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Commentary

Back to the future: natural history and the way forward in modern fungal ecology

ABSTRACT

The growing power and increasing availability of molecular tools for identifying fungi in environmental samples has revolutionized the way that fungal ecologists work. As a result, more people from around the globe have jumped into the fungal community sequencing endeavor. Paradoxically, as these extensive datasets accumulate we are often at a loss for interpretation due to the lack of basic autecology and natural history information for most fungi. As a result we are in danger of learning less and about more and more. I suggest that one way forward in fungal ecology is through a modern version of fungal natural history, with a focus on holistic understanding of individual species and ecosystems, but driven by modern genomic and molecular tools. By combining the extensive data generated through environmental sequencing with an intensive, molecular-based natural history we can create a synergy that will propel fungal ecology forward.

The growing power and increasing availability of molecular tools for identifying fungi in environmental samples has revolutionized the way that fungal ecologists work (Horton and Bruns 2001; Lindahl et al. 2013). We are now able to identify the presence of hundreds of co-existing species in minute samples of soil (<1 g) or plant tissue. More importantly, we are able to do this at a throughput – both in terms of sequence depth per sample (10,000 s) and number of samples (100 s) – at a per sample cost (<$10) that was unimaginable just a few years ago, when the first next generation sequencing (NGS) platforms hit the market (Fig 1). As a result, more people from around the globe have jumped into the fungal community sequencing endeavor, generating large datasets from the rainforest (McGuire et al. 2012) to the bottom of the ocean (Orsi et al. 2013) and from the skin on our backs (Findley et al. 2013) to the air that we breathe (Adams et al. 2013). These same tools have reconfigured the study of other 'microbial' groups where morphological taxonomy is of limited use – such as bacteria and viruses. From the pace of microbial discovery it is easy to draw parallels to the naturalist frenzy of 18th century Europe, when scientists like Linnaeus and Buffon were trying to collect and classify the visible dimensions of diversity on our planet.

As we synthesize results from across studies, and large scale sequencing efforts come to fruition, we are learning important things about the diversity and distribution of fungi at both small and large spatial scales. For example, contrary to previous expectations (Bisby 1943), most fungi are not cosmopolitan and have restricted geographic ranges (Kivlin et al. 2011; Sato et al. 2012; Meiser et al. 2013; Talbot et al. in press). Similarly, interesting large-scale diversity patterns are emerging. For example, ectomycorrhizal fungi appear to peak in diversity at mid-latitudes rather than the tropics (Tedersoo et al. 2012). Similar non-canonical patterns have been found in bacteria (Fierer et al. 2011), raising the question of whether the climate variables that correlate with macro-organism diversity are truly general factors controlling all organismal diversity. At smaller scales, we find that fungal diversity varies dramatically across habitats, from hundreds of species in a few grams of soil (Peay et al. 2013), to dozens of endophytes in a single tree (Zimmerman and Vitousek 2012) and near monodominance by single species of yeast in floral nectar (Beslisle et al. 2011). These discoveries raise important questions about the fundamental processes that control fungal diversity and distributions and how the unique biology of fungi contributes to generating the patterns we observe.

Despite all of this information, publication of fungal NGS studies appears to have lagged behind 16S studies of bacterial communities. For example, the search term "ecology" in the GenBank Short Reads Archive (Dec 2013) turned up 150 studies, of which 51 are bacterial and four are fungal. This may be, in part, because of the comparative difficulties in analyzing fungal ITS datasets, due to the lack of a standardized bioinformatics pipeline. However, with the incorporation of fungi into the QIIME platform tutorials (Caporaso et al. 2010), some recent papers on NGS guidelines (Nilsson et al. 2011; Lindahl et al. 2013) and the generation of a well curated ITS database (Kõljalg et al. 2013), the pace of dataset publication should increase rapidly. One consequence of this standardization and low-cost sequencing is that scientists without a background in mycology appear to be increasingly incorporating fungi into their research. This democratization of molecular techniques has eroded many of the taxonomic and methodological barriers that traditionally separated microbiologists and ecologists of various stripes. In particular, people traditionally working on bacterial communities are well poised to integrate fungal communities into their data streams. Perhaps not surprisingly, the ability to use molecular tools to detect fungi quickly and easily has likely been part of the growing

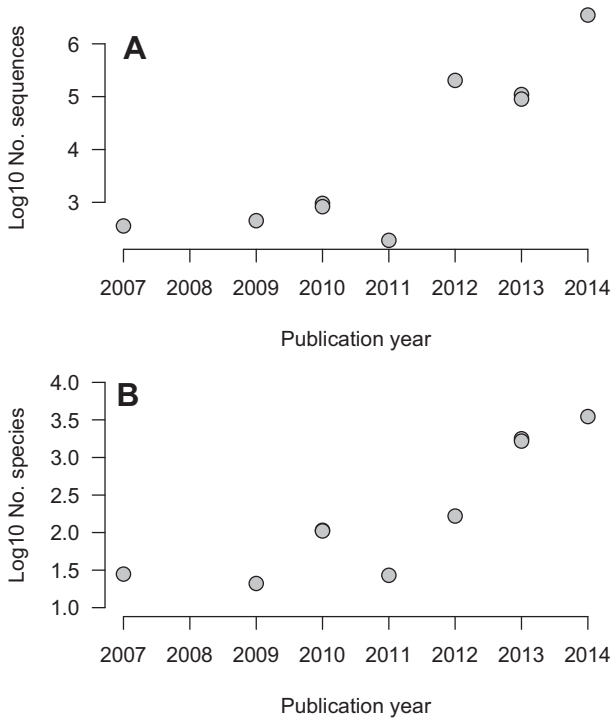

Fig 1 – A personal sequencing journey. Improving technologies are dramatically increasing the number of DNA sequences obtained and number of species detected in fungal community studies. Figure shows (A) the number of sequences obtained for fungal community ecology studies published by the author starting in 2007 up to the present. Most recent data point is an unpublished manuscript in process based on runs on the Illumina MiSeq platform; (B) the corresponding increase in the number of species uncovered with higher throughput methods. The increase in sequences and species richness makes unraveling the ecology of fungal communities evermore difficult without more autecological context.

appreciation of the fungal role in shaping ecosystems (e.g. Fisher et al. 2012; Liu et al. 2012; Clemmensen et al. 2013).

As should be clear from the previous paragraphs, all of these developments are having a positive effect on the field of fungal ecology. In some ways it is tempting to declare victory – we have the tools we always dreamed about and growing recognition of fungal importance amongst scientists and the general public. However, as conquering armies sometimes learn, winning the peace can be more difficult than winning the war. What do I mean by this? For the last two decades, arguably the biggest limit on our knowledge about fungal communities appeared to be sequencing power. That limit is now disappearing or gone. For even longer, academic mycologists were the majority of people that cared about fungal ecology. That is no longer the case. While these are both positive developments, I also think it indicates that the field may be approaching a crossroads. In a new age of unlimited sequencing power and widespread scientific participation, what is the most productive way to push fungal ecology forward?

There are, of course, many (and by no means mutually exclusive) paths that will move the field forward. The current trend appears to be riding the wave of increasing sequencing power to characterize fungal communities from a greater diversity of environments and at greater sequencing depth than previously possible. Metagenomics, metatranscriptomics (Baldrian et al. 2012), and even meta-proteomics (Schneider et al. 2012) will soon be providing an increasingly rich data stream for fungal ecologists to mine. This approach is certainly important, but it has limitations and will eventually reach the point of diminishing returns. This is primarily because environmental metagenomics *sensu lato* is an extensive source of data, rather than intensive source of data.

I say this for a number of reasons. First, Next generation amplicon sequencing of barcode genes can produce very comprehensive profiles of fungal community structure. However, we know the identity of most operational taxon units (OTUs) used in molecular studies only imprecisely and the usual blanket 97 % sequence similarity cutoffs used to delineate OTUs may obscure meaningful ecological differences (Kõljalg et al. 2013). In addition, the relationship between gene abundance and organismal abundance is not direct (Amend et al. 2010; Baldrian et al. 2012). Even with better algorithms and databases for taxonomic assignment, we still know very little about the detailed ecology of even OTUs for which a clear taxonomic assignment can be made. Well-designed sequencing studies can tell us a lot about the spatial distribution of fungi, but there is an important interplay between interpreting environmental sequencing and *a priori* knowledge of organismal ecology. For example, Lindahl et al. (2007) showed in a very elegant study that there was a strong correlation between the age of soil carbon substrates and the distribution of ectomycorrhizal and saprotrophic fungi. However, the most powerful conclusions from this work were based on *a priori* ecological knowledge that allowed the assignment of OTUs to trophic guilds, and even in this case only 25 % of the identified fungi could be thus assigned. Difficulty assigning trophic guild is not an uncommon problem, despite the fact that trophic mode is perhaps the most fundamental feature of an organism. A pioneering study by Vandenkoornhuyse et al. (2002) was unable to assign trophic guild for 94 % of the root-associated fungi they detected. This problem has only grown as NGS studies uncover greater and greater fungal diversity.

At some point the ecological detail we can learn from the OTU × sample data matrices (i.e. OTU tables) generated through NGS studies is limited, regardless of how good the environmental metadata is. This limit exists because the information in an OTU table is ultimately static – OTU tables tell us little directly about the nature of the interactions going on between the organisms present and between the organisms and their environment. For example, a significant portion of the fungal community detected through DNA sequencing may not even be metabolically active. Baldrian et al. (2012) found significant differences in the fungal community when sequencing DNA compared with RNA, suggesting that DNA is not an accurate representation of metabolic activity. Similarly, Nguyen (Pers Comm) has found that ectomycorrhizal spores buried in a closed container without host roots (i.e. no active hyphae could develop) could be amplified