

Interactive comment on “High concentrations of biological aerosol particles and ice nuclei during and after rain” by J. A. Huffman et al.

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Received and published: 10 March 2013

GENERAL REMARKS The authors have presented extensive details about aerosol composition and concentrations following rain events. The data are interesting and their publication would be a service to the scientific community. The extent of their impact on our understanding of the interaction of land surface covers, bioaerosols and atmospheric processes is not as palpable as the authors claim because some important details are missing in the presentation of the materials and methods and in the results. In the current version of the manuscript, the description of the experimental set-up, in terms of the exact placement of samplers and environmental probes in the forest canopy, is incomplete and makes it difficult to evaluate some of the phenomena observed and how they are related to atmospheric phenomena on a broader scale. Re-

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sults of the extensive microbial analyses could be presented in more detail to allow the reader to evaluate the validity of the conclusions. The conclusion should be strengthened to better highlight the novel conceptual contributions of this work and how it will boost our understanding of the link between biological ice nucleators and rainfall.

SPECIFIC REMARKS P 1769-1770 L 26, 1-2 : The authors should give more background information to explain the logic of their argument. They state that “however, evidence linking bioaerosols with increases in IN, especially . . . following precipitation, is limited”. Why would they expect there to be a link with IN after precipitation? Although this has been observed several times, perhaps it is surprising? A little bit more lead-in information would be useful.

P 1770 L 3-4: The authors state “there is an apparent disconnect between concentrations of IN active biological particles commonly found on vegetation and concentrations in the air above them (Garcia, et al., 2012)”. However, Lindemann et al (1982) were able to associate atmospheric bacterial concentrations to those of IN. The technique that they used allowed them to detect 0.001 bacterial cells /L of air. This level of detection was needed because INA bacteria were a very small fraction of the total bacteria and were present at concentrations very near the detection threshold. In the work of Garcia et al, their level of detection for INA bacteria was 0.1 gene copies /L. At 1 gene copy per bacterial cell, this means that they could only detect 0.1 INA bacterial cells /L of air. Hence, in order for them to have detected INA bacteria, the concentrations under their sampling conditions would have needed to be 100 times those observed by Lindemann et al. Hence the “disconnect” that they describe is most likely a problem of sensitivity of detection.

P 1770, Materials and Methods. In this section of the manuscript (including the relevant information from the supplemental materials section) I cannot find information to allow me to understand the position of the sampling devices relative to the plant canopy. They state that the work was conducted in the Manitou Experimental Forest. How tall is the canopy and what is the leaf-area index (roughly)? Is there an important

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understory? When rainfall is measured, is this the amount of rain observed on the ground inside the canopy or elsewhere? Likewise, in what part of the canopy was leaf wetness measured, and how did they account for the variability within the canopy? The authors state that inlets of samplers were placed at 4 m above the ground and in the supplemental materials they state that they are at 1 – 4 m above the ground. Given that they used the calculation of Lindemann et al for flux, then I suppose that the 1 m samples were used to calculate the difference in particle concentrations at the 2 heights. I don't think that the assumptions of this calculation are met for measurements within a canopy. The gradient method that they used for flux measurements has an important assumption of fetch that I think could not be met under a canopy (generally you need very large open fields with no obstacles to meet the assumption of fetch). The authors seem to be suggesting that the measured flux is linked to emission from the canopy, but can the measurements they made be extrapolated to what is happening above the canopy? In light of these remarks, Figure 5 is confusing and maybe even misleading because it does not necessarily illustrate what they have measured.

If the work had been conducted over a low canopy of an annual crop, for example, then the results would be relatively easy to interpret in light of experimental design. But for the work as reported here, there is likely to be a very important impact of canopy heterogeneity – both vertically and horizontally – on the measurements. When rain hits the canopy top, much of it is likely to “dribble” down through the canopy after the initial splashing, collecting particles as it successively comes into contact with leaves. As pine leaves are very fine and hydrophobic, rain drops are likely to roll right off. Secondly, there is often considerable litter in the understory, and when drops fall on the litter, splashing of the abundant associated microflora will occur. Rainsplash movement of spores within canopies can be very heterogeneous even for relatively short canopies. Furthermore there have been considerable studies in this area, mostly concerning the dissemination of plant pathogens. A few examples include: Paul et al 2004. Rain Splash Dispersal of *Gibberella zeae* Within Wheat Canopies in Ohio. *Phytopathology* 94:1342-1349. or, the very detailed chapter by Huber, Madded and Fitt on

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Rain-splash and spore dispersal: a physical perspective, Chapter 17 in Jone D.G (ed.) The Epidemiology of Plant Diseases, 1998 Kluwer Academic Publishers

Hence, a considerable improvement to this manuscript could be made by the addition of a figure that indicates precisely where in the plant canopy the measurements were made. This figure could then indicate the scenario of the processes of rainfall impaction and emission at the scale at which they were measured. It would add to the pertinence of statements such as that in the Results and Discussion section concerning the origin of the bioparticles observed after rainfall. For example, on pp 1776-1776, lines 28-29 and lines 1-7, the authors mention that bioparticles could have been emitted from wetted surfaces near the measurement location. What does “near” mean? The forces of agitation and wetting due to rainfall are known to release some spores on the scale of centimeters. Are the measurements made within centimeters of these sources, and if not then what trajectories and forces explain their abundance near the sampling inlets? (I am not casting doubt; I am just asking for clarification.)

P 1171 last line: In the sentence, “Fluorescent particles (Nf) detected by the UV-APS and WIBS can be regarded as a lower limit for the abundance of primary biological aerosol particles”, two things are unclear. Does “can be regarded as” really mean “are” (in fact are)? If so, please say it directly. Secondly, if this is the lower limit for abundance, it would be useful if the authors indicated precisely what this meant in terms of the number of particles /m³, for example.

P1773, paragraph beginning on line 6: There is no mention of detection limits for microorganisms in this work. In particular, for DNA analyses, how many “copies” (cells, spores) of a given microorganism need to be present in one m³ of air in order to be detected with these techniques?

P 1773, paragraph beginning on line 23: Other information is necessary to be able to interpret the results and compare with other works. For bacterial and fungal cultures, how long were the cultures incubated? In the case of bacterial cultures, was the incu-

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bation time sufficient to get the cells past the exponential phase of growth? A second very important piece of information that is needed concerns the densities of the suspensions tested in each well. For bacteria in particular, this would allow comparison with other reports concerning the number of ice nuclei/cell. This would also allow the authors to compare the activity in the strains they characterized here: does a negative reaction at -6°C , for example, for one strain have the same weight as a negative reaction for another strain (in light of the fact that they might have been tested at different cell concentrations). Finally, what was considered as a positive result? Was a single well frozen at a given temperature sufficient to be considered positive?

P1775, L 8-16: Here the authors mention that precipitation leads to increases in the concentration of coarse aerosol particles not embedded in rain droplets. It would have been interesting to know if these phenomena and the types of particles liberated are specifically linked to rainfall per se or rather to the mechanical agitation caused by rainfall. Interesting future experiments could involve supplemental mechanical agitation during certain rainfall events and during certain dry periods. Can the authors speculate about this based on existing literature concerning the role of mechanical forces in the concentration of particulate matter in aerosols?

P1776 L 5-6. Here the authors make a statement about the diameter of the particles related to the data presented in Fig 1e (Da) collected with the UV-APS. In the legend in Figure 2 they again refer to particle diameter, measured with the same equipment, but this time they call it aerodynamic diameter. This is confusing because for bio-particles in particular the correlation between these two parameters (physical diameter and aerodynamic diameter) is very poor (Reponen, T., et. al. 2001. Aerodynamic versus physical size of spores: Measurement and implication for respiratory deposition. Grana 40, 119-125). I think that the UV-APS device measures physical size of particles. If this is the case the authors should make this clear and avoid using the term aerodynamic diameter.

P 1777 L 4-5: Here the authors state “suggesting a net upward flux of fluorescent

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bioparticle emission after rainfall (50-500 /m/s)". As mentioned above, it is crucial to know the spatial pertinence of this estimate; where was flux estimated and is this estimate representative of conditions that can be extrapolated above the canopy? It would also be useful to see details about the flux calculations. They present the range of values, but it would be interesting to see if in fact net flux after rainfall is always upward and how variable are the measurements among all the replicates.

P 1777, paragraph beginning on line 8. In this paragraph the authors indicate that the identified microorganisms comprise a number of plant pathogens and human allergens. Identification of bacteria was based on sequencing the gene for 16S rRNA. To attribute a species name to the bacterium that is at the origin of the DNA, the authors most likely compared the sequence to the NCBI data base (this is not described in the methods). Likewise for fungi. These comparisons are associated with a probability of similarity to sequences deposited in the data base. It is a common phenomenon that sequences can be equally affiliated to several species. Likewise, many sequences often do not find a significant match at the species level. In any case, the information garnered from this approach only indicates the probability that the sequence resembles something deposited in the NCBI data base (and most of what is deposited represents soil habitats). This is why the details are very important here. How close were the matches to the putative plant pathogens and human allergens? And did they match at the species level or the genus level? A list of matches and their probabilities (at least of list of the specific matches that correspond to well-known plant pathogens and human allergens) would better substantiate their claim.

Overall, the data concerning microbial characterization have been presented in too little detail. The authors present these data to support important points about the processes underlying increases in IN abundance after rain. They went through a lot of work to obtain them. So it is unfortunate that more detail is not presented. It is also unfortunate that they make a cursory presentation of the results that corroborate their underlying hypotheses without allowing the reader to evaluate the strength of their conclusions.

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Out of curiosity, I recovered some the sequences for bacteria that they deposited in GenBank and Blasted them against the NCBI data base to see what types of bacteria they might have encountered. The sequences that I looked at corresponded to soil-associated bacteria such *Streptomyces* and *Arthrobacter*. This made me wonder if the relative abundances of soil bacteria were considerably less after rain than during dry periods. It would be very useful if the authors presented a more detailed analysis, perhaps by pooling the data for events after rainfall and those during dry periods and then presenting the relative frequencies of the different groups of micro-organisms encountered.

P 1778, paragraph beginning on line 9: Here the authors describe increases in the abundance of ice nucleators active at temperatures warmer than -15°C and the concomitant increases in particle sizes that are suggestive of biological particles. In parallel, they have isolated fungi and bacteria that are ice nucleation active in laboratory tests. This is precisely where it would be useful to know the rates of ice nucleation activity per biomass of the microorganisms. This information is critical for the authors to claim that these micro-organisms were present in the atmosphere at sufficient concentrations so that they could have contributed to the observed increases in ice nucleation activity. Toward the end of this paragraph the authors state: "Overall, the DNA analyses of aerosol samples collected during rain events showed higher diversity and frequency of occurrence for bacteria and fungi from groups that comprise IN active species (*Pseudomona*aceae; *Sordariomycetes*). Identification of both *Pseudomonas* sp. and *Sordariomycetes* directly from IN samples collected using the CFDC during rain shows conclusively that the biological particles were indeed active as ice nuclei." It should be noted that most bacterial species in the very large *Pseudomonadaceae* family ARE NOT ice nucleation active. INA is limited to strains of a few species within this family. Likewise for the over 10000 species in the *Sordanomycetes*: only one or 2 are INA. Even among the species of *Pseudomonas*, only a few are INA. The recent work of Joly et al (2013. Ice nucleation activity of bacteria isolated from cloud water. *Atmos. Environ.* (in press)) revealed that in clouds only about 10% of the fluorescent

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pseudomonads isolated from clouds had measureable INA. So the statement made by the authors is not well-supported by their generalizations. It would be better to indicate the specific species that contributed directly to this increase in INA and the rates (concentrations) at which they were present in the air.

P 1779, Conclusions. The first statement is accurate in light of the results of the work presented here. But, an added detail would be useful. To the phrase “bursts of bioparticle emission and massive enhancement of atmospheric bioaerosol concentrations”, it would be useful to delimit this increase in terms of space. Where is the massive enhancement occurring? Is it above the canopy or within the canopy in the forest system studied?

The second statement is something that has not been demonstrated here (in terms of spread and reproduction), but it is something that is well-known in the field of plant pathology, for example. In this light, it does not seem pertinent that it is a conclusion of this study.

Figure 5: This figure does not really add anything to the manuscript. It contains well-known information (pathogen spread, for example) and also does not present any specific details that have not been presented in other figures in other publications about the interaction of bioaerosols with landscapes and the atmosphere.

Interactive comment on Atmos. Chem. Phys. Discuss., 13, 1767, 2013.

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