

# Fungal Community Ecology: A Hybrid Beast with a Molecular Master

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*Fungi play a major role in the function and dynamics of terrestrial ecosystems, directly influencing the structure of plant, animal, and bacterial communities through interactions that span the mutualism-parasitism continuum. Only with the advent of deoxyribonucleic acid (DNA)-based molecular techniques, however, have researchers been able to look closely at the ecological forces that structure fungal communities. The recent explosion of molecular studies has greatly advanced our understanding of fungal diversity, niche partitioning, competition, spatial variability, and functional traits. Because of fungi's unique biology, fungal ecology is a hybrid beast that straddles the macroscopic and microscopic worlds. While the dual nature of this field presents many challenges, it also makes fungi excellent organisms for testing extant ecological theories, and it provides opportunities for new and unanticipated research. Many questions remain unanswered, but continuing advances in molecular techniques and field and lab experimentation indicate that fungal ecology has a bright future.*

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**F**ungi play pivotal roles in all terrestrial environments (figure 1). They are a major component of global biodiversity and control the rates of key ecosystem processes. Fungi are perhaps best known for their role as decomposers, dominating the decomposition of plant parts, and particularly of lignified cellulose. They produce a wide range of extracellular enzymes that break down complex organic polymers into simpler forms that can be taken up by the fungi or by other organisms. This process is an essential step in the carbon cycle; without it, plant detritus would quickly tie up available carbon and mineral nutrients. It is not surprising, therefore, that eliminating fungi results in a significant reduction in both carbon and nitrogen depletion from litter (Beare et al. 1992). In fact, fungal hyphae often account for the greatest fraction of soil biomass (Wardle 2002) and can reach lengths of hundreds to thousands of meters per gram of soil (Taylor and Alexander 2005).

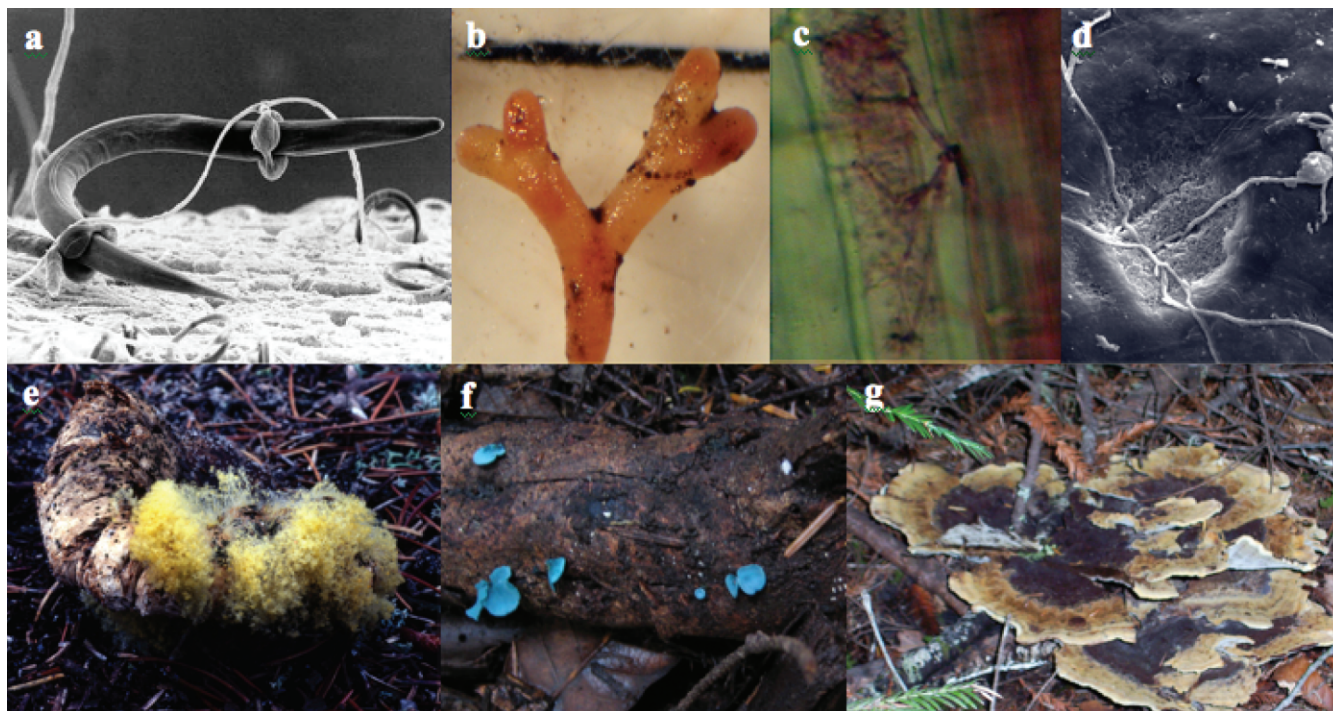
In addition to their central roles in carbon and nutrient cycling, fungi are also a major component of terrestrial food webs. Fungal mycelia serve as the primary carbon source in a number of soil food webs (Wardle 2002), and fungal fruiting bodies can serve as a significant food source for large vertebrates, including humans. Some fungi can also act as predators: Perhaps the best-known examples are nematode-trapping fungi, but fungi also trap, poison, or parasitize and

feed on other groups of soil invertebrates, including tardigrades, collembola, copepods, and rotifers (Thorn and Barron 1984). Interestingly, fungi express these behaviors in nitrogen-poor environments, suggesting that they seek nitrogen rather than carbon from their predation.

Fungi directly shape the community dynamics of plants, animals, and bacteria through a range of interactions. They are the most common and important plant pathogens, causing serious crop loss and shaping the composition and structure of natural plant communities in many significant but often underappreciated ways. For example, seedling mortality is often highest close to parent plants because of host-specific fungal pathogens that reside on the parents. Such distance-dependent mortality has been hypothesized as a major mechanism preventing competitive exclusion and maintaining plant species diversity (Gilbert 2002). The recent worldwide spread of the frog pathogen *Batrachochytrium*

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**Figure 1.** The many faces of the kingdom Fungi. (a) The nematode-trapping fungus *Arthrobotrys*. Photograph courtesy of Hedwig Triantaphyllou. (b) Fine root of *Pinus muricata* ensheathed by an ectomycorrhizal fungus. Photograph: Kabir Peay. (c) Intracellular root penetration by an arbuscular mycorrhizal fungus. Photograph courtesy of Shannon Schechter. (d) Unidentified endophytic fungus growing along the surface and into the stomata of a redwood needle. Photograph courtesy of Emily Lim. (e) Yellow sporangiophores of the mycoparasite *Syzygites* sporulating on a *Russula* fruit body. Photograph: Thomas Bruns. (f) Blue fruiting bodies of the ascomycetous wood decay fungus *Chlorociboria*. Photograph: Kabir Peay. (g) Fruiting body of a common root pathogen of coniferous forests, *Phaeolus schweinitzii*. Photograph: Kabir Peay.

*dendrobatidis* and its role in global amphibian declines also demonstrate the importance of vertebrate fungal pathogens (Lips et al. 2006). While some fungi are clearly parasites, the nature of many fungal interactions is uncertain and may change depending on the environment in which the interactions occur (Johnson et al. 1997). Endophytic fungi, which live ubiquitously inside the leaves, stems, and roots of plants, are a good example. Although some of these fungi produce secondary compounds that protect their hosts from herbivory, their overall effects on plant fitness can change dramatically depending on environmental conditions or herbivore pressure (Saikkonen et al. 2004). Because these fungi are often present in a quasi-quiescent state, their ecological roles remain poorly understood (Arnold et al. 2007).

The symbiosis between plant roots and fungi, referred to as mycorrhiza (literally, “fungus root”), is one of the most ubiquitous mutualisms in terrestrial ecosystems. These mycorrhizal associations enable plants to acquire mineral nutrients and water in exchange for photosynthetically derived sugars. It is likely that plant adaptation to life on land 400 million years ago was possible only with the help of mycorrhizal symbionts (Simon et al. 1993). Many plants depend heavily on mycorrhizae for mineral nutrition, and the absence of appropriate fungi can significantly alter plant community structure (Weber et al. 2005). Although most mycorrhizal

interactions are thought to be mutualistic, there are examples of mycorrhizal symbioses in which plants are parasitized by fungi (Johnson et al. 1997) or fungi are parasitized by plants, as in the case of certain nonphotosynthetic plants that have become parasites on mycorrhizal fungi involved in mutualistic interactions with other photosynthetic plants (Bidartondo 2005). Lichens, which are symbioses between fungi and algae or cyanobacteria, are also widespread and important, particularly in stressful abiotic environments, where they contribute significantly to biomass, nitrogen fixation, and mineral weathering. Other fungi form external mutualistic symbioses with insects, such as attine ants, some termites, wood wasps, and ambrosia beetles. The fungi break down otherwise indigestible plant material in return for a constant food source and a stable environment that is generally pathogen free (Currie et al. 2003).

Despite their ubiquity and clear importance in terrestrial ecosystems, the ecological study of fungal communities has long been held back by an inability to identify species in their vegetative states. Although reproductive structures can be diagnostic, they are not ideal for ecological studies because they are produced infrequently in the field, often harbor cryptic species complexes, and do not accurately represent species abundances (e.g., in a count that included only fruits, fungi would disproportionately dominate some tropical forests).

However, the recent adoption and dissemination of DNA- and ribonucleic acid (RNA)-based molecular tools has greatly reduced the barriers to sampling and identifying fungi from vegetative material. At the same time, improvements in techniques for measuring fungal biomass and nutrient uptake (e.g., isotopes, phospholipid fatty acids, and ergosterol) have confirmed the importance of fungi in key ecosystem functions, such as carbon and nutrient cycling (Hobbie and Hobbie 2006). In tandem, these developments have resulted in an explosion of studies that have propelled fungal ecology into the 21st century.

A number of excellent reviews have examined the current array of molecular techniques available to ecologists interested in working with fungi (Horton and Bruns 2001, Anderson and Cairney 2004, Bidartondo and Gardes 2005). The intention of this overview is not to add to this growing list, but instead to begin to synthesize the ecological results from the proliferation of studies that have used these tools to study fungal communities. Our purpose in doing so is to identify major research themes and theoretical advances in fungal community ecology, but also to point out key gaps that should be the foci of future research. Because of our own research interests, a disproportionate amount of material will be drawn from community studies of mycorrhizal and other soil fungi. This area of research was among the first to adopt many of the most commonly used molecular techniques and therefore provides a strong base from which to assess the current state of fungal ecology. The same molecular tools have, however, helped advance many other areas, including food webs (Hobbie and Hobbie 2006), population dynamics (Grubisha et al. 2007), and evolutionary ecology (Roy 2001, Bidartondo 2005). Here we focus specifically on a subset of community ecology topics—fungal diversity, niche partitioning, competition, spatial variability, and functional traits—but hope that our observations have broader implications for researchers working in other fields and on other fungi.

### The need for studying fungi with molecular tools

Fungi represent a hybrid between micro- and macroscopic lifestyles. Like those of other microbes, fungal communities are highly diverse and poorly described. Their vegetative bodies are composed of microscopic filaments that interact directly with the environment at the micron scale (box 1, figure 2). Fungal spores, often in the small-micrometer range (e.g., 10 to 20 micrometers), are produced in great numbers and are capable of long-distance dispersal. This microscopic aspect makes fungi nearly impossible to observe in their active, vegetative states and thus requires molecular tools for identification and quantification. On the other hand, fungi share many ecological similarities with macroorganisms. Like plants, for example, fungi are sessile and compete for space in order to control access to resources. And, although individual hyphae are microscopic, genets or ramets can come to occupy large spaces and can survive for many years (Smith et al. 1992). Unlike bacteria, fungi do not seem to exhibit a high frequency of horizontal gene transfer, so functional

traits are relatively stable, and species concepts are useful and reasonably well developed (Taylor et al. 2000). For these reasons, it is likely that much of the ecological theory derived from macroorganisms is applicable to the study of fungi. Because of their largely cryptic nature, however, it has only been with the application of molecular tools that fungal ecology has begun to successfully reconcile the micro-macro gap.

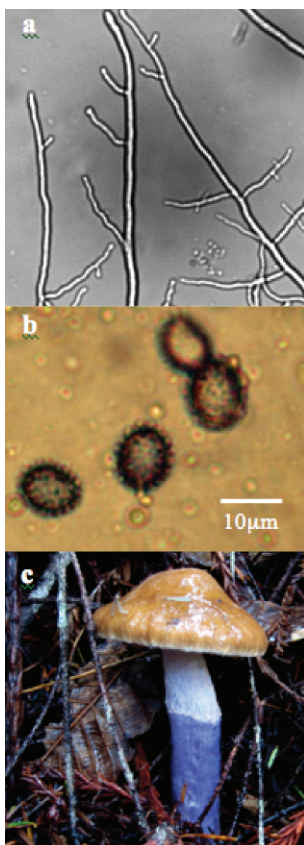
Before the advent of molecular tools, the identification of most fungal species and individuals depended on the haphazard availability of fruiting structures (e.g., mushrooms) in the field or the ability to culture fungi from environmental materials in the laboratory. While effective in some cases, these methods were not ideal for many reasons: cultures were time consuming, were biased toward fast-growing fungi, precluded many biotrophic fungi, could only be done on fresh materials, and in some cases were based on flawed morphological species concepts. The earliest applications of polymerase chain reaction (PCR)-based molecular techniques (box 2) to fungi were primarily for phylogenetic and population genetic research. However, the development of fungal-specific primers for amplification of the internal transcribed spacer (ITS) region of the ribosomal RNA genes (Gardes and Bruns

#### Box 1. Fungi 101.

*Hyphae* are the hallmark of the fungal lifestyle. These microscopic filaments, often on the order of 1 to 5 micrometers in diameter, are the primary building blocks of most fungi. Because of their filamentous nature, hyphae are ideal for growth into solid substrates, such as soil or wood, where they export hydrolytic enzymes and import necessary nutrients. Hyphae may successfully fuse if they belong to a single individual, but in most cases, hyphae from different individuals are not compatible except for the purposes of sexual reproduction. (This is referred to as *vegetative incompatibility*.) The aggregated hyphal network of an individual fungus is referred to as a *mycelium*.

*Spores* are the primary means of dispersal for most fungi. Fungi produce asexual, sexual, haploid, diploid, and dikaryotic spores, in an incredible diversity of shapes and sizes. Spores are produced in prodigious numbers and can potentially disperse large distances. Spore dispersal and germination syndromes are closely linked with fungal autecology, with many spores displaying dormancy that is broken only in response to signals such as heat, moisture, or host exudates.

*Fruiting bodies* are macroscopic, hyphal structures upon or within which spores are produced. The most obvious fruiting bodies, mushrooms, are what most people commonly recognize as a fungus, but most fungi lack them. When present these structures are often impressive, but they make up only a small portion of the fungal individual, analogous to the apples on an apple tree. Most fruiting bodies are ephemeral, although some can persist for many years. The exact controls on fruiting are not known, but production is often linked to seasonal or environmental cues, such as precipitation, temperature, or disturbance.



**Figure 2.** (a) Hyphal growth of *Neurospora crassa*. Photograph courtesy of Anna Simonin. (b) Spiny spores of *Russula*, a common ectomycorrhizal genus. Photograph: Kabir Peay. (c) Fruiting body of *Cortinarius vanduzerensis*. Photograph: Kabir Peay.

1993) opened the way for direct amplification of fungal DNA from complex substrates containing multiple sources of DNA, such as soil or plant tissue. Early studies demonstrated the utility of the molecular approach to fungal community ecology (Gardes and Bruns 1996), and in the past decade, an explosion of easy, cheap, high-throughput molecular methods has made these techniques more available to ecologists with little background in molecular biology. Although applying such techniques without understanding their inherent limitations can lead to problems (e.g., Avis et al. 2006), the shrinking technology gap has resulted in an ever-growing number of high-quality studies grappling with increasingly sophisticated ecological problems. These community-profiling techniques have become so widespread that in essence fungal community ecology is molecular.

In the following sections, we discuss a number of research areas in which the use of molecular techniques has provided significant new insight into fungal ecology. Although we focus on only a subset of topics, it is important to note that a wide array of molecular techniques has proven useful across many fields of fungal ecology. Examples of areas not covered in this review include the use of stable isotopes in the study of resource acquisition and allocation (Hobbie and Hobbie

2006), of microsatellites in the study of population genetics (Grubisha et al. 2007), of DNA-based phylogenies in evolutionary ecology (Roy 2001), and of fluorescent microscopy in population ecology (Bidartondo and Gardes 2005).

### How many fungi are there?

A prerequisite for most ecological studies is the ability to count all of the species present in the area or sample being studied. However, obtaining accurate estimates of species richness for microorganisms such as fungi has been challenging at both local and global scales. On the basis of pre-molecular estimates of fungal species richness in a few well-characterized ecosystems, Hawksworth (1991) estimated a fungal-to-plant species ratio of 6-to-1, and used this to extrapolate a global estimate of 1.5 million fungal species. Although DNA-based species concepts are not perfect (box 3), species enumeration with molecular tools suggests even greater dimensions of fungal richness. Specifically, PCR and direct sequencing of fungal DNA have repeatedly shown that large numbers of species are missed in culture studies (e.g., Allen et al. 2003, Arnold et al. 2007). In addition, morpho-species in poorly studied groups often harbor cryptic diversity (e.g., Arnold et al. 2007).

Taxon-specific PCR has also dramatically expanded the search for fungal diversity by allowing exploration of novel habitats where fungi cannot be cultured or do not produce fruit bodies. For example, an entirely new subphylum of fungi was discovered using molecular methods to examine the fungal community active beneath snowpacks in high alpine environments (Schadt et al. 2003), and a huge number of novel yeasts were found in beetle guts (Suh et al. 2005), a widespread but little-explored habitat. It is quite clear that cryptic species and new species discoveries would lead to an upward revision of Hawksworth's 6-to-1 ratio. One recent study found 49 fungal phylotypes (phylogenetically defined taxonomic units) from across all four fungal phyla on the roots of a single grass species (Vandenkoornhuys et al. 2002), and in the tropics, the fungal-to-plant species ratio has been conservatively estimated at 33-to-1 (Fröhlich and Hyde 1999). In Hawksworth's reestimation, allowing for cryptic species alone could more than triple the global species estimate to 5.1 million (Hawksworth 2001). Thus, molecular methods indicate that at the global level, the kingdom Fungi is one of the most species rich of the major eukaryotic lineages.

Molecular techniques have also allowed for reexamination of local species richness estimates in ecosystems previously thought to be well sampled from culture and fruiting body studies. Nowhere has this proven to be more striking than in soils. Because PCR and direct sequencing do not work on samples containing multiple target species, the exploration of soil diversity has been facilitated through the increasing ease of cloning technology (box 2). Whereas early culture-based studies led to the conclusion that soils were dominated by a few hundred globally distributed fungi, cloning studies easily document hundreds of species within a single plot and indicate high levels of local variability and endemism (O'Brien

### Box 2. Molecular techniques for analyzing fungal communities.

A wide range of molecular techniques are available to fungal ecologists. Here we briefly list some of the common and cutting-edge techniques used in fungal community profiling and quantification. Because each technique has strengths and limitations, care must be taken to match the proper technique to the specific research question. For more detail about specific techniques, see recent technical reviews by Horton and Bruns (2001), Anderson and Cairney (2004), and Bidartondo and Gardes (2005).

The *polymerase chain reaction* (PCR) is the basis for almost all community profiling techniques in molecular ecology. PCR techniques use probes (or *primers*) made up of approximately 20 DNA base pairs that are designed to match a specific region of the genome. A pair of primers can then be selected that bracket a larger target stretch of the genome (usually 500 to 1000 bases) and serve as the starting points for creating new copies of this region. The PCR process begins by using high temperatures (approximately 95 degrees Celsius) to separate double-stranded genomic DNA. When temperatures are reduced, primers attach to complementary nucleotide sequences, and a thermally stable DNA synthesis enzyme (Taq polymerase) uses the primers as the starting point to create new DNA templates. The thermal steps required to separate target DNA strands, anneal primers, and create new copies is referred to as a PCR cycle. The double-stranded nature of DNA allows for doubling in copy number with each PCR cycle and can create billions of copies from very low starting abundance after only 20 to 30 PCR cycles.

Selecting or designing primers is a critical step in PCR-based molecular techniques. Because the exact target sequence is unknown, PCR primers are often designed to match highly conserved regions of the genome that flank highly variable regions. Using this approach, PCR primers can be designed in such a way that they are “fungal specific” and, given a mixture of plant, animal, prokaryotic, and fungal DNA, can recognize sequences that are only conserved in fungi. Primers can be designed to target specific organisms at any taxonomic level, from kingdom to individual, depending on the nature of the research question.

*Direct sequencing* can be used to determine the exact nucleotide sequence from PCR products containing a single DNA template (e.g., from a fruiting body or a colonized ectomycorrhizal root tip). The resulting sequence (usually 500 to 700 base pairs) can be checked against online databases and an identity assigned based on match percentage or on phylogenetic methods of classification. Direct sequencing is a powerful, high-resolution technique but is limited by the requirement of single template samples and by the quality of data in bioinformatics databases. *Cloning* (the insertion of target DNA sequences into bacteria) can be used to separate mixed PCR products for sequencing, but is time-consuming and expensive, and thus less feasible for large numbers of samples.

*High-throughput sequencing* is becoming possible for ecological studies with the advent of next-generation technologies such as 454 pyrosequencing and Solexa (Roesch et al. 2007). These techniques were originally designed for genomic sequencing and can produce up to  $2 \times 10^7$  base pairs in a single four-hour run, compared with approximately  $5 \times 10^4$  base pairs per run with current Sanger sequencing technology. When coupled with the PCR of a common barcode gene, these techniques may provide high enough coverage to finally plateau sample accumulation curves for microbial communities. One limitation is that current sequence reads are quite short for this technology (approximately 200 to 250 bases), but this is predicted to improve with time. These techniques are also expensive (a single sample run can cost between \$10,000 and \$20,000), and current bioinformatics tools for fungi are insufficient to handle the large amount of sequence data produced.

*Restriction fragment length polymorphism* (RFLP) uses restriction enzymes to cut PCR products into smaller fragments. Because organisms vary in the location of restriction sites, different organisms will produce fragments with different size profiles. DNA is negatively charged and can be moved through a porous medium (such as an agarose gel) by applying an electric current. Because DNA fragments that differ in size migrate at different speeds, the fragments resulting from restriction digests can be separated and sized using this technique. This can be done manually through gel electrophoresis, or, if the DNA is fluorescently labeled, it can be automated with capillary electrophoresis machines used for DNA sequencing. Terminal RFLP (t-RFLP) is one popular variant of this technique that uses fluorescently labeled primers so that only the sizes of the two terminal fragments attached to the primers are determined. RFLP size profiles can be used to create fingerprints for known organisms and to determine whether or not they are present in an environmental sample. The taxonomic resolution of RFLPs is lower than that produced by direct sequencing, but RFLPs are relatively cheap and good for processing samples that contain template DNA from multiple target organisms (e.g., soil, leaves). However, the discriminatory ability of RFLPs can become extremely limited when species richness is high, and there are serious issues when trying to identify or count unknown organisms from mixed RFLP samples when no preexisting database is available (Avis et al. 2006).

*Quantitative real-time PCR* combines fluorescent primers with an *in situ* detector to monitor PCR cycling in real time. The purpose of this technique is to quantify the presence of target organisms rather than describe the entire community. Because the degree of fluorescence is based on the amount of double-stranded DNA present in the reaction, the amount of template present in a sample can be determined by comparing the fluorescence of unknown samples with standards that have known starting quantities. This technique can be used to determine the amount of biomass in a sample; however, the relationship between DNA quantity and biomass varies dramatically between different organisms and different genes as a result of variation in copy number, reaction efficiency, template quality, and other factors. For functional ecology studies, RNA can also be quantified to determine the amount of gene expression in a given sample. One limitation to this technique is that a single primer set can only target one taxonomic group. This means that researchers must either know the appropriate taxonomic level for the question at hand or else design multiple primer sets to compare different groups or species.

**Box 3. Defining a “molecular” fungal species.**

With the advent of molecular tools, fungal species are increasingly being defined by variation in DNA sequences. The ideal DNA-based identification employs data from multiple loci, as no single locus is universally reliable for species recognition (Taylor et al. 2000). However, in ecological settings, multilocus knowledge of species is rarely available; and this approach is problematic when all the sequences are drawn from a common environmental pool, such as soil or plant material, as the connections between loci are lost. For these reasons, a single locus, the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA gene, has become widely used for near-species-level identification (Horton and Bruns 2001). This region has four primary advantages over other regions: (1) it is multicopy, so the amount of starting material needed for successful amplification is extremely low; (2) it has well-conserved fungal specific priming sites directly adjacent to multiple highly variable regions; (3) there are many sequences already available for comparison, which greatly facilitates the identification of unknown samples; and (4) it correlates well with morphologically defined species in many groups (Smith et al. 2007). As a rule of thumb, approximately 97% sequence similarity is usually appropriate to distinguish species in environmental studies. However, there are groups in which ITS does not discriminate between closely related species, so it is important to recognize that use of the ITS as a surrogate for species is simply a convenient approximation.

et al. 2005, Fierer et al. 2007). Furthermore, the species documented in these studies still appear to be only a small fraction of the total species pool. Despite intense sampling efforts, no large-scale soil sequencing projects have managed to reach asymptotic estimates of fungal richness (O’Brien et al. 2005, Fierer et al. 2007). The relationship between species diversity and ecosystem function, species-area relationships, and biodiversity conservation are major areas of ecological research, and the inability to accurately estimate species richness is a major impediment in comparisons between sites or treatments in studies (Woodcock et al. 2006). Current sequencing technology is clearly inadequate for sampling such high-diversity fungal systems, but there is some hope that newly developed high-throughput sequencing techniques will soon provide a solution to this problem (box 2). Although such methods were primarily designed for genomic sequencing, they have performed well in studies of bacterial communities (Roesch et al. 2007). The volume of sequence data generated by these methods is immense, but researchers will require more sophisticated “community bioinformatics” tools before it is feasible to use these data in fungal community studies.

**The niche revisited**

Since the formal recognition of the competitive exclusion principle, the coexistence of species via niche partitioning has been a major topic in ecology. Although the utility of niche theory has been contested in recent years, studies on fungi in-

dicating that niche partitioning is an important determinant of fungal community structure. For example, many studies involving a diverse range of fungi (i.e., mycorrhizal symbionts, saprotrophs, and foliar epiphytes) have documented clear vertical stratification of species within a soil profile or tree canopy (Dickie et al. 2002, Gilbert et al. 2007, Lindahl et al. 2007). Although this is a strong pattern, identifying the parameters along which species are partitioned has been complicated by the fact that multiple environmental factors covary across these spatial gradients. Despite these challenges, studies using more advanced statistical methods to isolate the effects of plant species composition or model systems with simple plant communities have shown convincing evidence of niche partitioning based on soil chemistry (Parrent et al. 2006, Lekberg et al. 2007, Lindahl et al. 2007). In particular, nitrogen content, base saturation, carbon age, and soil moisture appear to be some of the most important determinants of soil fungal community structure.

While host changes may sometimes obscure abiotic niche partitioning, host specificity can also act as an important niche dimension itself. One of the primary advantages of using molecular techniques is that both the fungus and the plant host can be independently identified from the same DNA extract, so precise quantification of fungal-host partnerships is possible (Lian et al. 2006). Because of their economic importance, patterns of host specificity have probably been best characterized in fungal pathogens. For example, Roy (2001) used molecular phylogenies to examine patterns of host specificity for a set of crucifers and their flower-mimicking rust pathogens. Interestingly, the phylogenies showed that the pathogens were more likely to jump to new hosts that were in close geographic proximity rather than to those that were closely related. This suggests that dispersal limitation and local specialization are important factors in determining patterns of fungal host specificity. When dispersal is limited, highly specialized fungi may be at a disadvantage if hosts are rare across the landscape and thus are difficult to locate.

For this reason, it has been predicted that fungal host specificity should be low in high-diversity communities and vice versa (May 1991). This has important implications for predicting global fungal species richness based on ratios of fungi to plants (see above). Support for May’s contention has been provided by two non-molecular studies, one that showed polypores in a low-diversity mangrove swamp in Panama were highly specific to a single species of mangrove (Gilbert and Sousa 2002), and another that showed host generalism was common in wood-decay fungi in a diverse tropical rainforest (Ferrer and Gilbert 2003). However, few studies have examined host specificity in the tropics, in part because the taxonomy and identification of both tropical fungi and trees are challenging at best. Molecular tools have great potential for contributing to this area of research in the future. As an example, one polypore—an apparent exception to the pattern of high host specificity found in the mangrove study mentioned above—was later found through molecular methods to represent multiple species, one of which was a man-

grove specialist (Sarah Bergemann, Middle Tennessee State University, Murfreesboro, personal communication, 28 April 2008).

Molecular tools have also contributed greatly to researchers' understanding of host specificity patterns in fungal mutualisms. Mutualists are thought to have broader host ranges than pathogens (Borowicz and Juliano 1991). However, the importance of host preferences in mutualist communities was recently demonstrated in a study by Ishida and colleagues (2007) that characterized patterns of ectomycorrhizal host preference across plots of varying tree species richness by identifying both host and fungal DNA from colonized roots. The study found that host preferences led to significantly higher ectomycorrhizal richness in forests with greater tree richness, and thus showed that these preferences play an important role in maintaining fungal richness in mixed-species forests. Similar results have also been reported for arbuscular mycorrhizal fungi (Vandenkoornhuysen et al. 2003). Overall, host-fungal interactions pose a number of interesting ecological questions that remain to be answered and are well suited to exploration with molecular tools.

### Competitive interactions among fungi

Environmental conditions and host or substrate specificity set the fundamental niche of all fungi, but, as in many other organisms, competition appears to play a key role in determining the realized niche for many fungal species. Researchers' understanding of the effect of competition on species interactions and fungal assemblage structure has continued to improve in recent years thanks to an increase in studies using experimental approaches in conjunction with molecular identification. Recent work has demonstrated strong competitive interactions among pathogens and leaf endophytes (Arnold et al. 2003), ectomycorrhizal fungi (Kennedy and Bruns 2005), arbuscular mycorrhizal fungi (Lekberg et al. 2007), and saprotrophic fungi (Boddy 2000). The study of competition has a rich theoretical and empirical history in ecology, and researchers have only begun to test the ways in which fungi may or may not fit the competitive patterns observed in other organisms. For example, Kennedy and Bruns (2005) used a simple PCR-RFLP (restriction fragment length polymorphism) analysis to show that timing of host root colonization had a significant outcome on the competitive interactions between two morphologically indistinguishable ectomycorrhizal species. The use of more quantitative molecular methods such as real-time PCR has also facilitated the study of fungal competition by allowing researchers to directly quantify the amount of biomass belonging to different species in mixed-species treatments (Kennedy et al. 2007).

There are many central questions about the nature of fungal competition, however, that have yet to be addressed. For example, whether fungal competitive interactions are driven primarily by interference- or exploitation-type competition has been relatively well studied in saprotrophic fungi (Boddy 2000) but is less clear for mycorrhizal and pathogenic fungi. Differences in competitive strategies may also exist

between different fungal life stages (spore vs. mycelial), which may result in varying competitive outcomes depending on the time scale and environmental conditions being studied. In addition, most studies of fungal competition have focused almost exclusively on pairwise comparisons, but given the high diversity of fungal communities (see above), it is imperative that larger combinations of species be tested. Recently, for example, Kennedy and colleagues (2007) found that even a three-species comparison resulted in a different competitive outcome than was predicted from pairwise interactions between three ectomycorrhizal species. For symbiotic fungi (i.e., mycorrhizal and endophytic species), determining to what extent the outcome of the competition influences host performance is also an important future direction of study. There is considerable theoretical evidence that hosts could play a key role in driving fungal competitive dynamics (Kummel and Salant 2006), but there have been few direct tests of this idea. As this part of fungal ecology continues to expand rapidly, we believe that a tighter linkage between fungal life-history traits (e.g., mycelial production and foraging strategy, timing of spore germination, host range) and competitive strategies will provide significant insight into commonly observed ecological patterns in fungal communities, such as succession.

### Spatial and temporal variability in fungal communities

One challenge in studying fungal communities is that large spatial and temporal variability, coupled with high species richness, makes it difficult to observe taxa frequently enough to draw robust conclusions. The ubiquitous pattern of few dominants and many rare species has been consistently shown across a wide range of fungal lifestyles and in a variety of different ecosystems (Horton and Bruns 2001, Ferrer and Gilbert 2003, Arnold et al. 2007). The use of molecular tools has led to significant progress in quantifying the scale of this spatial and temporal variability. For example, Izzo and colleagues (2005) found that soil samples taken just 5 centimeters apart exhibited significant spatial and temporal turnover of ectomycorrhizal species. Such fine-scale variability is evident in the rapid decay of community similarity indices with distance, such that most ectomycorrhizal samples only exhibit spatial autocorrelation at less than 2 to 3 meters (Lilleskov et al. 2004). However, species composition at somewhat larger spatial scales (i.e., forest stand) shows greater stability, with most dominant species consistently recorded across multiple years (Izzo et al. 2005, Koide et al. 2007).

Attempts to scale up local spatial turnover to construct species-area relationships for fungi have had mixed results. Studies have found no (Andrews et al. 1987), weak (Green et al. 2004), or strong species-area relationships (Peay et al. 2007). The differences between these studies could be due to different patterns between the fungal groups studied (leaf epiphytes, soil ascomycetes, and ectomycorrhizal fungi, respectively), to different taxonomic criteria (morphology, ITS length, and ITS sequence, respectively) or to differences

in sampling efficiency (Woodcock et al. 2006, Peay et al. 2007). Although they are not normally considered at risk of extinction from habitat loss or fragmentation, fungi (and their ecosystem services) may be in jeopardy if habitat size proves to be a strong determinant of fungal richness.

The degree of spatial and temporal variability also seems to depend on the fungal structures being sampled. While root-tip composition appears to be relatively constant across seasons for ectomycorrhizal fungi, hyphal abundances are much more dynamic (Koide et al. 2007). Some species can have even abundances throughout the year, but many others have distinct maxima and minima depending on the season. Comparing between years, Parrent and Vilgalys (2007) found that changes in the composition of ectomycorrhizal hyphal communities were similar in magnitude to those observed from large experimental additions of nitrogen. The fact that assemblages are so dynamic is not necessarily due to a short life span, as individual fungi often live for years (Horton and Bruns 2001), and some, such as the pathogen *Armillaria*, may even live for centuries (Smith et al. 1992). Rather, it appears that fungal assemblages are heterogeneous at small spatial scales (Genney et al. 2006), and this heterogeneity, combined with rapid shifts in hyphal abundances, creates incredibly dynamic assemblages.

Although the high local variability of fungal communities may make some researchers despair of investigating them satisfactorily, it is also a fascinating phenomenon that requires explanation. There are many potential causes—for example, dispersal-driven neutral dynamics (Hubbell 2001), microscale fluctuations in abiotic conditions coupled with strong niche partitioning, and “rock-scissors-paper” competitive dynamics (Kerr et al. 2002)—and determining their relative importance in fungal assemblages would be a major contribution to the field of ecology. In addition, the fact that spatial scale matters implies that fungal ecologists need a better understanding of dispersal. We know that dispersal limitation is important for fungal population genetics and evolutionary ecology (Roy 2001, Grubisha et al. 2007), but we have very little idea of how it affects ecological patterns. For example, Peay and colleagues (2007) found evidence that fruit body production (a proxy for dispersal ability) was a good predictor of “tree island” colonization patterns. Establishing basic patterns of spore dispersal and viability at the landscape scale will be an important first step in linking dispersal to ecological patterns. However, dispersal is particularly challenging to study when propagules are microscopic, are hard to sample and identify, and can potentially travel long distances. Thus, new molecular tools or novel applications of existing ones will be key in answering questions about fungal dispersal.

### Functional ecology: A growing field for fungi

Functional ecology, which uses species traits to explain patterns of realized and fundamental niches, has emerged as a significant research focus in recent years (e.g., McGill et al. 2006). This area has been the provenance mainly of plant

ecologists, because of the large number of morphologically identifiable functional traits of plants (e.g., specific leaf area, seed size) and the relative ease with which they can be measured. Attempts to assign functional traits to fungal hypha on the basis of their morphology (Agerer 2001) have had some success, but, as with bacteria and archaea, functional differences among fungi are primarily biochemical and lack good morphological indicators. Cultured fungi can be assayed for enzymatic capacities (e.g., Lilleskov et al. 2002), but laboratory conditions seldom approximate field conditions and typically ignore the vast majority of unculturable fungi.

However, advances in genomics and quantitative PCR (box 2) have allowed the identification of some major functional genes and the *in situ* quantification of their expression. For example, laccase genes are thought to play an important role in the decomposition of high-lignin plant material. As predicted, a recent study using quantitative PCR demonstrated that laccase gene abundance increased as litter quality decreased and that these changes were linked to the presence of particular groups of wood decay fungi (Blackwood et al. 2007). While this approach targeted a single functional gene family, there are currently array-based technologies being developed that will allow simultaneous assays of multiple fungal lignin and cellulytic genes (Gentry et al. 2006; see also Bidartondo and Gardes [2005] for a detailed treatment of array-based techniques). Unfortunately, only a limited number of functional genes have been identified, but the rapid increase in fungal genomes available for comparison will provide more insight in this area. The ability to assign functional trait values to species (or species groups) is a critical link to interpreting changes in community structure along environmental axes and would strengthen researchers' mechanistic understanding of fungal community assembly. Successful quantification of functional traits will also allow fungal ecologists to begin addressing major questions in applied ecology, such as the degree of redundancy in high-diversity communities and the link between community structure and ecosystem function.

### From observation to experiment: Progress and pitfalls

Another challenge facing microbial ecologists, including those working on fungi, is that the vast majority of species found in natural environments are not easily culturable. As a result, many of the manipulative experiments central to ecological research are not feasible on natural assemblages under field conditions. For example, unlike plant or animal assemblages, fungal assemblages cannot be selectively weeded or fenced, because they often grow cryptically and lack species-distinguishing features in their hyphal or root-tip morphologies. Despite this difficulty, fungal ecologists are increasingly finding ways to use both natural and artificial manipulations to determine which factors drive the aforementioned community patterns.

Some of the earliest work involving molecular identification techniques examined shifts in ectomycorrhizal assem-

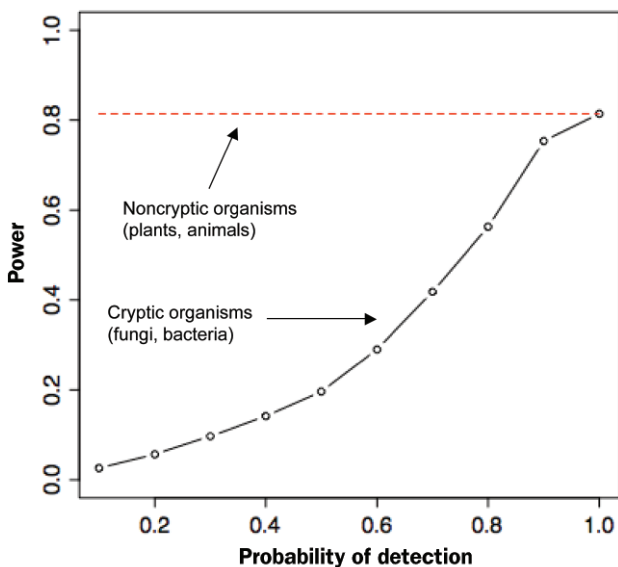


blages in response to disturbances. For example, studies in a California pine forest showed that stand-replacing fire induced a major shift in community assemblage, with the postfire community dominated by species that had disturbance-resistant propagules already present in the soil (Baar et al. 1999). While large shifts in assemblage patterns have been reasonably well documented for ectomycorrhizal fungi, the community response of saprotrophic and pathogenic fungi to major disturbances remains less clear. Arguably the most significant natural experiment, human-induced climate change, has been an area of active research in fungal ecology. Increasing carbon dioxide (CO<sub>2</sub>) levels may have strong effects on saprotrophic fungal communities by changing the carbon-to-nitrogen ratio of plant tissues, and may have similarly significant effects on pathogen and mycorrhizal communities by affecting host performance. A recent meta-analysis of global change experiments suggests that mycorrhizal abundance will most likely decrease with greater nutrient deposition (nitrogen, phosphorus) and increase with CO<sub>2</sub> enrichment (Treseder 2004). Large-scale field studies of this topic, however, have provided complex results. In a series of elegant studies at the Duke Forest in North Carolina, ectomycorrhizal species composition was found to change on CO<sub>2</sub>-

enriched plots, but biomass and species richness did not appear to be affected (Parrent et al. 2006, Parrent and Vilgalys 2007). While species composition did change between treatments, responses within larger taxonomic groups appeared idiosyncratic, making it hard to forge generalized predictions about how mycorrhizal assemblage structure will respond to global climate change.

Interpreting the results of nitrogen fertilization experiments has also proved difficult. Early studies on sporocarp abundance across nitrogen deposition gradients showed dramatic decreases in ectomycorrhizal species richness (Arnolds 1991), but molecular work on root tips and hyphae has shown that although assemblage structure changes, species richness does not necessarily decline (Parrent et al. 2006). Even though one study was able to link changes in abundance to species' ability to utilize organic nitrogen sources (Lilleskov et al. 2002), compositional changes within and across studies appear to be inconsistent with respect to larger taxonomic designations. This is an area where functional gene approaches may provide an important link to generalizing patterns across studies.

Although the observed fungal responses in these experiments are probably the result of multiple interacting factors, we believe a partial explanation for the lack of species richness effects is the inability to saturate sampling curves. As discussed previously, this issue is a major limitation when comparing species richness between treatments. Sampling effects may also limit the power to discern coherent responses from individual taxa or taxonomic groups. For example, the cryptic nature of fungal growth can dramatically reduce statistical power. Figure 3 shows that the ability to find a significant effect for a habitat parameter determining the presence or absence of a fungus is reduced dramatically when the fungus is difficult to detect. Even when the probability of detecting the fungus within a plot is 80%, the statistical power to determine differences between treatments is still only slightly better than a coin toss. Field manipulations are challenging precisely because they must simultaneously cope with all the complexity mentioned in previous sections: high species richness, co-correlated biotic and abiotic variables, high spatiotemporal variability, and few functional traits. However, ecological theories are of little use if they cannot be tested in



**Figure 3. Statistical power for cryptic versus noncryptic organisms. Statistical power is the probability that a true ecological effect will be successfully detected by a given study. It is affected by experimental design choices, such as sample size, and by the underlying properties of the phenomenon being studied, such as effect size and variability. Here we show that statistical power is also reduced dramatically when the target organism is cryptic (i.e., difficult to detect) rather than noncryptic (i.e., always detected when present). Each data point is based on 10,000 randomly simulated data sets in which the probability that our target fungus was present or absent was determined by a habitat parameter (e.g., soil nitrogen). We defined statistical power as the proportion of simulated data sets in which the habitat parameter was correctly found to be a significant predictor ( $p < 0.05$ ) of the presence of the fungus. The simulations were designed so that overall power was approximately 80% (dashed red reference line), the goal for most ecological studies. The simulations show that statistical power decreases rapidly with the degree of crypticism. Even at 80% detection probability, which is probably achieved only rarely in studies of bacteria and fungi, statistical power is still below 60%, slightly better than a coin toss. This illustrates some of the inherent difficulties in identifying even strong factors that structure fungal communities and the need for highly sensitive molecular tools that increase detection probability.**

the field; thus, we hope that improving molecular tools and manipulative techniques will lead to continued growth in the number of experimental field studies on fungi.

### Fungal ecology in the information age

Molecular tools have moved fungal ecology into the 21st century, but despite much progress, we still have more questions than answers about the ecological forces that structure fungal communities. One major impediment is that the available molecular data are fast outstripping our knowledge of fungal natural history. Natural history is not a substitute for rigorous experimentation and hypothesis testing, but it is integral to the generation of good ecological questions and interpretation of the data generated through experimentation. For example, Lindahl and colleagues (2007) found habitat partitioning among known ectomycorrhizal and saprotrophic fungi, but could assign only approximately 25% of the sequenced fungi to basic functional groups (i.e., mycorrhizal, endophytic, saprotrophic, or parasitic). Similarly, 94% of the root-associated fungi found by Vandenkoornhuyse and colleagues (2002) had no known ecological role. In addition to better natural history data, resolving these problems will require improving current bioinformatics databases and better understanding the link between phylogeny and ecology.

As more fungal communities are characterized using molecular data, there is an increasing need to develop better bioinformatics tools. The most popular current nucleotide search tool is the National Center for Biological Information (NCBI) Basic Local Alignment Search Tool, or BLAST, which allows users to search an enormous online database, GenBank, for sequences with the greatest similarity to their own. However, the NCBI GenBank database has numerous errors, poor-quality sequences, and many deposited sequences with little or no associated taxonomic information (Nilsson et al. 2006). In addition, the lack of third-party annotation means that it is not easy to update old submissions as new information becomes available. As a result, there have been some efforts to create new, high-quality sequence databases for use with environmental fungal samples. The Fungal Environmental Sampling and Informatics Network, or FESIN (Bruns et al. 2008), and the User-friendly Nordic ITS Ectomycorrhiza Database, or UNITE (Köljalg et al. 2005), aim to correct some of these problems, either by curating the GenBank data or by including only sequences from vouchered specimens identified by taxonomic experts. However, these efforts are still far behind bacterial projects, such as the Ribosomal Database Project and GreenGenes, which have greater batch processing and analytical capabilities.

While phylogeny is not explicitly considered in most ecological studies, it is often used implicitly to assign organisms to trophic or functional groups. This is particularly important in molecular studies of diverse groups with little formal taxonomic description, such as bacteria and fungi. Community ecology studies in general, and a number of recent studies on root-inhabiting fungi in particular, indicate that phylogeny is a good first-order approximation for major

ecological traits (e.g., Webb et al. 2002, Weiss et al. 2004). A number of groups have worked on using community phylogenetics in plant and bacterial communities (e.g., Webb et al. 2002, Lozupone et al. 2006), but little work has been done to develop such an infrastructure for fungal communities. Because the most commonly sequenced barcode gene, the ITS region, is unalignable at higher taxonomic levels (beyond genus in most cases), researchers need to develop fungal supertrees into which smaller group alignments based on ITS can be attached (e.g., Phylomatic; Webb and Donoghue 2005). The basic information necessary for the supertree approach has already been generated through the Assembling the Fungal Tree of Life, or AFToL, project (James et al. 2006) and the MOR project (Hibbett et al. 2005), which creates continuously updated fungal phylogenies as new sequences are deposited in GenBank. Thus, a little natural history and good phylogenetics have the potential to go a long way in helping to characterize the ecological role of fungi identified in environmental studies, even if the fungi have not been formally described.

### Conclusions

Fungal ecology is a rapidly growing, dynamic area of research. Molecular tools have led to great progress in understanding fungal ecology, but there is much yet to be learned. Based on our review, we have identified eight areas we believe will be fruitful venues of future fungal ecology research: (1) applying high-throughput molecular techniques to overcome sampling barriers and derive accurate estimates of local fungal species richness, (2) linking established patterns of niche partitioning with functional gene approaches to gain a mechanistic understanding of fungal community structure, (3) using functional genes to explore the link between fungal community composition and ecosystem function, (4) linking fungal competitive strategies with specific life-history traits, (5) quantifying landscape-scale patterns of fungal dispersal and linking these with ecological patterns such as succession, (6) determining the causes of fine-scale variability in fungal assemblages, (7) renewing a focus on fungal natural history and creating a broad phylogenetic framework that will give more meaning to molecular based identification, and (8) developing high-quality genetic databases and bioinformatics tools.

For ecologists, the unique life-history attributes of fungi represent an underexploited opportunity to bridge the theoretical and empirical gap between micro- and macroorganisms. In addition, their sessile growth form and host specificity make it more straightforward to delineate habitat units or ecosystem boundaries than it is for larger, more motile organisms (see, e.g., Andrews et al. 1987, Peay et al. 2007). Fungi can also be manipulated in the field (although cautious forethought should be given to the use of fungicides and the potential introduction of novel species), and the rapid growth and small space requirements of culturable fungi make them highly amenable to laboratory experimentation. While fungal ecology can be seen as a hybrid beast that straddles the

macroscopic and microscopic worlds, we believe it is a beast that can be tamed. Advances in molecular techniques, in tandem with field and lab experimentation, will make fungi excellent organisms to test many extant ecological theories and provide opportunities for new and unanticipated concepts in the field of ecology.

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