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The use of artificial media in fungal ecology

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Abstract

Can experiments conducted in agar really help us to understand the complexity of fungal systems? This question has been the focus of persistent and ongoing debate between fungal ecologists that favor reductionist versus holistic approaches. On one hand, artificial media are unrealistic and fail to reflect the heterogeneity and complexity of natural systems. But on the other hand, they offer simplified model systems that allow us to isolate mechanisms that would otherwise be obscured in natural systems. Following various technological advances that enable us to describe various aspects of complex fungal communities *in situ*, the dial appears to be tipping in favor of observational field studies, and the use of artificial media has declined. However, we argue that the loss of artificial media from experimental studies would impair our capacity to disentangle the complexities of fungal communities. Here, we outline the pros and cons of artificial media in fungal ecology and outline the types of questions that are best addressed using fungi growing in artificial media. We conclude that renewed emphasis on the value of artificial media could help us to generate the mechanistic understanding that might be critical to explaining the exciting patterns that are emerging from real-world fungal ecology studies.

Introduction

Since the dawn of this research field fungal ecologists have been divided along a spectrum of approaches. Those favouring holistic approaches choose to observe fungal communities in natural systems, enabling the identification of real-world patterns that might be explained by theory. On the other end of the spectrum, reductionists prefer to use simplified model systems to examine ecological mechanisms that might underpin those important ecological patterns. Although manipulative field experiments can serve as an intermediate between these approaches (Crowther et al., 2015; Hiscox et al., 2015), the majority of studies in fungal ecology fit at either end of this spectrum. As with most biological fields, the combination of these approaches is the best way to provide conceptual depth and to build a mechanistic understanding of fungal dynamics (Peay, 2014; Vilanova and Porcar, 2016). However, following a wide range of technological

advances (e.g. high-throughput sequencing, stable isotope and imaging techniques etc.) that enhance our capacity to describe fungal communities *in situ*, the scale has tipped drastically in favor of the holistic approaches, at the expense of mechanistic experimental studies (Peay, 2014; Vilanova and Porcar, 2016).

Traditionally, artificial media (ArtMed) such as solid agar or broth conditions have been used extensively as simplified model systems to perform manipulative experiments in fungal ecology. As such, much of the holistic versus reductionist debate has centered around experiments that make use of ArtMed. These model systems have been central to much of our basic understanding of fungal biology, providing a unique opportunity to observe fungi directly, and under highly controlled conditions. However, in search of increasing experimental realism, and facilitated by the emerging technological tools (e.g. genetic, stable isotope or remote sensing tools) there has been a concerted move away from the use of ArtMed in fungal experiments, towards natural substrates (e.g. plant roots, wood or soil) that more closely replicate natural systems (Fukami et al., 2010; Crowther et al., 2011; Hiscox et al., 2015) . Indeed, only 17% of the experimental studies published in *Fungal Ecology* in 2015 were conducted on artificial media, in comparison to 57% in 2010. As such, ArtMed are commonly being relegated to use as simple culture media. This declining use of ArtMed has pervaded the entire field of microbial ecology.

The recent shift towards natural substrates in experimental studies can certainly increase the strength of real world inferences. However, we suggest that the indiscriminate loss of ArtMed experiments might come at the expense of various mechanistic insights that can only be detected in such model systems, and this might place fungal ecology at a conceptual disadvantage compared to other fields of ecology. Despite a growing appreciation for the need to maintain more traditional, reductionist experimental research in light of emerging technological advances (Peay, 2014; Vilanova and Porcar, 2016), the use of ArtMed in experimental systems continues to fall. We believe that this stems partially from confusion over the appropriate use of ArtMed experimental systems. In this manuscript, we examine the pros and cons of using ArtMed in experimental fungal ecology, and we outline the types of questions that can effectively be addressed using

ArtMed. We hope that this manuscript can provide a framework for the effective use of ArtMed in experimental fungal ecology.

Pros and cons of artificial media

The major criticism of artificial media conditions is their inability to replicate accurately natural conditions. Most artificial media provide homogeneous, well-mixed conditions that inherently fail to imitate the structural or chemical heterogeneity of most natural resources. As a result, the growth, morphology or biochemical activity of fungal isolates on agar is often drastically different from that on soil, litter or plant roots. Any inferences made about the foraging, population or community dynamics of fungi in natural systems based on experiments conducted solely in artificial media cannot be strong. For example, if fungus X displays a phalanx foraging strategy when growing on agar, we cannot make strong inferences about the foraging or competitive dynamics of that fungus under any particular set of natural conditions (Boddy, 1993; Magan and Lacey, 1984). Second, liquid ArtMed generally provide homogenous, well-mixed, non-solid environmental conditions, and solid ArtMed typically present relatively uniform growing conditions at the centimeter scale. Homogeneous conditions are unrealistic in many microbial systems (e.g., soil or root-associated fungi), which exhibit high spatial heterogeneity at the millimeter or micrometer scale.

Although these criticisms certainly limit the application or scaling of results from artificial media to the real world, they do not limit their utility in all aspects of fungal ecology. Despite the limitations regarding structural realism, they can certainly capture certain aspects of fungal dynamics that cannot be isolated in natural complex systems. ArtMed provide unrivalled simplicity, and they are unique in their capacity to use, manipulate and replicate. The transparent nature of many ArtMed allows them to provide a unique window into the activity of otherwise concealed individuals. Finally, they can be manipulated to approximate ‘optimal’ growing conditions for the determination of ‘potential’ activity, in the absence of exogenous factors. Below we outline the types of ecological question that lend themselves to the use of ArtMed in fungal ecology.

Appropriate use of artificial media in fungal ecology

A possible factor contributing to the decline in ArtMed is the widespread misuse of ArtMed, and the prevalence of poorly designed ArtMed studies. Many studies use multiple cultures of the same isolate as true replicates in experiments (see bad examples by the authors: Boddy, 1983; Crowther et al., 2011b). This may be fine if the study intends to make precise inference at the level of the individual, but if the study aims to explore intra-, or inter-specific variation among fungal groups, or capture general patterns across fungal systems, then these different cultures can only represent technical replicates. In this case, all technical replicates taken from a single genetically identical isolate should be averaged into an individual point to avoid artificially inflating the sample size. These average points could be compared to other similar points (each representing a different isolate's mean response) in an ANOVA designed experiment, or used as an isolated example of that fungal group in a Regression-style experiment. Moreover, we suggest that future work in fungal ecology should prioritize the use of genetically distinct isolates, to avoid altogether the need to account for technical replication. Appropriate use of ArtMed in fungal ecology is critical if the interpretation and relevance of results and findings. Below we outline the types of questions that can be appropriately addressed with ArtMed, when using the correct study design.

When resource structure has no effect

A considerable amount of basic information about fungi is independent of growth substrate. Of course, this is true for many basic morphological features (basidiomycetes will produce basidia on all successful growth substrates), making ArtMed useful for identification and classification. But ArtMed are also valuable for identifying many biochemical, metabolic, reproductive, physiological and genetic aspects of fungal functioning that are consistent across growing conditions (Aguilar-Trigueros et al., 2017; Maynard et al., 2017; Samils et al., 2013). Although gene expression is highly context-

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4 dependent, the full genotype of a fungal individual will not vary considerably across
5 substrates and reproductive mechanisms are unlikely to change. Similarly, although
6 fungal growth rates and temperature-sensitivities can vary when growth on different
7 substrates, basic metabolic demands mean that the minimum and maximum growing
8 temperatures are generally consistent across different growing substrates (Aguilar-
9 Trigueros et al., 2017; Barcenas-Moreno et al., 2009; Carey et al., 2016; Crowther and
10 Bradford, 2013).

11 12 13 14 15 16 17 18 19 *When exploring 'potential' processes*

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22 If the study is intended to evaluate the potential existence of a process, then the substrate
23 of choice can be irrelevant. Of course, this can often be achieved using genetic
24 approaches, as the detection of functional genes might indicate the potential trait
25 expression. But ArtMed can provide a cheap, rapid and direct approach to detecting
26 potential processes. For example, when exploring *if* a fungus can produce lignin
27 degrading enzymes', then the detection of such enzyme production on any substrate can
28 be conclusive. Indeed, much of our mechanistic understanding of extracellular enzyme
29 production in fungi has been generated using ArtMed studies (Baldrian and Valášková,
30 2008; Crowther et al., 2011; Hiscox et al., 2010; Maynard et al., 2017; Žifčáková et al.,
31 2011). Similarly, fungi growing in ArtMed have been foundational to the development of
32 antibiotics, which have had implications that reach far beyond fungal ecology (Baym et
33 al., 2016; Gerardin et al., 2016). However, and importantly, although positive results on
34 any given substrate can confirm the existence of a process, the lack of detection cannot
35 categorically disprove the existence of that process. As in the first example, the detection
36 of fungal-mediated laccase activity can confirm the potential for laccase production from
37 fungus X, but a negative result cannot be used to discount the process, which might exist
38 under different growing conditions. In such settings, appropriate selection of nutrients in
39 the ArtMed (e.g., malt vs. lignin; organic vs. inorganic nitrogen) is critical for increasing
40 the robustness of the results, and for selectively identifying the process of interest.

Artificial media can even be useful for examining the potential responses to external biotic and abiotic cues, particularly when the research answers are categorical (i.e. the fungus can or cannot respond). For example, to assess whether moisture, grazing invertebrates or pressure variations in the air associated with sound can affect the growth of a fungus, the growing substrate can be irrelevant (Bastos et al., 2012). The specific nature (i.e. the magnitude or rate) of the fungal response on ArtMed might not reflect that observed under natural growing conditions, but a response observed in ArtMed can certainly confirm the existence of a mechanism that could then be examined in more detail across different natural substrates.

When disentangling multiple processes

A detailed understanding of how various environmental factors individually and interactively structure fungal physiology and behavior is needed to parameterize and construct mechanistic population-, community- and ecosystem-level models. Disentangling the effects of various biotic and abiotic conditions can be achieved under field conditions but highly controlled laboratory conditions can provide an unparalleled level of control, which is valuable for removing the confounding affects of other associated environmental changes. For example, the addition of nitrogen to soils can have many interactive effects, leading to secondary changes in pH, promoting community turnover, affecting individual physiology, or inducing progressive nutrient limitation (Manning et al., 2006; Morrison et al., 2016; Olander and Vitousek, 2000; Wallenstein et al., 2006). Thus, to assess how fungi respond to elevated nitrogen, such questions may be best addressed using artificial media, where these ancillary confounding factors can be tightly controlled for (Maynard et al., 2017).

Conceptual advances in ecological and evolutionary theory that are not specific to fungi

Fungi growing in ArtMed can often represent a valuable system to examine ecological principles that are not specific to the fungal system. Indeed, fungi in agar media can represent one of the most valuable systems to test many general ecological questions,

when the system or even taxa used are irrelevant. In this context, they represent simple model systems where different organisms can grow rapidly under a range of conditions, enabling researchers to explore fundamental ecological principles. The use of ArtMed was central to developments in various fields of research, including studies into the causes and consequences of adaptation and evolution (Fukami et al., 2007; McDonald et al., 2016); for evaluating the effects of gene deletion and gene expression (Bok et al., 2009; Garbeva and De Boer, 2009); for advancing our knowledge of population and community dynamics (Maynard et al., 2015; Maynard et al., 2017); for exploring the emergence of antibiotic resistance (Baym et al., 2016; Gerardin et al., 2016); or for quantifying the drivers behind diversity and coexistence (Kerr et al., 2002; Hol et al., 2015). If the ecological question addresses whether trait dissimilarity affects the coexistence of species, then an appropriate model system simply necessitates that different individuals can survive and interact within that given environment, and the realism of the environment is irrelevant.

When optimal conditions or standardization are paramount

In some circumstances, the requirement to standardize growing conditions across multiple species is more important than the need to replicate natural growing conditions. Like all organisms, fungi can show high levels of specialization to certain growing conditions. The comparison of two fungi on any given natural resource will therefore introduce bias that favors one of the individuals. As such, the use of ArtMed can provide a foreign substrate that can be manipulated to represent a common ground for the comparison across multiple individuals. This is particularly important during the emergence of trait-based approaches in fungal ecology (Lennon et al., 2012; Aguilar-Trigueros et al., 2014; Crowther et al., 2014;), where the comparison of trait values across individuals necessitates the measurement of ‘potential’ trait expression under non-limiting conditions (Pérez-Harguindeguy et al., 2013; Crowther et al., 2014). Given that all natural conditions exert a different set of physical or biochemical limitations that differentially affect the development of different species, they will all exert selective advantages for some fungi over others. As such, natural substrates do not represent a

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4 good opportunity to compare and contrast trait expression or performance across species.
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6 The use of ArtMed can provide a unique opportunity to approximate non-limiting
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8 conditions that facilitate the standardized comparison of multiple individuals or
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10 communities.

11 12 13 **Conclusions** 14

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17 Given the benefits of ArtMed under appropriate experimental studies, we argue against
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19 their indiscriminate rejection in fungal ecology studies. As a model system, these media
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21 are equivalent to the use of lab rats in human medical testing: even without providing
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23 definitive real-world effects, they can help to identify important mechanisms that can
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25 guide ongoing research. The rejection of ArtMed on the basis of limited realism would
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27 place fungal ecology at a conceptual disadvantage relative to other biological/ecological
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29 research fields, where the use of such model systems (including agar media) take a wide
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31 variety of forms. The important thing is that the implications of findings from ArtMed
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33 experiments should not be over-stated regarding the relevance for natural fungal
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35 communities. Undoubtedly, ArtMed experiments are usually only a first step in the study
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37 of any process, but without this first step, we are likely to miss many of the important
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39 mechanisms operating in fungal communities.

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41 There is an infamous question in biology, “Can a biologist fix a radio” (Lazebnik, 2004)?
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43 or alternatively posed as, “Can a neuroscientist understand a microprocessor?” (Jonas &
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45 Kording, 2017). The crux of this question is whether or not the standard, top-down,
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47 whole-systems approach used to understand complex biological systems can actually
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49 provide valid inferences about the system’s inner workings. Luckily, in fungal ecology,
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51 we are not faced with an either/or situation. We can embrace complexity and attempt to
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53 deconstruct the system from a top-down manner via realistic, *in situ* manipulative and
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55 observational experiments; and we can also simultaneously attempt to reconstruct and
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57 rebuild the system from the bottom up by using ArtMed to understand how individual
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59 components interact at the simplest of levels. Depending on the setting or the question,
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one approach may be more favorable or more likely to yield results. However, we argue that the most powerful strategy is to use these approaches in tandem.

We encourage careful consideration of the pros and cons of ArtMed when designing experiments in fungal ecology. In any situation where the benefits of ArtMed (e.g. ease of use or standardization) outweigh the negatives (unrealistic structure and heterogeneity), then the indiscriminate rejection of ArtMed will come at the cost of efficiency in experimental studies. The same considerations also apply to the use of natural substrates, because no single growing substrate can capture fungal dynamics displayed in all other substrates. Ultimately, given the complex nature of fungal communities, any solitary piece of information can only provide weak inference about fungal systems. A complementary set of approaches and perspectives is necessary if we are to maximize our chances of untangling the complexities of natural fungal communities (Peay, 2014; Vilanova and Porcar, 2016).

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Figure 1: Images of fungi growing and interacting in agar media. Panel A shows a range of fungi used in pairwise competitive interactions. Panel B shows fungi that have been growing at a range of different temperatures that are used to reflect an environmental gradient. Panel C shows three interacting cultures of the same fungal isolate.

Figure

