Arbuscular mycorrhizal fungi counteract the Janzen-Connell effect of soil pathogens

Minxia Liang,[1] Xubing Liu,[1,2] Rampal S. Etienne,[2] Fengmin Huang,[1] Yongfan Wang,[1] and Shixiao Yu[1,3]

[1]*Department of Ecology, School of Life Sciences/State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275 China*
[2]*Community and Conservation Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 11103, 9700 CC Groningen, The Netherlands*

Abstract. Soilborne pathogens can contribute to diversity maintenance in tree communities through the Janzen-Connell effect, whereby the pathogenic reduction of seedling performance attenuates with distance from conspecifics. By contrast, arbuscular mycorrhizal fungi (AMF) have been reported to promote seedling performance; however, it is unknown whether this is also distance dependent. Here, we investigate the distance dependence of seedling performance in the presence of both pathogens and AMF. In a subtropical forest in south China, we conducted a four-year field census of four species with relatively large phylogenetic distances and found no distance-dependent mortality for newly germinated seedlings. By experimentally separating the effects of AMF and pathogens on seedling performance of six subtropical tree species in a shade house, we found that soil pathogens significantly inhibited seedling survival and growth while AMF largely promoted seedling growth, and these effects were host specific and declined with increasing conspecific distance. Together, our field and experimental results suggest that AMF can neutralize the negative effect of pathogens and that the Janzen-Connell effect may play a less prominent role in explaining diversity of nondominant tree species than previously thought.

Key words: arbuscular mycorrhizal fungi; distance dependence; host specificity; pathogens; seedling survival; subtropical forest.

INTRODUCTION

Understanding the extraordinarily high diversity in the tropics and subtropics is one of the most challenging questions for ecologists. There is accumulating empirical evidence in plant community ecology suggesting that plant–soil feedbacks are primary determinants of plant species coexistence (reviewed by Bever et al. 2012). Soil microbes have large impacts on plant survival, population dynamics, and species distributions (Klironomos 2002, Schnitzer et al. 2011). Some microbes have a negative influence on plants, e.g., pathogens causing negative density- and/or distance-dependent effects (e.g., Augspurger 1984, Packer and Clay 2000, Bell et al. 2006, Bagchi et al. 2010, Liu et al. 2012). Others have a positive influence, e.g., microbial mutualists that may assist in uptake of soil nutrients or defense against herbivores (Read and Perez-Moreno 2003, Gange et al. 2005, van der Heijden et al. 2008). A complete understanding of the maintenance of plant diversity and community structure requires integrating microbial perspectives and ac-

counting for both positive and negative plant–soil interactions.

Soilborne pathogens have been found to play a dominant role in the Janzen-Connell effect (Augspurger 1984, Packer and Clay 2000, Bell et al. 2006, Mangan et al. 2010, Bagchi et al. 2014), which states that host-specific pathogens present in the soil around parent trees lead to higher disease-induced mortality for conspecific offspring; the negative effect of soil pathogens on seedling survival increases with increasing density of conspecific adults and seedlings (density-dependent effect) and decreases with increasing distance to conspecific adult trees (distance-dependent effect).

While there is clear evidence for a pathogen-mediated Janzen-Connell mechanism, most studies have overlooked (Packer and Clay 2000, Bell et al. 2006, Bagchi et al. 2010, Liu et al. 2012) or excluded (McCarthy-Neumann and Kobe 2008) the effects of arbuscular mycorrhizal fungi (AMF). AMF (Glomeromycota) are symbionts that infect the roots of most terrestrial plants and enhance host resource uptake in exchange for photosynthate (Smith and Read 1997). The principal role of AMF is thought to be in the facilitation of phosphorus (P) uptake by plants, especially in strongly P-limited soils as found, for example, in the tropics (Howeler et al. 1982). There is

ample evidence that AMF also have an important influence on the plant–pathogen interaction and are capable of reducing pathogenic infection (Smith 1988, Newsham et al. 1995, Borowicz 2001, Klironomos 2002, Hood et al. 2004, Herre et al. 2007). Some studies have found evidence for the host-specificity of AMF (Eom et al. 2000, Bever 2002, Bidartondo et al. 2002), suggesting that the beneficial effects of AMF should also decline with distance from a parent tree. However, almost all research on the effects of AMF on plant–pathogen relations has been performed on grass species (Newsham et al. 1995, Klironomos 2002, Schnitzer et al. 2011), economically important agriculture species (e.g., Evans et al. 2003), or a single tree species (Hood et al. 2004). Research on a broader range of species and more complex ecosystems is required (Hart et al. 2003), especially in tropical and subtropical forests, as survey work suggests that AMF community diversity is higher in these forests than in temperate grasslands or woodlands (Herre et al. 2005).

Although the density-dependent prediction of the Janzen-Connell hypothesis has been widely confirmed (e.g., Bell et al. 2006, Bagchi et al. 2010), multispecies analyses on distance dependence have yielded mixed evidence (e.g., Augspurger 1984, Condit et al. 1992, Blundell and Peart 1998, Queenborough et al. 2007). Indeed, a meta-analysis based on 75 unique species from at least 35 plant families found no general support for distance dependence (Hyatt et al. 2003), while a recent meta-analysis of experimental studies found significant support for both density- and distance-dependent effects (Comita et al. 2014). Because distance dependence is theoretically reasonable and has been detected by some other studies (e.g., Augspurger 1983, Packer and Clay 2000), and the observed spatial diversity pattern explained by the Janzen-Connell effect can potentially result from distance dependence but not from density dependence (Terborgh 2012), it is necessary to investigate factors that may obscure the distance dependence. In this study, we investigated whether the "negative" distance dependence driven by soil pathogens commonly exists in the wild, but might sometimes be neutralized by AMF that cause "positive" distance dependence.

We first conducted a four-year field census with two gravity-dispersed tree species and two wind-dispersed species in a subtropical forest, to determine whether newly germinated seedlings suffered distance-dependent mortality (i.e., decrease in mortality with distance from the conspecific tree). We then conducted two shade-house experiments with seedlings of six tree species, by manipulating soil microbes with only AMF, only pathogens, field soil (AMF + pathogens), and sterile soil treatments, to test the following hypotheses: (1) pathogens reduce seedling performance, and this detrimental effect decreases with increasing conspecific distance; (2) AMF promote

seedling performance, and this beneficial effect decreases with increasing conspecific distance; (3) the microbes cultured by adult trees have higher colonization or infection rates on roots in soil at closer conspecific distance; and (4) both pathogens and AMF are host specific and have no effect on seedlings of other species. We tested hypothesis 1 to confirm the distance-dependent effect of soil pathogens, which has been widely tested before but yielded mixed evidence. We proposed and tested the other hypotheses, in order to investigate the previously ignored role of AMF on distance dependence in the Janzen-Connell hypothesis and provide a more comprehensive analysis of plant–soil feedbacks.

Materials and Methods

Study site

The study site, Heishiding Nature Reserve (23°27′ N, 111°53′ E, 150–930 m altitude), is a 4200-ha protected area with the Tropic of Cancer running through its center, in Guangdong Province, south China. This region is covered by subtropical evergreen broad-leaved forests with a subtropical moist monsoon climate, receiving 1743.8 mm of rain annually with 79% of precipitation occurring between April and September. The mean annual temperature is 19.6°C and the mean monthly temperature ranges from 10.6°C in January to 28.4°C in July.

Field surveys

To detect distance dependence in natural communities and investigate the relationship between seedling survival and conspecific distance, we established 24 1.5 × 20 m transects at the field site and monitored each individual within them from 2009 to 2012. We chose four species that were not too closely related to reduce any phylogenetic correlation (Liu et al. 2012): *Cryptocarya concinna* Hance (Lauraceae), *Ormosia pachycarpa* Champ. ex Benth (Fabaceae), *Engelhardia fenzelii* Merr. (Juglandaceae, see Plate 1), and *Schima superba* Gardn. et Champ (Theaceae). These species are common but not dominant in the study area, allowing us to collect enough seeds for the experiment while providing sufficient distances between conspecific trees to test for distance dependence. Also, seeds of *C. concinna* and *O. pachycarpa* are gravity-dispersed, while *E. fenzelii* and *S. superba* are dispersed by wind. The wide range of traits of these selected species allows a general view on the plant–soil feedbacks. In October 2009, for each species, we randomly selected three focal trees at least 60 m away from the nearest reproductive conspecific, and laid out two transects in random directions radiating out from the focal tree, which was put in the middle of the 1.5-m axis. If the topography only allowed one transect, we selected more focal trees, until we had six transects for each species. We tagged all individuals within each transect and recorded species names, heights, diame-

ters at breast height (dbh; when ≥1 cm), and positions relative to the focal trees. We repeated the census twice every year in April and October, respectively, as most of the seedlings germinate during April (beginning of the wet season) and die before October (beginning of the dry season). A total of 9024 individuals from 164 species were recorded during the seven censuses.

Shade-house experiments

We conducted two shade-house experiments using newly germinated seedlings to evaluate the effect of AMF and pathogens on seedling survival and growth. We selected six common species for the shade-house experiments, including the four species mentioned in the previous section and two others: Ormosia glaberrima Y. C. Wu (Fabaceae; note that O. glaberrima and O. pachycarpa are congenerics and hence closely related) and Canavium album Raeuseh (Burseraceae). For the four species for which we conducted field surveys, we could only obtain seeds for O. pachycarpa and E. fenzelii but not for C. concinna and S. superba in 2010, so we chose two more species (O. glaberrima and C. album) that had sufficient seeds in 2010 for the shade-house experiments. We collected enough seeds for C. concinna and S. superba in 2011, so we added these two species in the experiments in the same shade house at 15% full sunlight. We collected seeds or fruits during autumn and winter throughout the study site. Seeds were surface sterilized (1 min 70% ethanol, 3 min 2.625% NaOCl, 1 min 70% ethanol, and 1 min distilled water) and kept at 4°C until March of the next year. Seeds were then left to germinate in plastic boxes filled with wet sterilized sand. We obtained sufficient newly germinated seedlings for all six species after 6–8 weeks.

We transplanted seedlings (1–2 weeks old) into pots containing sterilized field soil, where the field soil was collected from a common understory at the study site, thoroughly mixed, and sterilized with a dose of 25 kGy of gamma-radiation (McNamara et al. 2003). For each species, we randomly selected and transplanted seedlings into plastic pots (8 cm diameter × 10 cm height) that were filled with sterilized field soil. One week after the transplantation, we removed seedlings that were dead or growing poorly due to injuries during the transfer and replaced them with new ones. We conducted two experiments, in both of which we treated seedlings with one of the four microbial fractions: (1) AMF, (2) pathogens, (3) field soil (AMF + pathogens), and (4) sterile soil.

Experiment 1, host specificity of AMF and pathogens.—We conducted experiment 1 to test host specificity of AMF and pathogens by treating seedlings of each of the six target species with the AMF or pathogen fractions from soil beneath either conspecific or heterospecific adult trees. We transplanted seedlings of each of the six target species into pots containing sterilized field soil, and treated them with the four microbial fractions (AMF, pathogens, field soil, and sterilized soil) extracted from soil beneath either conspecific or heterospecific adult trees with a full reciprocal design ($n = 6$ seedling species × 6 soil sources × 4 microbial fractions × 20 replicates = 2880). For this purpose, we collected six soil samples at a distance of 0–2 m from each adult tree, thoroughly mixed the soil samples collected from three different trees for each species, and divided each soil sample into three 50-g subsamples. All subsamples were passed through a 250-μm sieve into three 100-mL suspensions separately before being inoculated, to eliminate larger organisms. Then we used the first suspension to extract (1) AMF and (2) soil pathogen communities using a wet-sieving method (Klironomos 2002). We collected AMF spores on a 45-μm sieve and surface sterilized them with 10% sodium hypochlorite to remove the pathogens; pathogens and other non-mycorrhizal microbes were collected from filtrate that passed through a 20-μm sieve. We used the second suspension as (3) field soil without any manipulation, to provide a best representative of the composition and abundance of AMF and pathogen communities in natural soil. We sterilized the third soil suspension by gamma radiation, and used it as (4) sterile soil to control for abiotic effects (see Appendix A for the detailed inoculating procedures). We inoculated each of our 2880 pots with 100 mL of suspension and covered it with a layer of 2 cm thick sterile soil.

The field soil treatment was the best representative of the microbial community in natural soil, but it may contain other organisms besides AMF and pathogens. To confirm that the effect of the field soil treatment was due to the interaction between AMF and pathogens, we conducted an additional experiment with an AMF + pathogen treatment (the addition of both AMF and pathogen fractions) and compared it to the field soil treatment (Appendix B). We found that the effect of the field soil treatment was indistinguishable from the AMF + pathogen treatment, strongly suggesting that the additive effect of the field soil treatment was indeed the interaction effect between AMF and pathogens, as in the AMF + pathogen treatment.

Experiment 2, distance-dependent effects of AMF and pathogens.—In this experiment, we aimed to separate the effects of AMF and pathogens extracted from soil at different conspecific distances (0, 5, 10, 15, and 20 m) on seedling survival and growth for each species. We randomly chose three of the field transects belonging to different adult trees for each of the six species as inoculum sources in early May. We collected soil samples at distances of 0, 5, 10, 15, and 20 m away from each adult tree, respectively. We thoroughly mixed the soil samples collected at the same distance from three different trees for each species, to make sure that we successfully collected the

target microbes and added exactly the same suspension to all replicates for each treatment, and divided each soil sample into three 50-g subsamples. We then used the same methods as in experiment 1 for extracting and inoculating soil microbes to arrive at the same four treatments (AMF soil, pathogen soil, field soil, and sterile soil), but now applied to soils at various parental distances.

There were 20–40 replicates for each treatment, depending on available seedling numbers. For each species, $n = 5$ distances $\times$ 4 microbial fractions $\times$ N replicates; $N = 25$ replicates for *C. concinna*, *O. pachycarpa* and *S. superba*, $N = 30$ for *E. fenzelii*, $N = 40$ for *O. glaberrima*, and $N = 20$ for *C. album*.

Seedlings were allowed to grow for 6 months for both experiments 1 and 2, and then we harvested and oven-dried them at 60°C for 48 h to determine the aboveground (shoots), belowground (roots), and total biomass. To examine the degrees of AMF colonization and pathogen infection on roots among treatments, we quantified the number of lesions per root for each treatment using the magnified intersection method (McGonigle et al. 1990). Mycorrhizal colonization was defined as typically aseptate hyphae, forming arbuscules and vesicles, and non-AMF infection defined as septate hyphae with frequent production of chlamydospores. Along with harvest at the end of the experiment, we randomly collected 50 root fragments of 1 cm length from each treatment, and washed them repeatedly with distilled water to remove any soil. Cleaned roots were stained with trypan blue (see the detailed staining procedures in Appendix C), and then each root segment was examined under a fluorescence stereomicroscope (SteREO Lumar.V12, Carl Zeiss, Germany) at 150× magnification to determine percentage of colonization by AMF (including hyphae, vesicles, and arbuscules) and infection rates by non-AMF (pathogenic, parasitic, and saprophytic fungi; McGonigle et al. 1990). For each treatment, the 50 root fragments were divided into five groups. We counted 200 intersections for each group, and the colonization or infection rate was calculated as the number of intersections where we observed AMF or non-AMF divided by total intersections. We also measured the contents of soil nutrients (total N, P, and K) from each distance, and confirmed that the wet-sieving suspensions did not influence the NPK contents of the soil among different distances (Appendix A: Table A1).

Data analysis

For the field survey data, to test whether the seedling survival was correlated with distance from the closest reproductive conspecific, we analyzed the first-half-year, first-year, and second-year survival of seedlings which were newly germinated during the censuses with generalized linear mixed-effects models (GLMM), in which we entered conspecific distances as the fixed factor and each seedling's recruitment year and location (transect and adult tree) as random variables, assuming a binomial error distribution and logit-link function.

For the first shade-house experiment where seedlings were treated with the four microbial fractions from soil beneath either conspecific or heterospecific adult trees, we calculated the log-transformed odds ratios comparing seedling survival in AMF, pathogens, and field soil pots vs. sterile soil pots, and the mean biomass for each treatment, respectively, to test how soil microbes of a given species affect seedling survival and growth of their own species or the other five species. Odds ratio is a measure of association which approximates how much more likely it is for the seedlings to survive among soil-biota-treated pots than among sterile soil pots. Log-transformed survival odds ratios that are significantly larger than 0 indicate positive feedbacks of soil microbes on seedling survival and negative values indicate negative feedbacks.

We analyzed seedling survival data for the second shade-house experiment using survival odds ratio regression models to determine AMF and pathogen feedbacks compared with sterile soil, and to test whether the survival odds ratio was significantly correlated with conspecific distance. A maximum likelihood approach is used in survival odds ratio regression, and the distribution of the log-transformed survival odds ratio is assumed to be normal (Jewell 2003, Liu et al. 2012). For each conspecific distance of each species, we calculated the log-transformed odds ratios comparing seedling survival in AMF, pathogens, and field soil pots vs. sterile soil pots, respectively. We analyzed the total biomass using analysis of variance (ANOVA) to compare seedling growth among the four soil microbial treatments for each soil–seedling combination. For each of the six species, we also fitted linear regression models with mean total biomass of surviving individuals as the dependent variable and conspecific distance as the independent variable (mean root and shoot biomass were also analyzed separately, see Appendix D). We used ANOVA to compare AMF percentage of colonization and non-mycorrhizal infection rates at different parental distances and soil microbial treatments for each species. All statistical analyses were performed using R 2.15.1 (R Development Core Team 2012).

RESULTS

Field surveys

In the field survey, the survival of seedlings that germinated during 2009–2012 was not significantly related to the distance to the closest reproductive conspecific for any of the four species (Table 1), indicating that the distance-dependent prediction of

TABLE 1. Logistic regression results between first-half-year, first-year, and second-year survival of newly germinated seedlings and conspecific distance for four subtropical tree species during 2009–2012 in the field survey in south China.

	First-half-year survival			First-year survival			Second-year survival		
Species	Estimate	z	P	Estimate	z	P	Estimate	z	P
Cryptocarya concinna	−0.036	−1.163	0.245	0.004	0.117	0.906	−0.014	−0.499	0.618
Ormosia pachycarpa	−0.076	−0.158	0.874	0.546	0.873	0.383	−1.235	−1.961	**0.049**
Engelhardia fenzelii	−0.034	−1.088	0.277	0.029	0.895	0.371	0.076	1.130	0.258
Schima superba	−0.045	−0.651	0.514	−0.092	−1.041	0.298	−2.631	−1.189	0.235

Notes: For each species, we analyzed individual conspecific seedling survival over the first half-year, the first year, and the second year using a generalized linear mixed-effects model (GLMM), in which we entered conspecific distance as the fixed factor, and each seedling's recruitment year and location (transect) as random variables, assuming a binomial error distribution and logit-link function. Bold text indicates significance at $P < 0.05$.

the Janzen-Connell hypothesis was not supported in the natural communities.

Experiment 1, host specificity of AMF and pathogens

In experiment 1, feedback effects of soil biota on seedling survival (Fig. 1) and growth (Fig. 2; Appendix D: Fig. D1) were demonstrated to be host specific. For each one of the six species, both seedling survival and growth were significantly limited when seedlings were treated with pathogens extracted from soils beneath conspecific trees compared to those in sterile soils, but there were no inhibition effects compared to sterile
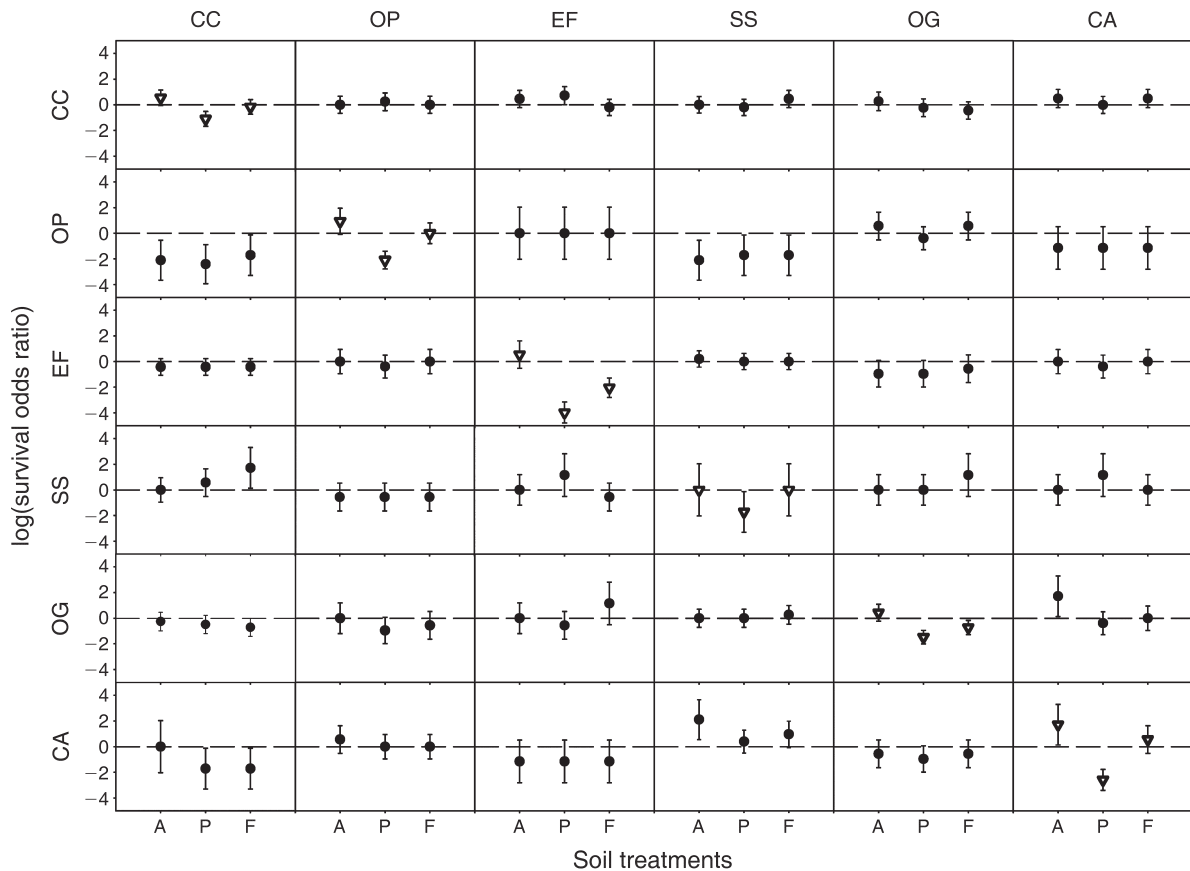

FIG. 1. Host-specific feedback effects of soil biota on seedling survival. The survival of six tree species in arbuscular mycorrhizal fungi (A), pathogen (P), and field soil (F) fractions obtained beneath adult trees of their own species (hollow triangles) or beneath the other five species (solid circles), compared to sterile soils. Rows indicate species of seedling tested, columns indicate species beneath which soils were collected; species studied were *Cryptocarya concinna* (CC), *Ormosia pachycarpa* (OP), *Engelhardia fenzelii* (EF), *Schima superba* (SS), *Ormosia glaberrima* (OG), and *Canavium ablum* (CA). Odds ratio is a measure of association which approximates how much more likely it is for the seedlings to survive among soil biota treated pots than among sterile soil pots. Log-transformed survival odds ratios above 0 indicate positive effects and ratios less than 0 indicate negative effects. Error bars represent standard errors.
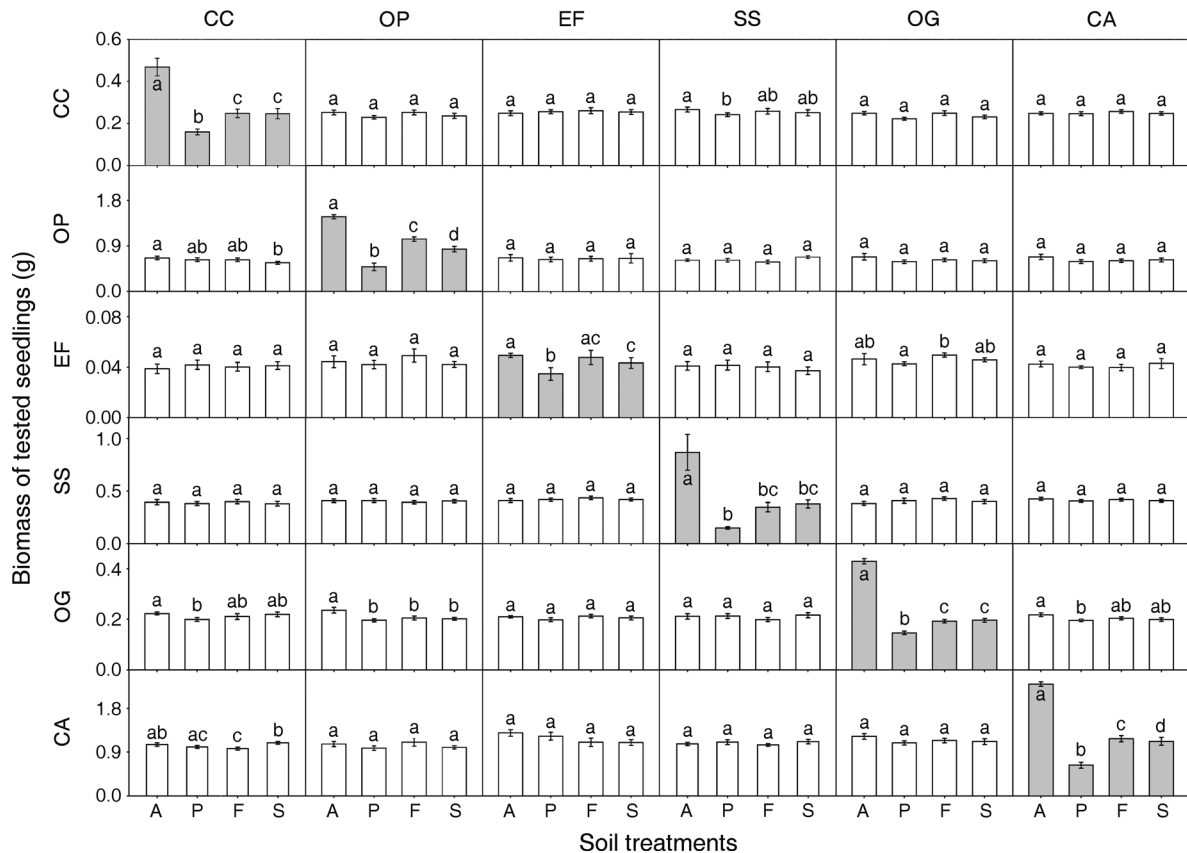
Fig. 2. Host-specific feedback effects of soil biota on seedling growth. The growth responses of six tree species to arbuscular mycorrhizal fungi (AMF), pathogens, field soil, and sterile soil (S) fractions obtained beneath adult trees of their own species (gray bars) or beneath the other five species (white bars). Bars represent the mean total biomass ± SE. Different lowercase letters represent significant differences among treatments ($P < 0.05$). Row and column arrangement, abbreviations are as in Fig. 1.

soils when seedlings were treated with pathogens from soil beneath the five heterospecifics (Figs. 1 and 2). A beneficial effect of AMF (a weak effect on seedling survival [Fig. 1] but a significant effect on growth [Fig. 2]) was detected when seedlings and AMF fractions were collected from the same species. There were a few exceptions where heterospecifics also had inhibition or beneficial effects on seedling survival, but these effects did not have a consistent trend among different species, while all conspecific soil and seedling combinations showed consistent trends for all six species (Fig. 1).

Experiment 2, distance-dependent effects of AMF and pathogens

In shade-house experiment 2, the rates of root infection by non-mycorrhizal (pathogenic/parasitic/saprophytic) fungi were much lower at larger conspecific distances when seedlings were treated with pathogens or field soils (Appendix A: Fig. A1). The percentage of mycorrhizal colonization also decreased with conspecific distance for the AMF treatment and the field soil treatment (Appendix A: Fig. A1). In

sterilized soils, we detected only minimal levels of pathogen infection and mycorrhizal colonization, which were not distance-dependent. Both the pathogen infection in the AMF treatment and the AMF colonization in the pathogen treatment almost vanished at all conspecific distances (Appendix A: Fig. A1), indicating that pathogens were successfully removed in the AMF treatment and there were no AMF in the pathogen treatment.

Most of the log-transformed odds ratios comparing seedling survival in AMF-treated pots and sterile soil pots were positive but were not significantly different from 0, and the log-transformed survival odds ratio was not correlated with conspecific distance for all six species (Fig. 3, left-hand column). In contrast, the pathogens had significantly negative effects on seedling survival for all species (Fig. 3, middle column). Moreover, these negative effects decreased with increasing conspecific distance (Fig. 3, middle column), except for *E. fenzelii* and *S. superba*. In field soils vs. sterile soils, the log-transformed survival odds ratios showed no significant departures from 0, except

Cryptocarya concinna

$y = 0.601 + 0.009x$
$R^2 = 0.040, P = 0.747$

$y = -1.103 + 0.049x$
$R^2 = 0.754, P = 0.049$

$y = -0.113 + 0.022x$
$R^2 = 0.204, P = 0.445$

Ormosia pachycarpa

$y = 0.698 - 0.021x$
$R^2 = 0.244, P = 0.398$

$y = -2.641 + 0.107x$
$R^2 = 0.762, P = 0.053$

$y = 0.089 + 0.020x$
$R^2 = 0.275, P = 0.364$

Engelhardia fenzelii

$y = 0.459 - 0.009x$
$R^2 = 0.037, P = 0.757$

$y = -2.882 + 0.058x$
$R^2 = 0.219, P = 0.427$

$y = -1.830 - 0.009x$
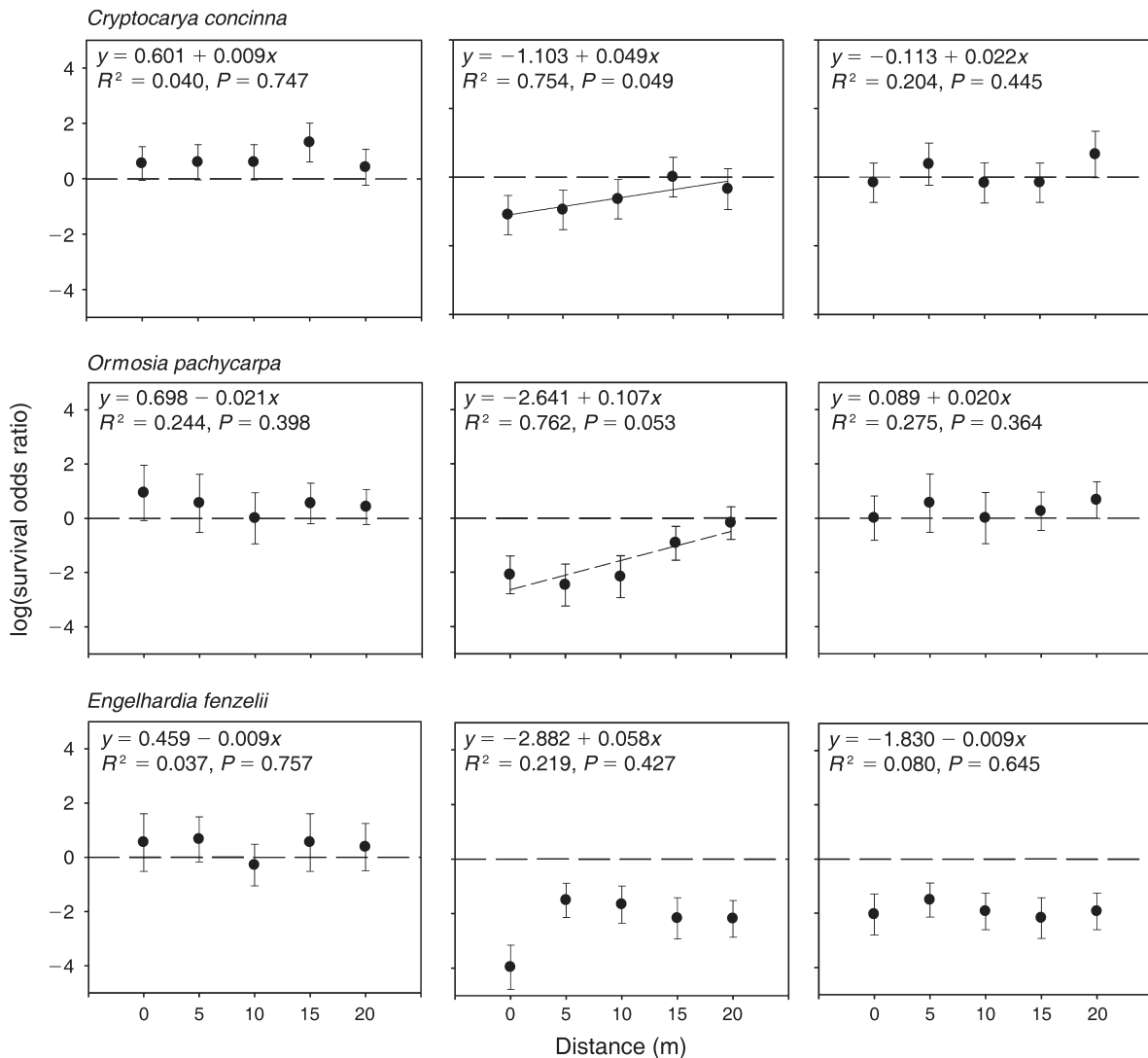$R^2 = 0.080, P = 0.645$

Distance (m)

FIG. 3. Effects of soil microbes at different conspecific distances on seedlings survival in a shade house. Effects of the soil microbe treatments are plotted against conspecific distance: log(survival odds ratio) of AMF-treated pots vs. sterile soil pots (left-hand column), of pathogen-treated pots vs. sterile soil pots (middle column), and of field soil pots vs. sterile soil pots (right-hand column). Ratios above 0 indicate positive effects and ratios that are significantly lower than 0 indicate negative effects. Odds ratio regression models were fitted for each panel, and P values of likelihood ratio tests check for significant departure from no relationship with conspecific distance. The solid regression lines indicate odds ratio regression significant at $P < 0.05$ and dashed lines indicate significance at $P < 0.1$. Error bars represent standard errors.

for *E. fenzelii*, which had significantly negative odds ratios at all distances, and *C. album*, which showed significantly positive effects that increased with increasing distance (Fig. 3, right-hand column). These results suggest that negative feedbacks of soil pathogens on seedling survival are ubiquitous and mostly distance dependent, whereas AMF reduces this effect and consequently the plant–soil microbe interactions show a variety of intensities and trends in field soils.

The mean biomass in AMF-treated soil was higher than in field or sterile soil, while in pathogen-treated soil it was lower than in field or sterile soil (Fig. 4; Appendix

D: Fig. D2). In AMF-treated pots, seedlings had the highest biomass at a distance of 0 m, and the mean biomass decreased with increasing conspecific distance (Fig. 4). Conversely, mean biomass was lowest at 0 m and positively correlated with conspecific distance in pathogen treated pots (Fig. 4), while they showed no significant relationships in field soils (except for *O. pachycarpa*; Fig. 4) and in sterile soils (Fig. 4). These results indicate that the intensities of the beneficial effect (AMF) and the inhibition effect (pathogens) on seedling growth were strongest beneath conspecific trees and decreased with increasing conspecific distance.
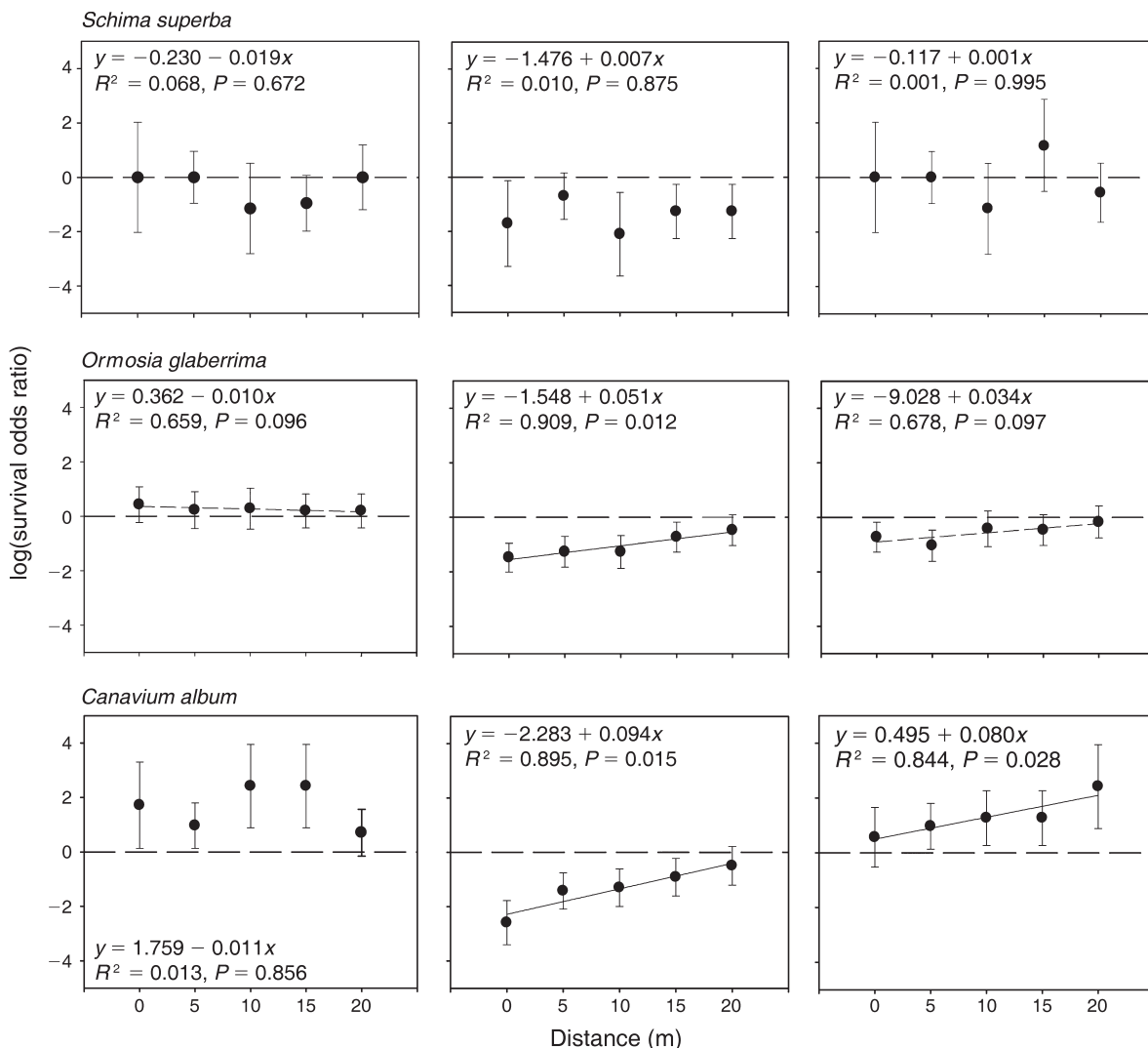
Schima superba

$y = -0.230 - 0.019x$
$R^2 = 0.068, P = 0.672$

$y = -1.476 + 0.007x$
$R^2 = 0.010, P = 0.875$

$y = -0.117 + 0.001x$
$R^2 = 0.001, P = 0.995$

Ormosia glaberrima

$y = 0.362 - 0.010x$
$R^2 = 0.659, P = 0.096$

$y = -1.548 + 0.051x$
$R^2 = 0.909, P = 0.012$

$y = -9.028 + 0.034x$
$R^2 = 0.678, P = 0.097$

Canavium album

$y = -2.283 + 0.094x$
$R^2 = 0.895, P = 0.015$

$y = 0.495 + 0.080x$
$R^2 = 0.844, P = 0.028$

$y = 1.759 - 0.011x$
$R^2 = 0.013, P = 0.856$

log(survival odds ratio)

Distance (m)

FIG. 3. Continued.

DISCUSSION

By separating feedback effects of AMF and non-mycorrhizal (pathogenic/parasitic/saprophytic) fungi on seedling performance of six subtropical tree species, we have demonstrated that, at least at relatively low adult densities, both the beneficial effect of AMF and the detrimental effect of pathogens on seedling survival and growth are host specific (Figs. 1 and 2), and that, in general, the effects of AMF and pathogens significantly decline with increasing conspecific distance (Figs. 3 and 4). While the conventional view of distance dependence in the Janzen-Connell hypothesis only concerns the interaction between tree hosts and soil pathogens, our findings suggest that the beneficial effect of AMF is also distance dependent and can neutralize the inhibition effect of pathogens (Fig. 5a, c). It thus explains our field surveys (Table 1), and the results of the field soil treatments in our second shade-house experiment (Fig.

3), as well as previous studies that found no net distance-dependent effect (Augspurger 1984, Blundell and Peart 1998, Hyatt et al. 2003, Queenborough et al. 2007). Specifically when soilborne microbes were experimentally separated, we detected a ubiquitous negative distance-dependent effect on both seedling survival (Fig. 3) and growth (Fig. 4) caused by soil pathogens. In contrast, the presence of AMF significantly increased seedling performance, especially seedling growth (Fig. 4). The two opposing effects apparently canceled each other, because the field soil treatment containing both AMF and pathogens showed no general distance-dependent effect.

The interaction between pathogens and AMF suggests that the Janzen-Connell mechanism may play a less important role in maintaining species diversity than previously thought. Studies using glasshouse experiments to quantify soil feedbacks may favor detection of

PLATE 1.   A newly emerged *Engelhardia fenzelii* seedling established under a heterospecific adult and surrounded by many fallen leaves of *Acer tutcheri*. As seeds of *E. fenzelii* are dispersed by wind, their seedlings could establish far away from the parents and consequently escape the density- and/or distance-dependent mortality caused by soil-borne pathogens, but may lose the beneficial effect of arbuscular mycorrhizal fungi. Photo credit: M. Liang.

negative rather than positive soil feedbacks, because negative feedbacks dominate on short time scales (Bardgett and Wardle 2010), and consequently these studies overestimate the Janzen-Connell effect. However, it does not mean that the Janzen-Connell mechanism has no effect at all. First, soilborne pathogens cause high mortality of the seedlings while AMF mainly promote seedling growth; hence the Janzen-Connell effect may still occur during seed dispersal and seedling germination (Jansen et al. 2012). Second, plants have different abilities to influence their abundances by changing the structure of their soil communities (Klironomos 2002), and pathogens may be more sensitive to host density or may accumulate faster than AMF.

Particularly, the pathogens' higher sensitivity to host density may explain the apparent contradiction between studies finding evidence for a distance-dependent effect (e.g., Augspurger 1983, Packer and Clay 2000) and studies finding no support (e.g., Hyatt et al. 2003), including our own previous work that supported distance dependence (Liu et al. 2012), and the current study that does not: in our previous work, we collected pathogens in soil under adults from highly abundant populations (Liu et al. 2012), whereas here we focused on the adults with relative low local abundance which have no other conspecific adults within 60 m. Similarly, Condit et al. (1992) found that only the few most

dominant species experienced a distance-dependent effect in large-scale forest plots.

The negative plant–soil feedback that is central to the Janzen-Connell effect increases alpha diversity by allowing local species coexistence, but reduces gamma diversity by promoting community convergence (reduced beta diversity). In contrast, positive plant–soil feedback has been hypothesized (reviewed by Bever et al. 2012) to reduce alpha diversity by allowing dominance of early-arriving species, but to increase gamma (regional) diversity by promoting community divergence (increased beta diversity) through the emergence of alternative stable states. Using a basic simulation model of plant community assembly, Fukami and Nakajima (2013) argued that complex plant–soil interactions including positive and negative plant–soil interactions simultaneously increase plant species diversity by delaying community convergence. Thus, while pathogens may play a key role in maintaining and structuring diversity in plant systems (e.g., Klironomos 2002, Mangan et al. 2010, Bagchi et al. 2014), AMF certainly have the potential to influence the diversity and distribution of host species (Grime et al. 1987, Kiers et al. 2000, Hood et al. 2004, van der Heijden et al. 2008). A more complete understanding of plant dynamics and community structure requires integrating both positive (AMF)
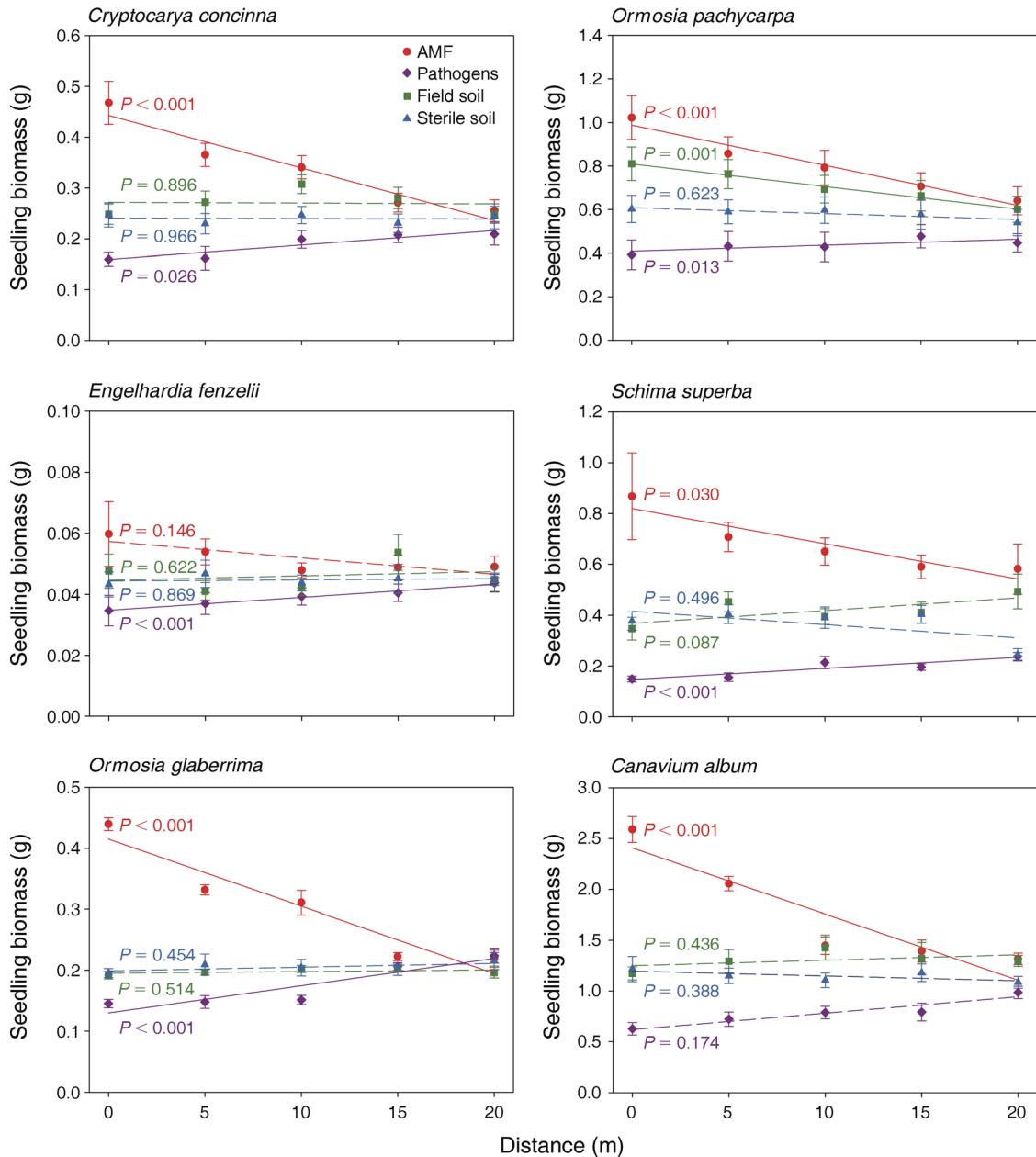
FIG. 4. The relationships between mean seedling biomass and conspecific distance in AMF, pathogen, field soil, and sterile soil treatments. The intensities of the beneficial effect (AMF) and the inhibition effect (pathogens) on seedling growth were strongest beneath conspecific trees and decreased with increasing conspecific distance. Solid lines indicate linear regressions significance at $P < 0.05$. Error bars represent standard errors.

and negative (pathogens) plant–soil feedbacks (Bever et al. 2012).

Thus, to reconcile our results with previous findings, we suggest a new perspective on distance dependence under the Janzen-Connell hypothesis (Fig. 5), in which the Janzen-Connell effect is absent for low-density populations because AMF and pathogen effects cancel, whereas a net Janzen-Connell effect appears at high population densities.

AMF have traditionally been considered to be relatively broad-spectrum (Hart et al. 2003), but their host specificity on root colonization and seedling growth has been found in grasslands and tropical forests (Bever et al. 1996, Kiers et al. 2000, Davison et al. 2011). Using data of 95 grassland species and their AMF, Reinhart et al. (2012) detected phylogenetic signals for arbuscular mycorrhizal colonization of roots and plant responses to arbuscular mycorrhizae.
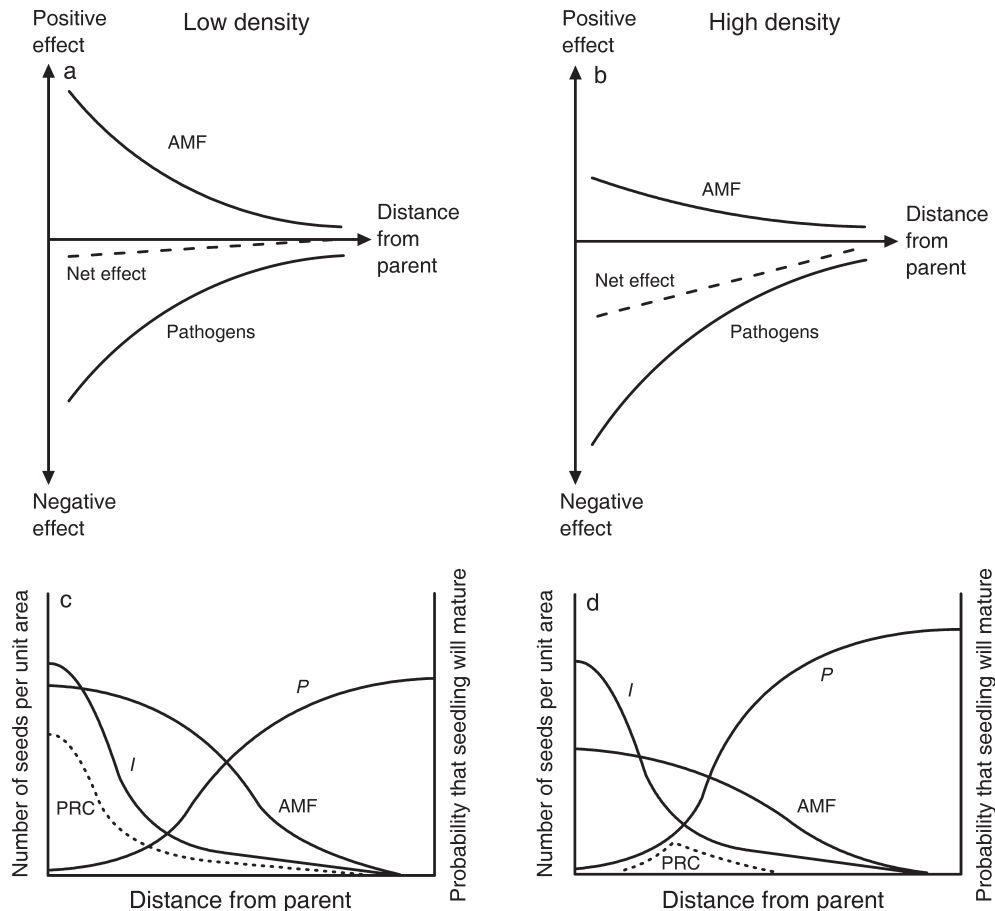
FIG. 5. Theoretical relationships between offspring recruitment and plant–soil feedbacks generated by the modified Janzen-Connell model considering both soil pathogens and AMF. (a, c) At low adult density, both the detrimental effect of pathogens and the beneficial effect of AMF on seedling survival and growth decline with increasing distance from parent tree and cancel each other, and consequently the Janzen-Connell effect plays a minor role in explaining diversity. (b, d) At high density, pathogens are more sensitive to host density and hence have a stronger effect than AMF, thus, the net effect of plant–soil feedback is negative and can promote species diversity through the Janzen-Connell mechanism. Panels (c) and (d) show the interacting effects of distance from parent, the number of seeds per unit area (I), the probability that a dispersed seed or seedling will escape pathogens (P), the probability that a dispersed seed or seedling will benefit from arbuscular mycorrhizal fungi (AMF), and the population recruitment curve (PRC; dotted line).

Our first shade-house experiment was performed to study this host specificity and any possible phylogenetic signal. The results revealed a clear host-specific effect for both soil pathogens and AMF. Soil microbes cultured by adults of a given species greatly affected seedling performance of conspecifics but not heterospecifics (Figs. 1 and 2), which confirmed that our selection of not-too-closely related species substantially reduced a possible phylogenetic Janzen-Connell effect. The congenerics *Ormosia pachycarpa* (OP) and *Ormosia glaberrima* (OG) did show similar patterns (compare OP–OG to OP–OP and OG–OP to OG–OG in Fig. 1), but the effect was not significant.

The proportions of mycorrhizal colonization and pathogen infection decreased with conspecific distance (Appendix A: Fig. A1), indicating that the concentrations of host-specific fungi declined with increasing

conspecific distance. Due to limited dispersal ability of soilborne fungi, a given host individual may culture its own soil microbial community around its root area. Seedlings establishing around conspecific trees suffer high mortality caused by soil pathogens (e.g., Augspurger 1983, Packer and Clay 2000, Bagchi et al. 2010), but our results also suggest that seedlings may benefit from host-specific AMF while fighting against pathogens. Once some seedlings escape or defeat the disease beneath conspecific adults, they may benefit from AMF in nutrition and growth and hence have a competitive advantage.

In the shade-house experiments, we thoroughly mixed the parent soil samples collected from three adult trees, to make sure that we successfully collected the target microbes and added exactly the same suspension to all replicates for each treatment, and to remove any

intraspecific effects, because our focus was on interspecific effects. This mixing procedure, if anything, will make our results conservative, because if there are strong intraspecific effects, this will boost the significance of the host specificity. In shade-house experiments 1 and 2, we used the field soil treatment, which was the best representative of the composition and abundance of the microbial community in natural soil but may meanwhile contain other organisms, to investigate the additive effect of AMF and pathogens. The additional experiment results showed that seedling performances had no difference in the field soil treatment and the AMF + pathogen treatment (Appendix B: Figs. B1 and B2), indicating that the additive effect of the field soil treatment was indeed the interaction effect between AMF and pathogens: the beneficial effect of AMF neutralized the inhibition effect of pathogens, as we hypothesized.

In summary, while it is generally accepted that dispersal away from the parent may promote seedling establishment and survival because of a higher probability of landing in light-gaps (Augspurger 1984), but may also increase the chance of landing in a hostile location (Nathan 2006), our study suggests that the plant–soil feedbacks are also double-sided: seedlings established far away from the parents can escape the pathogen damage but also lose the beneficial effect of AMF (see Plate 1). Thus, future research on plant–soil interactions should consider both pathogens and AMF.

LITERATURE CITED

Augspurger, C. K. 1983. Seed dispersal of the tropical tree, *Platypodium elegans*, and the escape of its seedlings from fungal pathogens. Journal of Ecology 71:759–771.

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

Bagchi, R., R. E. Gallery, S. Gripenberg, S. J. Gurr, L. Narayan, C. E. Addis, R. P. Freckleton, and O. T. Lewis. 2014. Pathogens and insect herbivores drive rainforest plant diversity and composition. Nature 506:85–88.

Bagchi, R., T. Swinfield, R. E. Gallery, O. T. Lewis, S. Gripenberg, L. Narayan, and R. P. Freckleton. 2010. Testing the Janzen-Connell mechanism: pathogens cause overcompensating density dependence in a tropical tree. Ecology Letters 13:1262–1269.

Bardgett, R. D., and D. A. Wardle. 2010. Aboveground–belowground linkages: biotic interactions, ecosystem processes, and global change. Oxford University Press, Oxford, UK.

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. 2002. 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.

Bever, J. D., T. G. Platt, and E. R. Morton. 2012. Microbial population and community dynamics on plant roots and their feedbacks on plant communities. Annual Review of Microbiology 66:265–283.

Bidartondo, M. I., D. Redecker, I. Hijri, A. Wiemken, T. D. Bruns, L. Domínguez, A. Sérsic, J. R. Leake, and D. J. Read. 2002. Epiparasitic plants specialized on arbuscular mycorrhizal fungi. Nature 419:389–392.

Blundell, A. G., and D. R. Peart. 1998. Distance dependence in herbivory and foliar condition for juvenile *Shorea* trees in Bornean dipterocarp rain forest. Oecologia 117:151–160.

Borowicz, V. A. 2001. Do arbuscular mycorrhizal fungi alter plant–pathogen relations? Ecology 82:3057–3068.

Comita, L. S., S. A. Queenborough, S. J. Murphy, J. L. Eck, K. Xu, M. Krishnadas, N. Beckman, and Y. Zhu. 2014. Testing predictions of the Janzen-Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. Journal of Ecology 102:845–856.

Condit, R., S. P. Hubbell, and R. B. Foster. 1992. Recruitment near conspecific adults and the maintenance of tree and shrub diversity in a neotropical forest. American Naturalist 140:261–286.

Davison, J., M. Öpik, T. J. Daniell, M. Moora, and M. Zobel. 2011. Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. FEMS Microbiology Ecology 78:103–115.

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.

Evans, H. C., K. A. Holmes, and S. E. Thomas. 2003. Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gileri*, in Ecuador and a preliminary assessment of their potential as biocontrol agents of cacao diseases. Mycological Progress 2:149–160.

Fukami, T., and M. Nakajima. 2013. Complex plant-soil interactions enhance plant species diversity by delaying community convergence. Journal of Ecology 101:316–324.

Gange, A. C., V. K. Brown, and D. M. Aplin. 2005. Ecological specificity of arbuscular mycorrhizae: evidence from foliar- and seed-feeding insects. Ecology 86:603–611.

Grime, J. P., J. M. L. Mackey, S. H. Hiller, and D. J. Read. 1987. Floristic diversity in a model system using experimental microcosms. Nature 328:420–422.

Hart, M. M., R. J. Reader, and J. N. Klironomos. 2003. Plant coexistence mediated by arbuscular mycorrhizal fungi. Trends in Ecology and Evolution 18:418–423.

Herre, E. A., D. A. 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* Biotic interactions in the tropics. D. F. R. P. Burslem, M. A. Pinard, and S. E. Hartley editors. Cambridge University Press, Cambridge, UK.

Herre, E. A., L. C. Meija, D. A. Kyllo, E. Rojas, Z. Maynard, A. Butler, and S. A. van Beal. 2007. Ecological implications of anti-pathogen effects of tropical fungal endophytes and mycorrhizae. Ecology 88:550–558.

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.

Howeler, R. H., L. F. Cadavid, and E. Burckhardt. 1982. Response of cassava to VA mycorrhizal inoculation and

phosphorus application in greenhouse and field experiments. Plant and Soil 69:327–339.

Hyatt, L. A., et al. 2003. The distance-dependence prediction of the Janzen-Connell hypothesis: a meta-analysis. Oikos 103: 590–602.

Jansen, P. A., B. T. Hirsch, W. J. Emsens, V. Zamora-Gutierrez, M. Wikelski, and R. Kays. 2012. Thieving rodents as substitute dispersers of megafaunal seeds. Proceedings of the National Academy of Sciences USA 109:12610–12615.

Jewell, N. P. 2003. Statistics for Epidemiology. Chapman and Hall/CRC Press, Boca Raton, Florida, USA.

Kiers, E. T., C. E. Lovelock, E. L. Kruger, 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.

Liu, X., M. Liang, R. S. Etienne, Y. Wang, C. Staehelin, and S. Yu. 2012. Experimental evidence for a phylogenetic Janzen-Connell effect in a subtropical forest. Ecology Letters 15: 111–118.

Mangan, S. A., S. A. Schnitzer, E. A. Herre, K. M. L. Mack, M. C. Valencia, E. I. Sanchez, and J. D. Bever. 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. Nature 466:752–755.

McCarthy-Neumann, S., and R. K. Kobe. 2008. Tolerance of soil pathogens co-varies with shade tolerance across species of tropical tree seedlings. Ecology 89:1883–1892.

McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild, and J. A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 115:495–501.

McNamara, N. P., H. I. J. Black, N. A. Beresford, and N. R. Parekh. 2003. Effects of acute gamma irradiation on chemical, physical and biological properties of soils. Applied Soil Ecology 24:117–132.

Nathan, R. 2006. Long-distance dispersal of plants. Science 313:786–788.

Newsham, K. K., A. H. Fitter, and A. R. Watkinson. 1995. Arbuscular mycorrhizae protect an annual grass from root pathogenic fungi in the field. Journal of Ecology 83:991–1000.

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

Queenborough, S. A., D. F. Burslem, N. C. Garwood, and R. Valencia. 2007. Neighborhood and community interactions determine the spatial pattern of tropical tree seedling survival. Ecology 88:2248–2258.

R Development Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org

Read, D. J., and J. Perez-Moreno. 2003. Mycorrhizas and nutrient cycling in ecosystems–a journey towards relevance? New Phytologist 157:475–492.

Reinhart, K. O., G. W. T. Wilson, and M. J. Rinella. 2012. Predicting plant responses to mycorrhizae: integrating evolutionary history and plant traits. Ecology Letters 15: 689–695.

Schnitzer, S. A., et al. 2011. Soil microbes drive the classic plant diversity–productivity pattern. Ecology 92:296–303.

Smith, G. S. 1988. The role of phosphorus nutrition in interactions of vesicular-arbuscular mycorrhizal fungi with soilborne nematodes and fungi. Phytopathology 78:371–374.

Smith, S. E., and D. J. Read. 1997. Mycorrhizal symbiosis. Second edition. Academic Press, London, UK.

Terborgh, J. 2012. Enemies maintain hyperdiverse tropical forests. American Naturalist 179:303–314.

van der Heijden, M. G. A., R. D. Bardgett, and N. M. van Straalen. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11:296–310.

SUPPLEMENTAL MATERIAL

Ecological Archives

Appendices A–D are available online: http://dx.doi.org/10.1890/14-0871.1.sm