Using aggregated field collections data and the novel R package "fungarium" to investigate fungal traits

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Project Goals: Fungi dominate the biological decomposition of wood and other lignocellulosic plant tissues in nature. These saprotrophs offer us a proven model for making energy, sustainably, from biomass. They also offer those with commercial interests a range of pathways for unlocking sugars embedded in lignin. Their strategies range from 'white rot' mechanisms that remove lignin to gain access to polysaccharides to 'brown rot' mechanisms that selectively extract sugars, leaving most lignin behind. This metabolic diversity could be harnessed, industrially, but research has generally been focused more toward white rot delignification pathways. White rot fungi can unsheath polysaccharides by selectively removing lignin, a capacity that historically attracted interest for the potential to extract intact fibers for papermaking. Modern bioenergy schemes, however, do not aspire for intact fibers - instead, the goal is to depolymerize polysaccharides to release fermentable sugars (saccharification), saving lignin as a co-product, if possible. This is a better fit for the carbohydrate-selective pathways of brown rot fungi, but our grasp of fungal brown rot metabolism lags behind what we know about white rot.

Our collaborative project is aligned to address these gaps, with the **goal** of producing an integrated regulatory model for brown rot. Our proposed objectives insure stand-alone advances, but will also synergize to push ideas forward in a systems context.

Objective 1 is to identify fungal gene regulation patterns that distinguish brown rot fungi from fungi with other decay modes (e.g., white rot). We are comparing fungi among relevant lineages but with varied carbohydrate-selectivities. We are culturing these strains on solid wood wafers, spatially mapping gene expression and then overlaying fungal/wood metabolite patterns to enable temporally-resolved functional genomics. These maps can isolate patterns unique to brown rot and can target characterization.

Objective 2 focuses on characterization, starting with a short list generated in an earlier transcriptomics study, and progressively adding objective 1 gene targets. We are using routine single-/multi-cellular *in vitro* transformation pipelines, but complementing this with efforts to develop a brown rot transformation system, enabling *in vivo* manipulations (e.g., Crispr-Cas9).

Objective 3 is to use metabolomics to map metabolite-expression feedback over time, providing networks of gene regulation. This approach promises to advance our understanding of this unique brown rot strategy, beyond current ROS-centric models toward a systems view.

Abstract:

Fungal traits offer predictive characters useful in ecology and for prospecting new strains for bioconversion. Archived sporocarp data, like the collection and observation records accessible through The Mycology Collections Portal (MyCoPortal), are well-suited for trait investigations considering that these records circumvent the need for field work, are geographically and temporally diverse, and often have detailed and trait-relevant environmental metadata.

There are, however, inefficiencies and inadequacies in the MyCoPortal online interface that affect dataset generation and trait searching, and many of the available records have outdated or misspelled taxon names as well as misspelled location names. Thus, we created the R package *fungarium*, which enables the efficient download of complete MyCoPortal datasets from within the R environment, enhances the identification of trait-relevant records, confirms or updates taxon names based on current scientific consensus while also accounting for spelling errors, and fixes misspelled location names. Utilizing this package and MyCoPortal data, we demonstrated methods for analyzing taxonomic, geographic, and temporal patterns in ecological traits, using fire-association as an example.

We found that fire-association, which was quantified via fire-associated enrichment factors (fire-associated records/total records), differed substantially between taxa and these differences were qualitatively supported by existing literature, as hypothesized. Fire-association varied between counties and years as well, but these patterns did not correlate with burned acreage as expected. This lack of correlation was linked to sampling bias within the MyCoPortal data and limitations of the burned acreage dataset used (i.e. Monitoring Trends in Burn Severity). However, both confounding factors are likely depend on the trait analyzed and external dataset used. Overall, the *fungarium* package and associated methods presented here effectively enable the use of archived sporocarp data for future ecological trait studies.

References:

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