Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2018-496-AC1, 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.

**Yalda Vasebi et al.**

vinatzer@vt.edu

Received and published: 3 March 2019

REVIEWER COMMENT: 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. RESPONSE: We really appreciate the detailed, extensive, and insightful comments provided by reviewer #1, Cindy Morris. We agree that we cannot a priori exclude that the leaves themselves are the source of INA. Therefore, when revising the manuscript, we will specify in the last paragraph of the introduction that



we focused in this manuscript on the hypothesis of bacterial or fungal sources of INA. We are planning to say: "... While the INPs in the sample could be of plant origin (Pummer et al 2015), here we tested the specific hypothesis that the INPs in this leaf litter sample were produced by strains of Lysinibacillus or by other bacteria or fungi. To test this hypothesis, we performed a comprehensive characterization of sample 70-S-14 in regard to its content in INPs and the composition of its microbial community." REVIEWER COMMENT: Authors do not give sufficient information about the efficiency of removal of particles retained on the filters in the quantification of their contribution to the INA of the litter. RESPONSE: We would like to point that for the litter we purposely measured INA of the filtrate and the retentate at each filtration step. This allowed us to determine if any INPs were retained by the filter, in other words, if there were any INPs that could neither pass through the filter nor could be resuspended from the filter. In the case of the 0.22 um filter, most INPs were found in the filtrate. Therefore, retention on the filter was not a problem. In the case of the 100kDa filter, comparing the activity of the filtrate with that of the retentate, we found that most INPs were in fact retained on the filter. Therefore, we already pointed out on page 6, line 20, that this was probably the reason for seeing a loss of INPs. To make this statement clearer, we will say in the revised manuscript "Intriguingly, resuspensions of the INPs from the 100kDa filter revealed a concentration of INPs in the 100kDa filter retentate that was even lower than that in the 100kDa filtrate. This could be due to the majority of INPs strongly binding to the filter." REVIEWER COMMENT: What if we knew precisely what contributed to long term maintenance of the INA in leaf litter – so what? It would be useful to offer thoughts on that question. RESPONSE: We agree with the reviewer that we did not adequately address this question. We are planning to modify the conclusion section and say: "... In conclusion, we think that combining the results from these recent studies with our new finding that 70-S-14 still contains viable Pa. ananatis and M. alpina with INA, supports a role of these organisms as important sources of atmospheric INPs. Importantly, finding that heat-sensitive sub-micron INPs are still active after 48 years in leaf litter suggests that leaf litter might represent an important reservoir of atmospheric

INPs. The relative importance of leaf litter compared to live plants and soil as a contributor to the atmospheric pool of INPs is thus an important question that needs to be investigated. REVIEWER COMMENT: 1. . . . Authors 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. RESPONSE: We completely agree with this comment and we will follow this advice in the revised manuscript as detailed in our response above. REVIEWER COMMENT: 2. . . . the reader was not reminded of one of the ways in which bacterial ice nucleation was discovered. In the chapter about the discovery of bacterial INA by Vali and Upper, Upper describes how dried powdered corn leaves had high levels of INA that led to frost damage . . . 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. RESPONSE: We already cited the original paper by Arny et al (1976) for the independent discovery of INA in P. syringae and we will specify in the revision that P. syringae was isolated from dried powdered corn leaves in this work. REVIEWER COMMENT: 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. RESPONSE: We will change this sentence in the revised manuscript and replace "is still producing" with "still contains". REVIEWER COMMENT: 4. . . . the authors state that the INA in the litter at the sampling site is as warm at -4.5 to -5âŮęC. In contrast, in the introduction the litter is described to have activity as warm as -1.3âŮęC. 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. RESPONSE: Since we could not assay the leaf litter using the exact same conditions as 48 years ago, any direct comparison of INA is difficult. However, we an-



swer the reviewer's question in the manuscript on page 6, line 10, where we report the observed difference between the data published in 1976 and now in detail: "Based on the earlier tests of ice nucleation on less aged 70-S-14 by Schnell and Vali (1976), it appears that sample of 70-S-14 lost between 1°C -1.5 °C of threshold ice nucleation activity and an order of magnitude in total INP concentration active at -10°C to -12°C over the 48 years of storage." REVIEWER COMMENT: 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 âŮęC 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. RESPONSE: As stated in our response to an earlier comment, we compared INA of the filtrate with INA of the retentate for each filtering step. For example, we clearly state in the manuscript that for the leaf litter sample the majority of the INPs were retained on the 100kDa filter. Since we measured how many INPs passed through the filter, we do not think that quantifying how many of the INPs were retained on the filter compared to how many INPs were resuspended from the filter would change the conclusions of the manuscript. REVIEWER COMMENT: 6. The authors state . . . 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. RESPONSE: We agree that our statement is based on the cited literature but that this possibility should be re-examined in the future. REVIEWER COMMENT: 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 un-ambiguously 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. RESPONSE: We agree that we were not clear in our rationale explaining the importance of identifying INA genes for determining the importance of leaf litter as source of atmospheric INPs. In the revised manuscript, we are planning to say "Identifying the genetic basis of biological INPs produced by additional bacteria and by fungi would instead allow determination of the presence of all these various INA genes in environmental samples, such as soil, plants, leaf litter, precipitation, and even clouds. Comparison of presence and abundance of various INA genes between samples could in turn help infer the migration of microbes with INA among environments and their relative contribution to atmospheric INPs." REVIEWER COMMENT: 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). RESPONSE: We agree with the reviewer and will change the type of leaves shown and make the leaves look more like litter. REVIEWER COMMENT: 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. RESPONSE: We agree with the reviewer and we will change the order in which we list taxa for Figures 3A and 3C.

---

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-496, 2019.