

GENERAL COMMENTS

This manuscript aims to illustrate how the life style of a fungus will impact its occurrence in the atmosphere. Indeed, it seems obvious that fungi that produce heavy, sticky spores will not be found as readily in the atmosphere as fungi that forcefully project massive amounts of dry and ultra-light spores into the air. Likewise, plants also vary in the dimensions of their production of pollen and seeds and this is reflected in their biogeography and in the epidemiology of allergies. Here the authors investigate if the abundance and dynamics of the fungal spores captured in the air reflect the strategies that fungi deploy to assure their life cycles.

The motivation for this investigation is not really clear. The authors discuss the importance of functional ecological studies, but via the generic approach used here, what new information do they hope to reveal? In the abstract and discussion they state that knowing the relationship between sporulation strategies and atmospheric abundance could help in forecasting spread of human and crop pathogens and the effect of climate change and land use on their spread. However, epidemiological studies of plant and human diseases have historically considered factors linked to spread and the climate sensitivities of specific species and genetic lines that are pathogenic and with regard to specific traits that the microorganisms are known to express. It is not clear how a general approach based on broad groups of fungi with presumed life history traits could contribute to this goal more so than detailed investigations on the specific species involved in epidemics.

The lack of a strong motivating question for this work is not what troubled me the most. As a microbial ecologist and plant pathologist, I was very troubled by how the authors categorized the fungi that they detected. These categories are fundamental to their analysis and conclusions. The authors grouped fungi into eight life history categories based on whether the principal substrate of the fungus was herbaceous or woody plants and if the fungus was a pathogen and/or a saprophyte. I see at least three major objections to this categorization:

1. Identification of the fungi was based on direct sequencing of DNA obtained from environmental samples and then a BLAST of the results into the NCBI data base to find the most probable result. This is a useful way to obtain information about the likelihood of an identity and it is widely used in microbiology. However, the NCBI data base contains a lot of sequences that have not been validated with other means of taxonomic identity. It is also overpopulated by the habitats and organisms with which researchers work most frequently - and likewise underpopulated with data from systems that are less frequently studied such as the atmosphere. Also, BLAST results can lead to multiple options for identity that are equally probable. Were there any cases where the choice among alternative identities would have changed the categorization? Furthermore, BLAST results are based on conserved gene sequences that are related to ancestry. However, they do not account for traits that can be acquired through horizontal gene transfer or mutations/recombinations or that are affected by epigenetic effects that do not change the ancestry but can alter phenotypic traits. Therefore, extrapolating a hypothetical identity obtained from sequencing to a specific ecological behavior can be very misleading. The amount of error associated with their extrapolations cannot be assessed because the behavior of the organisms has not been validated with other methods to check the validity of the extrapolations.
2. The rules by which the authors attributed the 8 categories to the different species and genera are not clear. Furthermore, are they confident that it is even possible to categorize entire genera into a single life style type? Also, there are arguments against some of the categories that the authors chose. For example, *Aspergillus fumigatus* was characterized as a saprophyte.

While this is true, it can also cause aspergillosis in humans and animals. Should this be taken into account in their categories? Why or why not? As another example, the authors classified *Cladosporium* spp. as « herbaceous plant fungi ». There are over 700 species of *Cladosporium* that are pathogens of herbaceous plants, of woody plants, or are saprophytes. Why did they choose to classify them as herbaceous plant fungi? Likewise, *Penicillium* spp. are predominantly soil saprophytes with some species able to cause disease on living plants but some can also degrade plant tissue after harvest with important losses for the citrus industry, for example. Why did the authors classify this as a herbaceous plant fungus? As a final remark, what is the point of separating “pathogens” from “saprophyte/pathogens”? If a fungus is not an obligate parasite, then it will have phases in its life cycle when it is not causing disease but it is nevertheless growing. *Heterobasidion annosum*, for example, can survive for many months in the soil before infecting a tree and eventually causing disease. Is this saprophytic phase of its life insignificant to the creation of fungal tissue (spores or mycelium) that could be released into the atmosphere? If so, then why do they believe this?

3. Even if the authors could assume that the identification of species or genus was accurate, the life styles of all of the different fungal taxonomic groups that they have encountered have not been investigated to the same extent. In other words, if they attribute *Sistotrema* spp. to the category of ‘saprophytic, pathogenic and plant surface inhabiting fungi associated with woody plants’ they assume that the life histories of this group of fungi have been sufficiently well-studied to have eliminated other hypotheses about possible life styles. Likewise, they assume that the data for the life style of this group of fungi are as complete as for other groups such as *Cladosporium* spp leading to the same level of confidence in the attribution of categories. Unfortunately, the life histories of many microorganisms have not been studied in a comprehensive way. This is particularly true for microorganisms (fungi and bacteria) that are considered to be plant pathogens but whose full life histories are poorly understood (Morris et al. 2009. Expanding the paradigms of plant pathogen life history and evolution of parasitic fitness beyond agricultural boundaries. PLoS Pathogens 5(12): e1000693. doi:10.1371/journal.ppat.1000693). Therefore, attribution of categories of life style to fungi based on their genus or even species identity can be misleading because research has been either insufficient or focused on presumed behaviors.

All of these reasons make it very difficult to agree with the categorization used in this study. These categories are the basis of the novel parts of this manuscript that distinguish it from Fröhlich-Nowoisky et al., 2012.

And finally, there is a paradox underlying their approach to putting fungi in categories. Individual organisms are constituted by a diverse collection of attributes that contribute to their survival. Which of these attributes would be the most important to defining an adaptive response to aerial dissemination? This seems to be the question that the authors want to ask. But they have not looked at any specific set of traits but rather they have looked at the assemblages of the ensemble of traits of individual organisms – that are summarized in their taxonomic identity. This makes me wonder if the taxonomic context of the fungus is not, in fact, the overriding factor in their analysis.

As I final general remark, I feel frustrated to not be able to make more constructive criticisms to the authors to help them improve their work. I am stuck in the perception of this work as being founded on overly broad and presumptive generalizations about the biology of organisms with complex lives where selective pressures from diverse environmental factors mold their evolution. I understand the need to simplify to be able to address questions of functional ecology. But the simplifications should

have solid justification and validation. It would also be more convincing if the simplifications and generalizations were compared to alternative generalizations to test the overall robustness of the analysis.

MAJOR SPECIFIC COMMENTS

On pg 2, starting on line 6 the authors discuss their vision of the relationship between taxonomic affinity, communities, niche and overall ecology. This part is confusing. A "community" is an assemblage of organisms that interact in a given habitat. In ecological theory, diversity (i.e. genetic diversity as well as phenotypic diversity) is considered a *sine qua non* attribute for stability. Within the community there is usually a great deal of niche overlap that leads to competition, antagonism and synergy. In light of these basic traits of communities, geographic distance is not a predictor of genetic distance if the whole of microbial communities is considered. Perhaps the authors are referring to the relationship between geographic distance and genetic similarity within a species rather than across all the diverse members of a community? But, based on the sentence that follows it seems as if they are considering the whole of microbial PBAP. Based on their text, I also have questions about their definition of niche. When they state that related organisms could have entirely different niches, what does "entirely different niche" mean? For example, would this mean a desert aerobic oligotrophic niche vs. an aquatic, anaerobic eutrophic niche? Could they give some examples of close relatives that are adapted to such extreme differences? Overall, this part on pg 2 illustrates the authors' interest in questions about how function – rather than taxonomic identity – could influence the dynamics of the PBAPs in the atmosphere. It makes me wonder why the authors did not identify functions or traits that they thought were of major importance and then test specific hypotheses with regard to these traits by using *bona fide* direct measures – or validated proxies – of the traits.

On pg 2, lines 18-19 the authors state that « a fungus is a pathogen or a decomposer of dead plant material both will require entirely different sporulation strategies ». However, there are many fungi that are BOTH pathogenic and decomposers of dead plant material. The wood rot fungi are perfect examples of this. Also, many of the fungi that are considered as necrotrophs of herbaceous plants, i.e. they live off dead plant tissue, are also plant pathogens, such as *Botrytis cinerea* and *Verticillium dahliae*. In fact, the dichotomy necrotrophs-biotrophs is often used in plant pathology to characterize pathogenic fungi and bacteria.

On pg 2, line 19 the authors state that « the habitat of a fungal species should play a large role »: Why do they refer to "the" habitat" of a fungal species? This suggests that each fungal species has a single habitat. However, many fungi can persist and multiply in numerous habitats: *Fusarium*, *Alternaria*, *Botrytis*, *Cladosporium*, *Penicillium* etc. are among the many examples of ubiquitous organisms that are adapted to live in a wide range of habitats. How do they account for this in their reasoning here? Likewise, on lines 21-22 the authors state that «On a regional to local scale the habitat and its microclimatic conditions, e.g., a sheltered forest atmosphere in comparison to open grasslands, will require differing strategies” : How do the authors account for ubiquitous organisms that are present in a wide range of habitats? Nature is generally composed of organisms that are "generalists" that have behaviors that allow them to be successful in a wide range of diverse situations, and of specialists that are less adaptable. It seems that the authors assertions only account for the specialists.

On pg 4, lines 23-24 the authors give details about how identities were attributed by indicating that “two *Aspergillus* OTUs (*Aspergillus* sp.(A.) and *Aspergillus* sp.(B)) were not combined to a single taxon, firstly due to the importance of *Aspergillus* and secondly as both OTUs displayed distinct differences in the size fraction distribution, leading us to believe them to be separate species”. Why are the criteria

of classification of these OUT different from others? I would think that the same criteria of cut-off should be applied to the identification and labeling of all sequences.

On pg 4, in the last paragraph starting on line 27 the authors state that “The low taxonomic ranks within the subsets allowed additional information on the taxa to be gathered from literature.” As mentioned in the general comments, this is a very simplistic view of the diversity/adaptability within species. It is particularly simplifying if one considers that there are various *Aspergillus* sp., for example, in their data base. What attributes would the authors give to an organism such as the *Aspergillus* that can be a saprophyte in the open environment, can live on a shower curtain, produces mycotoxins, is a plant pathogen, and is a human pathogen among other capacities – depending on the specific species. This vision is also contradictory with the introductory remarks of the authors where they suggest that closely related organisms can be adapted to very different niches. This premise would make it very difficult to attribute « life style » to a taxonomic group.

Furthermore, the classification of fungi into life style categories implies that the life style of all these fungi has been sufficiently investigated. Many of the fungi reported here are known to be able to cause plant disease. But, it is very rare in plant pathology to look for a given pathogenic fungus where it is not thought to cause disease. Also, plant pathology has not invested sufficiently in understanding synergies among microorganisms in disease causation. This is an area of study that might just now be emerging with the advent of microbiome studies. Therefore, some fungi might erroneously be considered as saprophytes because there is not any evidence that they cause disease alone – but they might cause disease in synergy with others. Hence, the authors are making conclusions about reports from the literature that are not all of the same comprehensiveness for all of the fungi reported here.

Given that the main classification of these fungi involves separating “woody” from “herbaceous” species, it would have been much more convincing if the authors had searched for abundance of genes for degradative pathways such as for lignin degradation, etc. If they have the DNA from their previous study they could do this. Of course, it does not mean that the genes are expressed, but it would not involve so many provocative assumptions.

On the whole of pg 7 the authors discuss the relationship between fungal taxonomic groups and aerodynamic size. How would fungi that produce spores of varying sizes for a same species or within a same genus be accounted for? For example, *Fusarium* spp. produce multiple sizes of spores from microconidia that are a few microns in diameter to macroconidia that are oblong to boomerang shaped and up to 50 microns long. Likewise, fungi that have alternating sexual and asexual cycles produce different types and sizes of spores that have different potentials for active release and for aerial dissemination. In fact, how many fungal genera have only one homogenous spore size and one release mechanism?

On pg 8, lines 13-15 in the discussion and analysis of their results, the authors state: “Of course, many fungi can survive on a vast array of different hosts and substrates, can be opportunistic pathogens or display complex life cycles, making an exact categorization challenging especially on genus level”. If the authors recognize that indeed this is challenging, did they test the robustness of their classifications on the conclusions? If they switched categories of fungi that could be attributed to multiple classes, how would this affect the results? Furthermore, how do their conclusions compare to those for a system of random classification of fungi? This would be a control for the relevance of the classification that they chose on the conclusions that they draw.

On pg 8, lines 26-27 the authors state: “When viewing the classification composition for the coarse and fine fractions separately, 52% of taxon occurrences in the coarse fraction are herbaceous-plant-associated, while they make up only ~42% in the fine fraction. » Are these differences statistically significant? What is the underlying variability associated with these frequencies?

On pg 9, the text in the first paragraph raises questions about the effect of their sampling site and the types of conclusions that can be drawn from a single site. It is difficult to measure the effect of the site surroundings on the results. Furthermore, the sampling site was not equidistant from the different types of sources (herbaceous vs woody, for example) that they consider. Wouldn't that influence the results if the classification of the fungal types really represents "far away sources" vs "close sources"? The authors need to account for their sampling location in their analysis and conclusions.

On pg 10, line 27, the text makes me wonder about what are the major findings of this manuscript. In discussing the frequent occurrence of *Aspergillus* and *Epicoccum* in the samples, the authors remark that "This may indicate that the sampled taxa are in fact pathogenic or at least inhabit the surfaces of plants. » It is already very well known that many species of these fungi generally live in association with plants, either in saprophytic or pathogenic phases on living plants and on dead plant material as well. The observations of the authors do not serve as a needed confirmation or demonstration of these well-known facts; their observations are simply a consequence of the ubiquity of these fungal genera on plants and also of the fact that plants contribute the greatest part of the microbial aerosols.

Likewise on pg 13, line 30, for the statement: "This should be of concern for diverse fields, such as food security, agriculture and human health" it can be argued that the effect of environmental conditions on fungal behavior, including sporulation, that is well-known from many epidemiological studies of plant and human pathogens. Therefore, how do the results presented in this manuscript represent new information?

On pg 10, lines 27-29 the authors make more statements about fungi that contradict well-founded observations. The authors state: "*Botryotinia fuckeliana* (anamorph: *Botrytis cinerea*) a pathogenic fungus with a very broad host range, mostly known to infect fruits,...." Indeed *B. cinerea* has a broad host range. But of the over 400 botanical species that it has been reported to attack, the majority are not fruits. Furthermore, for some of the fruit-producing plants that it attacks, it has the most prolific sporulation on the vegetative parts of the plants and not on the fruits themselves.The authors then state that the increases in *B. cinerea* abundance could be due to the following: "This most probably corresponds to an initial sporulation after winter dormancy, after which there is a decrease in May and steady increase up to a maximum September to November, which corresponds to the months where host fruits, such as grapes and apples, are ripe." Indeed this could be an explanation. But it could simply be due to the overall increase of vegetative plant tissue over this time frame. Did the authors look at the temporal dynamics of total leaf area in the region of their study? Did the authors also consult local chambers of agriculture or other sources that report the incidence of *Botrytis* grey mold on apples and grapes in their region during the duration of the study?

MINOR SPECIFIC COMMENTS

The first sentence in the Introduction states "one of the most important groups": What does "one of the most important" really mean? - in terms of abundance? of some type of activity? in terms of mass? Yes, of course one needs some sentence to start the introduction, but this type of statement has no real meaning given that "fungal spores" could be replaced with "bacterial cells" or "algae" and the value of the sentence remains equally vague and difficult to contradict or affirm.

On pg 2, line 5, for the statement about INA fungi that could be involved in atmospheric processes, it would be more appropriate to cite a paper that illustrates both of the criteria mentioned by the authors, i.e. INA and evidence that the spores of the fungi are in the atmosphere at cloud heights for the same fungus. The papers that they cite do not show both of these for the same fungus. There is a paper that shows this. It is for the rust fungi that have very warm temperatures of INA of their urediospores and have been found at altitudes up to 3 km (observed as early as the 1940's): Morris, et

al. 2013. Urediospores of rust fungi are ice nucleation active at > -10 °C and harbor ice nucleation active bacteria. *Atmos. Chem. Phys.* 13 (10):4223-4233.

On pg 2, lines 25-27 the authors state that “insight into the influence of meteorological factors on sporulation is of critical importance to understand and estimate the impacts of climate change, which is especially important for agriculture, due to the high abundance of fungal plant pathogens.” To put their concern into a context of active research, I encourage the authors to read the growing body of literature on this topic including:

Chakraborty, S. 2013. Migrate or evolve: options for plant pathogens under climate change. *Glob. Change Biol.* 19:1985-2000.

Pautasso, M., T.F. Doring, M. Garbelotto, L. Pellis, and M.J. Jeger. 2012. Impacts of climate change on plant diseases – opinions and trends. *Eur. J. Plant Pathol.* 133:295-313.

West, J.S., J.A. Townsend, M. Stevens, and B.D.L. Fitt. 2012. Comparative biology of different plant pathogens to estimate effects of climate change on crop diseases in Europe. *Eur. J. Plant Pathol.* 133:315-331.