

Community-level traits as a way to partly circumvent the culturing problem in mycorrhizal trait-based ecology?

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Traits are the intermediate by which species respond to environmental filters and influence ecosystem functions. With the myriad of biogeochemical processes controlled by fungi, the past decade has witnessed a rising interest in applying trait-based approaches, core to the toolkit of plant and animal ecophysiologists, to fungi. One of the first challenges to tackle when working on fungal ecophysiology is to circumscribe the very definition of what we consider a fungal trait. Traits are characteristics/features possessed by an *individual* that can influence how it interacts with its environment. Here the individual scale is both important, and problematic. Important because the very goal of comparative ecology is to measure traits on individuals belonging to known species. This allows to populate trait databases, and syntheses of such databases can reveal key trade-offs and trait syndromes that govern species' life-histories. The scale of the individual is problematic, however, because it is hard to define for soil fungi, and because a rare minority of fungi can be sampled at the individual scale in the environment (e.g., macroscopic sporocarps, ectomycorrhizal root tips, lichen thalli). Beyond this minority, the individual organisms can only be accessed/sampled through establishing fungal cultures, which probably represents one of the main bottlenecks in the development of fungal trait databases. In this issue, Zhang et al. (2022) show how interesting insights in fungal trait-based ecology can be gained by working at the community level.

In their study, Zhang et al. (2022) adapted a protocol developed by Neumann & George (2005) to capture mycorrhizal fungal hyphae using ingrowth bags. If we assume that most hyphae recovered through this technique are mycorrhizal, the washed hyphae can be characterized through various chemical/morphological downstream analyses. Measuring such traits for biomass recovered from whole communities is akin to estimating community-weighted mean (CWM) traits, which are central to many aspects of ecophysiology. Various paradigms/theories in community ecology assume some form of equilibrium between species and their environment (Leibold et al., 2004). If we assume (1) a heterogeneous environment, (2) species as reproductively isolated units competing for space/resources and (3) traits as determinants of their reproductive success, correlations between species traits and environmental parameters are naturally expected to arise (Shipley et al., 2011). Under specific stable environmental conditions, a species bearing certain traits should have a higher probability to (1) occur and (2) become abundant in such environment. At the community level, we thus expect a correlation between CWM traits (the sum of species mean traits weighted by their relative abundances), and environmental parameters (box 1). With mycorrhizal fungi, we can have a reasonable access to species' relative abundances through sequence-based profiles of communities, but the species \times traits matrix remains inaccessible. The shortcut taken by Zhang et al. (2022) is to take measurements of traits (here, hyphal C:N:P stoichiometry) at the community level directly.

Does the species \times traits become dispensable in mycorrhizal ecology? Certainly not. Bringing mycorrhizal fungi into cultures, identifying traits likely to represent important trade-offs in fungal resource management strategies (Chagnon et al., 2013), ensuring reproducible measurement of such traits and establishing common resources to share such traits (Kattge et al., 2020; Zanne et al., 2020) remains a priority of mycorrhizal ecophysiology. Opinions (Chagnon et al., 2013) and definitions (Chaudhary et al., 2020) will only be useful if followed by actual work to populate databases currently storing the very fragmentary data on fungal traits. We cannot leave aside this important work at the species and individual scales, because evolutionary trade-offs defining resource management and life history strategies emerge at those very scales, not at the community level (Grime & Pierce, 2012).

Trait-environment relationships, however, can inform us on the way environmental pressures may select for particular species characteristics, and in this regard, progress can be made over much shorter timescales than the work expected to rely on permanent culture banks and individual-level trait measurements. Zhang et al. (2022), for example, identified an increase in hyphal P concentrations in response to warming and drought treatments, illustrating hyphal stoichiometry as a potentially important “response trait” for mycorrhizal fungi. The upcoming challenge with stoichiometry is now to link form and function. What is the purpose of enhanced mycelial P for the fungus? Luxury uptake and storage as polyphosphates, which may confer bargaining power to the fungus? Increased cellular concentration of “growth-related molecules” (*sensu* Zhang et al., 2022) such as RNA? This remains to be elucidated. The same is true for nitrogen, which can be present in both growth- and function-related proteins, or in cell wall components slowing down necromass decomposition (Fernandez et al., 2019). This will influence how likely are fungal hyphae to contribute to soil organic pools of different turnover times (See et al., 2022; Klink et al., 2022).

We can probably identify many other traits that we expect to be (1) measurable at the community level and (2) associated with environmental filters. Spore size and wall ornamentations could be linked to dispersal dynamics (e.g., Chaudhary et al., 2020). Cell wall thickness could be linked with susceptibility to fungivory (as a constitutive structural defense), and could be expected to be associated with predation risk, but also community-level productivity. Generally, structural defenses are expected to be maximal under harsh conditions promoting conservative species with long-lived, constitutively defended tissues (Coley, 1988). Hyphal allocation allometry to the root vs. the soil habitats already has received considerable attention (e.g., Maherali & Klironomos, 2007), although the assumption that extensive soil foraging is associated with more efficient P return to host can be questioned (Jakobsen et al., 1992). Community-level allometric measurements could be coupled with soil nutrient availability along natural or experimental gradients could clarify this issue. Relative mycelial investments in the soil, however, is a multifaceted trait that bears implication for other aspects of fungal growth and dispersal, namely the colonization of new patches (emerging roots), the exposition to parasites/predators, and the interactions with non-mycorrhizal microorganisms potentially including hyphosphere mutualists. In other words, soil hyphae are not strictly foraging units, but may also serve dispersal, chemical warfare and interkingdom cooperation. This may decrease the probability of finding clear univariate linkages between hyphal allometry and single environmental filters such as nutrient availability. Other traits requiring our attention are biomass growth and turnover rates. Tissue maximal growth rate and lifespan are central to the definition of ecological strategies (e.g., Westoby et al., 2002; Darling et al., 2012). In principle, this can be measured at the community level for mycorrhizal fungi, although the experimental approach should be selected wisely. Traditional approaches to measuring biomass accumulation in mycorrhizal studies typically rely either on microcosms inoculated with fungal propagules, or on ingrowth bags. Both these approaches will select for colonists that can rapidly invade this new empty niche (a bulk sterile pot/ingrowth bag), thus biasing our estimates of growth rates in favor of those displayed by ruderal colonists (i.e., community-level trait not matching the community composition/structure). However, regarding biomass turnover rates, it could be envisaged to derive such estimate using stable isotope probing targeting a specific biomarker (e.g., NLFA 16:1 ω 5). The only drawback is that evaluating dilution rate of heavy carbon in such a biomarker rapidly makes the cost per sample prohibitive, hampering measurements of biomass turnover rates along environmental gradients, or in response to an experimental treatment.

Despite the technical difficulties associated with measuring community-level traits, or the challenges to

linking form and function, the approach put forth by Zhang et al. (2022) with hyphal stoichiometry are part of the equation to advance mycorrhizal ecophysiology, and should be extended to other traits. Meanwhile, the long-term objective for mycorrhizal ecophysiologicalists should still be to isolate and culture strains, and make these *permanent resources* for them and other research groups to measure traits in future studies. Intraspecific trait variation appears so important, at least for arbuscular mycorrhizal fungi (e.g., Munkvold et al., 2004; Antunes et al., 2011), that strain identity will be just as important as species identity in building trait databases. And as mycorrhizal ecophysiology matures, new traits will gain interest and have to be measured on those strains for which we have already measured a number of other traits. Plant and animal ecophysiologicalists have a permanent resource they can sample individuals from: it is called nature. Mycorrhizal ecologists are in need for such analogous resource: permanent culture banks. Thus, it seems that challenges lying ahead in mycorrhizal ecophysiology are multifaceted, encompassing the need for conceptual development, standard laboratory methods, but also creativity in getting long-term funding to maintain biological material.

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BOX 1. Emergence of traits x environment correlations at the community level

Community-level traits are a useful starting point linking environmental properties and traits of organisms even when we cannot easily sample individuals in nature, as is the case for microorganisms. Here, I present a simplified community assembly scenario showing how traits x environment correlations emerge at the community level under an environmental filtering / species sorting paradigm. Assume an environmental gradient consisting of 5 distinct environmental patches with contrasted conditions for an environmental parameter x (i.e., the red-blue scale in the large squares in the figure). Also assume a pool of 5 species with given values for a trait y , exemplified in the figure using a similar red-blue color scale, such that low values for trait y translates into high reproductive success in environments with a low value for parameter x . We start ($t = 1$, first row of panels in the figure) by randomly drawing 100 individuals from the species pool and placing them in each local community. Then, we simulate the extinction of a given number of individuals (here, 5 per time step) and replace them based on the reproductive success of the remaining species in the local community, as opposed to a zero-sum ecological drift assumed by a neutral model. This is rather in line with a species sorting paradigm where local community dynamics are assumed to reach an equilibrium faster than the environmental properties change, and with little/no impact of dispersal from neighbouring communities (i.e., source-sink dynamics) (Leibold et al., 2004). If, at each time step, we compute a community-weighted mean (CWM) trait y for each local community, and correlate CWM and local environmental property x , we see that community-levels traits rapidly mirror local environmental conditions (right panel).

