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 nesiota, 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-modified 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
and positive plant-soil feedback (Bever 2002a, 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 2002a, 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 2002a, 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 scrobiculata*, *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. arthusu*, and *G. l* all produce their spores in clusters surrounded by copious amounts of fungal mycelia and are commonly dispersed by rodents (Mangan and Adler 2002). *Glomus arthusu* 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. arthusu*, *G. fasciculatum*, and *G. l* 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 pyramidal* (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. pyramidal* 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 autoclave soil. Six-week old seedlings of *Eugenia* and *Virola* and three-week old seedlings of *Lueheae* and *Apeiba* were transplanted into 3.7-L pots containing a 3:1 steam-pasteurized 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 5-g subsample of soil using sucrose flotation (Daniels and Skipper 1982). Isolated spores were then identified and quantified.

**Do host-mediated 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 *Lueheae* 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 *Lueheae* 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 *Lueheae* inoculum). One-half of the pots containing each inoculum type received a single three-week-old *Lueheae* 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 *Lueheae* 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 *Lueheae* 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. l*, *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 *Lueheae* 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 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...
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 × AMF” contrast interaction for total biomass and 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).

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 semmannii, on Barro Colorado Island, Panama.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Total biomass (error df = 135)</th>
<th>Leaf area (error df = 133)</th>
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<tr>
<td></td>
<td></td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Host</td>
<td>1</td>
<td>2.68</td>
<td>0.104</td>
</tr>
<tr>
<td>AMF</td>
<td>6</td>
<td>102.57</td>
<td>&lt;0.001</td>
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<tr>
<td>Live AMF vs. sterile</td>
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<td>555.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Modified AMF communities</td>
<td>1</td>
<td>5.80</td>
<td>0.017</td>
</tr>
<tr>
<td>AMF single species</td>
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<td>18.91</td>
<td>&lt;0.001</td>
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<td>&lt;0.001</td>
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<tr>
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<td>Host × AMF single species</td>
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<tr>
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<tr>
<td>Initial leaf area × host</td>
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<td>8.52</td>
<td>0.004</td>
</tr>
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</table>

Notes: ANCOVA was used to examine log_{10}-transformed 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.
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 *Acaulospora 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.
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...
and not positive as observed in our study with negative (see Mills and Bever 1998, Klironomos 2002), would have expected the direction of feedback to be treatments was notably high. Moreover, if pathogenic whereas percent colonization of AMF in all live-inocula Luehea experiment occurred because the fungal species that AMF-mediated negative feedback observed in that AMF-mediated positive feedback. No evidence for AMF-mediated feedback was observed in Luehea because of this increase. In a previous study, Bever (2002 identity of the AMF species that was most important to subsequent individuals of the other host species (i.e., 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 properties 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 (2002a) 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 2002a). 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 2002b). 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 (Augsburger 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 2002a, 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–adult interactions may be different than that of feedback generated via seedling–seedling 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.

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