols collected at various background sites in Europe and used cellulose as a proxy for plant debris. They found biannual average concentrations of 33.4 to 363 ng per m^3 air (Sánchez-Ochoa *et al.*, 2007). Especially the leaves of birch trees could be an important source for INP as it is shed and produces annually. Decaying leaf litter is known to be a good source of INP (R.C. Schnell and Vali, 1973). Conen et al. (2016, 2017) showed that air masses passing over land can be enriched with INP derived from such leaf litter. Collectively, these studies underscore the importance of plants as sources of INP."

To further document our fit into this journal, we would like to point to the sizeable number of papers published in ACP concerning primary biological aerosols and their impact on heterogeneous ice nucleation (as e.g. Huffman et al. 2013 10.5194/acp-13-6151-2013, Hummel et al. 2018 10.5194/acp-2018-182), as well as submicron biological INP (Pummer et al. 2012 10.5194/acp-12-2541-2012, Augustin et al. 2013 10.5194/acp-13-10989-2013, Pummer et al. 2015 10.5194/acp-15-4077-2015), and the influence of biological residues on the ice nucleation activity of other particles (Conen et al. 2011 10.5194/acp-11-9643-2011, Tobo et al. 2014 10.5194/acp-14-8521-2014, Hill et al. 2016 10.5194/acp-16-7195-2016, O'Sullivan et al. 2016 10.5194/acp-16-7879-2016). We think that our data can contribute to all of these fields. Also we would like to stress the point that our data indicate that there could be a greater fraction of heat resistant biological INP in the atmosphere suggesting that heat treatment alone is not sufficient to completely discriminate between biological and non-biologial INP.

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.

Response: As the samples used are of natural origin, we have to assume impurities to be present. However, since we do not know the distribution of the INP throughout the tissue and the role of the bark in this process, stripping samples could affect the outcome tremendously without pointing to the role of impurities per se. Especially problematic are the secondary wood samples, which often exhibit a rough fractured surface and we would need to strip not just the bark, but also the outermost layers of wood contained underneath to ensure the removal of all layers, which were in contact with the surrounding environment.

The centrifugation and filtration helps minimize the possible effect impurities can have on our samples, as most biological and mineral material, which is known to be ice nucleation active will not pass through the 0.2 µm syringe filter (we added a remark about this in the sample preparation section (p4, l4-7) *"Afterwards it was centrifuged (3500 rpm/ 1123 g for 5 min) and the supernatant was pressed through a 0.2 µm syringe filter (VWR, cellulose acetate membrane, sterile), removing all bigger particles, as well as possible impurities from e.g. intact bacterial cells."*). Especially biological material is in some cases known to release INP into the aqueous phase, which are in the submicron size range. These INP however, were shown to trigger freezing at temperatures typically above -10°C. As we did not observe a single freezing event at such high temperatures, we assume biological impurities to be of minor importance in our samples. To further address this important problem, we added another paragraph to the discussion (p10, l1-13) to discuss the possible role of impurities on our samples.

"Since all of the analysed materials are of natural origin, we cannot rule out that some contamination could play a role in the INA of our extracts. Some bacteria have been found to act as INP (as e.g. Pseudomonas syringae (Maki et al., 1974)), however, these bacteria are typically in the size range > 1 µm (Monier and Lindow, 2003) and therefore easily filtered with the 0.2 µm syringe filter. Further, some lichen are known to be INA (Kieft, 1988), and some microorganisms release their small contained INP in the aqueous phase as e.g. Mortierella alpine (Fröhlich-Nowoisky et al., 2015), which cannot be filtered with used methods. However, most known ice nucleation active lichens and microorganisms as well as released INP typically freeze at significantly higher temperatures (above -10°C (Maki et al., 1974; Kieft, 1988; Pouleur et al., 1992; Murray et al., 2012; Fröhlich-Nowoisky et al.,

2015) than the freezing temperatures observed for our samples, with very little exceptions (Iannone et al., 2011). As the highest onset temperature observed in our measurements was -14.1 °C (TBC-L), and the onset temperature of birch pollen washing water was quite close to this value (-15.1 °C), and heat treatment did not affect the extracts of TBA, we do not suspect significant contamination of our samples. However, the INA of birches, especially if growing close to a road or in urban regions, could be affected by soot and other anthropogenic emissions, as soot can act as INP (DeMott, 1990; Murray et al., 2012)"

Pg 3, line27–The drying process was continued until the weight was constant. How did the authors define "constant?"
Response: As weight consistency between two weight measurements separated by at least 2 h with ongoing drying procedure between the measurements. This has been included in the methodology part (p3-4, l41-1).

"All samples were dried for at least twelve hours. Weight consistency was determined by two weighing steps separated by at least two hours of drying."

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.
Response: The suggested changes have been implemented in Figure 2.

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.
Response: The statement has been removed. We included a short discussion on polysaccharides (p10, l 15-18)

"The measured FTIR spectra indicate that the birch extracts are chemically similar to each other, and to pure birch wood. As plants do not only contain polysaccharides but several soluble carbohydrates (Magel et al., 2000), we assume those substances to play an important role in the chemical composition of our extracts. Fitting to this assumption, most of the bands found in our spectra could be assigned to carbohydrates and polysaccharides."

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"
Response: This has been changed.

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 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.
Response: Unfortunately, we cannot control the analyte concentration, as the contained mixture in the different extracts is too diverse to be easily assessed. However, we do not suggest that this is due to chemical or biological differences. Quite on the contrary, we believe our spectra show quite well that none of the analysed extracts contain fluorescent analytes active in the observed range, which

cannot be found in all other extracts too. We broadened the discussion on this point (see p10, l34-37)

"Throughout the different extracts we found the same peaks, which might stem from similarities in fluorescent analytes between pollen and branch extracts. Small differences in intensities and ratios could result from differences in the concentration of the active substances"

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?"
Response: We specified this paragraph (see p8, l33-39).

"The freezing temperature observed for the aqueous birch pollen extract (-17.1 °C see Figure 2), is in line with values reported in the literature for aqueous birch pollen extracts (reported freezing events are generally between -15 and -23 °C (Diehl et al., 2001; Pummer et al., 2012; Augustin et al., 2013; O'Sullivan et al., 2015)). Interestingly, most of our samples froze in that temperature range between -15 °C and -23 °C.). Half of the leaves (TBC-L, TBD-L, TBF-L, TBG-L, and VB), eight out of ten primary wood samples (TBA-P, TBB-P, TBC-P, TBE-P, TBF-P, TBG-P, TBI-P, and TBV-P) and all secondary wood samples exhibited a mean freezing temperature in this temperature window. Moreover, we observed heat resistance at 100 °C, similar to the results of Pummer et al. (2012)."

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.
Response: The main reason to include this citation was the comparison between a pure wood sample and our aqueous extracts. With this we like to show that our extracts exhibit most IR spectroscopic patterns found in pure wood by other working groups except for lignin, which is only very weakly soluble in water. Therefore these differences were expected. We tried to make the point of this comparison clearer (see p10, l25-28)

"Other than the lignin bands, our aqueous extracts show very similar spectroscopic features compared to the pure wood samples. These similarities between the spectra of our extracts and the spectrum of pure wood indicate that our extract method retrieves the majority of components, leading to a similar distribution of bands, with differing intensities due to differences in concentration."

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.
Response: Pollen of several tree species have been analysed by Pöhlker et al. 2013 (10.5194/amt-6-3369-2013) and showed that the different species can be differentiated on a family level by maxima and relation between maxima. In our presented fluorescence data, the maxima of the different tree extracts and the birch pollen extracts look very similar not just in the position of the maxima but also

in the relation between the different maxima. The only exception is the primary wood showing a slightly enhanced peak maximum at the 260 nm excitation wavelenght.

Page 9, line 24 – "suggest that birch tissues tested contained chemical substances similar to birch pollen." I disagree 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.

Response: We changed this to *"aqueous extracts of birch materials tested showed similarities to aqueous extracts of birch pollen"* (p11, l35-36) Further we included another paragraph in the discussion section about similarities between the different samples (p11, l7-12)

"In both, FTIR and fluorescence spectroscopy, we found strong similarities between birch pollen washing water and the different aqueous extracts from the TBA samples. Further comparison with whole pollen grains (for both FTIR and fluorescence spectroscopy), as well as with pure wood (for FTIR), as found in literature, shows strong similarities in the spectroscopic features of our different birch samples. As not just the band position, but also the intensity ratios are agreeable with each other, we assume this to indicate that we are able to extract the major components found in wood with our extraction method and that the pollen and wood samples extracts exhibit chemical similarities to a certain extend."

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.

Response: The suggested changes have been implemented.

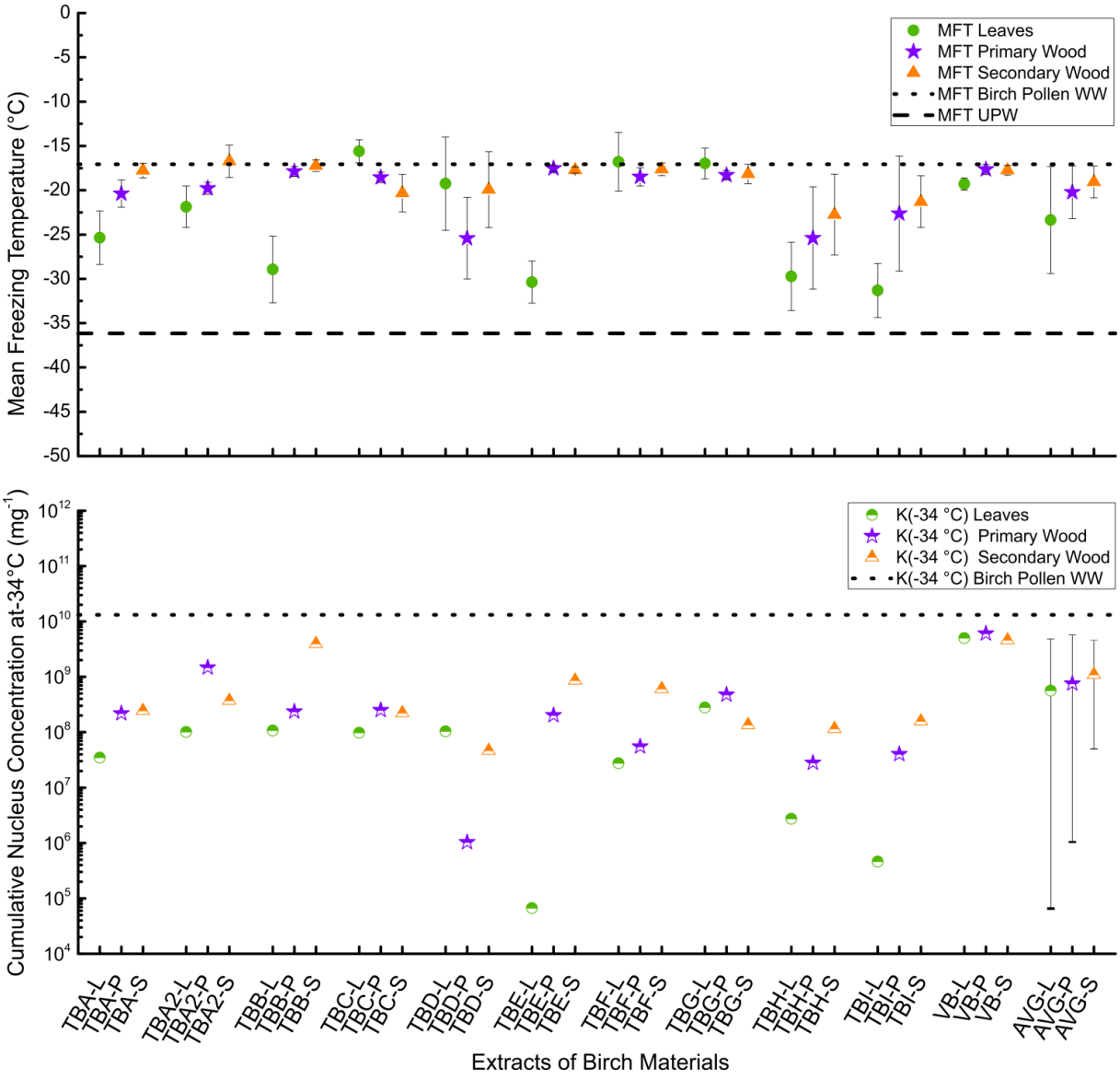

Figure 1: Top panel: Mean freezing temperature (MFT) of the different birch samples. Leaf extracts (L) are marked with a green circle, primary wood extracts (P) with a violet triangle, and secondary wood extracts (S) with an orange star. Further we introduced a dashed line for the MFT of ultrapure water (as a summary of regular measurements conducted over the course of the analysation of the presented samples, -36.2 °C, with a standard deviation of 0.5 °C (not plotted)), and a dotted line for the MFT of birch pollen washing water (-17.1°C with a standard deviation of 0.5 °C (not plotted)). The last three values on the right side represent the average of all mean freezing temperatures for leaves (AVG-L), primary wood (AVG-P) and secondary wood (AVG-S) with the corresponding standard deviation. Bottom panel: cumulative nucleus concentration at -34°C (K(-34 °C)) of the different birch samples per mg extracted sample. Assignment of the symbols is similar to the MFT plot. The dotted line refers to the K(-34 °C) of birch pollen washing water per mg extracted pollen ($1.3*10^{10}$ mg^{-1}). The last three values on the right side represent the average of all K(-34 °C) values. Error bars point to the area of trust, ranging from the highest to the lowest measured values.

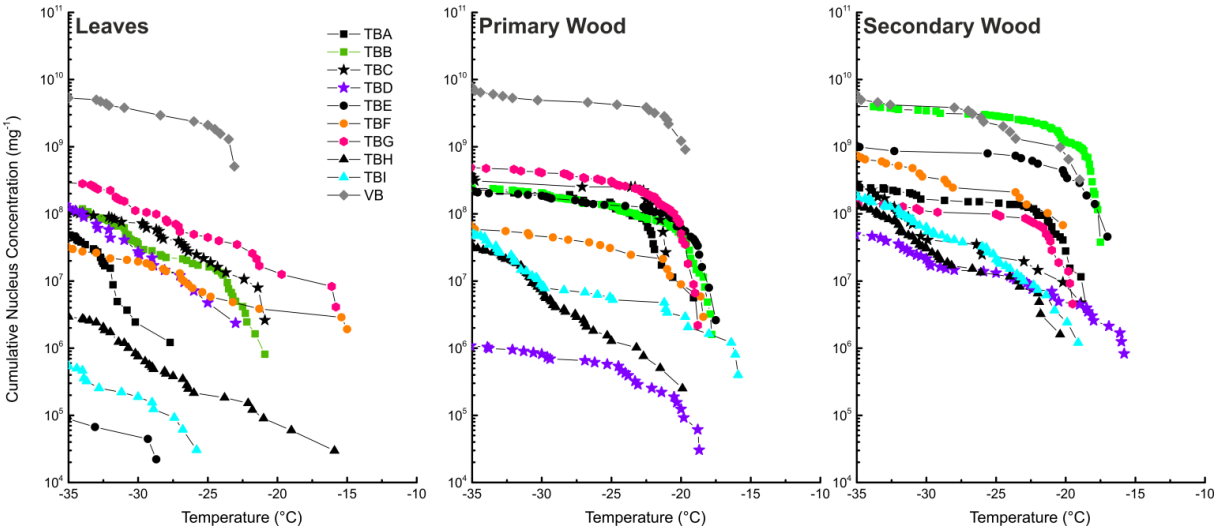

Figure 2: Cumulative nucleus concentration as a function of temperature for leaf extracts (right), primary wood extracts (middle), and secondary wood extracts (right). The diagram is cut off at -35°C, since we cannot contribute freezing events below this temperature to heterogeneous nucleation. The symbols used for the different data points are grouped. Birches growing in close proximity under similar conditions are marked with the same symbol (different fillings).

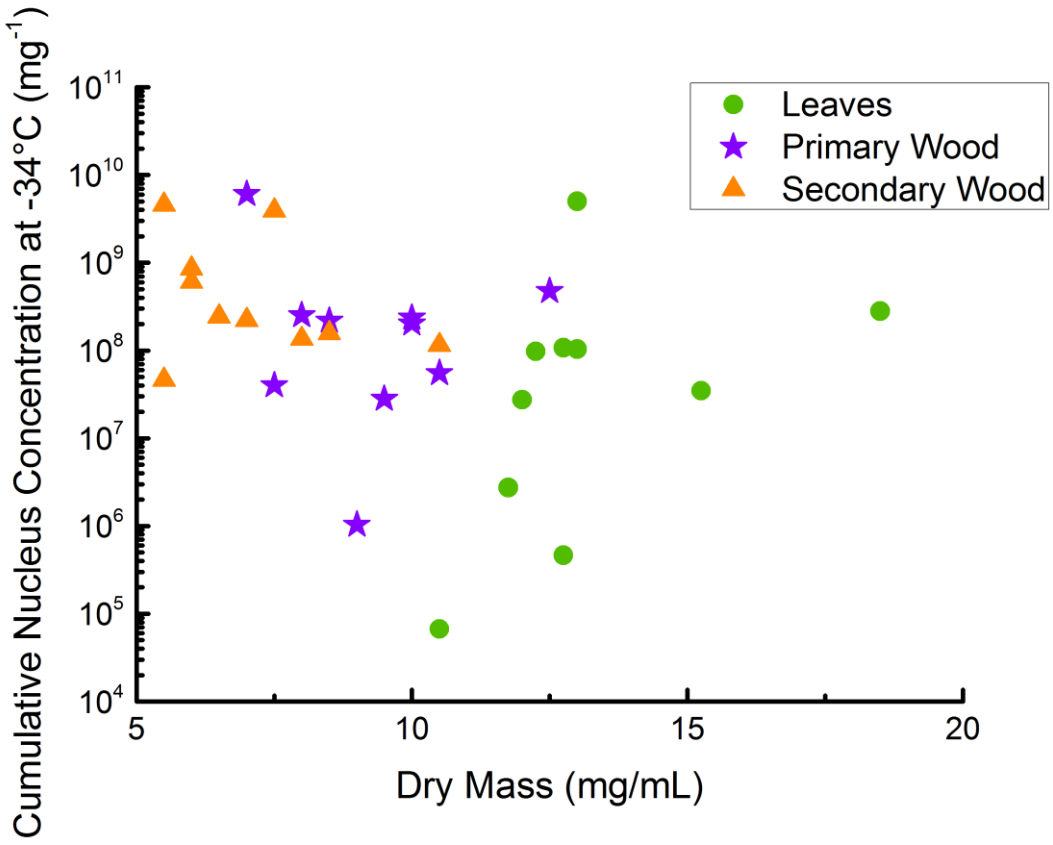

Figure 3: Scatterplot of dry mass (dry residues of the different filtered extracts) and cumulative nucleus concentration at -34°C per sample mass. The dry mass is the mass we were able to extract with the 50 mg/mL suspensions. The data show that secondary wood, which contained mostly the highest INM concentrations and lowest variations between different samples, also contained the lowest extractable mass. Therefore INM ratios in the extractable content of the different samples were highest in secondary wood samples.

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

similar techniques?

Response: We compared our data to the freezing temperature range of other known atmospheric INP and precipitation samples in the discussion section (p11, l14-24), where we compare our results to different substances and try to sum up what is known about the identity of the birch pollen derived INP.

"Only little INP are known to trigger freezing above -10°C, which are typically biological substances such as bacteria (Murray et al., 2012). Below -10 °C, birch pollen belong to the group of highest freezing temperatures, with onset higher than most mineral dusts, ash and soot samples (Murray et al., 2012). The vast majority of atmospheric INP and INP retrieved from precipitation samples exhibit freezing temperatures below -10°C (DeMott et al., 2010; Petters and Wright, 2015). The identity of the INP released from birches is still unclear. Pummer et al. (2013) showed that proteins, saccharides, and lipids are easily extracted aqueously from birch pollen. While Pummer et al. (2012) and Dreischmeier et al. (2017) speculate that the responsible molecules are carbohydrates, Tong et al. (2015) attributes the highest INA to extracted proteins. Hiranuma et al. (2015) showed that cellulose, which is ubiquitous in plants, exhibits INA in the right temperature range With our spectroscopic data, we found strong indicators for saccharides being present, including prominent bands which could be associated with cellulose. Further, we found bands in the most prominent protein regions, though those could be assigned to other molecule groups."

Further changes:

We excluded the Saxena reference in the introduction

Figure 2 was split into 2 panels. Further we included the K(-34 °C) per mg birch pollen as reference line (introduced in p6, l20-22)

"The dotted line in the lower panel refers to the K(-34 °C) value of birch pollen washing water ($1.3*10^{10}$ mg^{-1}). Presented data shows that the samples with the highest K(-34 °C) values (TBB-S, and all samples from the Viennese birch) contain similar amounts of INP per mg extracted sample."

We further included Sheil 2018 in the introduction (p 2, l 20-23)

"*While we know that forests influence the atmospheric water-cycle, the underlying processes are only poorly understood and characterized and it is important to further our understanding in this area, not just to enhance climatic predictions, but also to better understand the consequences of the changes in Earth's forests due to human activities (Sheil, 2018)."*

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