CHAPTER EIGHT

An overview of arbuscular mycorrhizal fungal composition, distribution and host effects from a tropical moist forest

EDWARD ALLEN HERRE AND DAMOND KYLLO
Smithsonian Tropical Research Institute
SCOTT MANGAN
Smithsonian Tropical Research Institute and Indiana University
REBECCA HUSBAND
Smithsonian Tropical Research Institute and University of York
LUIS C. MEJIA
Smithsonian Tropical Research Institute
AHN-HEUM EOM
Smithsonian Tropical Research Institute and Korea National University of Education

Introduction

Arbuscular mycorrhizal fungi (AMF) (Zygomycetes) are an ancient group, dating back to the invasion of land surfaces by plants. Currently, they are perhaps the most abundant soil fungi, and they form intimate relationships with the roots of the vast majority of terrestrial plant species across the planet. These fungal symbionts generally play a mutualistic role, aiding the host plant primarily by enhancing the acquisition of soil nutrients, particularly phosphorus (P). In addition, AMF species often affect plant hormone production/induction (Allen *et al*. 1980), resistance to root pathogens (Newsham *et al*. 1995); water uptake (Kyllo *et al*. 2003) and soil structure (Andrade *et al*. 1998; Rillig & Allen 1999). In return, all AMF species obligately depend on the host plant for photosynthetically fixed carbon. Given their obligate dependence, AMF are influenced by their hosts at essentially every phase in their life history – hyphal development, sporulation and spore germination (Hetrick & Bloom 1986; Sanders & Fitter 1992; Bever *et al*. 1996). On the other hand, the degree of mycorrhizal dependence often varies widely among the host plant species in a community (Janos 1980a; Azcon & Ocampo 1981; Hetrick *et al*. 1992; Kiers *et al*. 2000).

A central and still largely unanswered question is the degree to which host plant and AMF species influence each other's community composition in natural systems. Fundamentally, for community effects to occur, different combinations

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of host and AMF species must produce different outcomes of survival and growth. Furthermore, given such differential effects, the potential for either host plant or AMF species to affect the other's community composition will depend largely on the identities and distributions of the associated species in a given habitat. The most conducive conditions for reciprocal effects would be that AMF species are heterogeneously distributed and differentially associate with, and affect, the growth and survival of different hosts. The form of the interactions between particular plant and AMF species (e.g. negative or positive feedbacks) will determine net effects on the diversities and distributions within plant and AMF communities (Bever *et al*. 1997; Bever 1999).

AMF have long been considered to be a relatively homogeneous group, both functionally and morphologically. Until recently, the diversity and composition of AMF species have been largely discounted as factors that significantly affect aboveground diversity for at least three reasons. First, if the number of recognized AMF species (< 200) is roughly correct, then strict-sense specificity is impossible because there are least three orders of magnitude fewer mycorrhizal species than host plants they colonize. Second, it is clear that most AMF species are not host-specific colonizers (Janos 1980; Harley & Smith 1983; Clapp *et al*. 1995). Third, even at fairly small scales, AMF communities often contain a mix of many component species, and colonization of a given host's roots by multiple AMF species is both likely and observed (Allen 1996; Husband *et al*. 2002a, b). Thus, even if individual AMF species can produce different effects on particular hosts, one would expect colonization of roots by multiple AMF species to homogenize and blur any differential effects on host plants, and thereby diminish any community-wide influences. These observations and considerations suggest that it is unlikely that AMF communities can have much effect on aboveground community composition. However, it has also become increasingly obvious that this view is open to challenge.

More recently, many observations from temperate grasslands and microcosm experiments suggest that AMF are in fact likely to have a significant influence on the distribution and diversity of plant communities (Grime *et al*. 1987; Gange *et al*. 1990; Bever 1994; Mills & Bever 1998; van der Heijden *et al*. 1998a; Hartnett & Wilson 1999; Olff *et al*. 2000; Klironomos 2002; Castelli & Casper 2003). For example, many studies indicate that different AMF species or mixes clearly invoke varying growth responses in different host plant species (Mosse 1972; Schenck & Smith 1982; Talukdar & Germida 1994; Streitwolf-Engel *et al*. 1997; van der Heijden *et al*. 1998a, b; Smith *et al*. 2000; Bever 2002; Helgason *et al*. 2002; Klironomos 2002). Likewise, the species identity of the plant host affects spore abundances of AMF species in the soil (Johnson *et al*. 1992; Sanders & Fitter 1992; Bever *et al*. 1997; Eom *et al*. 2000) or tendency to form hyphal associations with roots (Husband *et al*. 2002a, b). All of these observations suggest that AMF communities indeed influence the composition of host plants, at least

in some temperate communities. But is this also true for more diverse tropical communities?

In contrast to most temperate and boreal tree species, which tend to form associations with ectomycorrhizae (Basidiomycetes), the vast majority of tropical tree species form associations with arbuscular mycorrhizae (Smith & Read 1997). However, basic biological information such as AMF floras (e.g. species lists based on spore morphology) is only beginning to become available for a few sites (e.g. Australia: Brundrett *et al*. 1999; Mexico: Guadarrama & Álvarez-Sánchez 1999; Nicaragua/Costa Rica: Picone 2000; Costa Rica: Lovelock *et al*. 2003). To date, studies examining the habitat associations of AMF communities in the neotropics have mostly concentrated on comparing AMF-spore compositions in soils of intact forests with those in adjacent disturbed soils such as pasture (Fischer *et al*. 1994; Johnson & Wedin 1997; Allen *et al*. 1998; Picone 2000; but see Lovelock *et al*. 2003). That shifts in AMF-spore communities are often detected across such starkly distinct habitats may come as little surprise (but see Picone 2000). Nonetheless, in contrast to some temperate studies documenting fine-scale differentiation (Bever *et al*. 1996; Pringle & Bever 2002), relatively little is known about the spatial scales in which changes in AMF communities can be detected within intact neotropical forests (Janos 1992; Husband *et al.* 2002a, b; Mangan *et al*. 2004; A. H. Eom *et al*., unpublished results).

At best, the existing tropical species lists can only be considered as partially reflecting true AMF diversity (e.g. Bever *et al*. 2001; Helgason *et al*. 2002; Husband *et al*. 2002a, b; see below). Further, even in those sites for which there are partial floras, little information is available on the distributions of the component AMF species with respect to relevant ecological factors – space, time and host species (but see Lovelock *et al*. 2003). To make any advances in understanding tropical AMF–host interactions it is necessary to combine multiple approaches for characterizing the species composition and ecological properties of AMF communities at a series of sites. Further, having ecological and physiological characterizations of at least the dominant components of the plant communities at these sites will aid the proper design and interpretation of experiments concerning interactions with the AMF community.

Here, after discussing limitations associated with each technique, we present results from a combination of spore- and molecular-based techniques to determine the diversity and identities of AMF in the soils and roots (respectively) of such a tropical forest (Barro Colorado Island, Republic of Panama). We then assess AMF distribution among different sites, times and hosts. We present evidence that different components of the AMF community are functionally distinct, and cannot be considered as ecological equivalents. Further, we show that different host species are not ecological equivalents with respect to their associations with the AMF community. It thus appears that the AMF community at this site possesses all properties that are prerequisite for influencing the composition and distribution of the aboveground plant community.

Sources of information and methods

Morphological (spore-based) sampling and production of pure cultures

Traditionally, AMF identities have been determined by the morphology of spores, and AMF diversities and distributions in the field have been estimated on the basis of spore counts. However, this method for assaying AMF communities can be problematic. To begin with, the identification of AMF species based on spore morphology is a difficult enterprise, requiring a great deal of training and expertise in order to establish an acceptable level of consistency of identification. Ideally, therefore, taxonomic comparisons among different AMF communities should be conducted by the same researcher. Beyond that, the AMF community itself is difficult to characterize through spore abundances. AMF spore densities and species composition often change seasonally (Lee & Koske 1994; Allen *et al.* 1998; Schultz *et al*. 1999). Further, the rate of AMF spore production is affected by many factors (e.g. water, nutrient levels, light levels, host identity, etc.). For any given set of these conditions, such sporulation rates can vary among different AMF species (Morton *et al*. 1995; Bever *et al*. 1996; Eom *et al*. 2000). Therefore, the relative abundances of the spores of different AMF species in the soil may bear little relation to the relative abundances of AMF populations that have colonized roots (Clapp *et al*. 1995). In fact, it is likely that AMF diversity estimates based on spore morphology underestimate true species richness. For example, Bever and coworkers (2001) have demonstrated that AMF spore communities of temperate grasslands are much more diverse than would be expected from a single soil survey. By examining the fungal community at a single site through extensive soil-sampling and an assortment of subsequent trapping approaches over many years, they increased their initial estimate of 11 morphospecies to at least 37 different recognizable AMF morphospecies, one-third of which had not been previously described. Interestingly, this outcome suggests that the diversity of the AMF community at this temperate grassland site is roughly equal to that of the plants.

The spore identifications discussed in this chapter have all been performed by one person, Ahn-Heum Eom, and represent three types of collections from on and near Barro Colorado Island (BCI). The first involved the repeated collection of the AMF communities from the base of at least two individual trees for each of seven host species. The collection periods were: early February (early dry season), mid April (late dry season), mid June (early wet season) and mid December (late wet season) in order to characterize: (1) AMF species composition in the BCI community, (2) relative host associations and (3) the seasonal variation in spore abundance. The second method involved collections taken from four points around a 2-m perimeter of three adult host tree species growing in close proximity (each other's nearest neighbours) at each of four sites on BCI. This allowed us to establish the relative importance of location and host in affecting AMF spore community composition (A. H. Eom *et al*., unpublished results). The third method involved structured sampling of AMF spores in soils of forested

mainland and island sites in the vicinity of Gatun Lake, Republic of Panama. This sampling allowed us to better understand spatial structuring of AMF communities with particular attention to potential influences of island size (Mangan *et al*. 2004). The sampling consisted of individual plots, each with 16 soil sampling points arranged in a 9 × 9 m grid. Three sampling plots were located on small islands (less than 1 ha), three on medium-sized islands (2–4 ha) and three on adjacent mainland sites. At each plot we also conducted chemical analyses of the soil, as well as complete inventories of all vegetation greater than 0.5 m tall.

Molecular sampling

Although it requires appropriate training, assigning species names based on spore morphology is standard practice. Unfortunately, identifying AMF species within roots is nearly impossible because species-distinctive characters are generally lacking. However, advances in molecular techniques now make it feasible to identify directly the AMF within root tissues by using the polymerase chain reaction (PCR) to target specific AMF sequences. Several PCR-based methods have been developed over recent years, the majority of which target the ribosomal RNA genes (see Clapp *et al*. 2002 for review). Even so, the analysis of ribosomal genes is not without problems when applied to roots because any such approach is limited by the available genetic markers and is currently very time-consuming. Both Simon *et al*. (1992) and Helgason *et al*. (1998) designed primers (VANS1 and AM1 respectively) for the small subunit (SSU) rDNA intended to amplify all known glomalean fungi. Unfortunately, the VANS1 site is not well conserved in the Glomales (Clapp *et al*. 1999) and the AM1 site is absent from several highly diverged lineages (Redecker *et al*. 2000). Thus, these techniques are likely to give conservative estimates of AMF diversity *in planta*. Furthermore, the delineation of AMF species is ambiguous. In some cases, individual AMF spores have been found to contain multiple, genetically distinct nuclei (Kuhn *et al*. 2001; Sanders 2002), and give rise to subsequent, functionally different cultures (see Hart & Klironomos 2002). However, in contrast to patterns obtained with some more variable loci (Sanders *et al*. 1995; Lloyd-Macgilp *et al*. 1996; Clapp *et al*. 2001), differences between SSU sequences found within single spores are usually small compared with differences found among different AMF species (Clapp *et al*. 1999; Schussler *et al*. 2001; L. C. Mejia, personal observations). Also, in one of the few attempts to compare directly the morphological and molecular data for a host species (a forest herb in the United Kingdom), the results were broadly in agreement (see Merryweather & Fitter 1998; Helgason *et al*. 1999).

In the first application of molecular techniques to tropical host–AMF associations, Husband *et al.* (2002a, b) used AM1 primers to obtain SSU ribosomal gene sequences associated with seedlings and saplings of *Tetragastris panamensis* and *Faramea occidentalis*. First, newly emergent seedlings from cohorts of two host

plant species were sampled from mixed seedling carpets at two different sites. Individuals from each cohort that survived to one year were then collected and all root systems were analysed. Second, at a third site, survivors from a cohort of *T. panamensis* were collected over two subsequent seasons, and, in the final collection, older (> 5 yr) saplings were also collected. Thus, one sample gave a picture of associated AMF of successful seedlings of two species at the beginning and end of their first year of survival at two sites (Husband *et al*. 2002a). The other gave a glimpse of AMF associations of seedlings of different ages sampled at the same time point, as well as AMF associated with the survivors in a cohort across two different years (Husband *et al*. 2002b).

Testing AMF effects on plant growth

In addition to sampling spores and roots to study the diversities and distributions of AMF, we have used two approaches for determining effects of different AMF inocula on the performance of host seedlings. Kiers *et al*. (2000) conducted three reciprocal inoculation experiments in the greenhouse using seedlings from six native tree species representing a range of life histories (early successional pioneers, a persistent understorey species and emergent species) typical of mature forest. Seeds were germinated in sterile soil and then either kept in sterile soil as controls or exposed to arbuscular mycorrhizal fungi in current association with naturally infected roots from adults of either the same or different species growing in intact forest. Using pure cultures of four AMF morphospecies and a complete AMF mixture, Eom *et al*. (unpublished results) inoculated two host plant species, *Luehea seemannii* and *Theobroma cacao* (a small-seeded pioneer and a large-seeded mid- to late-successional understorey tree species, respectively). The AMF species represented the three genera commonly found as spores at the site: *Glomus*, *Acaulospora* and *Scutellospora*.

Results

Tropical–temperate diversities

The factors that potentially affect the dramatic observed differences in aboveground plant diversity between temperate and tropical regions have been discussed extensively (Leigh 1999; Givnish 1999; Harms *et al*. 2000; Wright 2002). However, even the most basic estimates of belowground diversity are only now beginning to be obtained for sites in either region. Nevertheless, a comparison of both regions using the same survey techniques done by the same researcher or laboratory group is now possible for both morphological and molecular data. Eom has surveyed the spore communities of temperate grassland in Kansas (Eom *et al*. 2000) and tropical moist forest in Panama (unpublished results). In Panama, four forest sites on Barro Colorado Island (BCI) in which soils around three specific hosts were targeted, a total of 25 morphospecies were identified. In the additional study that included intensive sampling of grids and the inclusion of

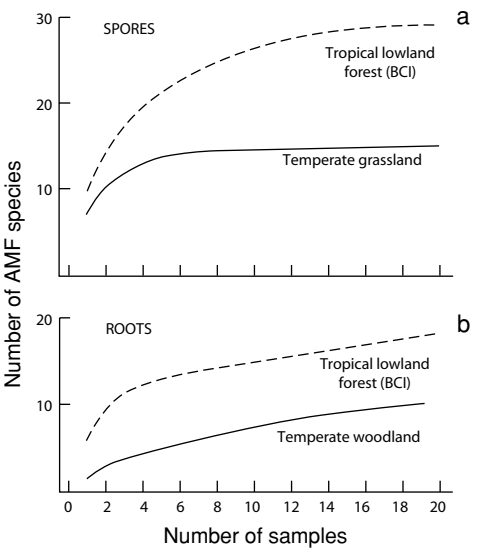

Figure 8.1 Comparison of BCI AMF species accumulation with accumulation in temperate sites where the same techniques have been used by the same researchers. (a) Morphological (spore-based) community surveys of BCI (each sample represents spores collected from a 10-g sample of soil) and a Kansas grassland (each sample represents spores collected from a 100-g sample of soil) from Eom *et al*. (2000), and (b) molecular survey of AMF species in roots (each sample represents AMF genotypes sampled from a single seedling root system; see Husband *et al*. 2002a). The curves for both the spore and root samples suggest conservatively that BCI has roughly twice the number of species of temperate regions where the same techniques have been applied.

several island and mainland sites around BCI, a total of 27 AMF morphospecies were encountered, with 17, 8, 1 and 1 from the genera *Glomus*, *Acaulospora*, *Sclerocystis* and *Scutellospora*, respectively (Mangan *et al.* 2004). Conservative comparisons of species accumulation curves from spore collections from both BCI and the temperate grassland (both sampled by A. H. Eom) suggest that the BCI AMF flora is roughly twice as large as that of the Kansas grassland (see Fig. 8.1a; Eom *et al.* 2000). This suggests that despite a higher overall AMF diversity at the BCI site, the ratio of AMF to plant species is much lower than at the temperate site.

The molecular surveys of roots from three host plant species show at least 30 different AMF types that exhibit genetic differences similar to those observed among closely related, named AMF species (see Husband *et al*. 2002a, b). A comparison of the AMF species accumulation curves from the same laboratory group using the same method for this tropical forest and temperate woodlands also suggests that the BCI AMF diversity is roughly twice as high (Fig. 8.1b). Further, the Shannon diversity index is appreciably higher for BCI ($H = 2.33$, $H_{max} = 3.135$, based on 48 roots from two host species) than for three temperate sites: a semi-natural woodland in England ($H = 1.44$, $H_{max} = 2.565$, based on 49 roots from five host species; Helgason *et al*. 1998), a temperate grassland ($H = 1.71$, $H_{max} = 2.890$, based on 47 roots from two host species; Vandenkoornhuyse *et al*. 2002), and temperate arable fields ($H = 1.16$, $H_{max} = 2.08$, based on 79 roots from four host species, calculated from Daniell *et al*. 2001).

Thus, both spores and molecular methods indicate that this tropical mycorrhizal community is relatively diverse compared with temperate sites where the similar procedures have been used to estimate AMF species diversity. However,

unlike the results of temperate grasslands where the number of AMF and plant species are approximately equal, our current results from BCI suggest that, even with more extensive sampling, the AMF species diversity will not approach the magnitude of the aboveground diversity. Further, spore surveys offer no reason to suspect new genera or explosive proliferation of recognizable morphotypes. Nonetheless, we note that the molecular data have been predominantly collected from the seedlings of only two host species. More-extensive sampling of hosts is likely to increase the number of species identified using molecular methods, particularly if there are even modest degrees of differential host affinity (see below).

Interestingly, a phylogenetic tree combining molecular results from both temperate and tropical ecosystems indicates that there is no clear temperate–tropical differentiation of AMF floras. Neither is there a differentiation between Old World and New World (Husband *et al*. 2002a, b). It is remarkable to consider that only a few base substitutions separate the *G. mosseae* found in a deciduous English woodlot and that found in a Panamanian moist forest. Either there was an early and rapid radiation of AMF that pre-dated the successive continental breaks and was followed by a dramatic deceleration of rates of genetic change, or AMF have previously unsuspected and prodigious capacities for long-distance/intercontinental dispersal. Perhaps some combination of pirates, botanists and backpackers may have introduced English AMF to the New World tropics, or vice versa.

Spatial diversities

Landscape scale

Relatively extensive sampling has been conducted in two tropical sites of Central America: La Selva, Costa Rica (see McDade *et al*. 1994 for site description) and BCI, Republic of Panama (see Leigh 1999 for site description). In Costa Rica, Lovelock and coworkers (2003) examined the AMF spore community with respect to possible variation associated with host tree species, soil type, seasonality and rainfall. We have conducted similar sampling in a tropical moist forest of Panama (Mangan *et al*. and Eom *et al*. unpublished results). A comparison of the two distant sites (> 500 km apart) indicates surprising differences in basic AMF composition.

In the Costa Rican forest, over 90% of the AMF spore community is comprised of only two species (*Acaulospora morrowiae* and *A. mellea*). This contrasts with the findings from BCI in two ways. First, it is species of *Glomus*, not *Acaulospora*, that dominate the AMF community in BCI soils. Second, the degree of dominance by a few species is much less marked. On BCI, six *Glomus* species make up roughly 70% of the total spore volume (and spore number). Lovelock and coworkers suggest that dominance by *Acaulospora* may correlate with ecosystem type (pasture vs. forest), but this seems unlikely given that both the dominance

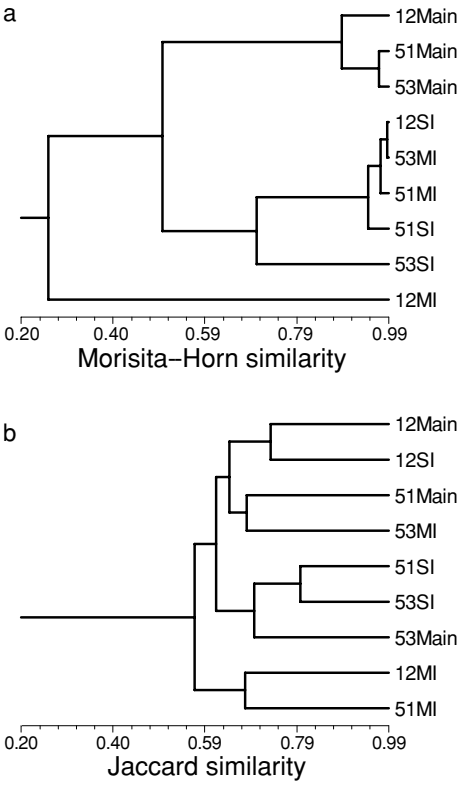

Figure 8.2 Similarities of AMF spore communities collected on mainland (Main), medium island (MI) and small island (SI) sites. The numbers refer to a geographical grouping of Main, MI and SI sites (see text). (a) Morisita–Horn similarities (based on relative abundances of AMF species). (b) Jaccard similarities (based on presence and absence of AMF species). Notice that analyses based on relative abundances clearly distinguish mainland (Main) from island (MI and SI) AMF floras, whereas the presence/absence analyses do not (see text).

by *Acaulospora* in Costa Rica and the dominance by *Glomus* in Panama occur in intact primary forest. Further, surveys of AMF spore communities from an intact Mexican seasonal forest also showed a dominance of *Glomus* (Allen *et al.* 1998). Possible explanations for this difference may be that La Selva has a greater total rainfall, and a less intense pattern of seasonality than BCI and the Mexican site. Another possibility is that, although the La Selva forest is more diverse overall, it is also more clearly dominated by a single tree species, *Pentaclethra macroloba* (Leguminosae) (McDade *et al.* 1994).

Intermediate and fine scale (mainland versus islands)

Within the vicinity of BCI, we found that the AMF spore community of any given mainland plot was more similar (Morisita–Horn index) to other distant (> 5 km) mainland plots than to nearby (within 0.7 km) island plots. This pattern reflects a more general pattern in all of our analyses of AMF distributions, and demonstrates the need to analyse relative abundance data in addition to presence/absence data when possible (see Fig. 8.2). Also, there was no decrease in AMF species richness (number of species, or Fisher's Alpha Index) either with decreasing forest size (size of the adjacent forest, mainland or island), or with decreasing species richness in the vegetation. In contrast, species richness of

vegetation did decrease with decreasing island size in a classical island biogeographical pattern that contrasted with the lack of such a decrease in the AMF diversity. Finally, within the smaller scale of the 9 × 9 m plots, we found no evidence for structuring of the AMF communities (no decay of Morisita–Horn community similarity with distance within plots; Mangan *et al.* 2004).

Differential host affinity

Spores

On BCI, we sampled AMF spore communities under the crowns of adults of three host tree species (*Luehea seemannii*, *Anacardium excelsum* and *Tetragastris panamensis*). These species were chosen because their life histories range from early pioneer to mature forest species, and because at each of four sites widely distributed across BCI, these trees were each other's closest neighbours. Each tree had four soil cores taken 2 m from the base for AMF community analysis. Both within and across sites, species composition of AMF spores was significantly influenced by the species of adult host (Eom *et al*., unpublished results). Although most AMF species were present at most sites, and under most trees, the relative abundances varied significantly with host tree species.

Minimally, these results suggest that AMF species vary in their rates of sporulation, and/or in total underground biomass, in response to different hosts. This further suggests differential fungal/host affinity, an interpretation that is consistent with the observation that, although mycorrhizal fungi from all inocula were able to colonize the roots of all host species, the inoculum potential (the infectivity of an inoculum of a given concentration) and root colonization varied depending on the identity of the host seedling and the source of the inoculum (Kiers *et al*. 2000). In Costa Rica, host plant species also affected AMF community composition, and again the differences were primarily due to changes in relative abundances (Lovelock *et al*. 2003). However, in an interesting contrast, the differences in La Selva AMF communities were primarily due to changes in the relative abundance of the two dominant species. On BCI, the pattern was quite different. There were no significant differences in the relative abundances of the top AMF species of the two common genera, *Glomus* and *Acaulospora*. However, there were significant host effects on seven less-abundant AMF species.

Molecular analyses of roots

We collected roots from cohorts of *T. panamensis* and *Faramea occidentalis* seedlings from a series of mixed seedling carpets, and analysed the AMF sequences (Husband *et al*. 2002a, b). The two hosts have distinct life histories. *Tetragastris panamensis* is a mid-to-late successional species associated with mature forest, whereas *F. occidentalis* is a persistent understorey species. These mycorrhizal communities showed significant spatial heterogeneity and non-random associations with the different hosts. It appears that distinct AMF species preferentially colonize and

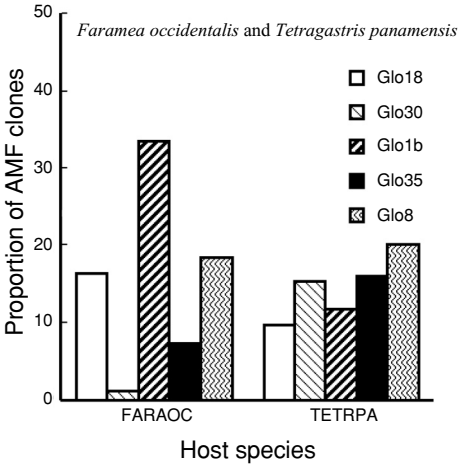

Figure 8.3 Comparison of the relative abundance of the five most common AMF species on six juvenile seedlings each of *Faramea occindentalis* (FARAOC) and *Tetragastris panamensis* (TETRPA) growing in a mixed-species stand at a single site on BCI. Notice that different AMF species dominate *Faramea* seedlings and *Tetragastris* seedlings (e.g. glo1b, glo30).

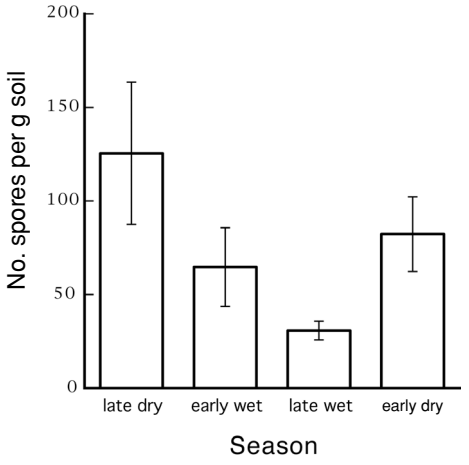

Figure 8.4 Density of spores at the base of at least two individuals for each of seven host tree species collected during four seasons on BCI. Notice that highest spore densities correspond to late dry season (mid-April), which immediately precedes the period of greatest seedling germination. This pattern reflects the seasonal pattern obtained at La Selva, in Costa Rica (see text, and see also Lovelock *et al.* 2003)

then differentially proliferate in roots of these two hosts (see Fig. 8.3; Kiers *et al.* 2000; Husband *et al.* 2002a, b).

Temporal patterns

Seasonal (spores)

At both BCI and La Selva, the overall density of spores was substantially higher at the end of the dry season than during the rainy season. Using four sampling periods on BCI, we find that overall spore density goes up just before the onset of the wet season (see Fig. 8.4; also see Mangan and Adler 2002). This corresponds with the period when the maximum germination of seeds is about to start (Garwood 1983). At both the BCI and La Selva sites, seasonality was also correlated with relative abundances of particular AMF species such that community composition would be different through time. For example, in La Selva,

A. morrowiae was the most abundant during the wet season, whereas *A. mellea* dominated during the drier season. However, season had no significant effect on community species diversity (Shannon's index) or richness (Lovelock *et al*. 2003). One possible explanation for these seasonal changes is that ecologically distinct AMF respond differently to changes in the abiotic environment, and as the environment changes over time, so do the dominant AMF. Such responses, plus changes in host phenology, are the implicit assumptions used previously to explain temporal variation in mycorrhizal communities (Lee & Koske 1994; Merryweather & Fitter 1998; Eom *et al*. 2000; Daniell *et al*. 2001; Mangan & Adler 2002). These possibilities require further examination.

Successional (molecular analyses of roots)

AMF species that dominate the roots of newly germinated seedlings are almost entirely replaced by previously rare species in the seedlings that survive a year (Husband *et al*. 2002a, b). In a second study, significantly different fungal populations were found to dominate 2-year-old seedlings and 5-year-old seedlings sampled at the same time point (Husband *et al*. 2002b). Both studies show a strong repeating pattern whereby the dominant mycorrhizal species are replaced by previously rare species in the surviving seedlings (see Fig. 8.5). Furthermore, both studies reveal a decrease in fungal evenness and diversity across plant age. Indeed, the repeated pattern both within host species and across sites suggests two non-mutually exclusive explanations. Either there is a succession of AMF types within a single host, possibly driven by differences in fungal life-history strategies (see Hart *et al*. 2001 for review); or individual AMF affect seedling recruitment, so that the most effective host–fungus combination has a higher probability of survival and is consequently enriched in the surviving population. The observation that the AMF combinations found in seedlings that survive for one or more years are not found in any of the earliest seedlings suggests that within-host succession of AMF plays the more important role (see Husband *et al*. 2002a, b). However, further experimental testing is needed to establish the relative importance of the roles of within-host succession and differential seedling survival depending on the identities of associated AMF.

Effects

Both pure culture and root inocula show that different AMF species or combinations generally produce different growth patterns (relative growth rates) in host seedlings, and that these effects vary interactively with AMF species (Kiers *et al*. 2000; Eom *et al.*, unpublished results; see Fig. 8.6). Further, both types of experiments also show that small-seeded pioneer plant species are more dependent on AMF for initial survival and growth (Kiers *et al*. 2000; D. Kyllo *et al.* unpublished results). Specifically, all of our experiments indicate that the tiny seedlings of small-seeded species soon die after germination in

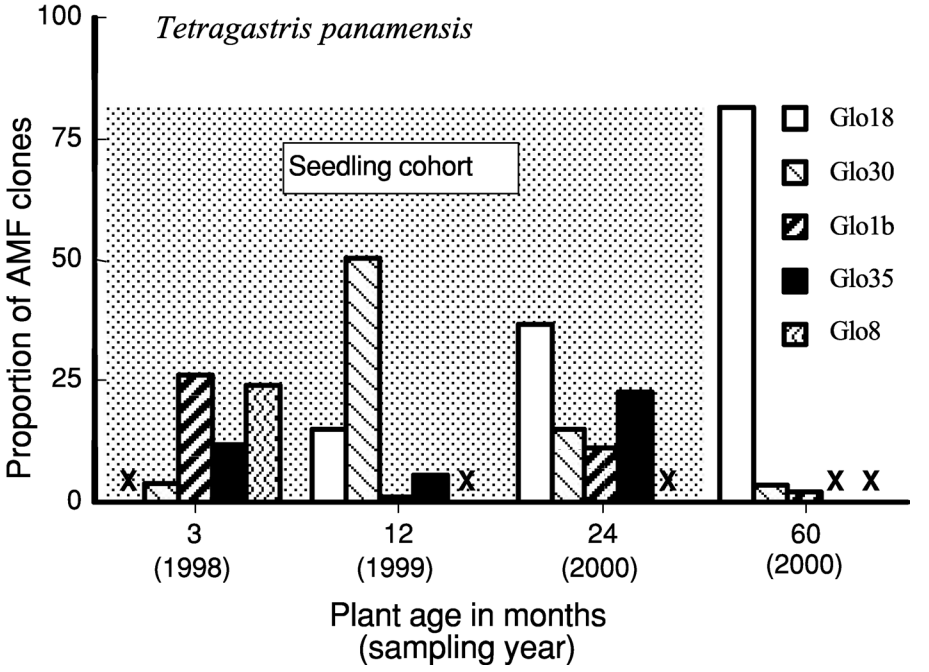

Figure 8.5 Sucession of AMF species associated with survivors of a cohort of *Tetragastris* seedlings between 1998 and 2000 (collected at 3 months, 12 months and 24 months). Further, during the 2000 collection, older saplings (older than 5 years) were also collected (60) for comparison of the AMF communities on the roots of the younger plants. Notice that the AMF species that dominate juvenile (3-month) seedlings (glo1b and glo8) decrease in abundance and are replaced by other species (e.g. glo18) in the older surviving seedlings and saplings. X indicates a zero count for the clone in the sample.

sterile soil. These are also the host species that show the most striking differences in growth when inoculated with different AMF sources. Consistent with these suggestions, recent greenhouse work with more than 80 woody species in Brazil clearly demonstrates that the early successional species generally show a much greater response to AMF than late successional species (Siqueira *et al*. 1998; Zangaro *et al*. 2003). Variation in response to different AMF species was greater in the host with greater mycorrhizal dependency, following a pattern suggested by van der Heijden (2002). It appears that having a relatively large seed provides some buffer against immediate dependence on AMF.

Two further interpretations are suggested by the combination of the effects on growth and the infectivity trials presented in Kiers *et al*. (2000). The experiments that use roots of adults as the source of inoculum for seedlings suggest that the AMF communities established in adult root systems do not necessarily optimize growth in conspecific seedlings (Kiers *et al*. 2000). If subsequent experimentation supports this pattern, then AMF associated with roots of adult

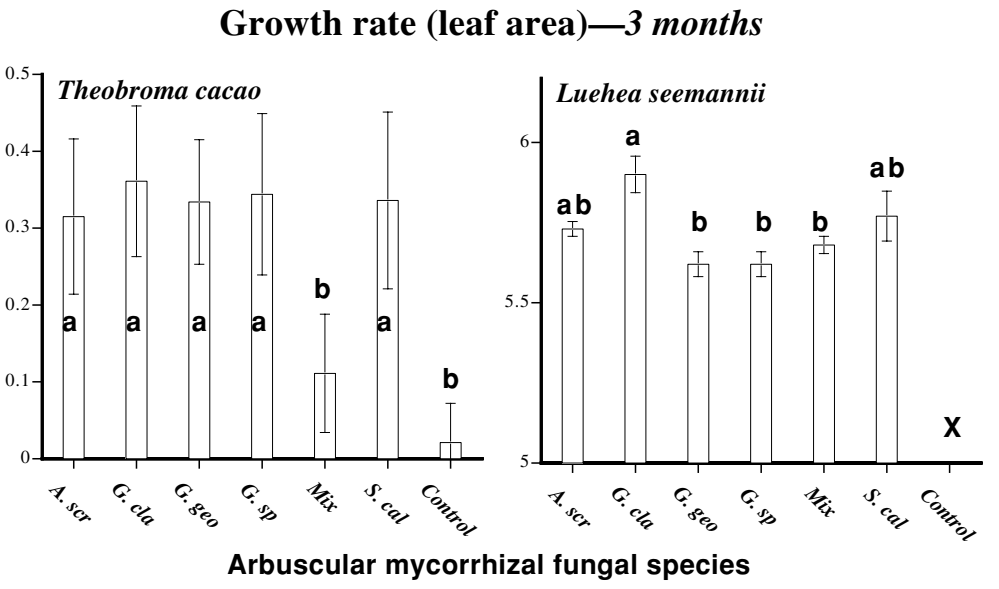

Figure 8.6 Growth responses of *Luehea seemannii* and *Theobroma cacao* seedlings in response to different AMF inocula. (Different letters refer to significantly different means $P < 0.05$). For both host species, AMF improved growth relative to controls, but the growth response was determined by the specific fungus–host combination. The pioneer species, *L. seemannii*, grew at varying rates depending on the AMF species and was obligately dependent on the mutualism for initial survival. Variation in response to different AMF species was greater in the host with greater mycorrhizal dependency following a pattern suggested by van der Heijden (2002). Although *T. cacao* showed no clear differences among different AMF species (and genera), the growth response of *T. cacao* was significantly lower for the mixed AMF species inoculum than single AMF species. The AMF species names are *Acaulospora scrobiculata* (A. scr), *Glomus clavisporum* (G. cla), *Glomus geosporum* (G. geo), *Glomus* sp. (G. sp.) and *Scutellospora calospora* (S. cal).

trees may contribute to the pervasive pattern of negative density dependence observed in the establishment of tropical seedlings (Augspurger 1984; Gilbert *et al*. 1994; Wills *et al*. 1997; Harms *et al*. 2000; Wright 2002). Finally, there is some suggestion that larger-seeded species show a higher level of differential affinity for certain AMF inocula (Kiers *et al*. 2000). Perhaps having a larger initial resource base also permits a seedling the luxury of being more 'choosy' concerning the AMF species with which associations are formed (see also Kitajima 2003). If so, we might expect differences in the tendency in larger-seeded species to form associations with particular AMF species that are more or less beneficial.

Discussion

Although preliminary, these results are nonetheless sufficient to begin to delineate the properties we might expect tropical mycorrhizal communities to

possess. For example, consistent with findings from other groups studying tropical AMF–host associations, it is now clear that different AMF species and mixes of species produce different effects on host growth (Nemec 1978; Kiers *et al.* 2000). Further, different hosts react differently to any given set of AMF inocula (Zangaro *et al.* 2003). Moreover, densities and distributions of AMF species are not random with respect to space, time or host. Therefore, neither the AMF nor the host plants are functional and ecological equivalents with respect to their biotic interactions with each other. This demonstrates that AMF and host-plant communities possess the prerequisite properties necessary for each to influence the species composition and distribution of the other. As is the case with other elements of the biotic environment (Augspurger 1984; Gilbert *et al.* 1994; Wills *et al.* 1997; Harms *et al.* 2000; Wright 2002), we should not expect that AMF communities provide a neutral background for the establishment and growth of host plants (see also Connell & Lowman 1989). The preponderance of the evidence both from BCI and other sites suggests that tropical AMF communities indeed affect aboveground community composition and distributions. However, some important pieces are still missing from the puzzle.

How do AMF species in roots correspond to spores in the soils?

We have presented both morphological data collected from AMF spores in soils and molecular data collected from AMF in association with roots. Both methodological approaches show higher AMF diversity in a tropical diverse forest than in temperate ecosystems, when the same methods are used by the same researchers. Sampling curves for both morphological and molecular data appear to approach an asymptotic limit at roughly 30–40 species. Both approaches demonstrate clear non-random AMF distributions with respect to space, time and host. But how do spores relate to what is in roots? More specifically, how does the molecular information correspond to morphological information? In one of the few examples to compare the morphological and molecular data directly for a host species, the results were broadly in agreement (Merryweather & Fitter 1998; Helgason *et al.* 1999). Is this also the case with the Panamanian samples?

Of the 30 AMF species that have been identified using the available sequences taken from field-collected roots, only one shows a match with a sequence obtained from the 12 spores from pure cultures of AMF species for which sequences have been obtained. This is particularly striking because spores were also collected from the sites where the roots were collected for molecular analyses. Therefore, our current understanding is that there are at least 41 genetically distinct AMF species in BCI soils and roots. Nonetheless, if the AMF that are present as spores and those that are present in roots represent samples from the same population, we would expect the overlap to be higher. Minimally, this suggests that the actual AMF diversity at this site estimated (at least 41) is even higher than we had directly estimated on the basis of either soil or root

samples. It is possible that, with even more intensive sampling (e.g. Bever *et al.* 2001), some of the species currently found only as molecular signatures in roots might be encountered as spores. However, if there is no further overlap between the spores and roots, the total number of AMF species at this site would be at least 57, and it is noteworthy that only a relatively small number of individual seedlings from two species of host were used for the molecular sampling.

Further, the lack of overlap between root- and soil-collected sequences also suggests that these represent ecologically distinct groups of AMF in this forest. One group (those that dominate the root systems) tends to persist in active association with roots and tends not to produce large quantities of spores, while another (those that dominate the spore comunity in the soil) appears to be more transient in the association with roots, and tends to produce relatively large quantities of spores. In essence, the AMF groups are analogues of old-growth and pioneer tree species, respectively (Reader *et al.* 2001; also see Dalling & Hubbell 2002). If this is true, we might also expect that the different sets of mycorrhizae show different life-history strategies and/or fundamentally different sets of relationships with hosts. Specifically, the root-associated 'old-growth' AMF species (at least some of which apparently form much longer-standing associations) might provide greater benefits to the host than the 'pioneer' AMF species that might effectively be weeds, and provide less benefit for the hosts.

What is the relative importance of AMF spores and hyphae for colonizing germinating seedlings?

We know that spores derived from the different AMF pure cultures produce different growth effects in seedlings. We know that different host species respond differently to a given AMF inoculum. We know that the relative abundances of spores of different AMF species show relatively little variation over spatial scales that appear to correspond roughly with areas dominated by roots of a single canopy emergent tree (81 m^2). This suggests that at spatial scales of this order we can expect relatively homogeneous AMF spore communities that are likely to benefit the growth of some species more than others.

However, our preliminary results show that the composition of AMF species associated with surviving seedlings changes consistently through time, and that AMF communities in older seedlings and saplings tend to be dominated by relatively few species. Further, we know that few of the AMF species that we have identified from spores correspond genetically to AMF species that we have found associated with roots. Moreover, we do not know whether the spores or the AMF hyphae running throughout the adjacent soils from the roots of large individual plants are more important for colonization of seedlings. Are newly germinated seedlings colonized primarily from spores, only to have those spore-derived AMF displaced by subsequent colonization by hyphae? This scenario implies a competition for available seedlings by AMF that have access to

widely different resource bases (spores vs. extensive hyphal systems connected to the carbon source of adult trees (see Grime *et al.* 1987; Kyllo 2001). A hypothesis to be tested is whether the AMF that tend to dominate older seedlings also correspond to those that dominate the roots of adults in the area. Specifically, the patterns revealed by our sampling thus far suggest that a forest floor consists of a patchwork of functionally distinct AMF communities that correspond roughly with the root systems of the different adult canopy trees. If this proves to be the case, and the results obtained by Kiers *et al.* (2000) from greenhouse experiments are relevant to the field, then the overall effect of AMF on host communities would be to maintain host diversities through negative density-dependence.

Ultimately, there are gaping holes in our view of AMF–host interactions in BCI that need to be filled. We need to develop an even more detailed view of what AMF species are dominating root systems of different host species of different ages. Are the AMF species associated with older seedlings and saplings largely a reflection of locally abundant AMF on existing, dominating root systems? Further, we only have pure cultures from about 12 of the genetically identified species available for experiments. Pure cultures will need to be produced from AMF species that currently have been found only in association with roots. These species will need to be characterized with respect to growth and survival effects on different hosts. Those effects will need to be placed in the context of the field samples that show how the various AMF species occur primarily as early or later associates in the roots of seedlings of particular host species, or predominately as spores. Nonetheless, despite the gaps in our knowledge, the preponderance of available evidence both from BCI and other sites suggests that neither hosts nor AMF species are functionally ecological equivalents, and that all prerequisites are fulfilled for AMF to influence the community composition and distribution of host plants. The possibilities are exciting. Studies of the ecological role of arbuscular mycorrhizal fungi in tropical forests are in their infancy.

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