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Published on: 01 Sep 2010 - Ecology (Ecological Society of America)

Topics: Luehea, Apeiba, Virola, Glomus and Glomeromycota

Ecology, 91(9), 2010, pp. 2594–2603

Specificity between Neotropical tree seedlings and their fungal mutualists leads to plant–soil feedback

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Abstract. A growing body of evidence obtained largely from temperate grassland studies suggests that feedbacks occurring between plants and their associated soil biota are important to plant community assemblage. However, few studies have examined the importance of soil organisms in driving plant–soil feedbacks in forested systems. In a tropical forest in central Panama, we examined whether interactions between tree seedlings and their associated arbuscular mycorrhizal fungi (AMF) lead to plant–soil feedback. Specifically, do tropical seedlings modify their own AMF communities in a manner that either favors or inhibits the next cohort of conspecific seedlings (i.e., positive or negative feedback, respectively)? Seedlings of two shade-tolerant tree species (*Eugenia nesiotica*, *Virola surinamensis*) and two pioneer tree species (*Luehea seemannii*, *Apeiba aspera*) were grown in pots containing identical AMF communities composed of equal amounts of inoculum of six co-occurring AMF species. The different AMF–host combinations were all exposed to two light levels. Under low light (2% PAR), only two of the six AMF species sporulated, and we found that host identity did not influence composition of AMF spore communities. However, relative abundances of three of the four AMF species that produced spores were influenced by host identity when grown under high light (20% PAR). Furthermore, spores of one of the AMF species, *Glomus geosporum*, were common in soils of *Luehea* and *Eugenia* but absent in soils of *Apeiba* and *Virola*. We then conducted a reciprocal experiment to test whether AMF communities previously modified by *Luehea* and *Apeiba* differentially affected the growth of conspecific and heterospecific seedlings. *Luehea* seedling growth did not differ between soils containing AMF communities modified by *Luehea* and *Apeiba*. However, *Apeiba* seedlings were significantly larger when grown with *Apeiba*-modified AMF communities, as compared to *Apeiba* seedlings grown with *Luehea*-modifed AMF communities. Our experiments suggest that interactions between tropical trees and their associated AMF are species-specific and that these interactions may shape both tree and AMF communities through plant–soil feedback.

Key words: arbuscular mycorrhizal fungi (AMF); Barro Colorado Island, Panama; belowground interactions; Glomus *spp.; light level; plant–fungal interactions; plant–soil feedback; specificity; tropical forest.*

Introduction

Soil organisms are notably diverse and influence the diversity and species composition of plant communities via species-specific interactions (Van der Putten et al. 2001, Reynolds et al. 2003, Wolfe and Klironomos 2005, Kardol et al. 2007). For example, negative plant–soil feedback is a frequency-dependent process, which can result from the accumulation of detrimental organisms (e.g., plant pathogens) that have species-specific effects in soils surrounding adult plants. This buildup, in turn, can decrease the growth and survival of conspecific juveniles rooted in close proximity to their adults (Augspurger 1984, Bever 1994, Mills and Bever 1998, Packer and Clay 2000, Klironomos 2002, Hood et al. 2004, Bell et al. 2006, Petermann et al. 2008). Consequently, diversity is maintained because juveniles of other plant species (i.e., heterospecifics) that are relatively less affected by these pathogens have a competitive advantage at these sites (Connell et al. 1971, Bever 1999). Indeed, demographic patterns of density dependence in tropical tree communities are consistent with the predicted outcomes of negative feedback (Wills and Condit 1999, Harms et al. 2000, Peters 2003). In contrast, positive plant–soil feedback can arise and reduce local plant diversity if other species-specific soil organisms (e.g., plant mutualists) accumulate near adults and promote growth and survival of conspecific juveniles (Bever 1999). Therefore, depending on the species composition of microbial communities and their interactions with host plants, both negative and positive plant–soil feedback have the potential to influence the composition of plant communities.

Arbuscular mycorrhizal fungi (AMF) have been suggested to be important drivers for both negative

Manuscript received 6 March 2009; revised 8 September 2009; accepted 29 October 2009; final version received 20 December 2009. Corresponding Editor: J. N. Klironomos.

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and positive plant–soil feedback (Bever 2002*a*, Klironomos 2002, Castelli and Casper 2003). These fungi form symbiotic relationships with plants in which the fungus often provides the plant with the ability to access scarce resources in return for carbohydrates. Although in most cases this relationship is mutualistic, the net benefit that a single plant species receives depends on the identity of the fungus with which it is associated (van der Heijden et al. 1998, Bever 2002*a*, Klironomos 2003, Herre et al. 2005). Likewise, levels of root colonization and spore production of a given AMF species depends on the identity of the host plant (Bever et al. 1996, Eom et al. 2000, Husband et al. 2002). Consequently, these species-specific host and AMF effects set the stage for AMF-mediated plant–soil feedback (Bever 1999). To date, studies that have examined the ecological importance of the composition of AMF communities to the ecology of plant communities have been restricted largely to temperate grassland systems. Whether similar AMF-mediated processes also occur in other systems such as those dominated by longer-lived hosts (e.g., trees) remains largely unstudied.

Neotropical forests serve as excellent study systems for the exploration of AMF-mediated processes in tree communities, because tropical AMF are diverse and heterogeneously distributed (Picone 2000, Lovelock et al. 2003, Mangan et al. 2004, Herre et al. 2005), and the majority of Neotropical trees, like temperate grasses, are dependent on AMF for establishment and growth (Siqueira et al. 1998, Zangaro et al. 2003). The high dependency of tropical tree seedlings on AMF may be particularly important because the set of factors that affect seedling dynamics in tropical forests is thought to be one of the most important ecological filters that determine the composition of reproductive adults (Connell et al. 2005).

In order for interactions between tropical tree seedlings and AMF to result in plant–soil feedback as demonstrated in temperate grasslands (Bever 2002*a*, Castelli and Casper 2003), these forests must be characterized by: (1) sufficient host-mediated divergence of AMF communities, and (2) differential response of tree species to different combinations of AMF species (Bever 1999). Although available studies in the tropics are few, spore communities around different species of Neotropical adult trees have been shown to vary depending on host identity (Lovelock et al. 2003; S. A. Mangan and E. A. Herre, *unpublished manuscript*). Further, molecular data show that host identity influences the AMF species that colonize and persist in the roots of tropical tree seedlings (Husband et al. 2002). Moreover, different AMF species and combinations of species have been shown to differentially influence the growth of tropical tree seedlings (Herre et al. 2005). However, no study has determined whether host-associated divergence in AMF community composition, combined with different AMF effects on host growth and survival, has the potential to generate positive or negative plant–soil feedback in tropical forest systems.

In this study, we examine whether tropical trees exhibit sufficient specificity in the ecological outcomes of their interactions with AMF to shape seedling communities through AMF-mediated plant–soil feedback. Specifically, four species of tropical tree seedlings were grown with identical AMF communities and under two light levels. This allowed us to determine whether different plant species differentially modify the composition of their associated AMF communities, and whether light mediates such modifications. A reciprocal experiment that controlled for variation in abiotic properties was then conducted with two of the four host species to test whether AMF communities previously modified by different host species result in differential growth of subsequently recruiting seedlings. Specifically, do tropical seedlings change their own AMF communities in a manner that either favors or inhibits the next cohort of conspecific seedlings relative to the heterospecific seedlings (i.e., positive feedback or negative feedback, respectively)?

Methods

Study system

This study examined the interaction of tree and fungal species that co-occur in soils of Barro Colorado Island (BCI), Republic of Panama (9°10′ N, 79°51′ W). The forest on BCI is seasonally wet and is composed both of young (<100 years old) and old (300–500 years old) stands. Annual rainfall is 2600 mm, with 90% of precipitation falling from late April to mid-December, which is then followed by a pronounced dry season (Windsor 1990). AMF spore communities of BCI and its surrounding land masses are diverse (Mangan et al. 2004, Herre et al. 2005), and primarily dominated by members of the genus *Glomus* (Mangan et al. 2004).

In this study, we used seedlings of four tree species occurring commonly on BCI and comprising two groups differing in seed size and shade tolerance. *Luehea seemannii* and *Apeiba aspera* (both from the family Tiliaceae) are small-seeded pioneer species that require light gaps to establish. *Eugenia nesiotica* (Myrtaceae) and *Virola surinamensis* (Myristicaceae) are larger-seeded species that are more tolerant to low levels of light. Growth of these four tree species has been shown previously to be highly responsive to the colonization of AMF (S. A. Mangan, *unpublished manuscript*). Hereafter, only the name of the genus is used when referring to each plant species.

The AMF community used in this study was composed of six AMF species: *Glomus fasciculatum*, *Glomus artruva*, *Glomus geosporum*, *Acaulospora scorbiculata*, *Scutellospora calospora*, and an unidentified species of *Glomus*, "*G.* 1." These species are common in the local soil community (Mangan and Adler 2002, Mangan et al. 2004: Appendix 1), and show life history differences that are reflected in differing dispersal

strategies. *Glomus fasciculatum*, *G. artruva*, and *G.* 1 all produce their spores in clusters surrounded by copious amounts of fungal mycelia and are commonly dispersed by rodents (Mangan and Adler 2002). *Glomus artruva* and *G. fasciculatum* are especially common in fecal pellets of the spiny rat *Proechimys semispinosus* (labeled A and C, respectively, in Mangan and Adler 2002). The remaining three AMF species do not produce their spores in large clusters and are unlikely to be dispersed actively by rodents.

To minimize the potential of contamination by other soil microbes, all species of AMF used in this study originated from refreshed second-generation pure cultures. Originally, spores of *G. artruva*, *G. fasciculatum*, and *G.* 1 were isolated from feces of spiny rats, and spores of the three remaining species were isolated from BCI soil using sucrose flotation. After isolation, spores from each AMF species were introduced separately to sterile roots of seedlings of *Ochroma pyramidale* (Balsa) and maintained as first-generation pure cultures. Second-generation cultures were established by placing fresh spores obtained from first-generation cultures onto sterile roots of *O. pyramidale* and grown in sterilized soil for five months.

Do host identity and light level promote shifts in AMF spore communities?

We established a pot experiment consisting of the four host species and two light levels in a shade house to test the effect of host identity and light on AMF sporulation. Seeds of the four host species were surface sterilized (10% Clorox for 10 minutes [Clorox Company, Oakland, California, USA]) and germinated in flats containing autoclaved soil. Six-week old seedlings of *Eugenia* and *Virola* and three-week old seedlings of *Luehea* and *Apeiba* were transplanted into 3.7-L pots containing a steam-pasteurized 3:1 forest soil and sand mixture. An identical mixture (300 mL total) of pure culture inoculum (e.g., soil and fine roots containing AMF spores and mycelia) consisting of an equal quantity (50 mL) of each AMF species was added to the rooting zone of each pot. Two light levels representative of understory and large gap environments (2% and 20% full sunlight, respectively) were created with neutral black shade cloth. Each light × host combination was replicated eight times, and replicates were arranged randomly within each light level.

We allowed seedlings to grow for eight months, which was equivalent to the length of the rainy season in which the majority of annual plant growth occurs. We then collected three soil cores (2.5 cm diameter × 10 cm depth) at equal distances around each stem. Soil from the three cores was mixed thoroughly and AMF spores were isolated from a 50-g subsample of soil using sucrose flotation (Daniels and Skipper 1982). Isolated spores were then identified and quantified.

Do host-meditated shifts in AMF communities influence seedling performance?

Immediately following harvest of the first experiment, we established a second experiment to investigate whether host-dependent shifts in AMF spore communities reflect changes that can influence the growth of subsequently recruiting seedlings. Because of limited available bench space, we were unable to examine all host and light combinations. Instead, performance of only *Luehea* and *Apeiba* seedlings was assessed when grown in either soil previously supporting a conspecific host or soil supporting the other host species under high light. Specifically, roots and soil contained within pots of *Luehea* and *Apeiba* of the previous experiment were thoroughly homogenized (separately for each host species) and served as the live AMF inoculum source. Live inoculum (100 mL) was added to the rooting zone of 2.6-L pots that were all filled with an identical 3:1 steam-pasteurized soil and sand mixture. To control for potential host-mediated shifts in nutrients in the source inoculum, an additional 100 mL of sterilized inoculum from the nontarget host was also added to each pot (e.g., 100 mL of live *Apeiba* inoculum mixed with 100 mL of sterilized *Luehea* inoculum). One-half of the pots containing each inoculum type received a single three-week-old *Luehea* seedling grown previously in sterile soil, while the remaining pots received a three-week-old sterile seedling of *Apeiba* (see Plate 1). In separate pots, seedlings of each host species were grown with 100 mL of sterilized inoculum of either *Luehea* or *Apeiba* to examine host performance in the absence of live AMF. Also, these sterile controls allowed for differences in abiotic properties between the two live inoculum sources to be assessed through the examination of performance of each host across the two inoculum sources after the AMF community was eliminated. Finally, both sterile *Luehea* and *Apeiba* seedlings were grown separately with inoculum obtained from pure, single-species cultures of AMF to determine the relative importance of the most abundant AMF species (as determined by spore counts in experiment 1) to performance of each host species. Specifically, 100 mL of roots and soil of *G.* 1, *G. geosporum*, or *A. scorbiculata* was combined with 100 mL of sterilized inoculum and added to the rooting zone of pots containing the common steam-pasteurized soil and a seedling of either *Luehea* or *Apeiba*.

We replicated all treatment combinations 15 times, except for the sterile controls, which were replicated 8 times per sterile inoculum source. We assigned equal numbers of each treatment combination to one of three blocks, with pots randomized weekly within each block. Plants were grown under neutral black shade cloth, adjusted to 20% full sunlight. Total leaf area was measured approximately every 10 days throughout the duration of the experiment. Plants were harvested after 72 days to determine total final leaf area and dry

biomass of roots, stems, and leaves. Roots of five randomly selected individuals from each treatment combination were stained and scored for percent colonization of AMF.

Statistical analysis

For the first experiment, we examined overall spore abundance between light levels and among plant species using multivariate analysis of variance (MANOVA), with spore number of each AMF species common to both light levels included as independent variables. In this model and the following analyses, total spore number per AMF species was rank-transformed to improve normality. We then used multivariate profile analysis, separately per light level, to examine the overall community composition of AMF spores. Specifically, the interaction of the host species main effect with the profile was examined; a host species × profile interaction would indicate a significant change in community composition of AMF species across host species (Bever et al. 1996). To identify which host species supported different AMF communities, we constructed pairwise contrasts within each MANOVA model. Specifically, the interaction with each possible pair of host species (per light level) and the profile was examined, with Bonferroni correction used to adjust for multiple comparisons. Finally, to identify which members of the AMF community changed across the suite of host species, univariate ANOVAs were conducted per AMF species and per light treatment. (Only the resulting Bonferroni-corrected multiple comparisons are presented; see Fig. 1.)

For the second experiment, we used analysis of covariance (ANCOVA) to determine the influence of both host identity and the different AMF treatments on final total biomass of *Apeiba* and *Luehea*. All ANCOVA models included AMF, host, and block as fixed effects. Also, to control for any differences in plant size at the onset of the experiment, initial leaf area was included as a covariate. Because initial leaf area was found to be a good predictor of total biomass at the end of the experiment for *Luehea* ($R^2 = 0.15$, $F_{1,87} = 14.73$, $P < 0.001$), but not for *Apeiba* ($R^2 < 0.01$, $F_{1,88} = 0.28$, $P = 0.598$), the interaction between initial leaf area and host was also included. In addition, repeated-measures ANOVA was used to examine treatment effects on changes in leaf area over the duration of the experiment.

In both the ANCOVA and repeated-measures ANOVA models, we decomposed the AMF main effect into three a priori orthogonal contrasts. The first contrast was used to assess the effect of AMF inoculation on overall plant growth by comparing biomass averaged among sterile plants with biomass averaged among plants grown in live AMF. The second contrast examined biomass averaged among plants grown in AMF communities previously modified by *Luehea* with biomass averaged among plants grown with AMF communities previously modified by *Apeiba*. The third contrast examined whether biomass differed across the three AMF single-species treatments. We then decomposed the AMF × host interaction into the same three contrasts to examine whether these comparisons differed depending on host identity. Dependent variables in all models were $\log_{10}$-transformed to meet the assumption of normality. All statistical procedures were conducted in SAS, version 9.1 (SAS Institute 1996).

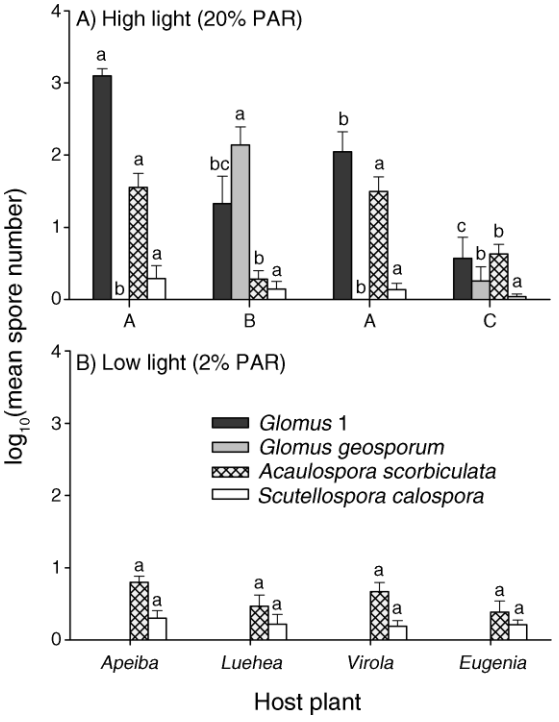

FIG. 1. AMF community composition of spores associated with each host species grown under (A) 20% PAR and (B) 2% PAR. Per light level, different uppercase letters below the *x*-axis in the upper graph indicate significant differences ($P < 0.05$) in AMF communities across the four host species (i.e., significant pairwise contrasts within the multivariate profile analysis). In the lower graph, there were no significant differences among the four host species. Per light level, different lowercase letters above bars indicate significant differences ($P < 0.05$) in spore abundance per AMF species across the four host species (not across different AMF species within each host species). Hosts are two pioneer trees (*Luehea seemannii*, *Apeiba aspera*) and two shade-tolerant trees (*Eugenia nesiotica*, *Virola surinamensis*) on Barro Colorado Island, Panama. Although $\log_{10}$-transformed means for spore abundance are presented for illustrative purposes, all analyses were conducted using rank-transformed spore numbers. Error bars indicate standard errors.

RESULTS

Do host identity and light level promote shifts in AMF spore communities?

We found that both light and host species identity influenced the composition of AMF spore communities. Spores of four of the six AMF species originally introduced into pots were common in soil under high

TABLE 1. The effects of host identity and arbuscular mycorrhizal fungi (AMF) and their interaction on growth of two pioneer tree species, *Apeiba aspera* and *Luehea seemannii*, on Barro Colorado Island, Panama.

Source	df	Total biomass (error df = 135)		Leaf area (error df = 133)	
		F	*P*	*F*	*P*
Host	1	2.68	0.104	0.08	0.778
AMF	6	102.57	<0.001	261.72	<0.001
Live AMF vs. sterile	1	555.15	<0.001	1516.62	<0.001
Modified AMF communities	1	5.80	0.017	4.47	0.036
AMF single species	2	18.91	<0.001	16.13	<0.001
Host × AMF	6	5.04	<0.001	23.13	<0.001
Host × live AMF vs. sterile	1	15.90	<0.001	123.09	<0.001
Host × modified AMF communities	1	3.01	0.085	4.64	0.028
Host × AMF single species	2	5.18	0.007	4.50	0.013
Block	2	2.67	0.073	1.49	0.229
Initial leaf area	1	9.45	0.003		
Initial leaf area × host	1	8.52	0.004		

Notes: ANCOVA was used to examine $\log_{10}$-tranformed final biomass, with initial leaf area and its interaction with host as covariates. Repeated-measure ANOVA was used to examine $\log_{10}$-transformed leaf area measured every ~10 days over the duration of the experiment (Fig. 3). The "live AMF vs. sterile" contrast examined differences between biomass averaged across all live AMF treatments and biomass averaged across sterile treatments. The "modified AMF communities" contrast examined differences between biomass of plants grown in AMF communities modified by *Apeiba* from those grown in communities modified by *Luehea*. The "single AMF species" contrast examined differences in overall plant growth across the three pure AMF strains. Contrasts within the host × AMF interaction term examined whether *Apeiba* and *Luehea* seedlings performed differently in these different AMF treatment combinations.

light, whereas spores of only two of the six species were present under low light (Fig. 1). For the two AMF species (*A. scorbiculata* and *S. calospora*) where spores were present in soil under both light levels, overall spore abundance was significantly lower under low light, and differed significantly across host species (light, Wilks' $\lambda = 0.75$, $F_{2,53} = 8.70$, $P = 0.0005$; host, Wilks' $\lambda = 0.59$, $F_{6,106} = 5.42$, $P < 0.0001$; light × host, Wilks' $\lambda = 0.83$, $F_{6,106} = 1.75$, $P = 0.1164$). Under high light, profile analysis revealed that AMF spore community composition was influenced significantly by host identity (profile × host, Wilks' $\lambda = 0.09$, $F_{3,26} = 11.66$, $P < 0.0001$), whereas host identity had no effect under low light (profile × host, Wilks' $\lambda = 0.87$, $F_{1,3} = 1.26$, $P = 0.3085$). Specifically, community composition was similar only in soils of *Apeiba* and *Virola* under high light; spore compositions differed significantly between all other pairwise comparisons (Fig. 1, top graph). Under low light, composition did not differ across any of the host species (Fig. 1, bottom graph). We found that host-dependent shifts in community composition of AMF spores under high light were a result of both change in relative abundances of spores, and the presence and absence of different AMF species. Spore abundance of three of the four AMF species differed depending on host species identity (Fig. 1, top graph). Furthermore, although spores of *G. geosporum* were common in soils of *Luehea* and *Eugenia*, spores of *G. geosporum* were absent in soils of *Apeiba* and *Virola* (Fig. 1, top graph).

Do host-meditated shifts in AMF communities influence seedling performance?

We found that both biomass and leaf area were influenced significantly by the different AMF treatments. Plants inoculated with live AMF (pooled across all live treatments) were significantly larger than plants grown in sterile soil (Table 1, Fig. 2A). When averaged across plant species, both total biomass and leaf area were significantly larger in *Apeiba*-modified AMF communities (Table 1, Fig. 2A). However, the magnitude of this effect was greater in *Apeiba* as suggested by the significant "host × modified AMF communities" contrast interaction for leaf area and near significant contrast interaction for total biomass (Table 1). Indeed, *Apeiba* seedlings were significantly larger in AMF communities previously modified by conspecific seedlings than in communities previously modified by *Luehea* ($t = -2.07$, $P = 0.005$; Fig. 2A), thereby providing evidence for positive feedback. However, total biomass of *Luehea* did not differ significantly between the two modified AMF communities ($t = -0.46$, $P = 0.628$; Fig. 2A). Likewise, leaf area of *Luehea* did not differ between modified AMF communities, whereas leaf area of *Apeiba* was significantly greater in *Apeiba*-modified AMF communities (Table 1, Fig. 3). We found no difference in biomass for either host species when seedlings were grown in sterilized *Apeiba*-modified communities and in sterilized *Luehea*-modified communities (AMF, $F_{1,23} = 0.86$, $P = 0.363$; Host, $F_{1,23} = 0.07$, $P = 0.799$; AMF × Host, $F_{1,23} = 0.17$, $P = 0.687$; Fig. 2A).

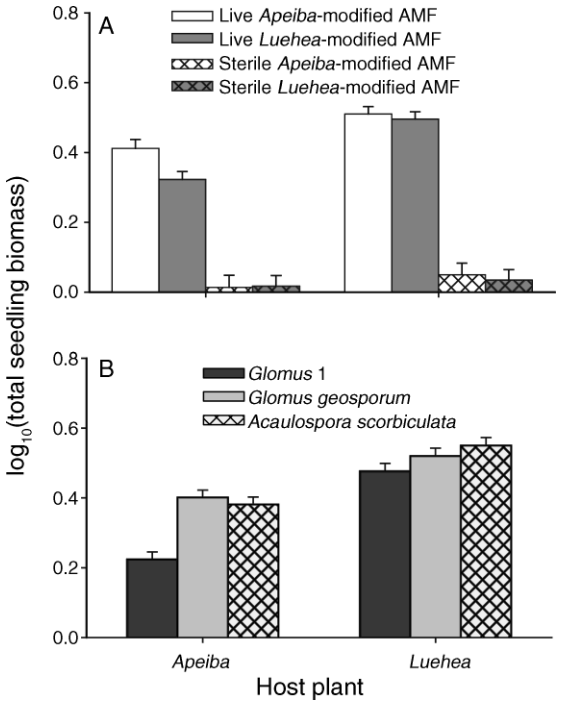

FIG. 2. (A) Total biomass of the trees *Apeiba aspera* and *Luehea seemannii* when grown in soils containing live or sterile AMF communities previously modified by either *Apeiba* or *Luehea*. (B) Growth response of *Apeiba* and *Luehea* seedlings to the three most common AMF species in host-modified soils of the first experiment. Letters indicate significant Tukey-adjusted differences ($P < 0.05$) in $\log_{10}$-transformed biomass per host species. Error bars indicate standard errors.

This finding suggests that growth differences in *Apeiba* across the two modified-AMF communities were most likely due to changes in AMF communities and not due to changes in abiotic soil properties (e.g., nutrients).

When pooled across host species, both total biomass and leaf area differed significantly across the three AMF pure cultures (Table 1, Fig. 2B). However, growth response across the AMF species depended on host identity (i.e., significant "host × AMF single species" contrast interaction; Table 1). *Glomus geosporum* and *A. scorbiculata* promoted greatest growth in *Apeiba*, while seedlings of *Apeiba* were significantly smaller when grown with *G.* 1. Total biomass of *Luehea* did not differ across the three AMF pure cultures (Table 1, Fig. 2B).

All examined seedlings grown with live AMF were well colonized. Colonization ranged from 93.7% in *Apeiba* grown with *G. geosporum* to 52.6% in *Apeiba* grown with *A. scorbiculata*. Three of the 20 examined seedlings grown in sterile soil had low levels of colonization (~2%); the remaining seedlings showed no sign of AMF infection. No apparent root infection by pathogenic fungi was detected in any of the seedlings examined.

DISCUSSION

Our study provides three lines of evidence that interactions between tropical tree seedlings and AMF are species specific. First, when different species of seedlings were initially provided with identical AMF communities, the composition of the spore communities diverged as a function of the species of host plant when seedlings were grown under higher light. Second, *Apeiba* showed clear differences in growth across single-species AMF isolates. Third, soil containing AMF modified previously by *Apeiba* seedlings favored the growth of *Apeiba* seedlings of the next cohort, relative to soil containing AMF modified previously by *Luehea* seedlings. Combined, these findings suggest that tree seedlings-AMF interactions are likely to result in plant–soil feedbacks.

Influences of host and light on AMF spore communities

Species composition of AMF spore communities have been shown to be correlated with the species identity of adult trees near which the soils were sampled (Merryweather and Fitter 1998, Lovelock et al. 2003; S. A. Mangan and E. A. Herre, *unpublished manuscript*). However, these patterns could also be due to other soil factors related to host identity (e.g., nutrients, water availability, etc.). The present study is the first to demonstrate consistent *changes* in spore communities that are associated with seedlings representing different tropical tree species, when they are initially provided with identical AMF communities. This interpretation is consistent with molecular data showing that the composition of AMF communities colonizing roots of tropical tree seedlings depend on host identity (Husband et al. 2002). Combined, these studies suggest that host identity may be an important determinant of AMF community composition in both the roots and as spores

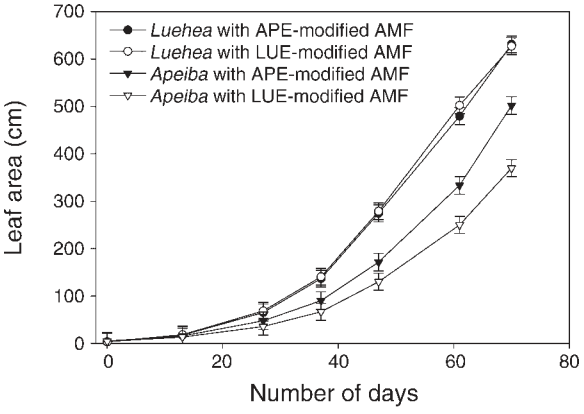

FIG. 3. Change in *Apeiba* and *Luehea* leaf area over the duration of the experiment when seedlings were grown with AMF communities previously modified by conspecific or heterospecific seedlings. Although actual leaf area is presented, repeated-measures ANOVA was conducted using $\log_{10}$-transformed leaf area. Error bars indicate standard errors.

PLATE 1. Seedlings of *Apeiba aspera* and *Luehea seemannii* shortly after planting them in pots containing different AMF communities of pure AMF cultures (second experiment). Photo credit: S. A. Mangan.

in the adjacent soils throughout the long life-span of trees.

In addition to a host effect, we observed a strong effect of light on AMF spore production. Of the six AMF species comprising the original AMF community, four of those species sporulated when their hosts grew under high light, with none of the *Glomus* species sporulating under low light. Reduction of AMF sporulation under low light was most likely due to lower availability of host carbon to AMF (Ferguson and Menge 1982). Aboveground biomass of each plant species was greatly reduced under low light (ranging from 4% of high-light biomass for *Apeiba* to 13% of high-light biomass for *Virola*; data not shown). Similar reduction in other fungal activity such as AMF root colonization under low light has been reported previously in tropical tree seedlings (Whitbeck 2001, Gehring 2003).

Further, we found that hosts differentially modified their associated AMF spore communities when grown under higher light. This interaction between host and light level on AMF spore composition was driven largely by change in relative abundances of spores of *Glomus* 1 and *A. scorbiculata* across the four host plants, coupled with the absence of *G. geosporum* spores when associated with *Apeiba* and *Virola* seedlings. These changes did not appear to be correlated with plant relatedness, life history, or root morphology. The similarity in spore communities between *Apeiba* and *Virola* was high despite both species differing distinctly in shade tolerance, seed size, and root architecture (*Apeiba* produced abundant fine roots, whereas *Virola* produced coarser roots). In contrast, AMF composition differed strikingly between *Apeiba* and *Luehea* despite both species being small seeded, shade intolerant, and having similar root morphologies (both belong to the Tiliaceae).

Some aspects of AMF life history, however, did correlate with patterns of fungal sporulation. No spores of *G. fasciculatum* or *G. artruva* were produced under any of our host or light treatments. Spores of both species are formed in large clusters (sporocarps) surrounded by loose masses of fungal hypha, which are dispersed frequently by small mammals (Mangan and Adler 2002). Interestingly, both *G. fasciculatum* and *G. artruva* can produce abundant spores in pure culture grown for a similar time period and under light levels similar to the high-light level of this experiment (S. A. Mangan, *personal observation*). The lack of sporulation of these AMF species when grown in mixed-species communities suggests a higher cost for the production of

sporocarps, and that these sporocarpic AMF species were outcompeted by the other species. Similar competition–dispersal trade-offs are well documented in other taxa (Coomes and Grubb 2003). Further studies that examine larger arrays of plant and fungal species are required to better understand the influence of the life histories of both taxa on the functional specificity of tropical tree–AMF interactions.

Influence of host-modified AMF communities on seedling performance

Differences in seedling growth across the two host-modified AMF communities are expected to occur if hosts exhibit differing sensitivities to individual AMF species. We found no differences in growth in *Luehea*, either when grown separately with the three AMF strains or when grown in the two modified AMF communities. In contrast, we found that *Apeiba* did differ between the two host-modified AMF communities. *Apeiba* also exhibited differing sensitivities to the three pure strains tested that comprised the original AMF community. Increased growth of *Apeiba* associated with AMF communities modified previously by an earlier cohort of *Apeiba* is consistent with AMF-mediated positive feedback. No evidence for AMF-mediated feedback was observed in *Luehea* because of its lack of differential response to individual community members.

We found no evidence that measured growth responses were due to factors other than AMF. We detected no differences in seedling growth across pots containing sterilized *Luehea* and *Apeiba* inoculum. This observation confirmed that it was not abiotic properities of these inocula that drove the observed growth differences. Although it is possible that soil organisms other than AMF contributed to the observed growth differences, microscopic examination of roots of both *Apeiba* and *Luehea* showed little infection by non-AMF fungi, whereas percent colonization of AMF in all live-inocula treatments was notably high. Moreover, if pathogenic fungi (which may not show obvious signs of root infection) were contaminants in our study, then we would have expected the direction of feedback to be negative (see Mills and Bever 1998, Klironomos 2002), and not positive as observed in our study with *Apeiba*.

Our study provides evidence that *Apeiba* seedlings changed their AMF community in a manner that promoted increased seedling growth of the next cohort of *Apeiba*. However, we were unable to determine the identity of the AMF species that was most important to this increase. In a previous study, Bever (2002*a*) demonstrated that two short-lived, herbaceous hosts from a temperate grassland community each modified that AMF community in a manner that favored subsequent individuals of the other host species (i.e., AMF-mediated negative feedback). Functionally, AMF-mediated negative feedback observed in that experiment occurred because the fungal species that had the highest growth rates when associated with a particular host species, in turn, provided that host species with the least growth benefit (Bever 2002*a*). In contrast, the direction of feedback in our study was positive. Therefore, we expected that AMF species with the highest growth rates when associated with *Apeiba* would also provide *Apeiba* with the greatest growth advantage. However, we found that spores of *G*. 1 were most abundant in *Apeiba*-modified AMF communities, whereas seedling growth of *Apeiba* was the lowest when grown in association with only *G*. 1. Further, *G. geosporum* was absent as spores in *Apeiba*-modified AMF communities, but pure cultures of *G. geosporum* promoted the greatest growth of *Apeiba* seedlings. Our observation that the spore abundance of the different AMF species in the modified soils did not correspond to their benefit to the host when grown in pure culture suggests an inability to estimate fungal abundance in roots based solely on spore production (Clapp et al. 1995, Sanders 2004, but see Bever 2002*b*). The development of molecular techniques that allow for accurate assessment of AMF relative abundance (and not just presence or absence) is needed to fully understand the relationship between spore abundance and fungal density in roots.

Scale and ecological implications

Previous studies that have investigated the importance of soil-borne organisms in forested systems have largely examined interactions between adult trees and their juvenile seedlings. Such studies have generally found that seedling survival and growth are reduced in soil collected near conspecific adults due to an accumulation of soil-borne fungal pathogens (Augspurger 1984, Packer and Clay 2000, Hood et al. 2004, Bell et al. 2006). Differences in seedling performance when grown in soils near and far from a conspecific adult tree may be of no surprise considering the large amount of time in which fungal pathogens are allowed to accumulate. In contrast, Packer and Clay (2003) provided evidence that fungal pathogens accumulate quickly in soils under young seedlings, with such accumulation inhibiting growth of the next generation of conspecific seedlings. However, our study is the first to demonstrate that AMF communities associated with different species of tree seedlings also changed quickly, and enhanced growth of conspecific *Apeiba* seedlings was mostly likely due to overall effects caused by this change. Therefore, AMF-mediated plant–soil feedback generated solely via seedling–seedling interactions has the potential to influence the dynamics of tree seedling communities. Field-based studies are required that experimentally address the relative importance of both soil-borne pathogens and AMF in shaping the composition of seedling communities.

Studies to date have largely provided evidence consistent with AMF-mediated negative feedback (Kiers et al. 2000, Bever 2002*a*, Castelli and Casper 2003), and

not positive feedback as we show here. Of particular interest is whether the AMF communities associated with seedlings change over time as they mature into reproductive adults (see Husband et al. 2002). If so, then the direction of AMF-mediated feedback generated via seedling–seedling interactions may be different than that of feedback generated via seedling–adult interactions. Unlike short-lived hosts, trees undergo physiological changes that span many decades. For example, trees not only remain nonreproductive for long periods of time, but they are faced with vastly different light regimes as they grow towards the canopy. Prolonged physiological changes may set the stage for succession of associated AMF communities within an individual tree. Therefore, the suite of AMF that is most beneficial at the seedling stage may not be the same suite that is most beneficial to adults (Kiers et al. 2000, Herre et al. 2005). In our study, positive feedback was detected among *Apeiba* seedlings of closely aged cohorts that were undoubtedly physiologically similar. In contrast, microorganisms associated with roots of adult trees at the study site inhibited growth of conspecific seedlings relative to those of heterospecific seedlings (Kiers et al. 2000), thereby suggesting negative feedback. If this pattern was indeed driven by AMF, as suggested by Kiers et al. (2000), then it appears that the direction of AMF-mediated feedback may indeed shift from positive to negative with maturation of the focal tree involved in the modification of AMF communities.

In conclusion, AMF community composition is increasingly being recognized as an important determinant for temperate plant communities. This is the first study to experimentally examine the complete cycle of plant–soil feedback mediated by AMF in a forested ecosystem. These findings emphasize the potential importance of such processes to the ecology of both tree and AMF communities.

Acknowledgments

We are indebted to Dora Alvaréz, Camila Pizano, Charlotte Jander, Andrea Vincent, and Robert Horan for their assistance. We thank two anonymous reviewers for improving this manuscript. This work would not have been possible without the logistical support and funding provided by the Smithsonian Tropical Research Institute (STRI predoctoral fellowship to S. A. Mangan and a soil initiative grant to E. A. Herre). The National Science Foundation provided grant DIGG-0308779 to S. A. Mangan and James D. Bever, and grants DEB-0049080 and DEB-0616891 to James D. Bever. Funding was also provided by Indiana University, Sigma Xi, and the American Mammalogy Society to S. A. Mangan.

Literature Cited

Augspurger, C. K. 1984. Seedling survival of tropical tree species: interactions of dispersal distance, light-gaps, and pathogens. Ecology 65:1705–1712.

Bell, T., R. P. Freckleton, and O. T. Lewis. 2006. Plant pathogens drive density-dependent seedling mortality in a tropical tree. Ecology Letters 9:569–574.

Bever, J. D. 1994. Feedback between plants and their soil communities in an old-field community. Ecology 75:1965–1977.

Bever, J. D. 1999. Dynamics within mutualism and the maintenance of diversity: inference from a model of interguild frequency dependence. Ecology Letters 2:52–62.

Bever, J. D. 2002*a*. Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. Proceedings of the Royal Society B 269:2595–2601.

Bever, J. D. 2002*b*. Host-specificity of AM fungal population growth rates can generate feedback on plant growth. Plant and Soil 244:281–290.

Bever, J. D., J. B. Morton, J. Antonovics, and P. A. Schultz. 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. Journal of Ecology 84:71–82.

Castelli, J. P., and B. B. Casper. 2003. Intraspecific AM fungal variation contributes to plant–fungal feedback in a serpentine grassland. Ecology 84:323–336.

Clapp, J. P., J. P. W. Young, J. W. Merryweather, and A. H. Fitter. 1995. Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. New Phytologist 130:259–265.

Connell, J. H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. Pages 298–312 *in* P. J. den Boer and G. R. Gradwell, editors. Dynamics of poplations. Center for Agricultural Publishing and Documentation, Wageningen, The Netherlands.

Connell, J. H., I. Debski, C. A. Gehring, L. Goldwasser, P. T. Green, K. E. Harms, P. Juniper, and T. C. Theimer. 2005. Dynamics of seedling recruitment in an Australian tropical rainforest. Pages 486–506 *in* E. Bermingham, C. W. Dick, and C. Moritz, editors. Tropical rainforests past, present, and future. University of Chicago Press, Chicago, Illinois, USA.

Coomes, D. A., and P. J. Grubb. 2003. Colonization, tolerance, competition and seed-size variation within functional groups. Trends in Ecology and Evolution 18:283–291.

Daniels, B. A., and H. A. Skipper. 1982. Methods for the recovery and quantitative estimation of propagules from soil. Pages 29–35 *in* N. C. Schenck, editor. Methods and principles of mycorrhizal research. American Phytopathological Society, St. Paul, Minnesota, USA.

Eom, A. H., D. C. Hartnett, and G. W. T. Wilson. 2000. Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. Oecologia 122:435–444.

Ferguson, J. J., and J. A. Menge. 1982. The influence of light intensity and artifically extended photoperiod upon infection and sporulation of *Glomus fasciculatus* on Sudan grass and on root exudation of Sudan grass. New Phytologist 92:183–191.

Gehring, C. A. 2003. Growth responses to arbuscular mycorrhizae by rain forest seedlings vary with light intensity and tree species. Plant Ecology 167:127–139.

Harms, K. E., S. J. Wright, O. Calderón, A. Hernández, and A. E. Herre. 2000. Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. Nature 404: 493–495.

Herre, E. A., D. Kyllo, S. A. Mangan, R. Husband, L. C. Mejia, and A. H. Eom. 2005. An overview of arbuscular mycorrhizal fungi composition, distribution, and host effects from a tropical moist forest. Pages 204–225 *in* D. F. R. P. Burslem, M. A. Pinard, and S. E. Hartley, editors. Biotic interactions in the tropics: their role in the maintenance of species diversity. Cambridge University Press, Cambridge, UK.

Hood, L. A., M. D. Swaine, and P. A. Mason. 2004. The influence of spatial patterns of damping-off disease and arbuscular mycorrhizal colonization on tree seedling establishment in Ghanaian tropical forest soil. Journal of Ecology 92:816–823.

Husband, R., E. A. Herre, S. L. Turner, R. Gallery, and J. P. W. Young. 2002. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time

and space in a tropical forest. Molecular Ecology 11:2669–2678.

Kardol, P., N. J. Cornips, M. M. L. van Kempen, J. M. T. Bakx-Schotman, and W. H. van der Putten. 2007. Microbe-mediated plant–soil feedback causes historical contingency effects in plant community assembly. Ecological Monographs 77:147–162.

Kiers, E. T., C. E. Lovelock, E. L. Krueger, and E. A. Herre. 2000. Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. Ecology Letters 3: 106–113.

Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67–70.

Klironomos, J. N. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301.

Lovelock, C. E., K. Andersen, and J. B. Morton. 2003. Arbuscular mycorrhizal communities in tropical forests are affected by host tree species and environment. Oecologia 135: 268–279.

Mangan, S. A., and G. H. Adler. 2002. Seasonal dispersal of arbuscular mycorrhizal fungi by spiny rats in a neotropical forest. Oecologia 131:587–597.

Mangan, S. A., A. H. Eom, G. H. Adler, J. B. Yavitt, and E. A. Herre. 2004. Diversity of arbuscular mycorrhizal fungi across a fragmented forest in Panama: insular spore communities differ from mainland communities. Oecologia 141:687–700.

Merryweather, J., and A. Fitter. 1998. The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta*. II. Seasonal and spatial patterns of fungal populuations. New Phytologist 138:131–142.

Mills, K. M., and J. D. Bever. 1998. Maintenance of diversity within plant communities: soil pathogens as agents of feedback. Ecology 79:1595–1601.

Packer, A., and K. Clay. 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. Nature 404: 278–281.

Packer, A., and K. Clay. 2003. Development of negative feedback during successive growth cycles of black cherry. Proceedings of the Royal Society B 271:317–324.

Petermann, J. S., A. J. F. Fergus, L. A. Turnbull, and B. Schmid. 2008. Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. Ecology 89:2399–2406.

Peters, H. A. 2003. Neighbour-regulated mortality: the influence of positive and negative density dependence on tree populations in specie-rich tropical forests. Ecology Letters 6: 757–765.

Picone, C. 2000. Diversity and abundance of arbuscular-mycorrhizal fungus spores in tropical forest and pasture. Biotropica 32:734–750.

Reynolds, H. L., A. Packer, J. D. Bever, and K. Clay. 2003. Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. Ecology 84: 2281–2291.

Sanders, I. R. 2004. Plant and arbuscular mycorrhizal fungal diversity—are we looking at the relevant levels of diversity and are we using the right techniques? New Phytologist 164: 415–418.

SAS Institute. 1996. The SAS system for Windows, Version 6.11. SAS Institute, Cary, North Carolina, USA.

Siqueira, J. O., M. A. C. Carneiro, N. Curi, S. C. da Silva Rosado, and A. C. Davide. 1998. Mycorrhizal colonization and mycotrophic growth of native woody species as related to successional groups in Southeastern Brazil. Forest Ecology and Management 107:241–252.

van der Heijden, M. G. A., T. Boller, A. Wiemken, and I. R. Sanders. 1998. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79:2082–2091.

Van der Putten, W. H., L. E. M. Vet, J. A. Harvey, and F. L. Wäckers. 2001. Linking above- and belowground multi-trophic interactions of plants, herbivores, pathogens, and their antagonists. Trends in Ecology and Evolution 16:547–554.

Whitbeck, J. L. 2001. Effects of light environment on vesicular-arbuscular mycorrhiza development in *Inga leiocalycina*, a tropical wet forest tree. Biotropica 33:303–311.

Wills, C., and R. Condit. 1999. Similar non-random processes maintain diversity in two tropical rainforests. Proceedings of the Royal Society B 266:1445–1452.

Windsor, D. M. 1990. Climate and moisture variability in a tropical forest: long-term records from Barro Colorado Island, Panama. Smithsonian Contributions to the Earth Sciences 29:1–14.

Wolfe, B. E., and J. N. Klironomos. 2005. Breaking new ground: soil communities and exotic plant invasion. BioScience 55:477–487.

Zangaro, W., S. M. A. Nisizaki, J. C. B. Domingos, and E. M. Nakano. 2003. Mycorrhizal response and successional status in 80 woody species from south Brazil. Journal of Tropical Ecology 19:315–324.