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Biogeosciences Discussions

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

C. Morris (Referee)

cindy.morris@avignon.inra.fr

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This study represents the results of a titanic effort of global sampling of aerosols and subsequent molecular characterization of the fungal component of the particulate matter collected. The inclusion of continental (urban and rural) as well as oceanic sampling sites in both the Northern and Southern Hemispheres has yielded fascinating data. However, in their analyses and conclusions the authors do not take into account some well-known biological properties of fungi and some principles of assessment of microbial biodiversity. This leads to certain conclusions that are disputable and others that are statements of obvious facts. These points, as well as a few other specific remarks about organization of the manuscript, are described below.

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General remarks:

The introduction does not present any real compelling reason for this study. This is not a weakness that could make-or-break this manuscript. But the authors have rallied together an incredible work force for sampling. There must have been some great passion that motivated this effort; I was very surprised to not find this passion in the introduction. There is no obvious statement of hypothesis or goal other than describing the diversity. How did the authors intend to go beyond current knowledge of the abundance and diversity of fungi in the atmosphere?

The major part of the results and conclusions of this manuscript emanate from the assessment of the diversity of fungi via molecular characterization. Data are used for calculating the number of species detected from a range of different fungal phyla and classes and for calculating a range of diversity indices that are based on the abundances of these different species (such as the relative proportion of an individual species, or the number of species detected only once (singletons) or twice (doubletons)). It is debatable if the data can be used for quantifying diversity according to all of the indices presented. Firstly, it is not clear if the sampling at the different sites is comparable. Although the authors present rather detailed information on each sampling site and the associated sampling procedures, some critical comparative information is lacking. Firstly it would have been useful to have comparative information on the sampling efficiency for each of the samplers relative to the size of fungal spores, for the cut-off diameter (cut-off diameter is presented for the sampler used at the German site (P7077 L15) but not for the other samplers), and for the total air volume that the fungal diversity represents at each site. It is important to take into account that differences in sample volume, and hence in the total number of spores collected, can greatly influence the number of species detected (the general principle is that the more individuals analyzed the more species detected). Secondly, the method of PCR amplification and cloning does not necessarily lead to representation of sequences in the colonies of the cloning vector at the same frequency that they are in the initial sample. This is because

there can be competitive interference among sequences for amplification during PCR and during cloning. One consequence is that some sequences might be missed or very poorly amplified, hence the number of species detected is not reliable. But this error can be considered to be equivalent to a detection threshold limit (and these are the type of limits of techniques that we learn to live with). However, the amplitude of the error in quantification of the frequency of sequences is unknown. If it depends on the density of the different sequences and their competitors during amplification, then it is not a constant that can be considered to be comparable among samples.

Most of the important conclusions of this manuscript are based on measures of number of species of the different phyla and classes. The diversity indices that involve quantification of relative abundance of the different species are not used in this manuscript for any of the major conclusions, but simply to state that the level of diversity is comparable to systems studied by other authors (P7085 L4-11). In a supplementary table (S1) the authors have tabulated all the different diversity indices calculated for the different sites. Furthermore, they present means of the indices for continental, marine and coastal sites. In spite of all these calculations, the authors do not use these indices to formally test the hypothesis, for example, that there is less diversity in marine air compared to the continental and coastal sites – making me wonder if they felt confident in the use of these indices. I would suggest that if they present the values of these indices, then they need to bring to the reader's attention the associated limits. These and other considerations for the quantification of microbial biodiversity can be found in more detail in: Morris C.E., et al. 2002. Microbial biodiversity: approaches to experimental design and hypothesis testing in the primary scientific literature from 1975 to 1999. Microbiol. Molec. Biol. Rev. 66 :592-616.

To explain the different biogeographies of Ascomycota and Basidiomycota fungi, the authors conduct simulations of residence times and atmospheric transport using existing atmospheric simulation models. Estimations of residence times and transport are based on spore size. They use the term "aerodynamic diameter" but also "spore

size", and it seems as if the spore property used in these calculations is effectively the physical diameter in μm of the spore. The spore dimensions used are those corresponding to the predominant fungal spores reported for the species they identified. It is well-known that theoretical estimations of particle trajectories in the atmosphere predict that larger particles have shorter trajectories than smaller particles. Hence, their results on the increasing relative abundance of Ascomycota with increasing distance is not a surprise given the spore dimensions used in their models. However, these theoretical models ignore an important biological property of fungal spores – their buoyancy. For example, spores of the Ascomycota fungus Erysiphe spp (powdery mildews) and the Basidiomycota fungus Puccinia spp (the rust fungi) are known for their capacity to be disseminated in the air across hundreds of km in spite of their rather large diameter (ca. 20 μm in both cases). In fact, the urediospores of rusts have nearly the same residence times in the air as bacteria and generally need rainfall to be washed out, otherwise they can remain in the atmosphere almost indefinitely. Their buoyancy is due in part to wing-like structures on the spores. This is one of numerous examples. If the calculations made by the authors were based on the effective aerodynamic diameter of the spores that accounted for the propensity of the spores for flight, then the calculations would be interesting. In most cases of modeling, the effective aerodynamic diameter has not been reported in the literature for fungal spores of different species and the real physical diameter is used as a proxy (as is the case for all previous efforts to model this phenomenon). This leads to classical predictions that one can expect. The effect of size on simulated transport of microorganisms in the atmosphere is treated in detail in a recent paper. (Wilkinson D.M. et al 2011. Modelling the effect of size on the aerial dispersal of microorganisms. Journal of Biogeography, doi:10.1111/j.1365-2699.2011.02569.x). If the authors choose to maintain the results of their simulations in their revised manuscript, they should point out the assumptions of their analyses and make reference to how their analysis is complementary to the information contained in Wilkinson et al as well as in other previous works.

In the discussion, the authors suggest the possible interactions of air borne fungi with

atmospheric processes: ice nucleation and cloud condensation. They cite Diel et al to state that large particle size makes for effective ice nucleators. Although size above several microns has an overriding role on the capacity of a particle to act as a CCN (overriding the impact of the chemical composition on cloud condensation activity), it is surprising that they claim the effect of size on INA. Size of the water binding site has a positive effect on INA, but ice nucleation sites are generally specific sites and the whole particle is not necessarily concerned. Secondly, the authors cite Bowers et al (2009) indicating that their data suggest that there are more INA fungal species than currently described. Bowers et al (2009) report ice nucleation activity of their samples at levels below 100 ice nuclei / m3. They sequenced nearly 5000 microbial ribosomal RNAs from their samples, but their samples represented populations of 10e5 and 10e6 individuals/m3. Hence, they were not able to detect microorganisms whose abundance was on the same order as ice nucleation activity. The organisms responsible for the ice nucleation activity in their samples are most likely not among the taxonomic groups constituting the dominant part of the populations that they described and therefore they have not eliminated the usual suspects as candidates.

The concluding statement of the discussion - "we suggest that air flow patterns in the global atmospheric circulation, as well as spore size-driven selection, may be important for the evolution and spread of fungi" – is the most troubling part of this manuscript. It gives the impression that this work is detached from the fundamental and founding literature on aerobiology. Yet the authors have cited some of this work in a supplementary section on emission and transport of fungal spores, and in particular: Gregory, P.H. (1961; 1973) The Microbiology of the Atmosphere. New York: Interscience Publishers, Inc. This work is a several hundred-page tour de force of most of the major concepts in aerobiology richly illustrated with abundant data. Several of its pages are dedicated to the microbiology of oceanic air, and can serve as an important basis of comparison. In conjunction with the work of E. Stakman (for example: Stakman, E., and Christensen, C.M. (1946) Aerobiology in relation to plant disease. Botanical Review 12: 205-253.) and the subsequent research that was inspired up to about the early 1990's, this body

of research has clearly establish that dissemination capacity is linked to the evolution of pathogens. Dissemination is also a fundamental principle of population genetics (gene flow), a body of concepts that describe how organisms evolve. I encourage the authors to clearly anchor their conclusions in this body of knowledge and to specify how their findings are complementary and go beyond it.

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Specific remarks:

1) As mentioned above, it would be very useful to add a summary table comparing the different sampling sites (above ground height; sampler type, efficiency, and total volume of air sampled in particular) to make it easier for the reader to determine the degree to which samples can be compared. This table should also indicate which samples are continental, coastal and oceanic.

2) In section 2.4 on DNA analysis, the authors indicate that they have eliminated possible chimers from the analysis. How frequent was the occurrence of chimers? Likewise they indicate that sequences corresponding to contaminations originating from the filters were eliminated from the analyses. The supplementary information about background DNA on blanks was very useful.

3) P7085 L14-27, Here the authors present data about proportions of AMC and BMC. They should clearly define how they calculate 'proportion'. I assume that it is: [total number of AMC species (independent of the frequency of occurrence of each species)/ total number of species detected in the sample]

4) P7088 L14, The statement "diversity and spread of ecosystems" is not clear. What is the spread of an ecosystem?

5) In the supplementary information, the authors discuss the impact of different sampling methods and conditions. This type of analysis is critical for putting the results into perspective and the whole of this should be in the main manuscript.

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