

## ***Interactive comment on “Biogeography in the air: fungal diversity over land and oceans” by J. Fröhlich-Nowoisky et al.***

### **Anonymous Referee #2**

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We know surprisingly little about fungal diversity in the atmosphere even though scientists have known for centuries that airborne fungi can be relatively common. This knowledge gap persists because those techniques that have been used for many decades to describe fungal diversity undoubtedly miss a large portion of the fungal diversity found in the atmosphere. Thus I was quite excited to read this manuscript as the authors definitely set out to tackle a key knowledge gap in our understanding of airborne microbial diversity. However, there seem to be a number of issues with the manuscript as it is currently written. Some of these issues could be addressed in a revised version of the manuscript, but some of the issues would require conducting a completely new suite of analyses.

The Introduction seems a bit cursory. There is no reason to write a long Introduction

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(I'm a firm believer in maintaining brevity in manuscripts), but key details seem to be left out. For example, I would like to see a few sentences describing the questions this work addresses and how this work fits into what we already know about microbial distributions in the atmosphere (there is scarcely any mention of the reasonably large body of literature on this topic).

A lot of the text explaining the sampling sites and methodologies used at each site could probably go into a supplement. I think the key information (e.g. site locations, general characteristics, particle sizes collected at each location) could be summarized in a single paragraph, with the rest of the information added to one of the supplemental tables.

What is meant by 'several' PCR reactions (page 7081, line 17-18)? Two reactions, four, five?

The PCR set-up is highly unconventional and I worry that this strongly impacts the reported results. Why use so many different primers? – I recognize that primers have biases, but using multiple primers on the same samples does not necessarily solve this problem. Were the same primer combinations used on all samples? –if not, it is very tricky to compare results across the samples. Furthermore, nested PCR is notoriously problematic as any PCR biases will only be magnified. Essentially I don't have much faith in the molecular methods employed here and they are definitely not using standard methodologies.

Page 7081, lines 24-26: I wouldn't call this phenomenon 'interesting', it just means that the amplification efficiencies of some primer pairs are likely higher than other primer pairs.

Section 2.5: It is not clear how this modeling fits into the rest of the manuscript. What questions does the modeling exercise address? Again, without a more thorough Introduction it is not clear how this part of the manuscript fits with the rest of the manuscript.

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The diversity estimates reported in Table S1 are quite misleading as the data were not rarefied prior to calculating diversity levels. In other words, one cannot compare alpha diversity levels in microbial communities without first controlling for sequencing effort and comparing all communities at the same level of sequencing effort (x sequences per sample). There is a large body of literature on this topic. Furthermore, the sites differed in the number of samples collected and there was no control for this in the diversity calculations (everything else being equal, more samples should result in higher estimated diversity levels – the so-called ‘collectors curve’). By not controlling for sampling extent and sequencing depth across sites, it is impossible to effectively compare diversity levels as done here. Is it surprising at all that the German site with >1300 sequences and >40 samples had far higher estimated fungal diversity levels than all the other sites??

The authors actually recognize that their diversity levels are not to be trusted, see page 7087, “Note, however, that the probability of detecting rare species is limited by the. . . .” but then they ignore their own advice and try to compare diversity levels across the collected samples.

There is no support for the statement on page 7085, lines 8-10 “. . . are similar to the values commonly obtained. . . .” and this statement would probably surprise many people as most would probably assume that fungal diversity in the atmosphere would be lower than bacterial and fungal diversity levels in soil. Frankly I don’t think this statement is valid and to show that it is valid would require more than a cursory comparison of diversity values, but I’d be happy to be convinced otherwise.

I’m confused by the phrase ‘species richness of different groups’ – I think the authors really mean ‘relative abundance of different groups’ (see pgs. 7085-7086 and Figures 1, 2, S1 and S2). Why not report the results as % of sequences - that would be far more relevant (and maybe this is what was done – I’m just confused). This is one of the critical aspects of the paper, yet I’m not sure if the authors are reporting % of species of richness (e.g. # of species that are Basidio. vs. Asco.) or % of sequences (e.g. # of

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sequences that are Basidio vs. Asco.). These are obviously two very different things.

The last two paragraphs of the paper are pure speculation – no measurements of fungal ice nucleation or cloud condensation were taken at all and the (relatively) lengthy discussion of fungal IN and CCN has little to no relevance to the data actually presented in this paper. I recognize that this sounds harsh, I would just encourage the authors to focus on what is unique and novel about this study (and there is plenty!) instead of these over-reaching speculations.

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**BGD**

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