

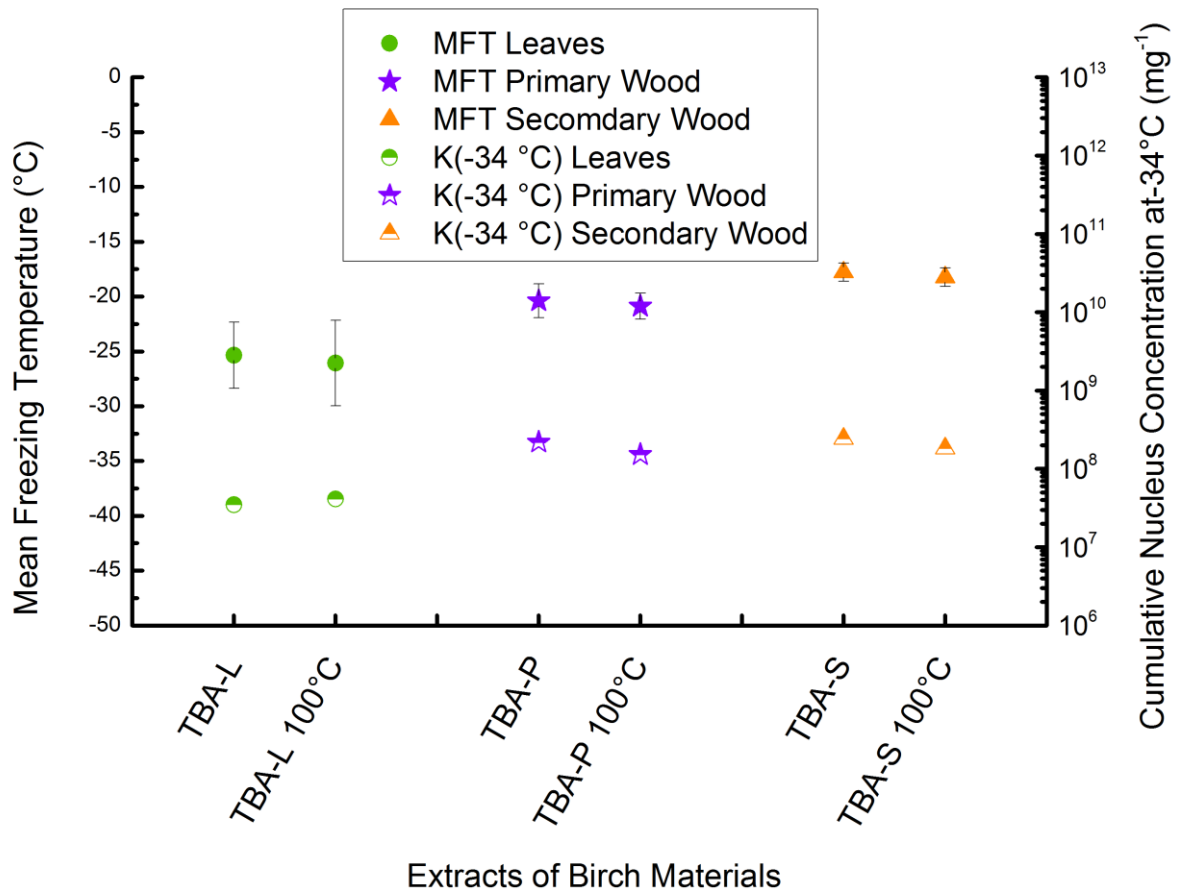
The authors would like to thank the referee for the time and effort in reviewing our manuscript „Birch leaves and branches as a source of ice nucleating macromolecules”

*a.) Spectral analysis of extracts lead to the conclusion that birch leaves, twigs and branches contain chemical substances similar to those in birch pollen, which implies that INP in either material carry of the same sort of ice-nucleating macromolecules (INM). If so, leaf, twig, and branch INM should equally withstand denaturation at temperatures up to 445-460 K, which clearly distinguishes birch pollen INM from bacterial and fungal INM that are already denatured at much lower temperatures (Pummer et al., 2015, <https://doi.org/10.5194/acp-15-4077-2015>). Did you test the heat tolerance of your samples? If so, what was the result?*

Response: We conducted heat experiments at 100 °C, which showed no changes in the INA of the different extracts from the birch TBA. We included these results in section 3.2 (p7, 11-18):

*“To analyse the similarities to birch pollen washing water, all three extracts of TBA were treated at 100 °C following the protocol introduced by Pummer et al (2012). Therefore 100 µl of each extract were applied on a clean glass slide and put in an oven set to 100 °C. After an hour the dry residues were resuspended in 100 µl of ultrapure water each and analysed for INA. The results of this experiment are given in Figure 1 as MFT and K(-34 °C) values. The corresponding values of the untreated TBA extracts are plotted for comparison. We find no major changes in the mean freezing temperatures (TBA-L -25.4 °C, TBA-L treated -26.1°C; TBA-P -20.4 °C, TBA-P treated -20.9 °C; TBA-S -17.8 °C, TBA-S treated -18.2 °C) or K(-34 °C) values (TBA-L  $3.5 \cdot 10^7 \text{ mg}^{-1}$ , TBA-L treated  $4.1 \cdot 10^7 \text{ mg}^{-1}$ ; TBA-P  $2.2 \cdot 10^8 \text{ mg}^{-1}$ , TBA-P treated  $1.5 \cdot 10^8 \text{ mg}^{-1}$ ; TBA-S  $2.4 \cdot 10^8 \text{ mg}^{-1}$ , TBA-S treated  $1.8 \cdot 10^8 \text{ mg}^{-1}$ ).”*

and with the corresponding Figure 5:



**Figure 1: Results of the heat treatment of the different TBA extracts. Leaves are marked with green circles, primary wood with violet stars and secondary wood with orange triangles. The left value belongs to the untreated sample, the right value to the sample treated with 100 °C for an hour. Filled symbols represent the mean freezing temperature and correlate with the left Y-axis, half filled symbols represent the cumulative nucleus concentration as -34 °C per mg extracted sample and correlate with the right Y-axis.**

and in the discussion section about the similarities to birch pollen washing water (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).”*

*b.) Another issue I would like to see addressed with regard to the nature of the INM is whether they could be a form of cellulose. This issue could be discussed with reference to the FTIR spectra in Figure 5 and also with regard to the slope of the cumulative nucleus spectra (Figure 3), as compared to similar spectra available for cellulose (e.g. Hiranuma et al., 2015, doi:10.1038/ngeo2374).*

Response: As the INP are contained in quite low concentrations, it is challenging to use the FTIR spectra for qualitative analytics of the INP. However, we added a section concerning the possible identity of the INP in the discussion section (p11, l14-24).

*“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 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.”*

*c.) In the Discussion you write that INM could be “: :washed into the soil during rainfall: :” (page 7, lines 29-30). Leaves and twigs are usually covered by a thin layer of wax to protect against desiccation. I wonder whether INM sitting in the tissues below the protective outer layer could be washed off. Wouldn't leaves and twigs first need to be shed and to disintegrate for INM to be washed off in larger numbers?*

Response: We changed this into *“Cracks and wounds on the surface could allow the INP to be washed off the surface of twigs and leaves into the soil. This marks a potential to influence the INA of mineral dust and soil particles and act as INP in the atmosphere.”* (p8, l26-28) and added a small discussion on the importance of further studies on this topic. (see p8, l 30-31)

*“Further studies on possible release pathways of the INP from birches into the surrounding environment are necessary to quantify such effects.”*

*d.) In Section 2.1 you introduce the altitudinal gradient along which you sampled the trees. Later in the paper there seems no further reference to this gradient. Instead, you relate results to the proximity of the trees to road or river. Is altitude irrelevant for the production of INM? Could similarity in terms of INM in a particular kind of location result from a genetic proximity of the trees (i.e. seeds spreading along a road or a river)?*

Response: We found no correlation between altitude and INM production. We expanded the discussion on this point based on your suggestions (see p9, l28):

*“Other than roads and rivers in close proximity, the tree altitude was not correlated to INA.”*

*Page 2, line 9: Please be more precise. Concentrations reported by Christner et al. (2008) were quite low (at -10 C: 4 to 490 INP/L) compared to other studies (up to 500'000 INP/L at -10 C; Petters and Wright, 2015, [dx.doi.org/10.1002/2015GL065733](https://doi.org/10.1002/2015GL065733)). What the paper by Christner et al. (2008) indeed has clearly shown was the large fraction (95%) of biological INP in the total number of INP.*

Response: This has been changed and the Petters and Wright citation has been included (p 2, l 6-11)

*“Precipitation can contain large amounts of INP. Petters and Wright (Petters and Wright, 2015) combined data from a large number of measurements and found a high variability in concentration in the range between -5 and -12 °C, which is assumed to be biological, with a maximum of approx. 500*

000 per L water. Christner et al. (2008) analysed snow and rain samples from the United States (Montana and Louisiana), the Alps and the Pyrenees, Antarctica (Ross Island) and Canada (Yukon), where they found rather low INP concentrations, but biological INP to represent the majority of the contained INP.”

*Page 2, line 20: ‘mechanism’ seems more appropriate here than ‘tool’ (same in line 35).*

Response: The suggested changes have been implemented.

*The term “tissue” you use to denominate your samples does not seem correct to me. As I understand, you processed entire leaves and sections of twigs and branches, which you call primary and secondary wood. Branches, for example, are made up of several types of tissue (xylem, phloem, sclerenchyma, cortex, epidermis). I would find it more appropriate to not talk about “tissue” in your context but say that you analysed material from different parts of the trees (leave, twig, branch).*

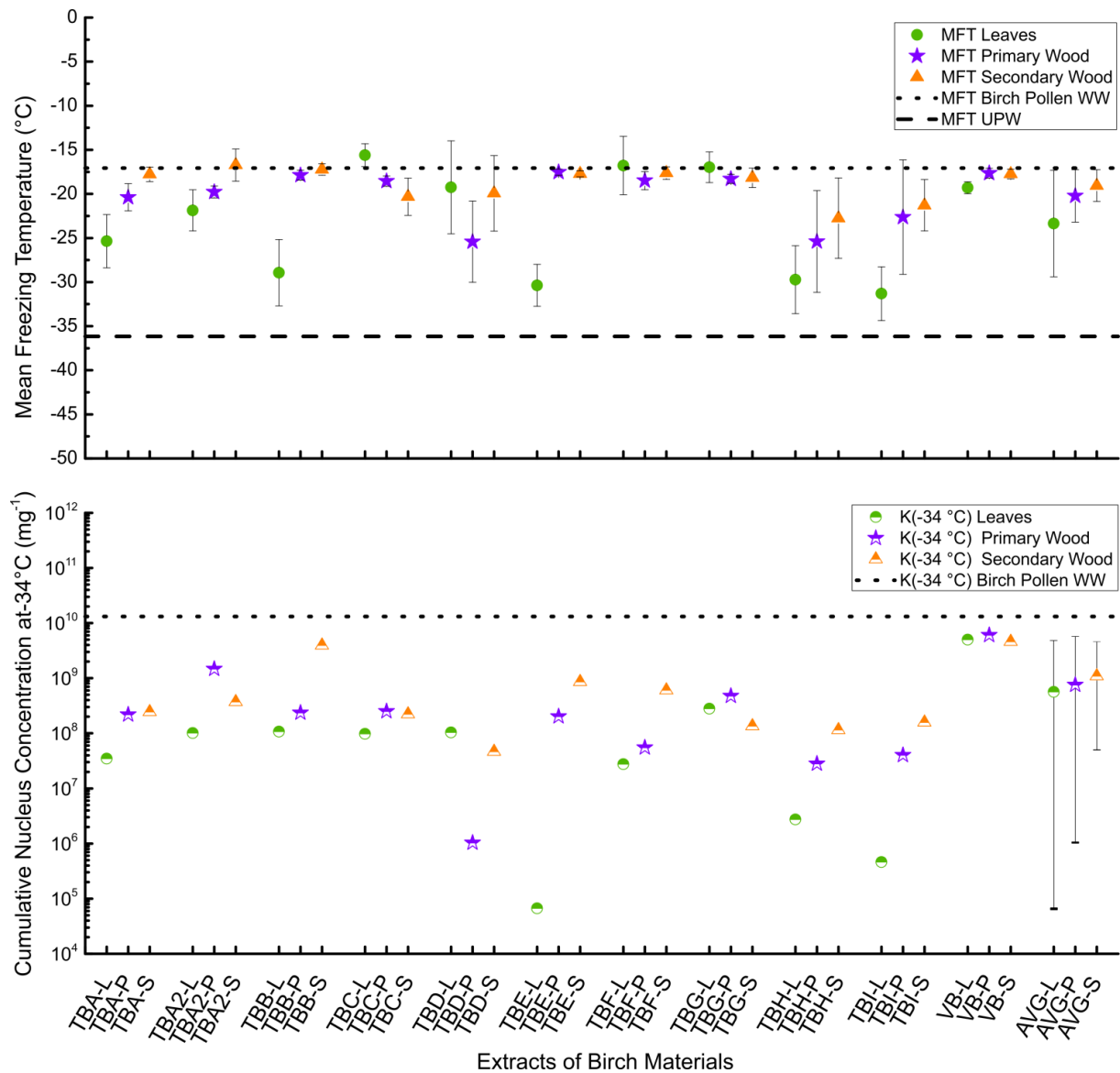
Response: The suggested changes have been implemented and the terms have been changed throughout the manuscript.

*Trees differ in MFT and cumulative nucleus concentration in leaves. How reproducible are these values? Did you prepare and analyse, perhaps during the preparatory phase of your study, two or more samples from the same tree, i.e. did you process from one or several trees two sets of leaves or two sets of twig material?*

Response: We analysed a second branch from the birch TBA according to the described protocol. While we found minor differences for the primary and secondary wood samples, we found a significantly enhanced freezing temperature in the leaves of the second twig. The concentration of INP in the leaves remained constant. We included this in our results section (p6, l28-34):

*“To examine the INP distribution within a tree, a second branch of TBA was prepared and measured according to the described protocol. Resulting data are presented in Figure 2 and marked with a 2 (TBA-L2, TBA-P2, and TBA-S2). Primary and secondary wood extracts are well in line regarding their freezing temperatures (TBA P -20.4 °C, TBA-P2 -19.8 °C; TBA-S -17.8 °C, TBA-S2 -16.7 °C), however, the primary wood from the second analysed branch contained higher INP concentrations (TBA P  $2.2 \cdot 10^8 \text{ mg}^{-1}$ , TBA-P2  $1.5 \cdot 10^9 \text{ mg}^{-1}$ ; TBA-S  $2.4 \cdot 10^8 \text{ mg}^{-1}$ , TBA-S2  $3.7 \cdot 10^8 \text{ mg}^{-1}$ ). Leaves varied in their freezing temperatures and cumulative nucleus concentrations (TBA-L -25.3 °C and  $3.5 \cdot 10^7 \text{ mg}^{-1}$ , TBA-L2 -21.8 and  $1.0 \cdot 10^8 \text{ mg}^{-1}$ ).”*

as well as in Figure 2:



**Figure 2:** 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 analysis 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 ( $1.3 \cdot 10^{10} \text{ mg}^{-1}$ ). 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.

and the discussion section for the variability of the INA of leaves (p9, I5-6).

*“We observed a high variability of INM in leaves. Even for leaves of two branches of the same tree, we found differences in their freezing temperatures.”*

**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 ( $1.3 \cdot 10^{10} \text{ mg}^{-1}$ ). 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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