Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2018-496-RC1, 2019

Biogeosciences Discussions

Interactive comment on "Comprehensive characterization of an Aspen (*Populus tremuloides*) leaf litter sample that maintained ice nucleation activity for 48 years" *by* Yalda Vasebi et al.

Morris (Referee)

cindy.morris@avignon.inra.fr

Received and published: 23 January 2019

GENERAL DESCRIPTION OF THE WORK AND REMARKS The goal of the work reported in this paper is to reveal the basis for the stable ice nucleation activity (INA) that has been maintained in a nearly 50-year-old sample of leaf litter of Populus tremuloides that has been stored by one of the authors. The authors have focused on the hypothesis that the INA is of microbial origin because the level of activity detected in the litter was absent in the green leaf tissue and that it increased during leaf decay (as described in previous works on these substrates). In the original description of the INA

of this material (Schnell and Vali, 1976 as cited in the manuscript), the INA component passed through a filter with 0.1 μm-diameter pores. This led the authors of the current manuscript to propose that bacteria such as Pseudomonas syringae that are not known to produce cell-free ice nuclei would not be at the origin of the INA of this plant material. They suggest that other microorganisms or pollen that do produce cell-free INPs might be responsible.

To characterize the possible causes of the INA of this leaf litter, the authors conducted tests of INA on a series of filtered washings and on the filter retentate – with or without boiling. They also conducted metagenomics analyses and direct microbial cultivation to describe the microorganisms that were associated with the litter. They present corroborative information to defend various hypotheses of which microbial components could be implicated in the INA of this leaf litter.

The methods are of very good quality and the results are clearly presented. However, I think that it is unfortunate that the authors so readily evacuated hypotheses about origins other that microbial particles or components as the source of INA. Also, they do not give sufficient information about the efficiency of removal of particles retained on filters in the quantification of their contribution to the INA of the litter. I suggest that the authors modify the manuscript to take into account the comments below.

Also, in the Discussion and Conclusions the authors do not indicate the significance of their findings. What if we knew precisely what contributed to long term maintenance of INA in leaf litter – so what? It would be useful to offer thoughts on that question.

SPECIFIC REMARKS 1. In the introduction the authors have not sufficiently covered the ensemble of hypotheses about the possible origins of the INA of the leaf litter. Why do they fully exclude material of plant origin? I understand that these materials will not "multiply" during the decay of the litter. But what if they were a product of decay of the leaf material via microbial activity? They should look more closely at the article by Pummer et al, 2015 (Ice nucleation by water-soluble macromolecules. Atmos. Chem.

Phys., 15, 4077-4091 – not cited in their manuscript) at all of the possible chemistry involved in this activity. They should modify the introduction to indicate that they focus on the hypothesis of microbial origin but that other hypotheses are viable. This is particularly important in light of the ambiguity of their results vis-à-vis microorganisms as the source of the INPs in the litter.

2. In the introduction I was also surprised that the reader was not reminded of one of the ways in which bacterial ice nucleation activity was discovered. In the chapter about the discovery of bacterial INA by Vali and Upper (in "Biological Ice Nucleation and its Applications" 1995. APS Press, Lee et al (eds)) C. Upper describes how dried, powdered corn leaves (prepared from field-grown corn) had high levels of INA that led to frost damage of corn when applied in the field as a means to inoculate plants with the northern leaf blight pathogen. This powder was maintained in the refrigerator between field seasons and maintained INA and microbial viability. It led to one of the independent discoveries of the INA of P. syringae in the 1970's.

3. Pg 3, ln 15: The authors state that the litter "is still producing" prodigious numbers of ice nuclei. Do they mean that the litter still "contains" high numbers of ice nuclei? If they do indeed mean "producing" then it would be useful to describe the dynamic process of increase of the ice nuclei in the litter at the sampling site.

4. For the same line as mentioned in remark #3, the authors state that the INA in the litter at the sampling site is as warm at -4.5 to -5°C. In contrast, in the introduction the litter is described to have activity as warm as -1.3°C. It would be useful if the authors provide information about the activity initially described for the sample they are characterizing (#INP/g tissue at warmest temperature detected). This would help defend their basic assertion of the long term maintenance of the INA of this sample.

5. In the Methods section the authors do not give any information about the efficiency with which particles retained on the filter are removed by washing. The efficiency of removal will have an impact on their calculations of the contributions of larger particles

to the INA of the litter. I suspect that it is very difficult to assess the washing efficiency. Hence, it would have been interesting if the authors had tested filters directly, with a quantitative method (Conen et al, 2012. Atmospheric ice nucleators active > 12 °C can be quantified on PM10 filters. Atmos. Meas. Tech. 5:321-327). It would greatly improve this manuscript if the authors could compare the estimates they make from washing filters vs testing them directly. They could conduct such comparisons for one type of sample in order to assess the error.

6. The authors state in the Discussion and Conclusions that "most Pseudomonas species do not shed the INA protein as part of extracellular vesicles" (pg 9, ln 9). Has this question been comprehensively evaluated? How many strains have been tested and under what conditions? Perhaps this possibility needs to be re-examined.

7. The Discussion and Conclusion ends with remarks on the need for universal tools to find genes for INA. What is the justification of this part? As the results do not unambiguously point to a microbial origin for the INA, this is not the obvious perspective for this work. Furthermore, they state that such tools would be useful to know which organisms contributed most to INPs in the atmosphere. Again, I do not see the link with leaf litter. The authors do not show or mention studies indicating that leaf litter is what carries INPs into the atmosphere.

8. I am surprised by the graphic in Fig 1. Why did the authors draw leaves? They state that they are working with litter. This is misleading. Furthermore, in this graphic, the leaves are oak leaves. But they are working with litter from Aspen trees (that have a completely different form from oak leaves).

9. In figure 3, the legend for Fig 3B is logical, i.e. the order of the colors/names in the legend parallels that in the bar. However, those of Fig 3A and 3C are in the inverse order. Please change the top-to-bottom order of these latter two legends.

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