Anonymous Referee #3

Received and published: 12 March 2013

For clarity and easy visual distinction, the referee comments are copied here in black and the authors' responses are offset in blue below each referee statement. Page and line numbers refer to online ACPD version.

The authors have presented a very detailed study concerning biological aerosols in a forest ecosystem. They showed that biological particles increase dramatically during rain and are closely correlated with atmospheric ice nucleation. Additionally, they found two new species of ice nucleation active fungi. This research is new and highly interesting for the readership of ACP and should be published after some very minor changes.

We thank the referee for his/her helpful comments and for the recommendation that the manuscript should be published after some very minor changes.

General remarks:
Many biological particles probe fluorophores (i.e. NAD(P)H, riboflavin, tryptophan etc.). This is particular true for bacteria and fungal spores. However, only few bacteria are good ice nuclei and most fungal spores do not even show any ice nucleation activity. So it would be very interesting to know the fraction of fluorescent particles emitted during or after rain which really show an enhanced ice nucleation activity. Additionally, it would be interesting to know the exact numbers by species which have been identified as active nuclei.

The referee asked about the fraction of fluorescent particles that showed enhanced ice nucleation activity. This was addressed somewhat by Prenni et al. (2013) in a companion study and in more detail by Tobo et al. (2013) in a recently submitted paper and was thus beyond the scope available for this manuscript. Prenni et al. (2013) points out that IN made up a significant fraction of the fluorescent particle number through the temperature range studied, but with a strong temperature dependence (analysis limited to particles < 2.5 μm). For example, the rain event on 10 August, when the CFDC was operating at -15 ^{o}C, IN made up only 2% of the total fluorescent particle number concentration; this number increased approximately linearly with decreasing temperature reaching 27% at -25 ^{o}C. These values represent upper limits for the fraction of biological particles that can serve as IN during the observed rain events for the size range noted.

The referee also asked about the exact numbers, by species, of biological particles that have been identified as active nuclei. This would indeed be a great result of the study, but unfortunately these data are not available. The best hope for this would be to apply qPCR (quantitative polymerase chain reaction) to the high-volume air samples collected continuously at the site. It may be possible to estimate airborne concentrations of a few species from these samples, but this is technically challenging and beyond the scope of what we were able to provide for this paper. Additionally, as referee Dr. Morris pointed out, the fraction of organisms exhibiting ice activity is usually very low with respect to the total numbers of that species present. So even if we can eventually provide an estimate of the airborne concentrations of a given species, this will not necessarily provide the number that could influence ice formation in the atmosphere. The referee's comment is an interesting suggestion, however, and we will continue working in this direction.

Probably there are a lot of biological particles which do not probe fluorophores but which are excellent ice nuclei. This could be leaf litter, starch particles and fragments

from pollen, polysaccharides, humic-like substances, etc. Particularly after rain fall these particles increase due to wash out effects or biological reproduction processes triggered by the enhanced humidity. Can you quantify these non-fluorescent particles? Can you assign them?

If I understand the referee's comment, he/she would like us to identify and quantify the concentration of non-fluorescent biological particles that could still serve as ice nuclei. This is again a very interesting idea, but very difficult to estimate, and so the short answer to the referee's comment is – no, we cannot currently quantify or assign/identify these particles. Pöhlker et al. (2012) comprehensively reviewed the fluorescence characteristics of fluorophores expected to be in aerosol particles of biological origin. They point out that the story of fluorescence is a complicated one and that detecting PBAP at a single excitation wavelength certainly misses classes of particles. Further, Huffman et al. (2012) pointed out that particles likely to be biological in nature exhibited fluorescence below the UV-APS detection limit and thus were not characterized as FBAP. The identification of these particle classes is challenging, however, because fluorescence is a function of metabolic state, atmospheric aging, and degradation processes. Ultimately, more fundamental laboratory work will be necessary to make the overall picture clear enough to be able to address the referee's comment.

Specific remarks:
Explain all abbreviations when first time used in the text (e.g. m.a.g.l., DAPI, FBP, PCR, RH, etc.). Does "IN" mean "ice nucleation" or "ice nuclei"? Please, look for clearness.
Avoid introducing abbreviation in tables. It is much better to establish them in the text.
When possible use regular units and not codes, e.g. LPM (L min-1).

All abbreviations have now been defined upon first usage within the text. All instances of LPM have now been changed to L min^{-1}. IN means "ice nuclei," as defined in the manuscript text. The confusion may arise when it is used as "IN active," (or INA) typically spoken as "ice nucleation active." This is the commonly observed way of writing the acronym and words.

Page 1773, 2nd paragraph: This text is extremely difficult to read for a non-biologist. Since most readers are chemists or physicists you might rewrite this paragraph.

The balance between appropriate detail and readability for readers of all scientific disciplines potentially interested in this manuscript has indeed been challenging. We understand the referee's wish to reduce biological jargon in the methods section, but, as evidenced by the comments of Referee #2, a high level of detail here is necessary to adequately describe the experiment for biologists working closely with atmospheric scientists. We removed one sentence and made limited clarifying changes, but ultimately decided to keep most of the existing text as it was written. We also slightly re-organized the section by consolidating all fungal information together and all bacterial information together in the hopes that this would increase the readable clarity. Our expectation is that atmospheric chemists and physicists interested in the overall message will likely skip reading this section in detail, but that it is available for readers wanting to know about the biological methods.

When quoting Pummer et al. 2012 you might also quote their latest work in Augustin et al. Atmos. Chem. Phys. Discuss., 12, 32911-32943, 2012.

The Augustin et al. reference has been added.

You might also quote the model and the conclusions of Sesartic et al. Environ. Res.

Lett. 8 (2013) 014029 (8pp).

The Sesartic et al. publication has been cited as well.

References:

Huffman, J. A., Sinha, B., Garland, R. M., Snee-Pollmann, A., Gunthe, S. S., Artaxo, P., Martin, S. T., Andreae, M. O. and Pöschl, U.: Biological aerosol particle concentrations and size distributions measured in pristine tropical rainforest air during AMAZE-08, Atmospheric Chemistry and Physics, 12, 11997-12019, 2012.

Pöhlker, C., Huffman, J. A. and Pöschl, U.: Autofluorescence of atmospheric bioaerosols - fluorescent biomolecules and potential interferences, Atmospheric Measurement Techniques, 5, 37-71, 10.5194/amt-5-37-2012, 2012.

Prenni, A. J., Tobo, Y., Garcia, E., DeMott, P. J., Huffman, J. A., McCluskey, C. S., Kreidenweis, S. M., Prenni, J. E., Pöhlker, C. and Pöschl, U.: The impact of rain on ice nuclei populations at a forested site in Colorado, Geophysical Research Letters, 227-231, 10.1029/2012gl053953, 2013.

Tobo, Y., Prenni, A. J., DeMott, P. J., Huffman, J. A., McCluskey, C. S., Tian, G., Pöhlker, C., Pöschl, U. and Kreidenweis, S. M.: Biological aerosol particles as a key determinant of ice nuclei populations in a forest ecosystem, Journal of Geophysical Research, In Review, 2013.