

Interactive comment on “Characterization of active and total fungal communities in the atmosphere over the Amazon rainforest” by A. M. Womack et al.

Anonymous Referee #2

Received and published: 21 May 2015

General comments:

The work by Woomack et al was motivated by the following questions: What are the compositions of the total and active airborne fungal communities? How similar are they compared to each other and compared to fungal communities found in terrestrial environments? The authors collected air samples and used DNA and RNA sequencing for determination of the total and active species composition. Then they compared these data with mass balance model calculations. Their findings show differences in the composition of the communities with more basidiomycotic fungi in the total and more ascomycotic fungi in the active community.

C2336

I was excited to read the manuscript as it seemed to be a great data set, but there are a number of issues with the manuscript as it is currently written. I would like to see the following issues addressed:

Specific comments

Introduction: The authors should carry out a thorough literature search on fungal ice nuclei and rewrite this part of the introduction.

page 7178, line 26: reference needed for the 50Tg yr⁻¹ statement

page 7179, line 2-3: non of the cited references actually showed that fungal spores and fragments affect precipitation

page 7179, line 11-13: reference needed for the statement that vegetative cells are more active than spores. In contrast to bacterial ice nuclei, most of the fungal ice nuclei seem not to be anchored in the fungal cell wall and can be easily washed of the mycelium/spores. These cell-free ice nuclei should be mentioned.

page 7179, 13-16: Please rewrite. The mycelium forming state is the vegetative form of the fungus. Pouleur et al 1992 studied suspensions of *Fusarium* cultures (containing mycelium and spores) as well as filtrates (containing cell-free ice nuclei).

Page 7179, line 19-20: Please cite the right reference. Iannone et al., 2011 worked with *Cladosporium* and did not study *Penicillium*.

Page 7180, line 7-15: It is not clear what the authors try to say here. “Recent estimates of the ice nucleation capacity of fungal bioaerosols based on culture-based approaches – the abundance of CFU-have a low ice nucleation efficiency” . Iannone et al, did not estimate the ice nucleation capacity based on the abundance of CFU. However, there are studies published where atmospheric fungi were cultured and screened for their ice nucleation activity (e.g. Huffman et al., 2013, ACP, Pummer et al., 2013, BG). These studies should be cited and discussed.

C2337

Methods: Please add information about blank samples for the entire study. How many and what kind of blanks were taken during sampling? How were the samplers cleaned (sterilized) between different samples? How was the cellulose nitrate filter pretreated to ensure that it's DNA free before filtering? How many of such filter blanks were included in the extraction? What are the results of the analysis of blank samples?.....

Results and discussion: Page 7186, line 22-24: The authors refer to figure S1, but there are no data from Haga et al, 2014 in this figure. Please correct.

Page 7186, line 25: Please correct as Iannone et al., 2011 did not work with *Penicillium* or add the right reference for *Penicillium*.

Page 7187- 7188: Can the authors clarify why they focus only on the lichen forming fungi as ice nucleation active? Some known ice nucleation active other fungi like *Fusarium* spp. and *Isaria farinosa* belong to the class Sordariomycetes, the most abundant (sequence or OTU level?) class found in this study. Did the authors find *Fusarium* or *Isaria* in their data set? What about ice nucleation active fungi of other classes or phyla like *Penicillium* sp., *Acremonium implicatum* (new name *Sarocladium implicatum*) or *Mortierella alpina*? Can the authors give some more details? I suggest also including these fungi in figure S1.

Page 7189: It is indeed surprising that the lichen fungi found in the active community were not found in the total community. For me it is not clear why the authors used LSU-amplicon sequencing for DNA but shotgun sequencing for RNA? Can the authors clarify?

Figure captions: All captions must be optimized. It is not possible to understand the figures with the current captions.

Figure 1: Is that on sequence or OTU level?

Figure 3: In the figure is written that it is DNA and RNA. Do the authors actually mean sequences or OTU? What kind of error is shown here?

C2338

Figure S1: This figure seems incomplete and partly wrong. Why are there several *Penicillium alli* or *Puccinia* sp.? What are the differences? Both cited references do not say anything about ice nucleation active *Penicillium alli*. Please add the missing references. Photobionts are not fungi and should be deleted from this figure. I suggest to include data from other studies like e.g. Huffman et al., 2013, Haga et al., 2014, Fröhlich-Nowoisky et al., 2015, ...

Tables: Table S4: I suggest to add the number of OTU for each study. Furthermore the information of the sequencing method would help (amplicon (which region)? Metagenome? Metatranscriptome?,...)

Other comments/typos: Methods, page 7182, line 27: typo: it should be extension

Interactive comment on Biogeosciences Discuss., 12, 7177, 2015.

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