

Arbuscular mycorrhizal fungal diversity and natural enemies promote coexistence of tropical tree species

BENEDICTE BACHELOT,^{1,6} MARÍA URIARTE,² KRISTA L. MCGUIRE,^{2,3} JILL THOMPSON,^{4,5} AND JESS ZIMMERMAN⁴

¹Department of Biology, Duke University, 130 Science Drive, Durham, North Carolina 27701 USA

²Department of Ecology, Evolution and Environmental Biology, Columbia University, 1200 Amsterdam Avenue, New York City, New York 10027 USA

³Department of Biology, Barnard College, Columbia University, New York City, New York 10027 USA

⁴Department of Environmental Sciences, University of Puerto Rico, Río Piedras Campus, San Juan, Puerto Rico 00936 USA

⁵Centre for Ecology & Hydrology, Bush Estate Penicuik, Midlothian, EH26 0QB United Kingdom

Abstract. Negative population feedbacks mediated by natural enemies can promote species coexistence at the community scale through disproportionate mortality of numerically dominant (common) tree species. Simultaneously, associations with arbuscular mycorrhizal fungi (AMF) can result in positive effects on tree populations. Coupling data on seedling foliar damage from herbivores and pathogens and DNA sequencing of soil AMF diversity, we assessed the effects of these factors on tree seedling mortality at local (1 m²) and community (16 ha plot) scales in a tropical rainforest in Puerto Rico. At the local scale, AMF diversity in soil counteracted negative effects from foliar damage on seedling mortality. At the community scale, mortality of seedlings of common tree species increased with foliar damage while rare tree species benefited from soil AMF diversity. Together, the effects of foliar damage and soil AMF diversity on seedling mortality might foster tree species coexistence in this forest.

Key words: arbuscular mycorrhizal fungi; coexistence; community compensatory trend; Janzen–Connell hypothesis; seedling mortality; tropical forest.

INTRODUCTION

Negative feedbacks are integral components of most species coexistence theories: if one species becomes too dominant, its performance declines, which, in turn, reduces its abundance; the opposite occurs for species that drift to low abundances (Chesson 2000). In highly diverse tropical forests, negative feedbacks operate through the action of seedling natural enemies, which show some degree of host preference (the Janzen–Connell [JC] hypothesis; Janzen 1970, Connell 1971, Comita et al. 2014). At the local scale (e.g., seedling neighborhood), the JC hypothesis predicts that seedling survival and recruitment should be low at high conspecific seedling densities and near adult conspecific trees, allowing heterospecific species to recruit. At the community scale (e.g., forest stand or plot), tree species coexistence might also be maintained if numerically dominant (common) tree species experience greater mortality than rare species. This process, known as a community compensatory trend, arises from differences in sensitivity to conspecific density (stabilizing effects) coupled with species-specific average fitness differences that are density-independent (Connell et al. 1984, Chesson 2000; equalizing effects).

In contrast to the large body of research on negative feedbacks (see review in van der Putten et al. 2013), the

role of mutualisms in species coexistence in tropical forests has received far less attention. Yet, interactions among trees, natural enemies, and arbuscular mycorrhizal fungi (AMF) could counteract JC effects (Hood et al. 2004, Liang et al. 2015), and influence tropical tree species coexistence. AMF provide trees with increased nutrient and water uptake and protection against enemies (Smith and Read 2008). The local effects of AMF associations on tree seedling growth vary strongly across AMF and tree species (Kiers et al. 2000, Herre et al. 2005), so that AMF diversity might influence seedling mortality. Locally, high AMF diversity may increase the probability of symbiosis with a beneficial AM species (i.e., positive selection effect). Alternatively, positive effects of high AMF diversity could result from functional complementarity among beneficial AMF (Naeem and Li 1997). However, high AMF diversity could also lead to a strong negative effect on seedling survival due to selection of a parasitic AMF. Several studies in grasslands have linked aboveground plant community productivity with AMF diversity and found evidence for both positive complementarity and selection effects (van der Heijden et al. 1998, Maherali and Klironomos 2007, Wagg et al. 2015). In tropical forests, only two experimental studies have investigated the effect of AMF diversity on net plant productivity (Lovelock and Ewel 2005) or on seedling growth (Siqueira and Saggin-Júnior 1998); they found positive correlations.

At the community scale, positive effects of AMF diversity on trees may help maintain tree species coexistence if AMF diversity enhances fitness of rare species

Manuscript received 20 July 2016; revised 19 October 2016; accepted 30 November 2016. Corresponding Editor: Christine V. Hawkes.

⁶E-mail: bmb43@duke.edu

more than that of common species. This may occur via two mechanisms. First, common species tend to have a high number of specialized AMF interactions while rare species associate with a few generalist species (Montesinos-Navarro et al. 2012). Second, seedlings of common species are more abundant and clustered and, as a result, may more easily be colonized by their species-specific AMF. These differences in AMF colonization rates and degree of specialization suggest that common tree species might not gain as much benefit from increasing AMF diversity as rare tree species, fostering tree species coexistence.

On the other hand, the positive effects of AMF diversity on trees may negatively influence tree species coexistence if common tree species extract more benefits than rare tree species. This may occur via three mechanisms. First, rare tree species might not be able to afford the costs of AMF associations (Bever 2003, Bever et al. 2009). Second, common tree species might support larger mycorrhizal networks than rare species thereby extracting more benefits from AMF than rare tree species (Verbruggen et al. 2012). Third, AMF might also be distance and density responsive (Liang et al. 2015). If common tree species are more clumped and closer to each other than rare tree species (Connell et al. 1984, Condit et al. 2000), distance responsive AMF could lead to greater associations with common tree species.

A few experimental studies in tropical forests have examined the combined effects of enemies and inoculation by AMF on seedling mortality (Hood et al. 2004, Pizano et al. 2011, Liang et al. 2015). These studies demonstrated that the presence of AMF could counteract negative distance-dependent JC effects in experimental settings. Yet, they did not investigate the simultaneous effects of fungal diversity and conspecific density effects on seedling mortality or examined consequences of fungal inoculation on seedling mortality at the community level.

Here, we examine the combined effects of aboveground pressure from natural enemies and AMF diversity in soil on seedling mortality and tropical tree species coexistence by asking two questions: (1) At the local scales, is mortality of tropical seedlings affected by foliar damage from natural enemies and AMF diversity? and (2) How do the effects of foliar damage and AMF diversity on seedling mortality change with tree abundance at the community scale?

METHODS

Study site

This study took place at the 16-ha Luquillo Forest Dynamics Plot (LFDP; 18°20' N, 65°49' W) in northeast Puerto Rico (elevation 333–428 m above sea level; Zimmerman et al. 2010). Mean annual rainfall is 3,500 mm, classifying the forest as subtropical montane. Since 1990, all free-standing woody stems >1 cm dbh (diameter at 1.3 m height) have been mapped, identified

to species, and measured approximately every 5 years (Zimmerman et al. 2010).

Tree community data

In 1998, 213 2-m² seedling plots were established throughout the plot (Uriarte et al. 2005). In 2007, 3 × 120 1-m² seedling plots were established on each side of a north-south running trail. Seedlings in these plots have been mapped, identified to species, and measured (root collar diameter) annually since 2007. Here, we used one of each of the subplots within 120 clusters of three 1-m² seedling plots as well as 117 of the 213 2-m² seedling plots ($n = 237$ plots total). For the 2-m² plots, we used seedlings and soil data present in one-half of the plots (1 m²) to make them comparable to the 1-m² plots. We used seedling census data from 2012 as our baseline (t_0) in this study and revisited the plots in 2013 to evaluate seedling mortality (t_1), restricting our analysis to individuals taller than 10 cm in 2012. We used the 2011 tree census to calculate community abundance of each tree species i as the sum of basal conspecific tree basal area (BA_i) within the 16-ha plot.

Seedling foliar damage

In May–July 2012 (beginning of the rainy season), we used a digital camera (Nikon D3100, Tokyo, Japan) with a microlens (18–55 mm VR lens), to photograph a maximum of five leaves of every seedling present in each of the 237 seedling plots. We excluded liana, fern, and palm seedlings. In total, we photographed 823 seedlings representing 42 tree species. From these photos, we estimated the proportion of leaf area with damage for each leaf. Damage included missing leaf area as well as evidence of galls, leaf mines, or necrotic spots caused by pathogens. Herbivory and pathogens caused most of the damage, whereas galls and leaf mines were less frequently observed (Bachelot et al. 2015c). Proportion of leaf damage at t_0 was quantified as an ordinal variable ranging from 0 to 100 by binning damage for each seedling into 20 categories: 0–5%, 5–10%, 10–15%, ..., 95–100% leaf damage. We acknowledge that this one-time measurement of damage does not represent the exact amount of damage that happened in 2012 as some leaves might have been removed or lost prior to our assessment of herbivory. Thus, our analyses rest on what is likely to be an underestimate of actual damage.

Shade tolerance

Shade tolerance is associated with variation in mortality rates. We evaluated species-specific shade tolerance using average sapling (trees ≥ 1 but ≤ 10 cm DBH) survival between 1995 and 2000 (Appendix S3: Table S1). This time period experienced high sapling mortality following pulses of recruits after Hurricane Hugo (Uriarte et al. 2009).

Arbuscular mycorrhizal fungal communities

In May–July 2012 (beginning of rainy season), we sampled soils in the same 237 seedling plots to characterize the AMF community (as described in Bachelot et al. 2016). We used a soil core (2.5 cm diameter) to collect the top 20 cm of soil from the corners of the plots. Supplies were cleaned with 70% ethanol between plots to prevent contamination. Samples were stored in a freezer (−20°C) before being shipped overnight to the laboratory. Soil samples were defrosted, sieved, and extracted using the PowerSoil DNA isolation kit (MoBio, Carlsbad, California, USA). Fungal DNA extracts were sequenced using barcoded high-throughput sequencing on the Illumina MiSeq platform (Illumina Inc., San Diego, California, USA) at the University of Colorado, Boulder, Colorado, USA. The first internal transcribed spacer region (ITS1) of the fungal rRNA gene was amplified using the primer pair ITS1-F and ITS2 (McGuire et al. 2013). DNA data were successfully obtained for 214 seedling plots. All reads were de-multiplexed, quality filtered, and processed using the QIIME v. 1.5.0-dev pipeline (Caporaso et al. 2010). Overall, we obtained between 1318 and 6,208 reads per soil sample before quality filtering and 606 and 3,737 reads per soil sample after quality filtering (Appendix S3: Table S2). We used a 97% similarity threshold to cluster sequences into similar operational taxonomic units (OTUs), using the open reference-based procedure in QIIME. For this, we used UCLUST (Edgar 2010) to cluster sequences with the UNITE November 2012 database (Abarenkov et al. 2010), and sequences that did not cluster with the database were clustered de novo. We excluded sequences that were less than 75% similar to any sequences in UNITE prior to OTU clustering as an additional quality control measure, assuming these sequences are artifacts or contaminants. In total, we identified 553 unique AMF OTUs. After taxonomic identification, we used the Shannon index to calculate AMF diversity present in soil (spores and hyphae) at t_0 for each of the sampled 214 seedling plots (Hill et al. 2003), using the number of reads as a measure of abundance.

Statistical analyses

After restricting the data to plots where fungal DNA was successfully amplified and sequenced (214 plots) and to seedlings of woody tree species that were at least 10 cm height in 2012 (t_0), we performed analyses on data from 181 seedling plots. We used a Bayesian regression model to predict mortality (Ψ_{ijp,t_1}) of seedling i of species j in plot p between 2012 (t_0) and 2013 (t_1), using the following abiotic and biotic variables measured in 2012 (t_0 ; see Appendix S1): seedling root collar diameter (Size_{i,t_0}), foliar damage per cm² (Damage_{i,t_0}), conspecific seedling abundance (Consp_{i,t_0}), soil arbuscular mycorrhizal fungal diversity (AMF_{p,t_0}), and heterospecific seedling abundance (Het_{i,t_0}). This model implies that AMF diversity influences seedling survival. This is a fair assumption given that AMF

community composition and diversity in the study site are more strongly structured by edaphic conditions (Bachelot et al. 2016) and precipitation (Hall et al. 2013) rather than by biotic factors. Conspecific seedling abundance accounts for all other negative and positive density-dependent effects (e.g., belowground natural enemies, competition) not captured by foliar damage data. Finally, to account for different time intervals between censuses for individual seedlings, we incorporated the number of days between the 2012 and 2013 censuses of each seedling (Time_i) in the mortality model as follows

$$\begin{aligned} \text{Mortality}_{ijp} &\sim \text{Bernoulli}(\Psi_{ijp,t_1})\Psi_{ijp,t_1} \\ &= \text{Inverse-logit}(\beta_1 \times \text{Size}_{i,t_0} \\ &\quad + \beta_2 \times \text{Damage}_{i,t_0} + \beta_3 \times \text{Consp}_{i,t_0} \\ &\quad + \beta_4 \times \text{AMF}_{p,t_0} + \beta_5 \times \text{Time}_i + \mu_j + \gamma_p) \end{aligned} \quad (1)$$

where γ_p is a plot random effect. To answer the first question, we assessed whether the 95% credible intervals (CIs) of damage (β_2), conspecifics (β_3), and AMF diversity (β_4) did not overlap 0. To account for fitness differences across species, we used a species-specific intercept (μ_j) as follows:

$$\mu_j \sim \text{Normal}(m + \alpha \times \text{Tolerance}_j, \sigma) \quad (2)$$

where m is the average species mortality, α is the effect of shade tolerance (Tolerance_j) on species-specific average mortality, and σ is the variance (Appendix S1). Removing shade tolerance from the model did not affect the results (Appendix S3: Fig. S2).

We also fitted the mortality model described above to three groups of tree species separated according to their abundance at the community level (sum of basal area in 16-ha plot). We defined three abundance categories using the following breaks in the distribution of the weighed sum of conspecific tree basal area (BA_j) across the full LFDP census 2011 data set (Appendix S3: Fig. S1): rare (0–785 cm²/16 ha), intermediate (868–5,834 cm²/16 ha), and common (10,587–36,656 cm²/16 ha; Appendix S3: Fig. S1, Table S3). We selected this breakdown because it maximizes evenness in the number of seedlings and species across abundance categories. Yet, this breakdown assigns more species in the rare category (12) than in the common category (7), consistent with tropical tree species distributions that contain more rare than common species. There are two main advantages of using categories rather than continuous abundance as presented in the Appendix S2 (Table S4): (1) we do not need to make an assumption on the shape of the community trends, which might be non-linear (Bachelot et al. 2015c), and (2) it enables us to draw conclusion on rare species whose sample sizes would be too low otherwise. To evaluate whether the main effects of conspecific density, foliar damage, and AMF diversity on mortality differ across abundance categories, we used pairwise Kolmogorov-Smirnov tests. This method compares whether two independent samples arise from the

same distribution and is therefore well adapted to compare posterior distributions obtained from different data sets (here, different sets of species).

To investigate community trends due to foliar damage, conspecific seedling density, and AMF diversity, we focused on the coupled effects of average fitness (μ_j) and the effects of foliar damage ($\beta_2 \times \text{Damage}$), conspecific seedling density ($\beta_3 \times \text{Consp}$), or AMF diversity ($\beta_4 \times \text{AMF}$) respectively. For example, there is a community compensatory trend if mortality due to average fitness and foliar damage in rare species is lower than in common species over a range of foliar damage

$$\begin{aligned} & \text{Inverse-logit}(\mu_{\text{rare}} + \beta_2 \times \text{Damage}_{\text{rare}}) \\ & < \text{Inverse-logit}(\mu_{\text{common}} + \beta_2 \times \text{Damage}_{\text{common}}) \end{aligned} \quad [\text{Inequality. 1}]$$

Finally, we ran species-specific analyses using the same model as previously described (Eqs. 1 and 2) within species and without the species-specific intercept. Each species-specific model was as follows:

$$\begin{aligned} \text{Mortality}_{ip} & \sim \text{Bernoulli}(\Psi_{ip,t_1}) \Psi_{ip,t_1} \\ & = \text{Inverse-logit}(\beta_1 \times \text{Size}_{i,t_0} + \beta_2 \times \text{Damage}_{i,t_0} \\ & \quad + \beta_3 \times \text{Consp}_{i,t_0} + \beta_4 \times \text{AMF}_{p,t_0} \\ & \quad + \beta_5 \times \text{Het}_{i,t_0} + \mu + \gamma_p) \text{Time} \end{aligned} \quad (3)$$

This second model provided us with a second test of community trends. Once fitted, each species-specific models were used to predict individual mortality due to fitness differences (μ), conspecific seedling density ($\beta_3 \times \text{Consp}_{i,t_0}$), foliar damage ($\beta_2 \times \text{Damage}_{i,t_0}$), and AMF diversity ($\beta_4 \times \text{AMF}_{p,t_0}$). We then fitted a generalized linear model to predict “predicted mortality” with respect to species community abundance with a binomial link and the length of the Markov chain Monte Carlo (MCMC) chains for the number of trials. A significant effect of species community abundance on seedling mortality would imply the presence of a community trend.

Highly skewed variables were log-transformed (conspecific density, sum of basal tree area, seedling size, and foliar damage). Size was z-transformed within species to correct for different size distributions and improve convergence (Gelman et al. 2013). The remaining variables were z-transformed across all individuals prior to analyses to allow for comparison of their effect size (Gelman et al. 2013). Analyses were performed using R and STAN statistical software (software available online).^{7,8} We used non-informative priors for each variable, and we ran three MCMC chains with 10,000 iterations. Significance of each parameter was assessed by non-overlap of the 95% CIs with 0. We assessed convergence, using \hat{R} (Brook and Gelman 1997). Goodness of fit was evaluated using Bayesian P value of the average mortality rate (Gelman et al. 2013) and posterior predictive checks. Finally, the

spatial structure of the residuals was assessed statistically by using a Mantel test between the residuals and the locations with 9999 permutations.

RESULTS

All mortality models converged ($\hat{R} = 1.00$ for each model), fitted the data well (Bayesian P value of mean = 0.49, 0.48, 0.47, and 0.48 for the all data together, low, medium, and high abundance model, respectively), and captured any spatial structure present in the residuals variance in seedling mortality across plots (Mantel test, $P = 0.22, 0.45, 0.08,$ and 0.53 respectively).

Foliar damage was highly variable across individuals ranging from 0% to 100.00% of leaf damage (median of 20.00%) and across species (median of 20.05% ranging from 1.00% to 60.00% of leaf damage). In 2013, 23.3% of seedlings, which were present in 2012, had died. On average, there were 20 seedlings per plot, ranging from 1 to 298 individuals. Conspecific seedling density also ranged broadly across seedlings from 0 to 23 individuals/m² (median of 2 individuals/m²). Soil AMF diversity (Shannon index) exhibited variation across seedling plots ranging from 0 to 3.1 (median of 2.22).

Seedling mortality for rare tree species was low (9.7%) relative to intermediate (14.4%) or common (27.2%) tree species. However, these differences in mortality rates across abundance categories were not significant (ANOVA, $F_{2,39} = 1.5, P = 0.25$). Densities of conspecific seedlings were similar for seedlings of rare and common tree species (Appendix S3: Fig. S3, $t = -1.0, \text{df} = 193, P = 0.31$). However, intermediate tree species experienced significantly lower conspecific seedling densities than did rare tree species (Appendix S3: Fig. S3, $t = -3.4, \text{df} = 168, P < 0.001$) or common tree species (Appendix S3: Fig. S3, $t = -7.2, \text{df} = 685, P < 0.001$). Foliar damage was significantly different across the three abundance categories; rare tree species experienced lower amount of foliar damage than did common tree species (Appendix S3: Fig. S3, $t = -3.1, \text{df} = 199, P = 0.002$) and tree species of intermediate abundance (Appendix S3: Fig. S3, $t = -2.5, \text{df} = 247, P = 0.01$). However, intermediate-abundance species experienced similar foliar damage as common species (Appendix S3: Fig. S3, $t = 0.5, \text{df} = 491, P = 0.63$). Finally, average AMF diversity did not differ among rare, intermediate, and common tree species (Appendix S3: Fig. S3, ANOVA, $F_{2,39} = 0.8, P = 0.50$).

At the local scales, is mortality of tropical seedlings affected by foliar damage from natural enemies and AMF diversity?

As expected, seedling mortality increased with natural enemy pressure measured as the amount of foliar damage (parameter β_2 in Eq. 3), and with conspecific seedling density (i.e., parameters β_3 in Eq. 2, Fig. 1A and Appendix S3: Table S5). Heterospecific density had no effect on seedling mortality (i.e., parameters β_5 in Eq. 2, Fig. 1A).

⁷ <http://www.r-project.org>

⁸ <http://mc-stan.org/rstan.html>

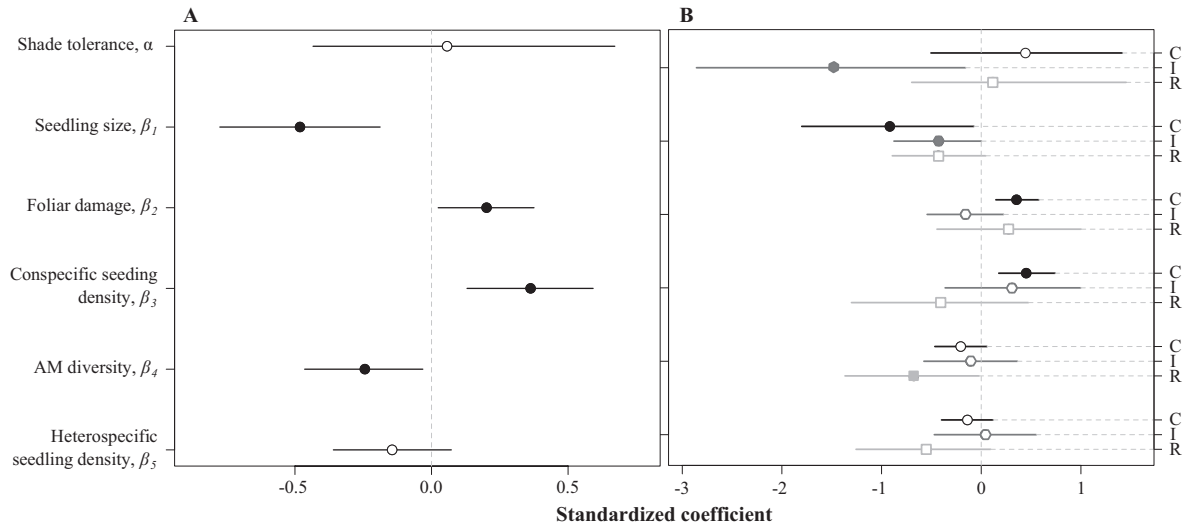


FIG. 1. (A) Posterior distribution for all parameters in the full mortality model. Solid circles indicate that the 95% credible interval (CI) of the factors do not overlap 0, whereas empty circles stand for factors whose 95% CI overlap 0. (B) Posterior distribution for all parameters in the mortality model for each abundance category. Abundance categories (common in black circles, intermediate in dark gray hexagons, and rare in light gray squares from the top to the bottom) were defined using breaks in the distribution of the weighed sum of basal tree area across the full LFDP census 2011 data set (Appendix S3: Fig. S1). Solid circles indicate that the 95% CI of the factors do not overlap 0, whereas empty circles stand for factors whose 95% CI overlap 0.

Seedling mortality decreased with AMF diversity (parameter β_4 in Eq. 2; Fig. 1A), suggesting that high AMF diversity is beneficial for seedlings. The magnitude of the conspecific seedling density and foliar damage effects together was greater than the magnitude of the AMF diversity effect, suggesting that AMF diversity may not fully counteract local negative JC effects.

As expected, seedling mortality decreased with seedling size (parameter β_1 in Eq. 3; Fig. 1A).

How do the effects of foliar damage and AMF diversity on seedling mortality change with community-scale tree abundance at the community scale?

Consistent with our expectation of a community compensatory trend disadvantaging more abundant tree species, differences in average fitness (parameter μ in Eq. 2; Appendix S3: Table S5) and in sensitivity to foliar damage (parameter β_2 in Eq. 2) led to high seedling mortality for common tree species (Appendix S3: Figs. S4 and S5). This was not the case for rare and intermediate-abundance tree species. The differences of the effects of foliar damage between common and rare/intermediate species were significant ($P < 0.001$, Kolmogorov-Smirnov [K-S] tests).

The effect of conspecific seedling density on seedling mortality (parameter β_3 in Eq. 2) increased with tree species abundance (Fig. 1B). Combined with fitness differences among tree species (parameter μ in Eq. 2; Appendix S3: Table S5), the effect of conspecific seedling density resulted in high seedling mortality for common tree species (Appendix S3: Figs. S4 and S5). The 95% CI of the effect of conspecific density did not overlap 0 for common species (Fig. 1B), and the effect sizes were

significantly different between rare and common species and between intermediate and common species (both $P < 0.001$, K-S tests).

Consistent with our hypothesis that rare species should extract the greatest benefit from AMF diversity, we found that high AMF diversity only decreased seedling mortality of rare tree species (parameter β_4 in Eq. 2; Fig. 1B). This AMF diversity effect combined with fitness differences among tree species (parameter μ in Eq. 2; Appendix S3: Table S5) resulted in low seedling mortality for rare tree species (Appendix S3: Figs. S4 and S5). Finally, the effect of AMF diversity on rare seedling mortality was significantly lower than the effects on both intermediate and common species (both $P < 0.001$, K-S tests).

The species-specific analyses revealed similar trends. Common species were disadvantaged due to high average mortality (Fig. 2A; Appendix S3: Tables S6 and S7, equalizing effect) and strong mortality due to conspecific seedling density (Fig. 2B; Appendix S3: Tables S6 and S7, stabilizing effect). However, the stabilizing effects of foliar damage and AMF diversity were not significant at that level of the analysis (Fig. 2C, D; Appendix S3: Tables S6 and S7).

DISCUSSION

At the local scale, we found that foliar damage increased seedling mortality whereas AMF diversity in soil reduced it. This result was consistent with recent experimental findings from other tropical forests showing that AMF can counteract local negative effects of soil pathogens (Hood et al. 2004, Liang et al. 2015). At the community scale, we found that seedlings of common species experienced

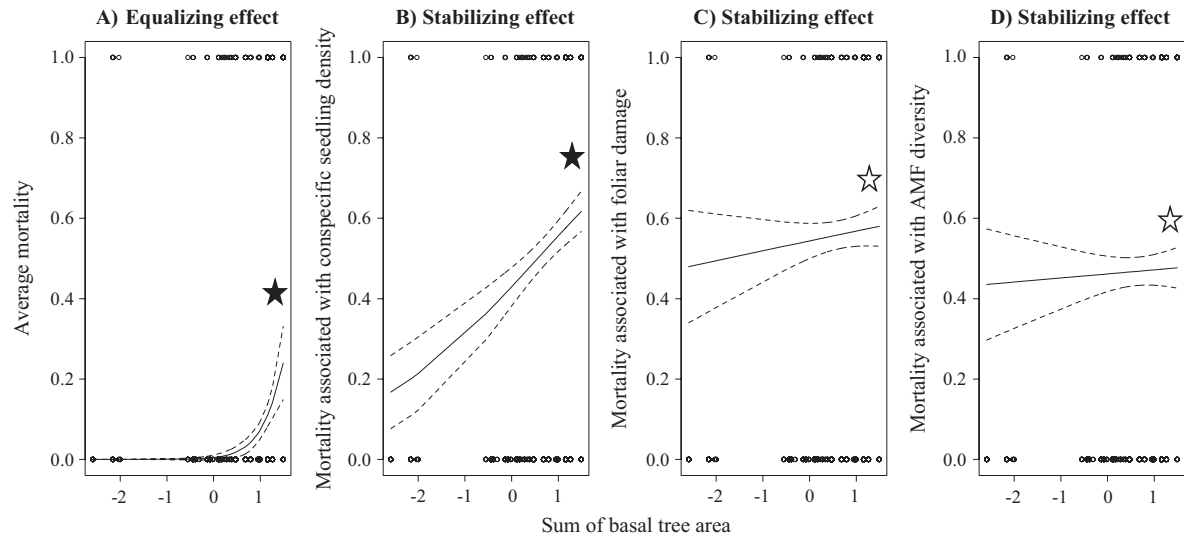


FIG. 2. Community trends are detected by investigating how the effects of a variable depicted in Fig. 1 (conspecific density, foliar damage, or arbuscular mycorrhizal fungi [AMF] diversity) coupled with fitness differences change along species abundance measured at the community scale (sum of basal tree area, log-transformed z -transformed and log-transformed). (A) The average mortality predicted by species-specific analyses showed a significant compensatory community trend, indicated by a solid star. Common species died on average more than rare species, consistent with an equalizing effect. (B) Mortality due to conspecific seedling density also displayed a significant compensatory trend since common species suffer more than rare species (stabilizing effect). (C) Mortality due to foliar damage behaved as a compensatory trend since common species suffer more than rare species (stabilizing effect). Yet this trend was not significant, as indicated by an open star. (D) Mortality associated with AMF diversity also followed a compensatory trend since common species suffer more than rare species (stabilizing effect). Yet this trend was not significant.

greater foliar damage from enemies, potentially promoting coexistence. High conspecific seedling density, our proxy for the sum of other density-dependent effects, also disadvantaged common species at the community scale. The positive effects of soil AMF diversity on seedling survival of rare tree species at the community scale further magnified these effects of foliar damage and conspecific densities. Therefore, at the community scale, natural enemy pressure and AMF diversity in soil act together to promote tree species diversity in this tropical forest.

Foliar damage increases seedling mortality at the local scale and in common species but not in rare tree species at the community scale

Consistent with our hypothesis, seedling mortality at the local scale (1 m²) increased with foliar damage. Herbivore insects and pathogens were likely to drive this pattern since they caused most of the observed foliar damage (Bachelot et al. 2015c). This result is consistent with a study from Costa Rica (Bachelot and Kobe 2013) and a recent experimental study (Bagchi et al. 2014) that found that insecticide reduced seedling mortality. At the community scale (16-ha plot), we found that the effect of foliar damage changed across tree abundance categories. The 95% CI of these effects did not overlap zero for common tree species, consistent with stabilizing effects of natural enemies and with a compensatory community trend (Connell et al. 1984). The lack of an effect of foliar damage on seedling mortality of rare species might arise

from low amount of damage. In contrast, seedlings of intermediate abundance tree species, whose mortality also did not increase with foliar damage, experienced significantly more damage than rare species. The lack of an effect of foliar damage on seedling mortality of intermediate abundance tree species suggests that these seedlings may be more tolerant to damage than seedlings of common tree species, which experience similar levels of damage as seedlings of intermediate abundance tree species. Future investigations of the JC hypothesis should consider incorporating trait data on tolerance to damage (e.g., regrowth traits) into seedling mortality models to account for tolerance differences across tree species.

High conspecific seedling density reduces seedling survival at the local scale and in common species but not in rare tree species at the community scale

Consistent with the JC hypothesis, we detected a significant effect of conspecific seedling density on seedling mortality at the local scale. Seedling mortality increased with high conspecific seedling densities. This result lends support to a recent meta-analysis that concluded that JC effects are widespread in tropical ecosystems (Comita et al. 2014). However, JC studies in situ usually focus on patterns of mortality across conspecific seedling densities without incorporating measures of natural enemy pressure. Here, we simultaneously investigated the effects on conspecific seedling density and foliar damage. The presence of a significant effect for both conspecific seedling density and

foliar damage suggests that foliar damage alone might not act in a density dependent fashion at the local scale (1 m²).

Tree species abundance alters the magnitude of conspecific seedling density effects on seedling mortality. In particular, we found that seedling mortality of rare and intermediate-abundance tree species did not change with conspecific seedling density. However, high conspecific seedling density increased seedling mortality of common species, consistent with stabilizing effects of negative feedbacks and with a community compensatory trend (Queenborough et al. 2007, Bagchi et al. 2014, Bachelot et al. 2015a). Our results contrast with a number of studies either failed to detect a community compensatory trend (Metz et al. 2010) or found a common tree species advantage, the opposite of a community compensatory trend (Comita et al. 2010, Mangan et al. 2010). Mangan et al. (2010) attributed advantage of common trees species to negative effects of soil microbes on rare species. Contrary to Mangan et al.'s (2010) study, we found that conspecific seedling density heightened seedling mortality of common species, as expected by our hypothesis. Our results remain unchanged when accounting for effects of conspecific and heterospecific adult trees on the soil biota, which could reflect covarying soil biota (Appendix S3: Fig. S6). The discrepancy between the two studies might suggest differences in active soil biota, which are agents of negative density dependence, between our sites and Barro Colorado Island (Panama).

High AMF diversity decreases seedling mortality at the local scale and in rare species but not in common tree species at the community scale

As expected, AMF diversity reduced seedling mortality, which suggests that seedlings benefit from growing in the presence of diverse AMF communities. At the local scale (1 m²), AMF diversity counteracts the local JC effects by enhancing survival, in agreement with results from greenhouse experiments using AMF inoculation of tree seedlings (Hood et al. 2004, Liang et al. 2015). These studies (Hood et al. 2004, Liang et al. 2015) also found that the counteracting effects of AMF was local and declined with distance from conspecific trees. Our results are also consistent with previous studies that linked AMF diversity with grassland ecosystem productivity (Maherali and Klironomos 2007, Wagg et al. 2015). These studies found a positive correlation between AMF diversity and aboveground plant productivity due to complementarity of beneficial effects between AMF taxa (Maherali and Klironomos 2007) or selection mechanisms by which one highly beneficial AMF occurred in diverse AMF community (Wagg et al. 2015). Our study does not allow us to disentangle these two effects. However, our work highlights the importance of AMF diversity for seedling dynamics in tropical forests.

Only rare tree species benefitted from soil AMF diversity, consistent with our hypothesis that AMF diversity would promote tropical tree coexistence at the

community scale by fostering the persistence of rare tree species (Bever 2003, Urcelay and Díaz 2003). This result emphasizes the importance of mutualisms in the persistence of rare tree species as it has recently been shown in grasses (Chung et al. 2015). However, earlier results of AMF fungal effects on forb species contradict our findings, showing larger AMF benefits on the growth of a common forb species (*Pulsatilla*) compared to a rare species of the same genus (Moora et al. 2004). In a similar manner, Hartnett and Wilson (2002) found that AMF diversity tends to enhance the dominance of common species of temperate grasslands. AMF communities and their effects on plant communities are highly context-dependent and vary across ecosystems and plant functional groups (Hoeksema et al. 2010, Davison et al. 2015, Lin et al. 2015) so these differences are not surprising. It is important to note that AMF diversity in soil represents existing diversity in spores and hyphae. Such diversity is not necessarily correlated with diversity of active AMF in seedling roots and is likely to change between dry and rainy seasons (Husband et al. 2002, Herre et al. 2005). Our study was conducted during the dry season and it is possible that our results may not be the same in the wet season. Finally, future experimental work should aim at understand the mechanisms behind the effects of AMF diversity and if they arise from a mass (abundance), complementarity (between AMF species), or selection (one very beneficial AMF) effect.

The lack of a significant effect of AMF diversity on intermediate and common tree species could arise because of the complex costs and benefits of AMF associations for trees (Pfeffer et al. 1999, Johnson and Graham 2013, Bachelot et al. 2015b). As AMF diversity increases, the probability of associations with costly AMF also increases (selection effects). Therefore, the effect of AMF diversity on tropical seedling mortality might be neutral due to the competing selection effects of beneficial and costly AMF.

To conclude, we showed that AMF diversity in soil and natural enemy pressure might act together to promote tree species coexistence by rescuing rare tree species and disadvantaging common tree species. Although, identities of specific AMF and their functional roles were not identified, our study highlights the role of AMF diversity in soil in the maintenance of tropical tree coexistence in some forests. The lack of a community trend of the effects of AMF diversity on seedling mortality in the species-specific analyses might be the result of low within species sample size, leading to low power to detect small effect sizes. This emphasizes the need to increase the size and temporal coverage of the data set. Our study also brings out future research directions: coexistence theories should aim at combining mutualism and negative feedbacks, and experimental work should further investigate the effects of AMF diversity on seedling mortality across tree species abundance.

ACKNOWLEDGMENTS

This work was supported by grants from the Institute of Latin American Studies at Columbia University to M. Uriarte,

K. McGuire, and B. Bachelot; from Columbia University to B. Bachelot; and grants BSR-8811902, DEB 9411973, DEB 0080538, DEB 0218039, DEB 0620910, and DEB 0963447 from NSF to the Institute for Tropical Ecosystem Studies, University of Puerto Rico, and to the International Institute of Tropical Forestry USDA Forest Service for the Luquillo LTER program. DNA data are available at <http://www.ncbi.nlm.nih.gov/sra> with the following accession number: SRP052581. We are grateful for Liza Comita, Rachael Eaton, Jesse Lasky, Duncan Menge, Bob Muscarella, Naomi Schwartz, and Ben Taylor for useful comments on the manuscript. We are also grateful for Allen Herre and anonymous reviewers whose feedbacks improve greatly this study.

LITERATURE CITED

- Abarenkov, K., et al. 2010. The UNITE database for molecular identification of fungi—recent updates and future perspectives. *New Phytologist* 186:281–285.
- Bachelot, B., and R. K. Kobe. 2013. Rare species advantage? Richness of damage types due to natural enemies increases with species abundance in a wet tropical forest. *Journal of Ecology* 101:846–856.
- Bachelot, B., R. K. Kobe, and C. Vriesendorp. 2015a. Negative density-dependent mortality varies over time in a wet tropical forest, advantaging rare species, common species, or no species. *Oecologia* 179:853–861.
- Bachelot, B., M. Uriarte, and K. McGuire. 2015b. Interactions among mutualism, competition, and predation foster species coexistence in diverse communities. *Theoretical Ecology* 8:297–312.
- Bachelot, B., M. Uriarte, J. Thompson, and J. K. Zimmerman. 2015c. The advantage of the extremes: Tree seedlings at intermediate abundance in a tropical forest have the highest richness of above-ground enemies and suffer the most damage. *Journal of Ecology* 104:90–103.
- Bachelot, B., M. Uriarte, J. S. Zimmerman, J. Thompson, J. W. Leff, A. Asaii, J. Koshner, and K. McGuire. 2016. Long-lasting effects of land use history on soil fungal communities in second-growth tropical rain forests. *Ecological Applications* 26:1881–1895.
- 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.
- Bever, J. D. 2003. Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytologist* 157:465–473.
- Bever, J. D., S. C. Richardson, B. M. Lawrence, J. Holmes, and M. Watson. 2009. Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. *Ecology Letters* 12:13–21.
- Brook, S. P., and A. Gelman. 1997. General methods for monitoring convergence of iterative simulations. *Journal of Computational and Graphical Statistics* 7:434–455.
- Caporaso, J., J. Kuczynski, and J. Stombaugh. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7:335–336.
- Chesson, P. 2000. Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics* 31:343–366.
- Chung, Y. A., T. E. X. Miller, and J. A. Rudgers. 2015. Fungal symbionts maintain a rare plant population but demographic advantage drives the dominance of a common host. *Journal of Ecology* 103:967–977.
- Comita, L. S., H. C. Muller-Landau, S. Aguilar, and S. P. Hubbell. 2010. Asymmetric density dependence shapes species abundances in a tropical tree community. *Science* 329:330–332.
- 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., et al. 2000. Spatial patterns in the distribution of tropical tree species. *Science* 288:1414–1418.
- 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. Boer and G. R. Graadwell, editors. *Dynamics of numbers in populations*. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands.
- Connell, J. H., J. G. Tracey, and L. J. Webb. 1984. Compensatory recruitment, growth, and mortality as factors maintaining rain forest. *Ecological Monographs* 54:141–164.
- Davison, J., et al. 2015. Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. *Science* 127:970–973.
- Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26:2460–2461.
- Gelman, A., J. B. Carlin, H. S. Stern, D. B. Dunson, A. Vehtari, and D. B. Rubin. 2013. *Bayesian data analysis*. CRC Press, New York, New York, USA.
- Hall, S. J., W. H. McDowell, and W. L. Silver. 2013. When wet gets wetter: decoupling of moisture, redox biogeochemistry, and greenhouse gas fluxes in a humid tropical forest soil. *Ecosystems* 16:576–589.
- Hartnett, D. C., and G. W. Wilson. 2002. The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. *Plant Soil* 244:319–331.
- Herre, E. A., D. A. Kylo, S. A. Mangan, R. Husband, L. C. Mejia, and A.-H. Eom. 2005. Biotic interactions in the tropics. Pages 204–225 in D. F. R. P. Burslem, M. A. Pinard, and S. E. Hartley, editors. *An overview of Arbuscular Mycorrhizal Fungi composition, distribution, and host effects from a Tropical Moist Forest*. Cambridge University Press, Cambridge, UK.
- Hill, T., K. Walsh, J. Harris, and B. Moffett. 2003. Using ecological diversity measures with bacterial communities. *FEMS Microbiology Ecology* 43:1–11.
- Hoeksema, J. D., et al. 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology Letters* 13:394–407.
- 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.
- Janzen, D. H. 1970. Herbivores and the number of tree species in tropical forests. *American Naturalist* 104:501–528.
- Johnson, N. C., and J. H. Graham. 2013. The continuum concept remains a useful framework for studying mycorrhizal functioning. *Plant and Soil* 363:411–419.
- 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.
- Liang, M. I. L., X. U. L. Iu, R. A. S. E. Tienne, F. E. H. Uang, and Y. O. W. Ang. 2015. Arbuscular mycorrhizal fungi

- counteract the Janzen-Connell effect of soil pathogens. *Ecology* 96:562–574.
- Lin, G., M. L. McCormack, and D. Guo. 2015. Arbuscular mycorrhizal fungal effects on plant competition and community structure. *Journal of Ecology* 113:1224–1232.
- Lovelock, C. E., and J. J. Ewel. 2005. Links between tree species, symbiotic fungal diversity and ecosystem functioning in simplified tropical ecosystems. *New Phytologist* 167: 219–228.
- Maherali, H., and J. N. Klironomos. 2007. Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science* 316:1746–1748.
- 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* 466:752–756.
- McGuire, K. L., et al. 2013. Digging the New York City Skyline: soil fungal communities in green roofs and city parks. *PLoS ONE* 8:e58020.
- Metz, M., W. Sousa, and R. Valencia. 2010. Widespread density-dependent seedling mortality promotes species coexistence in a highly diverse Amazonian rain forest. *Ecology* 91:3675–3685.
- Montesinos-Navarro, A., J. Segarra-Moragues, A. Valiente-Banuet, and M. Verdu. 2012. The network structure of plant–arbuscular mycorrhizal fungi. *New Phytologist* 194: 536–547.
- Moora, M., M. Opik, R. Sent, and M. Zobel. 2004. Native arbuscular fungal communities mycorrhizal influence the seedling performance of rare differentially and common *Pulsatilla* species. *Functional Ecology* 18:554–562.
- Naeem, S., and S. Li. 1997. Biodiversity enhances ecosystem reliability. *Nature* 390:507–509.
- Pfeffer, P. E., D. D. Doups, G. Becard, and Y. Shachar-Hill. 1999. Carbon uptake and the metabolism and transport of lipids in and arbuscular mycorrhiza. *Plant Physiology* 120: 587–598.
- Pizano, C., S. A. Mangan, E. A. Herre, A.-H. Eom, and J. W. Dalling. 2011. Above- and belowground interactions drive habitat segregation between two cryptic species of tropical trees. *Ecology* 92:47–56.
- Queenborough, S. A., D. F. R. P. 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.
- Siqueira, J., and O. Saggi-Junior. 1998. Arbuscular mycorrhizal inoculation and superphosphate application influence plant development and yield of coffee in Brazil. *Mycorrhiza* 7:293–300.
- Smith, S. E., and D. J. Read. 2008. *Mycorrhizal symbiosis*. Third edition. Academic, London, UK.
- Urcelay, C., and S. Diaz. 2003. The mycorrhizal dependence of subordinates determines the effect of arbuscular mycorrhizal fungi on plant diversity. *Ecology Letters* 6:388–391.
- Uriarte, M., S. Canham, J. Thompson, J. K. Zimmerman, and N. Brokaw. 2005. Seedling recruitment in a hurricane-driven tropical forest: light limitation, density-dependence and the spatial distribution of parent trees. *Journal of Ecology* 93:291–304.
- Uriarte, M., C. Canham, J. Thompson, J. K. Zimmerman, L. Murphy, A. Sabat, N. Fetcher, and B. Haines. 2009. Natural disturbance and human land use as determinants of tropical forest dynamics: results from a forest simulator. *Ecological Monographs* 79:423–443.
- van der Heijden, M., J. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396: 69–72.
- van der Putten, W. H., et al. 2013. Plant–soil feedbacks: the past, the present and future challenges. *Journal of Ecology* 101:265–276.
- Verbruggen, E., M. G. A. Van Der Heijden, J. T. Weedon, G. A. Kowalchuk, and W. F. M. Rø-Ling. 2012. Community assembly, species richness and nestedness of arbuscular mycorrhizal fungi in agricultural soils. *Molecular Ecology* 21: 2341–2353.
- Wagg, C., C. Barendregt, J. Jansa, and M. G. A. van der Heijden. 2015. Complementarity in both plant and mycorrhizal fungal communities are not necessarily increased by diversity in the other. *Journal of Ecology* 103:1233–1244.
- Zimmerman, J. K., L. S. Comita, J. Thompson, M. Uriarte, and N. Brokaw. 2010. Patch dynamics and community metastability of a subtropical forest: compound effects of natural disturbance and human land use. *Landscape Ecology* 25: 1099–1111.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.1683/supinfo>