Biogeosciences Discuss., 8, C4386–C4395, 2011
www.biogeosciences-discuss.net/8/C4386/2011/
© Author(s) 2011. This work is distributed under
the Creative Commons Attribute 3.0 License.

Biogeosciences
Discussions



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

**J. Fröhlich-Nowoisky et al.**

j.frohlich@mpic.de

Received and published: 14 November 2011

We thank the anonymous referee#2 for constructive comments and suggestions, which are highly appreciated and have been taken into account upon revision of our manuscript. Detailed responses are given below.

Comments by Referee#2

Referee#2: The Introduction seems a bit cursory. There is no reason to write a long Introduc-tion (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).

Response: To clarify the reason for this study we changed the text as follows:

Introduction, paragraph 1:"The biogeographic distribution of microorganisms is a subject of continued discussions in microbial ecology (Bass-Becking, 1934; Finlay, 2002; Papke et al., 2003; Whitaker et al., 2003; Green et al., 2004; Whitfield, 2005; Martiny et al., 2006; Vos, 2008; Womack et al., 2010). One of the major issues debated is, whether only the environ-ment drives biogeography as Baas-Becking postulates (Bass-Becking, 1934) or if other, e.g., historical events like dispersal limitations also can cause biogeographic distribution patterns. Recent studies reported evidence for regional distribution patterns of microorganisms in soil and water (Papke at al., 2003; Whitaker et al., 2003; Green et al., 2004; Martiny et al., 2006; Whitfield, 2005; Vos, 2008), but their global distribution remains largely unknown. The ma-jority of biogeographic studies have focused on terrestrial and marine environments (Womack et al., 2010), but little is known about biogeography in air, although air is the primary medium for the dispersal of microorganisms, connecting all ecosystems at the Earth's surface."

Introduction paragraph 3:"Recent studies using DNA analysis, however, suggest that the species richness of BMC may actually be higher than that of AMC (Fröhlich-Nowoisky et al., 2009; Hunt et al., 2004). ). The question, however, remains if the species richness of fungi in the atmosphere is generally higher for BMC than for AMC or if there are biogeographic regions in the air as suggested by Womack et al., 2010. Here we investigate . . ."

We added following references:

Bass-Becking, L. G. M.: Geobiologie of Inleiding Tot de Melieukunde, Van Stockkum & Zoon, The Hague, 1934.

Finlay, B. J.: Global Dispersal of Free-Living Microbial Eukaryote Species, Science, 296, 1061-1063, 10.1126/science.1070710, 2002.



BGD

8, C4386–C4395, 2011

Interactive Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

Papke, R., Ramsing, NB, Bateson, MM and Ward, DM.: Geographic isolation in hot spring cyanobacteria, Environ. Microbiol. , 5, 650-659, 2003.

Vos, M. V. G.: Isolation by distance in spore-forming soil bacterium Myxococcus xantus, Current Biology, 18, 386-391, 2008.

Whitaker, R. J., Grogan, D. W., and Taylor, J. W.: Geographic Barriers Isolate Endemic Populations of Hyperthermophilic Archaea, Science, 301, 976-978, 10.1126/science.1086909, 2003.

Referee#2: 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.

Response: In order to avoid confusion we prefer not to split the sampling descriptions in multiple parts. Instead we followed the suggestions of referee C. Morris and added the information about sampler type, the type of sample (size range), and the total volume of air in Ta-ble S1.

Referee#2: What is meant by ‘several’ PCR reactions (page 7081, line 17-18)? Two reactions, four, five?

Response: “Several PCR reactions” means at least 4 reactions for one DNA extract. As de-scribed in the manuscript the reactions were performed with different primer pairs listed in Table S11 except for the samples collected in Mainz, Germany, where more primer pairs were used (Fröhlich-Nowoisky et al., 2009).

To avoid confusion we changed the text as follows:

“With the DNA extract from each of the filters listed in Tabs. S2-9, at least 4 PCRs were per-formed to amplify fungal DNA for sequence analysis.”



BGD

8, C4386–C4395, 2011

As written in the manuscript for the samples from Germany we used more primers.

"PCR reactions were performed with the primer pairs listed in Table S10, except for the sam-ples collected in Mainz, Germany, where more primer pairs were used (Fröhlich-Nowoisky et al., 2009)."

Referee#2: 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.

Response: The same primer combinations were used on all samples except a few sequences were obtained for the Germany data set due to coamplification using other primer pairs (see Fröhlich-Nowoisky et al., 2009).

In an earlier study (Fröhlich-Nowoisky et al., 2009) we have tested and applied multiple PCR primer pairs; these and other experimental details were identified as key elements for efficient amplification of DNA from AMC, BMC, and other fungi. Consequently, we have been able to detect a wide range of species that are well-known from cultivation-based studies (e.g., Cladosporium spp., Penicillium spp., Alternaria spp., and Candida spp.) as well as non culti-vable species (e.g., Blumeria graminis and Puccinia spp.) and previously unknown species. Other DNA-based studies (e.g., Boreson et al., 2004; Despres et al., 2007, Fierer et al., 2008) of airborne fungi have neither found the expected high abundance of Cladosporium nor a high species richness of BMC, which we suppose to be due to limitations of the applied methods (primers).

Boreson, J., Dillner, A. M., and Peccia, J.: Correlating bioaerosol load with PM2.5 and PM10cf concentrations: a comparison between natural desert and urban-fringe



BGD

8, C4386–C4395, 2011

aerosols, At-mos. Environ., 38, 6029-6041, 2004. Després, V. R., Nowoisky, J. F., Klose, M., Conrad, R., Andreae, M. O., and Pöschl, U.: Characterization of primary biogenic aerosol particles in urban, rural, and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes, Biogeosciences, 4, 1127-1141, 2007. Fierer, N., Liu, Z. Z., Rodriguez-Hernandez, M., Knight, R., Henn, M., and Hernandez, M. T.: Short-term temporal variability in airborne bacterial and fungal populations, Appl. Environ. Microbiol., 74, 200-207, 2008. Fröhlich-Nowoisky, J., Pickersgill, D. A., Despres, V. R., and Pöschl, U.: High diversity of fungi in air particulate matter, Proc. Natl Acad. Sci. USA, 106, 12814-12819, 10.1073/pnas.0811003106, 2009.

Referee#2: 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.

Response: Following up on the reviewer suggestion we deleted "interesting".

Referee#2: 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.

Response: We used the model calculations to demonstrate the plausibility of transport effects on the AMC/BMC ratio. In fact, we included these calculations only after discussions with reviewers of earlier versions of this manuscript revealed that the result is not obvious to read-ers less well-versed in atmospheric transport issues.

We also included a more detailed introduction to clarify the aim of our study.

Referee#2: 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





CC BY

(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??

Response: We agree that further investigations and statistical analyses are desirable for fully unravelling the diversity in air particulate matter. The diversity indices are meant to characterize the overall dataset and we focused on species richness.

For clarification we have added the following statement and reference.

"Due to well understood limitations of these parameters mentioned by Morris et al. 2002, we focus on the relative proportions of the species richness of different groups of fungi in the investigated samples and the resulting biogeographic patterns."

We also added the reference:

Morris, C.E., Bardin, M., Berge, O., Frey-Klett, P., Fromin, N., Girardin H., Guinebretière, M.-H., Lebaron, P., Thiéry, J.M., Troussellier, M.: Microbial biodiversity: approaches to ex-perimental design and hypothesis testing in primary scientific literature from 1975 to 1999, Microbiol. Mol. Biol. Rev. 66: 592-616, 2002.

The quantification of the frequency of sequences was not the aim of our study. The principal aim of our study was to explore the overall species richness of airborne fungi. Each species was counted as one in each location, independent if we obtained 1 or more sequences of a certain species (e.g., due to fungal blooms, due to the multicopy nature of the amplified re-gion, or due to the presence on multiple filters).

The numbers of sequences per sample are given in Tables S2-S9. As described in



BGD

8, C4386–C4395, 2011

Interactive Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

the material and method section we used restriction fragment length polymorphism to select as many different clones as possible for sequencing reactions which results in different numbers in se-quences per sample.

The impact of the sampling methods was discussed in supplementary text. Following up on the suggestion of reviewer C. Morris the supplementary text and corresponding references were added to the main body of the manuscript (material and method section).

Referee#2: 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.

Response: The diversity indices are meant to characterize the overall dataset. In our discus-sion we focused on the species richness of AMC and BMC and not on comparing the diver-sity levels across the samples.

To clarify this we changed the text as follows:

"Due to well understood limitations of these parameters mentioned by Morris et al. 2002, we focus on the relative proportions of the species richness of different groups of fungi in the investigated samples and the resulting biogeographic patterns."

Referee#2: 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.

Response: The cited references present similar values of the statistical parameters.

"..are similar to the values commonly obtained for fungi in soil and on plants as well as



BGD

8, C4386–C4395, 2011

Interactive Comment

for bacteria in soil (Maria et al., 2002; Hill et al., 2003; Richard et al., 2004; Satish et al., 2007; Fröhlich-Nowoisky et al., 2009)"

As mentioned above we changed the text for clarification.

"Due to well understood limitations of these parameters mentioned by Morris et al. 2002, we focus on the relative proportions of the species richness of different groups of fungi in the investigated samples and the resulting biogeographic patterns."

Referee#2: I'm confused by the phrase 'species richness of different groups' – I think the au-thors 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 sequences that are Basidio vs. Asco.). These are obviously two very different things.

Response: We did not report the results as % of sequences (number of all obtained sequences for AMC/BMC) as this would not represent the correct ratio of AMC and BMC species. The principal aim of our study was to explore the overall species richness of airborne fungi. Each species was counted as one in each location, independent if we obtained 1 or more sequences of a certain species (e.g., due to fungal blooms or due to the multicopy nature of the amplified region or due to the presence on several filters). As described in material and methods (sec-tion 2.4) the sequences were grouped into operational taxonomic units which represent differ-ent species. The total number of different species represents the species richness (Table S12). The species were attributed to the fungal groups AMC and BMC and we focused on the pro-portions of the species richness of these groups (AMC vs BMC) and not on the relative abundance.

We included the description of how to calculate a proportion of AMC and BMC in the text.

“. . ..we focus on the relative proportions of the species richness of different groups of fungi in the investigated samples and the resulting biogeographic patterns. The relative proportion of AMC and BMC discussed below are defined as the ratio of AMC or BMC to the total number of species detected in the samples.”

Referee#2: 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.

Response: Fungal ice nucleation is discussed in this study, as members of fungal species that can act as ice nuclei (see cited references) were found in our samples. Table S10 gives an overview of these selected species and the relative frequency of occurrence.

For clarification the section about ice nucleation activity has been rewritten. We added a paragraph describing the relation between surface area and ice nucleation activity and a statement about ongoing investigations.

We also included “then”, to clarify that our statement is an if/ then statement and included a recent reference.

“ If fungal spores and other bioparticles are relevant as giant CCN (cloud condensation nuclei) or IN, as suggested by several studies (Bowers et al., 2009, Christner et al., 2008; Pratt et al., 2009; Prenni et al., 2009; Iannone et al, 2011),then. . ..”

Iannone, R., Chernoff, D. I., Pringle, A., Martin, S. T., and Bertram, A. K.: The ice nucleation ability of one of the most abundant types of fungal spores found in the atmosphere, Atmos. Chem. Phys., 11, 1191-1201, doi:10.5194/acp-11-1191-2011, 2011.



BGD

8, C4386–C4395, 2011

Interactive Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

CC BY

Interactive comment on Biogeosciences Discuss., 8, 7071, 2011.

BGD

8, C4386–C4395, 2011