

SYNTHESIS

Exploring the evolutionary ecology of fungal endophytes in agricultural systems: using functional traits to reveal mechanisms in community processes

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Abstract

All plants, including crop species, harbor a community of fungal endophyte species, yet we know little about the biotic factors that are important in endophyte community assembly. We suggest that the most direct route to understanding the mechanisms underlying community assembly is through the study of functional trait variation in the host and its fungal consortium. We review studies on crop endophytes that investigate plant and fungal traits likely to be important in endophyte community processes. We focus on approaches that could speed detection of general trends in endophyte community assembly: (i) use of the 'assembly rules' concept to identify specific mechanisms that influence endophyte community dynamics, (ii) measurement of functional trait variation in plants and fungi to better understand endophyte community processes and plant–fungal interactions, and (iii) investigation of microbe–microbe interactions, and fungal traits that mediate them. This approach is well suited for research in agricultural systems, where pair-wise host–fungus interactions and mechanisms of fungal–fungal competition have frequently been described. Areas for consideration include the possibility that human manipulation of crop phenotype and deployment of fungal biocontrol species can significantly influence endophyte community assembly. Evaluation of endophyte assembly rules may help to fine-tune crop management strategies.

Introduction

We now know that plants host a diversity of microbes. This is a paradigm shift away from conceptualizing plants as organisms beset by herbivores and pathogens – or engaged only in two-way mutualisms. We are now beginning to consider how the *in planta* microbial community figures into pair-wise models of host and pathogen. All wild and agricultural plant species surveyed to date harbor diverse communities of fungi, often at high density in tissue (e.g., Stone et al. 2000; Arnold 2007). Most members of these communities are endophytes, defined in this review as fungi documented to reside in living plant tissue that is apparently asymptomatic (Stone et al. 2000; Schulz and Boyle 2005). Designation as an endophyte therefore depends on an asymptomatic host–fungal interaction at the time of study. It does not, however, predict the endophyte's potential for shifts within the plant to

biotrophic or necrotrophic pathogenesis, to mutualism or to commensalism. It follows that the influence of some endophyte species on host health is not static and can range from beneficial to detrimental. Some species provide benefits to the host including protection against pathogens and herbivores, while others impose costs, for example, by decreasing photosynthetic efficiency under drought conditions (Pinto et al. 2000; Clay and Schardl 2002; Arnold et al. 2003).

Although by definition an endophyte does not cause visible symptoms, its potential trophic and phenotypic range in the host plant is not stuck in neutral gear, but can change over both short and long time scales. Expression of symbiotic phenotypes may be plastic; for example, latent pathogens can reside in plant tissue as commensals until conditions are amenable to disease development (e.g., Dodd 1980). Alternatively, we could hypothesize that endophyte lineages may have evolved over time as

commensals, for example, as a result of the loss of traits that confer pathogenicity. Endophytes may indirectly affect host health by altering the potential of other species in the community to act as pathogens or mutualists (e.g., Fravel et al. 2003). A plant individual can harbor anywhere from several to hundreds of fungal species, and with little known about the potential beneficial or detrimental effects of each species on the host, we can only speculate on the potential for single or synergistic interactions (Stone et al. 2000; Schulz and Boyle 2005; Arnold 2007).

One system in which host–endophyte relationships have been well studied is the interaction between fungal species in the family Clavicipitaceae and their grass hosts (for reviews see Clay and Schardl 2002; Belesky and Bacon 2009). Many species provide fitness benefits to the host, often increasing tolerance to environmental stressors, although the direction of this relationship can change with environmental conditions and plant–fungus genotype combinations (e.g., Meijer and Leuchtman 2000; Clay and Schardl 2002). The primary mode of transmission for the Clavicipitaceae endophytes is vertical, in seed, and colonization is often extensive in host tissue. This interaction does not represent the majority of fungal endophyte–host plant interactions. Most plant species harbor a phylogenetically diverse assemblage of endophyte species, also often at high density in tissue, but are primarily transmitted horizontally, from plant to plant, rather than vertically (Schulz and Boyle 2005; Arnold 2007). For the purposes of our review, the focus is on nonmycorrhizal endophyte species outside of the Clavicipitaceae.

One area of endophyte biology that has advanced rapidly is description of endophyte communities associated with different host species (for wild host species see Carroll 1995; Stone et al. 2000; Wilberforce et al. 2003; Arnold 2007; for agricultural host species see Carter et al. 1999; Seghers et al. 2004; Manici and Caputo 2009; Saunders and Kohn 2009). That endophyte species diversity varies significantly among plant species, including crop species, is evident from the literature (Carter et al. 1999; Franke-Snyder et al. 2001; Arnold 2007; Hoffman and Arnold 2008). Our research focus has been on biotic factors influencing the assembly of endophyte communities in maize, where we have found that communities can also vary significantly among plant genotypes and phenotypes (Saunders and Kohn 2009). That communities differ predictably among plant species and genotypes suggests that plant traits can mediate endophyte community processes, but the specific mechanisms are poorly known.

We are intrigued by the experimentally accessible question of how host phenotype influences assembly of fungal endophyte communities. Plant traits such as defense compound production and tissue lignification can influence

host colonization by known pathogens, and would therefore be expected to affect the species diversity of fungi within plants (VanEtten et al. 2001). Equally important to consider are the fungal traits that have putatively evolved in response to plant traits that discourage colonization. Interactions between microbial species are also expected to influence community processes. That microbial biocontrol agents can be deployed to control plant pathogens highlights the influence of one microbial species on the abundance of other species in the community. Effects of biocontrol agents on fungal community members other than the target pathogen are common (Brimner and Boland 2003), but the influence of biocontrol species on community processes is typically unknown.

Previous research in fungal community ecology and agricultural disease management has laid the foundation for asking exciting questions about the mechanisms that underlie fungal community dynamics, and the influence of the endophyte community on host health. In this review we examine fungal endophyte communities in agriculture with a focus on the following three areas of research that hold potential for describing general patterns in endophyte community ecology. (i) Application of the ‘assembly rules’ model to understand the dynamics of agricultural endophyte assemblages. This model provides a powerful framework with which to tease apart the specific mechanisms that contribute to community assembly. (ii) Use of plant and fungal functional trait variation to identify the phenotypes that significantly influence endophyte community processes. A given plant phenotype may effect the resident endophyte community, or may have a sphere of influence that extends to the endophyte communities of neighboring plants. The interplay between host and fungal phenotype is likely one of the most important factors in successful establishment for primary colonizing fungi. (iii) Identification of fungal traits important in mediating microbe–microbe interactions. Secondary colonizers will not only face challenges posed by the host, but also those presented by fellow members of the microbial community. Variation in fungal traits important in microbe–microbe interactions will influence endophyte community processes. We conclude by suggesting research questions that may help to describe mechanisms in endophyte community processes. Maize, an emerging model crop system for endophyte community research, will be a focal point for discussion.

Mechanisms that influence endophyte community dynamics: assembly rules, habitat filters and species interactions

Each individual plant harbors an endophyte community that is a subset of the species pool, the ambient fungal

species aggregate in the environment outside of the plant. What determines which species co-occur within the community? Research indicates that communities can be the result of random assembly events, or the result of predictable, trait-based assembly processes, and the field is divided as to the importance of the two (Hubbell 2001; Fargione et al. 2003). For our purposes here, the ‘assembly rules’ concept in ecology offers a most useful perspective. Community ecology theory proposes that ‘filters’ mediate community assembly through a series of processes that result in the co-existence of particular species at a site (Diamond 1975; Weiher and Keddy 1999). These processes can be roughly divided into two categories: habitat filtering and species interactions. Specific environmental variables are expected to act as habitat filters by preventing establishment of species that lack the phenotype required to survive. If a species is able to tolerate the abiotic conditions in the environment, establishment may then depend on the outcome of interactions with other species in the community.

Typically, the distinction between habitat filters and species interactions is a useful way to discriminate between the influence of abiotic and biotic factors in community processes. For plant-associated fungi, habitat filters may also include biotic variables, because the environment being colonized is a living host. Therefore species are likely to be challenged by abiotic habitat filters and two distinct biotic factors: plant-imposed habitat filters and interactions with fellow microbial community members (Fig. 1). Potential abiotic habitat filters include water availability and UV exposure, while plant traits such as biochemical defenses and tissue lignification may act as plant-imposed filters. Interactions among microbial species may range from competitive to facilitative, with the outcome often influenced by the timing of colonization. Interestingly, once a fungal species is established *in planta*, it may prevent subsequent species from colonizing, acting in essence as a plant defense mechanism. These factors are likely to work on different scales, with abiotic habitat filters acting at the scale of the plant neighborhood and plant-imposed filters at the level of the individual plant and potentially at the level of the plant neighborhood. Fungal–fungal interactions are expected to occur between localized colonies within plant tissue. Abiotic filters, plant-imposed filters and fungal–fungal species interactions may or may not act in a nested fashion.

The environmental filtering hypothesis utilizes the ‘assembly rules’ concept to predict the formation of the community (Weiher and Keddy 1999). Under this hypothesis, species within communities are constrained by environmental factors; the overall species abundance within a community is driven to a trait average optimal

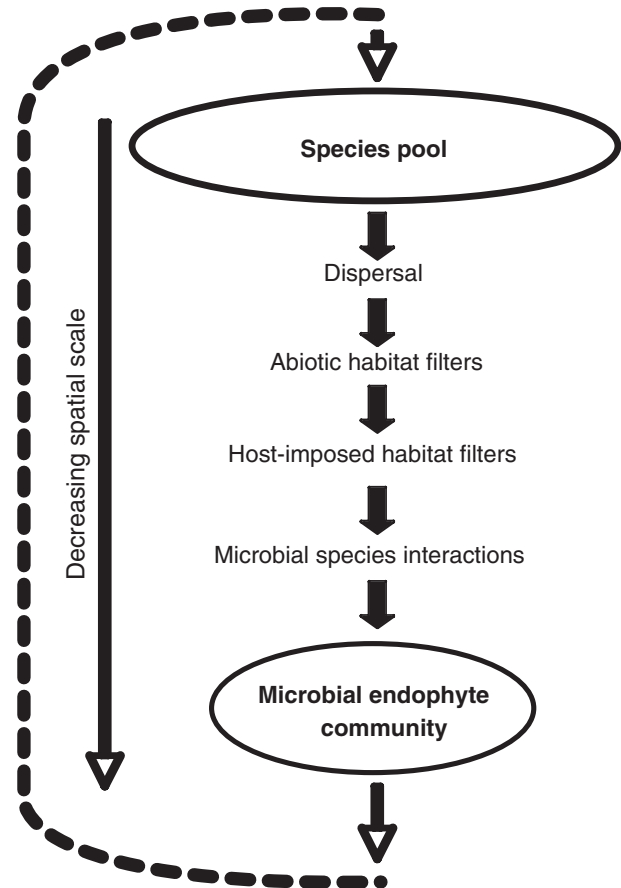


Figure 1 As species move from a regional species pool to become established as part of a community (the assemblage of fungi within an individual host), they must have the ability to disperse to the habitat and pass through environmental stressors, including abiotic habitat filters and plant-imposed habitat filters that prevent some species from colonizing. Once in the host environment, the species may experience competitive or facilitative interactions with other microbial species. The endophyte community assemblage may influence composition of the species pool; inoculum will reside on plant matter until it is dispersed by wind, water or animal to a new host (dotted line).

for the specific environment. This hypothesis has found success in predicting community structure of plants, where functional traits such as specific leaf area, stem mass and height have proven informative (Shipley et al. 2006). Such an approach may also be useful in understanding endophyte community assembly, and would likely rely heavily on physiological traits. The choice of traits to be evaluated is important, and specific to the questions being asked. For example, Saunders and Kohn (2009) evaluated the role of host defense compounds in endophyte community assembly by comparing endophyte communities from host genotypes that either could or could not produce the compounds. Host genotypes that

produce defense compounds had endophyte communities dominated by species with a higher level of tolerance to the toxins than did host genotypes that did not produce the compounds.

Community phylogenetics is an example of an ecological tool that could be widely useful in agricultural studies. Inference of community-wide phylogenies is a powerful approach that can be used to develop hypotheses about which fungal traits are most important in community assembly. It has long been noted that closely related species are ecologically similar, indicating that the traits important to success in a given environment are evolutionarily conserved (Darwin 1859). When functional traits are highly conserved, species are expected to compete when they co-occur (Cavender-Bares et al. 2009). Thus, community phylogenies can aid in the detection of community assembly processes. For example, if species within a community are phylogenetically clustered (more closely related than expected by chance), this may indicate that trait conservatism has resulted in environmental filtering. For plant-associated fungi, phylogenetic clustering may be the result of similarities in responses to abiotic factors or similarities in host range. Conversely, over-dispersion of fungal species distributions (more distantly related than expected by chance) suggests competition between closely related species. Although relatedness of fungal pathogens is widely recognized in agricultural studies (e.g., Elmer et al. 2007; Ward et al. 2008), phylogenetic tools provide a statistical approach for hypothesis development. In agricultural systems, community phylogenetic data could be evaluated in concert with known mechanisms of fungal colonization to either reveal previously unrecognized fungal traits, or to assess the role of known fungal colonization mechanisms.

After phylogenetic analysis has revealed potential fungal traits of interest, experiments comparing fungal phenotypes will help to confirm the roles of various traits on community assembly. Such studies should expose traits important to endophyte success within a particular host species or genotype. In a previous review, Rodriguez et al. (2009) delimited four broad functional classes of endophytes based on life history characteristics such as host range, type of transmission (vertical or horizontal), and plant organs colonized. We suggest that investigation of community assembly rules will aid in describing additional functional classes. Such studies could inform hypotheses on life history strategies of endophyte species; for example, if the relative roles of abiotic stressors and microbial competition are of interest, the response of fungal species to microbial competition could be assessed along an environmental gradient. Experiments that isolate the influence of abiotic habitat filters, plant-imposed habitat filters and

species interactions may reveal the relative importance of these factors in community assembly and in plant–fungal interactions.

Influence of plant-imposed habitat filters and the interplay between plant and fungal phenotype on endophyte community assembly

Which plant traits mediate endophyte community assembly? We know that variation in plant traits such as lignin quantity, cell wall thickness and production of defense compounds can influence the ability of fungal species to colonize and proliferate within plant tissue (Knogge 1996; VanEtten et al. 2001). Correspondingly, fungal species have evolved strategies to overcome colonization barriers, and species vary widely in both ability to colonize and the colonization strategies that are employed (Canhoto and Graca 1999; Osbourn 1999; Huckelhoven 2007; Kikot et al. 2009). For example, some plant pathogens are able to colonize by actively penetrating host tissue. Penetration is often mediated by the production of enzymes that degrade wax and cell walls, thus enabling penetration of host tissue (Kollattukudy 1985; Torto-Alalibo et al. 2009). Other species may colonize via open wounds or stomata and are able to successfully colonize without tissue penetration. Therefore, variation in plant traits such as leaf chemistry and susceptibility to herbivore damage are expected to influence the assemblage of fungal species that can successfully colonize the host.

How does the interplay between host and fungal traits influence fungal community dynamics? We know that host defense traits can select for colonization by a fungal species with an adaptive phenotype, but experiments have primarily been conducted on single host–fungal pairs, or on a single host and 2–3 fungal partners. A better understanding of how host trait variation influences fungal community assembly will be facilitated by studies that assess how plant functional trait variation affects both the species diversity and functional trait diversity of fungal communities. The ability of an individual plant to affect endophyte dynamics can also extend beyond its own resident endophyte community to affect community assembly of endophytes associated with neighboring plants. For example, antifungal compounds that are released into soil by one plant can influence the endophyte communities of surrounding plants, constituting a plant-imposed habitat filter that is mediated by neighbors of the host. In the next section we highlight several studies that either aim to identify plant traits that influence endophyte community dynamics, or hold promise for the screening and identification of important plant-imposed habitat filters.

Effects of plant traits on the resident endophyte community: plant defense compound production can impact assembly of maize endophyte communities

Endophyte communities from conspecific plant genotypes that differ in production of defense compounds can harbor predictably different endophyte communities, evidence that plant defense compounds can act as plant-imposed habitat filters (Bailey et al. 2005; Saunders and Kohn 2009). Secondary compounds produced by plants are incredibly diverse. Over 10 000 compounds have been described to date, and most that have been tested for biological activity have antifungal and/or antibacterial properties (Dixon 2001). Plant defense compounds are therefore likely to be one of the most common plant-imposed habitat filters encountered by plant-associated fungi. Predictably, fungal species commonly exhibit high tolerance to the toxins produced by their host species, evidence that defense compounds impose significant selective pressure on plant-associated fungi (Carter et al. 1999; Osbourn 1999; VanEtten et al. 2001; Bailey et al. 2005; Saunders and Kohn 2009). Detoxification of host defense compounds is one of the most common fungal tolerance mechanisms; in some host species, fungi that can detoxify can colonize and establish in greater abundance than those that cannot (Carter et al. 1999; VanEtten et al. 2001; Saunders and Kohn 2009).

Intraspecific variation in host defense compound production can significantly influence fungal endophyte community assembly in maize. Maize produces benzoxazinoids (BXs), leading to accumulation of a class of toxic byproducts, the benzoxazolinones, for example, 2-benzoxazolinone (BOA), in plant tissue and in soil (Krogh et al. 2006). BX byproducts have known toxicity against fungi, bacteria, insects and plants (Niemeyer and Perez 1995). Maize commonly harbors several species in the genus *Fusarium* that have relatively high levels of tolerance to BOA (Glenn et al. 2001; Saunders and Kohn 2008; Saunders and Kohn 2009). Many of these species, including *Fusarium verticillioides* (Sacc.) Nirenberg, *F. subglutinans* Wollenw. & Reinking and *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg, present an economic and health risk; as endophytes in grain they produce mycotoxins (secondary compounds that have adverse effects on animals) that cause toxicosis in domesticated animals and may pose risks for cancers and other human health problems (Ueno et al. 1997; Marasas 2001). Concentration of BXs is a highly variable trait; maize has undergone selective breeding aimed at increasing BX concentrations to protect against damage from the European corn borer [*Ostrinia nubilalis* (Hübner)] and other herbivores (Barry and Darrah 1991).

Results from our field studies compared fungal endophyte communities from BX producing and nonproducing genotypes. While endophyte species diversity did not differ between the host genotypes, BX production provided a colonization advantage to *Fusarium* species (Fig. 2; Saunders and Kohn 2009) confirmed in second field study (M. Saunders, A.E. Glenn and L.M. Kohn, unpublished). Abundance of *Fusarium* species was up to 35 times higher in mature leaves of BX producers than of nonproducers. Production of BXs also influenced the distribution of BOA tolerance levels within endophyte communities. In mature leaves, plant genotypes that did not produce BXs had significantly more BOA sensitive isolates than the BX-producing genotypes, indicating that tolerance to BOA provided an ecological advantage not only to *Fusarium* species, but also to additional fungal endophyte species with high BOA tolerance. This plant-imposed habitat filter, in combination with the functional trait of BOA tolerance among some fungal species, was shown to shape the composition of the endophyte community. We suggest that breeding for elevated concentrations of BXs in maize may have unintentionally increased colonization by *Fusarium* species. Our results highlight endophyte functional traits and plant-imposed habitat filters as key factors in understanding fungal community assembly.

Does host variation in insect resistance influence composition of the fungal community?

Some of the same species of *Fusarium* that commonly reside in roots and shoots of maize as endophytes produce toxins that accumulate in the grain. Studies con-

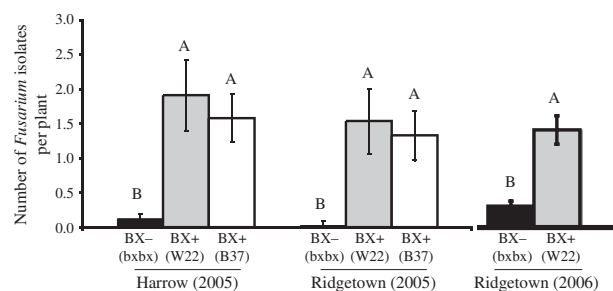


Figure 2 Mean number of *Fusarium* isolates per plant obtained from 9-week-old leaf tissue of maize genotypes that produce the benzoxazinoid defense compounds (BX+ genotypes = W22 and B37) and a genotype that does not produce BXs (BX- genotype = bxbx). In 2005, plants were grown in Harrow and Ridgetown, Ontario, Canada, and in 2006, plants were again grown in Ridgetown, Ontario. Tukey–Kramer HSD tests were conducted to compare mean number of isolates per plant obtained from bxbx (BX-), W22 (BX+), and B37 (BX+) genotypes. The same letter above two columns indicates no significant difference between means. Vertical bars, ± 1 SE.

ducted over the past 10 years indicate that transgenic Bt maize accumulates significantly lower concentrations of *Fusarium*-produced mycotoxins in grain than do isogenic genotypes without the transgenes (Hammond et al. 2004). Bt maize hybrids contain the Cry1Ab protein originated from *Bacillus thuringiensis* Berliner. Presence of the protein protects plants from herbivore damage (Pilcher et al. 1997). Mycotoxin concentration is extremely variable among maize crops, and there are several known factors that contribute to the susceptibility of maize to infection and mycotoxin contamination. Insect damage in maize is positively correlated with infection by mycotoxin-producing *Fusarium* species, and mycotoxin contamination of grain. Thus, Bt maize suffers less insect damage ultimately resulting in lower levels of contamination with mycotoxins, such as the fumonisins, compared to isogenic maize hybrids (Munkvold and Desjardins 1997; Sobek and Munkvold 1999). The correlation is likely due to suppression of both herbivory and its collateral effects; the herbivores vector fungal spores, the inoculum, and they create an infection court in wounds resulting from herbivory. Deployment of Bt maize has led to a significant decrease in economic loss from mycotoxin contamination; in 2004 it was estimated that farmers in the US would recover \$17 million dollars annually if Bt maize were deployed (Wu et al. 2004).

The impact of Bt maize on endophyte communities is unknown. We have included this example because wound-infecting endophyte species are common, and we hypothesize that plant variation in resistance to insect damage is likely to have a cascade of influence on the fungal community. We do not know which endophyte species, if any, occupy the niche space previously occupied by the wound-infecting *Fusarium* species, but it seems likely that a different suite of host-entry mechanisms is required for colonization in herbivore-resistant maize. The association between herbivore damage – or the lack of it – and penetration and subsequent colonization of endophyte species merits further investigation, especially where wound-infecting species are ‘keystone’ with respect to hindering or facilitating subsequent colonization by other species.

Host phylogeny may highlight plant functional traits important in endophyte community assembly

The evolutionary history of host plants should provide clues about plant traits regulating endophyte community processes. Use of community phylogenetic tools has proved useful for understanding dynamics of fungal pathogens. For example, Gilbert and Webb (2007) detected a phylogenetic signal in host range of foliar pathogens of tropical trees. The likelihood that a fungal

species could infect two plant species decreased as phylogenetic distance between host species increased. In a parallel vein, ectomycorrhizal fungal communities from eight plant hosts were compared, and taxonomically close host species (e.g., congeneric pairs), had more similar mycorrhizal communities than did host species of different genera (Ishida et al. 2007). That closely related host species have similar relationships with their fungal inhabitants suggests that evolutionarily conserved traits act as plant-imposed habitat filters. Studies that explicitly test for a phylogenetic signal in the association of plants with their endophyte species can be used to develop hypotheses about which traits are important in mediating endophyte community assembly. For example, closely related hosts may share a chemical or physical defense trait that is not shared with a more distantly related plant species. Follow-up experiments that compare plant genotypes or species that vary in the traits of interest could then be conducted to determine if specific plant phenotypes are correlated with particular endophyte assemblages. This could be especially useful in explaining the endophyte community dynamics in closely related crop plants, where much is known about specific host phenotypes.

Beyond the resident endophyte community: influence of plant functional traits on endophyte assemblages within neighboring plants

Here, we present two conceptual models developed for pathogens and herbivores and consider their pertinence to fungal endophytes. The plant traits that act as habitat filters in pathogen colonization can have a spatial sphere of influence that extends beyond the individual plant to the neighbors of the focal host. Plants can experience ‘associational susceptibility’ or ‘associational resistance’ when presence of plant neighbors causes a change in the amount of herbivore- or pathogen-induced damage that is sustained (Power and Mitchell 2004; Burdon et al. 2006). An example of associational resistance in agriculture is the use of crop species mixtures to decrease pathogen transmission. Co-occurrence of such plants in a crop field can result in a spatial dilution of the unwanted pathogen. A mix of distantly related host species is usually deployed. For example, clover reduces infection of wheat by *Septoria tritici* Desm. by providing a barrier to water-dispersed propagules (Bannon and Cooke 1998). In contrast, some species mixtures result in associational susceptibility, such as when a ‘reservoir’ host accumulates a high density of infective propagules that are in turn transmitted to neighboring plants (Power and Mitchell 2004; Burdon et al. 2006).

Exudation of defense compounds from roots into soil is an example of a plant-imposed habitat filter that can

have a spatial sphere of influence that extends to the endophyte communities of plant neighbors. Some root exudates can induce a defense response in neighboring plants, thereby providing a form of associational resistance. Root exudates can also cause associational susceptibility when the causative plant reduces the abundance of an organism that is a mutualist of its plant neighbors. Garlic mustard [*Alliaria petiolata* (M. Bieb.) Cavara & Grande], which is nonmycorrhizal, can dramatically reduce the activity of mycorrhizal fungi in soil by releasing fungitoxic compounds into soil, thereby suppressing the growth of surrounding tree species (Stinson et al. 2006; Wolfe et al. 2008). We tested the influence of BX producing maize plants on the endophyte communities of neighboring plants that cannot produce BXs (M. Saunders, A.E. Glenn and L.M. Kohn, unpublished). Maize genotypes that do not produce BXs were grown in a field with two BX-producing genotypes (triculture), as well as in monoculture. Communities in roots of BX nonproducing plants grown in monoculture had higher endophyte infection density and higher endophyte species diversity than those grown in triculture. Previous work has shown that BXs can be released into soil, where they persist (Krogh et al. 2006). Our results are consistent with what would be expected if BXs were present in soil, where they influenced the common microbial community. We did not measure BXs in the field and so can only conclude that the genetic composition and physiological activity of the host plants had a striking affect on endophyte species diversity.

Interactions between fungal species can modify endophyte assemblages

After a fungal species has become established within plant tissue it may pose an additional barrier to subsequent fungal colonizers. Therefore, for secondarily colonizing fungi, it is not only host plant traits that may preclude colonization, but also traits of the fungi that are already established in host tissue. Fungal species within plant tissue may either completely prevent colonization by additional fungi, or may interact with species once all are established in host tissue. It follows that the outcome of such species interactions will influence community assembly. Biocontrol research efforts have resulted in descriptions of several mechanisms of fungal competition (Baker and Cook 1974; Duffy and Raaijmakers 2003). We know that effects of fungal biocontrol species often extend beyond the target pathogen to nontarget fungal species (Brimner and Boland 2003). *Trichoderma* species are used to control soil-borne diseases such as *Sclerotinia sclerotiorum* (Lib.) de Bary and *Fusarium oxysporum* Schldl., but can also penetrate the

resting spores of the mycorrhizal species, *Glomus intraradices* Schenk and Smith (Sivan and Chet 1993; Inbar et al. 1996; Rousseau et al. 1996). The impact of biocontrol agents on nontarget fungal species is further evidence that microbe–microbe species interactions are likely to impact fungal community assembly, and that ‘keystone’ endophyte species can cause dramatic changes in fungal community structure as well as host health.

Fungal competition within host tissue can occur with direct or indirect contact. Within host tissue competition requires either direct contact, or residence within the same small area of substrate. For example, variation in fungal traits such as production of antifungal compounds and resource use are expected to modify competition within host tissue. Alternatively, species may engage in ‘long-distance’ competition by initiating the host defense response. Fungal–fungal interactions can also be facilitative, such as when one species mobilizes nutrients for another, or ameliorates a toxic environment (Lawrey 2000; Saunders and Kohn 2008). The following subsections describe several mechanisms of competition and facilitation. We draw attention to functional traits that have been documented to mediate the outcome of two- or three-way interactions and could therefore be interesting in a community interaction context. Research on the use of fungal species as biocontrol agents has provided a wealth of data on mechanisms of microbe–microbe competition, inviting further research aimed at impacts on endophyte community dynamics (e.g., Baker and Cook 1974; Mandeel and Baker 1991; Benitez et al. 2004; Minerdi et al. 2009).

Microbial competition can be mediated by production of antifungal compounds

The outcome of competitive interactions between fungal species is often attributed to the production of biologically active compounds produced by fungi. An *in vitro* study found that approximately 80% of fungal endophyte species produce secondary compounds with herbicidal, antibacterial or antifungal activity (Schulz et al. 2002). Overall, data from *in vitro* studies show that endophyte species can compete directly by producing antifungal compounds that diffuse in substrate, and prevent other species from encroaching. Some fungal species can detoxify the antifungal compounds produced by their microbial competitors, for example, *Fusarium graminearum* Schwabe can detoxify the toxic compound 6-pentyl- α -pyrone, produced by *Trichoderma harzianum* Rifai (Cooney et al. 2001). Ability to detoxify fungicides of fungal, bacterial, plant and synthetic origin varies widely among fungal species, and is likely to be important in community assembly. The response of fungi to plant and

synthetic toxins is well documented and indicates three primary mechanisms of tolerance: detoxification, structural alteration of the target of the toxin, and activation of membrane transporters that exude toxins from the cell (VanEtten et al. 2001). It is likely that all three mechanisms are also employed in response to fungal-produced bioactive compounds. It is possible that the interplay between production of antifungal compounds by some fungal species and the ability to tolerate or resist them by others is a major driver of fungal–fungal competition. This is an experimentally tractable question that could be addressed by evaluating natural variation in antifungal compound production and resistance or by creating microcosm communities with characterized phenotypes.

Interestingly, some endophyte species produce volatile compounds with antifungal activity, resulting in direct competition at spatial scales expected to be larger than scales of toxicity from nonvolatiles within a host or substrate (Stinson et al. 2003; Steinebrunner et al. 2008). For example, the suite of low molecular mass, volatile, antifungal compounds produced by *Muscodor albus* Worapong, Strobel & W.M. Hess and related species influence microbial growth without direct contact between microbes (Stinson et al. 2003). Tolerance of fungal species to the volatiles is variable, and the influence of the compounds on whole fungal communities is unknown. To our knowledge, fungal mechanisms of defense against volatiles have not been studied in detail. As more cases of volatile defense compounds accrue, it appears that this mechanism of competition may be widespread (Stinson et al. 2003; Steinebrunner et al. 2008; Strobel et al. 2008; Minerdi et al. 2009). Identification of the mechanisms of volatile defense would be a first step in contrasting the importance of short- versus long-range fungal competition.

Competition for space or nutrients

Some fungal species may compete by simply occupying all of the tissue available at a particular site on the plant. Mandeel and Baker (1991) suggested that the root surface has a limited number of infection sites, and that protection of the root would increase with abundance of a fungal biocontrol species. There is some evidence that this can occur. For example a comparison between pathogenic and nonpathogenic strains of *Fusarium oxysporum* showed that as primary colonizers, root colonization was qualitatively the same among pathogens and nonpathogens with both extensively colonizing root tissue and decreasing or preventing colonization by additional fungi (Olivain and Alabouvette 1997, 1999). Resource competition is also expected to be prevalent among fungi, and can either occur when one species depletes available nutrients to the

detriment of other species, or when one species blocks another species from access to nutrients. The later form of resource competition is inherently associated with other forms of competition. For example, nutrient availability of one species may be restricted by another species via production of antifungal compounds. This example illustrates the importance of colonization timing in community assembly, where priority effects are expected to play a significant role. A species also may demonstrate primary resource capture by growing on previously uncolonized substrate, or may obtain nutrients as a secondary colonizer, a strategy that likely involves interaction with a living tenant (Cooke and Rayner 1984; Boddy 2000). For the primary colonizing species, traits such as wide dispersal and rapid spore germination, mycelial growth and metabolism are important for success (Cooke and Rayner 1984). Competitive ability, such as that mediated by production of antifungal compounds, is crucial for survival of secondary colonizers, and is perhaps the most important type of interaction for many fungal species.

Competition via mycoparasitism

Fungal endophytes are subject to parasitism by other fungi, bacteria and viruses. Typical interactions characteristic of mycoparasitism are hyphal coiling, formation of a resistance sheath, and invasion into host hyphae. Ability to form these structures and penetrate fungal tissue can vary markedly within and among species. Several species in the fungal genus *Trichoderma* are exploited as biocontrol agents because they antagonize target pathogens via mycoparasitism, production of antifungal toxins and acquisition of space and nutrients (Benitez et al. 2004).

Relationships between endophytic fungi and ecto- and endosymbiotic bacteria can have broad impacts on the virulence, metabolism, and development of fungi. Of particular interest is the discovery of an avirulent *Fusarium oxysporum* strain that has biocontrol potential against plant disease caused by pathogenic strains of *F. oxysporum*. The ectosymbionts modulate the biology of the fungus, silencing its virulence and altering its developmental morphology (Minerdi et al. 2008). The association results in release of volatile organic compounds that may act as a long distance antagonism mechanism limiting the growth of pathogenic *F. oxysporum* strains (Minerdi et al. 2009). It is therefore possible that presence of the bacterium may alter the spatial scale at which *F. oxysporum* can influence endophyte community assembly. In contrast, the endofungal bacterium, *Burkholderia rhizoxinica* Partida-Martinez et al. confers phytopathogenicity to the fungal host (*Rhizopus microsporus* Tiegh.) by producing a phytotoxin (Partida-Martinez and Hertweck 2005;

Partida-Martinez et al. 2007; Lackner et al. 2009). Thus, while the ectosymbiotic bacteria of *F. oxysporum* silence fungal virulence, the endosymbiotic *Burkholderia* confers the phytopathogenicity of *R. microsporus*, in both cases due to aspects of secondary metabolism. Similar interactions have been documented between fungi and endosymbiotic mycoviruses, which can render previously pathogenic fungal strains avirulent (Nuss 2005).

How do microbes that parasitize fungal endophytes influence endophyte community processes? Profound changes in fungal physiology mediated by parasitic microbes are likely to influence endophyte community processes, either by changing host suitability or by altering the ability of the parasitized fungus to directly compete with microbial community members. Some fungal species engage in active combat and mutual parasitism (Vajna 2003); such pairings could be used to investigate both the traits important in ability to parasitize and to resist invasion, and the influence of such traits on community assembly.

Fungal species indirectly influence endophyte community assembly by initiating the host defense response

A primary colonizing endophyte may also influence colonization of other microbes by inducing a 'priming' reaction in the plant (Trillas and Segarra 2009). Priming occurs when the plant immune system registers a microbial invader by detecting a well-conserved trait (e.g., chitin), which triggers induced resistance that is characterized by an increased capacity to activate a defense response to a later infection (Conrath et al. 2002). Fungal biocontrol species can instigate priming (Trillas and Segarra 2009). For example, Djonovic et al. (2007) found that a hydrophobin-like elicitor of the fungal endophyte *Trichoderma virens* (J.H. Mill., Giddens & A.A. Foster) Arx can induce systemic resistance in maize. We should anticipate that priming induced by an endophyte species used in biocontrol could have collateral effects on the endophyte community as well as on the target pathogen. A primary colonizer could gain a competitive advantage if it were able to first induce priming and then tolerate or avoid the plant resistance mechanisms that were induced. Many other studies have found that inoculation with a biocontrol endophyte protects against pathogen colonization in circumstances where endophyte and pathogen are very unlikely to interact directly, indicating that endophyte-stimulated induced resistance may be common (for review see Terry and Joyce 2004). Investigation of the interaction between priming, pathogen abundance and the microbial community may help to explain why the outcome of biocontrol treatments can be difficult to predict. It is possible that ability to colonize virgin host tis-

sue and induce plant defenses is an adaptive colonization strategy. Is the identity of a primary colonizer simply a matter of chance or the result of adaptation to newly emerging tissue? Studies that investigate both the ability of different endophyte species to colonize and induce priming, and the downstream effects of endophyte-induced priming on the community would help to answer this question.

Facilitation between fungal endophyte species

Facilitation can occur between fungal species, although it is rarely documented (Lawrey 2000; Tiunov and Scheu 2005; Pan and May 2009). To evaluate if facilitation occurs between fungal endophyte species, we asked if detoxification of host defense compounds by one maize endophyte species could facilitate the growth of species less tolerant to the compounds. We found that under *in vitro* conditions, *F. verticillioides* could facilitate growth of less tolerant maize endophyte species (Saunders and Kohn 2008). *F. verticillioides* has a high level of tolerance to BOA, and converts it to a less toxic product. On synthetic medium, *F. verticillioides* was able to detoxify BOA and increase the growth rate of commonly co-occurring maize endophyte species. However, in field-grown maize *F. verticillioides* interacted competitively with community members by preventing species with lower BOA tolerance from colonizing root tissue (M. Saunders, A.E. Glenn, L.M. Kohn, unpublished manuscript). Interestingly, facilitation achieved via habitat amelioration has been documented for lichen-dwelling fungal species. The lichenicolous fungus *Nectria parmeliae* (Berk. & M.A. Curtis) D. Hawksw can only colonize the lichen *Punctelia rudecta* (Ach.) Krog after a lichenicolous *Fusarium* species has colonized and degraded the antifungal compounds produced by the lichen (Lawrey 2000).

Few studies have assessed the outcome of endophyte-endophyte species interactions across entire communities (Pan and May 2009). Evaluating endophyte communities associated with several genotypes of maize, Pan and May (2009) used null models to test for species that were found together more or less often than would be expected by chance, a hypothesized signal of facilitation and competition. Fungal communities in shoot tissue were assessed using both a culture-dependent and a culture-independent approach. Data from the culture-dependent approach suggest that interspecific facilitation is common, while data from the culture-independent approach, depending on the scale of analysis (individual leaf or individual plant), either did not detect significant pairwise interactions between species, or detected competition.

This highlights a unique challenge of working with microbial communities; natural communities can only be

characterized by isolating microbes directly from tissue (culture dependent), or by isolating DNA from plant tissue and obtaining sequence data (culture independent). Both have the potential to bias the resulting community data: culture-based studies will likely yield those species that grow well on the same substrate, and molecular methods may exclude particular species due to PCR bias (Arnold et al. 2007; Pan and May 2009; Avis et al. 2010). A combination of culturing fungi directly from tissue and obtaining genomic DNA directly from plant tissue to use in next-generation sequencing will give the most comprehensive view of endophyte community dynamics by allowing for the saturation of species area curves while maintaining the option for manipulative experiments. Future studies that evaluate the net outcome of endophyte–endophyte species interactions across whole communities will help to determine the extent to which facilitation and competition influence community assembly.

Conclusions

Most plant species harbor a diverse assemblage of fungal endophytes, yet our knowledge of why particular species assemblages are found in specific host species is limited. It has been suggested that research in community ecology will be more likely to result in the development of general rules if quantification of functional trait variation is combined with data on variation in the abiotic environment and evolutionary history of the species of interest (McGill et al. 2006). Research documented here indicates that this approach will likely prove fruitful in understanding endophyte ecology. Assignment of fungal phenotype values assessed *in vitro* should be undertaken with care, however, because many environmental factors, such as temperature and light exposure can influence the phenotype being tested. For example, we found that starting population size, colony age, and nutrient availability influenced the growth rate of endophyte species grown in the presence of BOA, but none influenced the BOA tolerance threshold of isolates, suggesting that the binary trait of plus/minus growth was less plastic than growth rate.

Although somewhat beyond the scope of this review, it is notable that abiotic factors such as fertilizer application, temperature and seasonal moisture regimes can influence endophyte community assembly (Suryanarayanan et al. 2002; Seghers et al. 2004; Gonthier et al. 2006). The interaction between host genotype and environment can also significantly impact endophyte communities (Elamo et al. 1999; Pan et al. 2008; Pan and May 2009; Saunders and Kohn 2009). Variation in abiotic factors are likely an important component in any model of endophyte community assembly – and certainly a key set of variables

to consider when assessing response to global change, predicting disease dynamics, or managing biocontrol efforts.

Much of the research presented here demonstrates that manipulation of host phenotype via crop breeding programs can have a huge impact on fungal endophyte colonization. Such influence can be desirable, as is seen in the reduction of mycotoxin contamination of Bt maize, or disadvantageous, as in the unanticipated increase in abundance of mycotoxin-producing *Fusarium* species that colonize BX-producing maize genotypes. *In vitro* studies indicate that microbe–microbe interactions can be mediated by the production of secondary compounds. Overall, production of toxins by plants and microbes has emerged as a significant player in these dynamics. Research on fungal communities associated with maize highlights a suite of factors that may impact community assembly, including production of defense compounds by the plant, herbivore resistance and presence of mycotoxin-producing endophytes in plant tissue. We suggest that answers to several questions will be particularly important in better understanding endophyte community dynamics:

1. Which host and fungal traits are important in endophyte community assembly? How does the distribution of host and fungal traits change with abiotic conditions, for example, across environmental gradients?
2. Which plant traits influence the available species pool of potential endophytes in a poly-species or poly-genotype plant neighborhood? What is the interaction between crop genetic diversity, endophyte colonization and pathogen abundance?
3. What is the influence of well-described mechanisms of fungal competition, such as mycoparasitism and fungicide production, on endophyte community assembly?
4. How can concurrent use of host and fungal phylogeny inform hypotheses about fungal community assembly rules?
5. To what extent and by what mechanisms do selective breeding and agronomic practices influence the assembly of endophyte communities? Can this information be used to optimize crop management and biocontrol strategies?

Community assembly dynamics will be key in the development of general models for endophyte biology in crop and wild plants. The field of microbial ecology has blossomed as molecular approaches such as next generation sequencing have facilitated more complete descriptions of endophyte communities. Use of such tools will be most fruitful when combined with trait-based studies that connect host and fungal phenotype. There is now a need to explore relationships between species diversity and functional traits, and to use phylogenetic tools to identify both the plant traits that act as plant-imposed filters and the fungal traits adaptive to such filters. We

see this as the most immediate route to understanding host–fungal relationships and endophyte community ecology. We also predict that more studies will factor the endophyte community into investigations of plant disease epidemiology. Such an approach could improve biocontrol models toward the goals of predicting variation in treatment response and improving biocontrol practice.

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