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Interactive comment on "Surfaces of Silver Birch (*Betula pendula*) are Sources of Biological Ice Nuclei: *In-vivo* and *In-situ* Investigations" *by* Teresa M. Seifried et al.

Anonymous Referee #2

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Plants are a source of ice nucleating particles found in the atmosphere. What fraction of emitted particles is synthesized by plants and what fraction is generated by microorganisms thriving on their surfaces is an open question. Another open question concerns the mechanisms by which rainfall aerosolises either kind of particle. Through the analysis of ice nucleating molecules (INMs) washed-off different parts of birch trees (in vivo) and in rain sampled below birch canopies and in open areas (in situ) the present study provides further proof that plants are sources of such entities released to the environment. Sampling and analysis were done very well. Results are clearly presented and the manuscript is overall a good read.

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Of major concern to me are interpretations that are biased by the lower limit of detection being around 100'000 INMs cm-2, as gleaned from Figure 5. Though the design of the freezing assay provides for exploring freezing temperatures approaching homogenous freezing, it becomes increasingly 'blind' towards the warmer side of the temperature range because of the small sample volumes analysed. Not taking this fact into account leads to the questionable interpretation that the phytobiome on the surfaces of birch trees is a minor contributor to the population of INMs released to the environment (e.g. lines 229 to 231 and lines 242 to 243). Certainly true for temperatures below about -20 °C, this interpretation is most likely not true for temperatures above -10 °C or so. Support for this guess can be found in Figure 5, Trees D and G, where INM concentrations on leaves start to overtake those of other parts at around -17 °C. If data at warmer temperatures would be available, they would probably show increasingly larger ratios of leaf INM concentrations to those of wood or bark towards the warmer end of the temperature range. Therefore, I would suggest to either mention this possibility in the 'blind spot' of the assay, or to explicitly limit interpretation to temperatures below -20 °C.

Another issue that I would like the authors to address concerns the conclusion of '...similarities between in-vivo prepared extracts and in-situ sampled rainwater.' (line 291). It is not entirely clear to me. By looking at Figures 5 and 6, I see similarities in the shape of INM spectra for Trees G and H (leafless), but not for Trees E and F (with leaves). Latter have INM spectra in rainwater with approximately linear slopes on the log-scale, while the spectra of sampled material from these trees are mostly horizontal between -34 °C and -25 °C, then diving off towards warmer temperatures. I think the manuscript would benefit from additional discussion of similarities and dissimilarities of the INM spectra.

Minor comments

Please provide information on INM concentrations found or not found in the ultrapure water used in the assays (laboratory blank) and also give an estimate for the lower limit

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of detection (around 100'000 INMs cm-2 ?).

Line 100: consider replacing 'pole testing drill' with 'increment borer', which is the correct technical term (see website of the instrument producer, http://www.haglofcg.com/index.php/en/products/instruments/survey/389-increment-borers).

Why did you choose to report K for-34 °C and not (also) for a warmer temperature? The -34 °C are so close to homogenous freezing, that the relevance of INMs at this temperature in what happens in nature seems questionable to me. Where K(-34 °C) values are mentioned, perhaps add in brackets also K values for a warmer temperature (e.g. -25 °C?).

Line 183: Please say what 'y' and 'x' stand for, including their units. If 'y' is K(-34 °C) in [cm-2] and 'x' is 'distance from surface of the stem' in [cm], then the concentration of INMs halves every 0.6 cm from the surface towards the core of the stem. Expressed this way, the information contained in the equation would be more amenable.

Line 190: '...samples did not show any ice nucleation activity,...' This statement depends on the detection limit of the freezing assay. To be more precise, you could say something like '...samples had K(-34 °C) values below about 10^-5 cm-2,...'

Line 199: What do you mean with 'blank samples'? Samples of ultrapure water or precipitation samples collected in an open area, outside the influence of a tree crown?

Lines 220 and 221: '... concentration too low to be captured with our freezing assay.' Again, I think it is important to state in the methods section the lower limit of detection and re-iterate it in a context like in these lines. Ice propagates quickly in or around plants (e.g. Hacker and Neuner, 2008, https://doi.org/10.1657/1523-0430(07-077)[HACKER]2.0.CO;2). Hence, a single freezing event (i.e. INM) can affect the entire plant.

Lines 235 and 236: ' This could be either due to the sample collector been situated

too far from the tree, due to the interaction between rain and the tree's surface being insufficient, or that the western part of the tree exhibits fewer INMs.' I find the second assumption most convincing. Does rain typically come with westerly winds? If so, particles detached during a storm would mainly be found to the East of the tree.

Lines 244 and 245: One of the litter samples analysed by Schnell and Vali (1973) was re-analysed recently and, in addition to P. Syringae, further ice-nucleating species were identified in it (Vasebi et al., 2019, https://doi.org/10.5194/bg-16-1675-2019).

Lines 254 to 274: This is a courageous extrapolation! The number of INMs potentially released by trees is impressive. But, would these INMs not have to be lofted to the height of cirrus clouds to become activated? Although the INMs themselves are small, the question remains whether they are aerosolised as such or associated with larger particles? I think this issue needs attention in future studies. It would be useful to see the same extrapolation for INMs active at a warmer temperature (e.g. -25 °C ?).

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