Associations among arbuscular mycorrhizal fungi and seedlings are predicted to change with tree successional status

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Abstract. Arbuscular mycorrhizal (AM) fungi in the soil may influence tropical tree dynamics and forest succession. The mechanisms are poorly understood, because the functional characteristics and abundances of tree species and AM fungi are likely to be codependent. We used generalized joint attribute modeling to evaluate if AM fungi are associated with three forest community metrics for a sub-tropical montane forest in Puerto Rico. The metrics chosen to reflect changes during forest succession are the abundance of seedlings of different successional status, the amount of foliar damage on seedlings of different successional status, and community-weighted mean functional trait values (adult specific leaf area [SLA], adult wood density, and seed mass). We used high-throughput DNA sequencing to identify fungal operational taxonomic units (OTUs) in the soil. Model predictions showed that seedlings of mid- and late-successional species had less leaf damage when the 12 most common AM fungi were abundant compared to when these fungi were absent. We also found that seedlings of mid-successional species were predicted to be more abundant when the 12 most common AM fungi were abundant compared to when these fungi were absent. In contrast, early-successional tree seedlings were predicted to be less abundant when the 12 most common AM fungi were abundant compared to when these fungi were absent. Finally, we showed that, among the 12 most common AM fungi, different AM fungi were correlated with functional trait characteristics of early- or late-successional species. Together, these results suggest that early-successional species might not rely as much as mid- and late-successional species on AM fungi, and AM fungi might accelerate forest succession.

Key words: arbuscular mycorrhizal fungi; functional traits; generalized joint attribute modeling; Luquillo Forest Dynamics Plot; Puerto Rico; succession; tropical forests.

Introduction

Arbuscular mycorrhizal (AM) fungi play an important role in the tree community dynamics of tropical forests (Dickie et al. 2015), but their importance for successional processes is uncertain. Several studies have demonstrated that AM fungi increase nutrient acquisition (Clemmensen et al. 2015), increase water uptake (Cooper and Tinker 1978), improve plant protection against natural enemies (Bi et al. 2007, Jung et al. 2012), and may promote tropical tree coexistence (Liang et al. 2015, Bachelot et al. 2016*a*). Tree species differ in their AM fungal responsiveness (e.g., Hoeksema et al. 2010) that is the growth difference with and without AM fungal inoculation (Janos

2007), and AM fungal dependency (e.g., Weremijewicz and Seto 2016) that is the inability to grow without AM fungi at low availability of phosphorus (Janos 2007). Therefore, AM fungi might be expected to influence tree community composition (e.g., Merrild et al. 2013, Van Der Heijden et al. 2015). In addition, interspecific differences in tree responsiveness to AM fungi may arise from differences in growth strategies (Koziol and Bever 2015) that are linked to differences in tree functional traits (Siqueira et al. 1998, Zangaro et al. 2003, Eissenstat et al. 2015). Similarly, differences in AM fungal dependency across species might arise from functional trait differences (Weremijewicz and Seto 2016). As a result, tree taxonomic and functional community composition may interact with AM fungal community composition (Peay et al. 2013) and create feedbacks that could influence tree successional dynamics (Janos 1980*a*, *b*, García De León et al. 2016).

We propose that differences in tree functional traits (measured by community-weighted means) related to

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their successional status and growth strategy (slow- vs. fast-growing species) might explain differences in AM fungal effects among tropical tree species. Evidence for such differences would be manifested in correlations between AM fungal communities and tropical tree communities. Early-successional species are characterized by large specific leaf area (SLA, Wright et al. 2010) and low wood density to support rapid growth. Furthermore, early-successional species tend to be fast colonizers with small seeds (Westoby et al. 2002). In contrast, late-successional species tend to produce large seeds (Westoby et al. 2002), and to display conservative traits that may include low SLA, high wood density, and slow growth (Poorter 2007).

Differences in adult tree functional traits with successional status might explain differences in the association of AM fungi with tree species along a forest succession gradient. On one hand, fast-growing early successional species typically have small seeds that might have limited carbon to invest in mycorrhizal associations. During forest succession, small-seeded pioneer species are replaced by slow-growing species with larger seeds that establish extensively AM-colonized root systems (Janos 1980, Huante et al. 1993). Therefore, AM dependency might increase along forest succession, if seed size is positively correlated with AM fungal dependency. On the other hand, requirements for rapid growth could promote a decrease in AM fungal dependency during succession (Allen and Allen 1990). Rapid growth of early successional species under strong nutrient or water limitation may only occur with AM fungal inoculation and facilitation (Walker et al. 2010). In contrast, slow growth of late successional species might not require AM fungal inoculation.

In tropical ecosystems, early empirical evidence suggested an increase in AM dependency along succession (Janos 1980a), but recent empirical evidence (e.g., Zangaro et al. 2013) is consistent with the hypothesis of decreasing tree dependency on AM fungi during succession (Allen and Allen 1990). In general, AM spores in the soil are more abundant early in succession (Fischer et al. 1994, Aidar et al. 2004, De Castro et al. 2008, Stürmer and Siqueira 2011, Zangaro et al. 2012, 2013). Inoculation potential of roots (Aidar et al. 2004, Zangaro et al. 2012, 2013) and root colonization (Aidar et al. 2004, Pasqualini et al. 2007, Zangaro et al. 2012, 2013) also decline with successional stage. Following the general pattern of decreasing AM dependence during succession, evidence suggests a decrease in AM responsiveness during succession: pioneer seedling growth rate is usually faster with (compared to without) AM fungi, whereas late-successional seedling growth rate does not depend on colonization by AM fungi (Fischer et al. 1994, Kiers et al. 2000, Matsumoto et al. 2005). However, this pattern could arise because large-seeded late successional species responses might be restricted by pot size and short experimental duration (Janos 2007). It is important to point out that mycorrhizal responsiveness and dependency may not follow the same pattern along succession (Janos 2007). In

this study, we are investigating how mycorrhizal responsiveness changes along succession.

Associations between AM fungi and seedlings belonging to different tree successional status groups may be obscured by biotic and abiotic factors that alter fungal and plant communities. Fungal taxa are characterized by different degrees of host preference (Husband et al. 2002), which could result in strong associations between adult trees, seedlings, and fungi and determine successional trends. The Janzen-Connell (JC) hypothesis predicts that specialized natural enemies might be more abundant near parent trees (Janzen 1970, Connell 1971). Empirical work highlighted that fungal pathogens acted as agents of JC effects (e.g., Bagchi et al. 2010, 2014). Additionally, AM fungi were found to counteract JC effects (Liang et al. 2015) including in our study site (Bachelot et al. 2017). In particular, Liang et al. (2015) found experimentally that AM fungi promoted seedling growth near adult trees, but the effect declined with distance from the adult tree. Overall, this suggested that the adult tree community should also be considered when modeling seedling and fungal communities.

Fungal community composition also reflects soil characteristics (Garbeva et al. 2004, Lauber et al. 2008), including in our study site (Bachelot et al. 2016b). In a previous study, we analyzed which abiotic and biotic factors were associated with the fungal soil communities identified from fungal DNA extracted from soil samples (Bachelot et al. 2016b). We found that these fungal communities were principally correlated with soil edaphic factors (soil moisture, soil pH, total soil carbon, available soil iron, and available soil aluminum, but not available soil phosphorus extracted with Mehlich 1 extraction solution), light availability, and land use history (Bachelot et al. 2016b). Therefore, observational studies may help us assess associations between AM fungi and plant community-weighted mean functional traits, seedling abundance, and foliar damage across tree successional status by taking into consideration important abiotic and biotic factors that might alter fungal and plant communities.

Seedlings represent an important bottleneck in the tree life cycle, because they suffer high mortality rates and are sensitive to abiotic and biotic conditions (Poorter 2007). Therefore, seedlings might be disproportionately influenced by interactions with fungi, leading to an interdependent response to the environment. In other words, seedling responses to the environment do not "cause" the response of the fungal communities; rather, seedlings and fungi interact and jointly respond to the environment. In this study, we made use of a Long-Term Ecological Research plot in which seedling dynamics is recorded annually (Zimmerman et al. 2007). In order to limit research impact on this long-term dynamic plot, we assessed the fungal community present in the soil surrounding the seedlings, rather than harvesting the seedlings to identify fungi in the roots. This approach cannot identify exact associations between seedlings and fungi,

but more importantly it enables us to fully describe the fungal community rather than only describing fungi in the roots, thereby allowing characterization of the co-dependence structure of the community.

Understanding of the role of fungal communities on ecosystem functioning and forest dynamics has been greatly improved by next generation sequencing, which enables the quick description of fungal communities in soil using specific fungal DNA primers. Such an approach identifies a large number of fungal operational taxonomic units (OTUs), or fungal phylotypes, based on DNA sequence similarity. The statistical analysis and synthesis of evidence using a large number of fungal OTUs and the responses of multiple plant hosts of different successional status, however, has been impeded by a lack of probabilistic models (Clark and Hersh 2009, Hersh et al. 2012). Soil fungal community data have generally been analyzed using one or two approaches. The first is univariate, treating each OTU as an independent response. This approach misses the fact that fungal taxa respond to each other as well as to the abiotic and biotic environment. Moreover, environmental DNA data are composition data; the abundance of any one taxon recorded as the number of reads in the sample induces codependence. The alternative multivariate analyses (e.g., Bachelot et al. 2016b, Paliy and Shankar 2016) are descriptive and do not provide probability statements in terms of a joint distribution of compositional data, confidence intervals, or predictive distributions. To complicate further interpretation of statistical analyses, not only fungi, but also characteristics of the plant hosts are response variables, leading to one mutually dependent response to the environment, composed of many OTUs and status of the host. Probabilistic interpretation requires a method of analysis that covers the joint responses of host plants and soil fungal communities. Here, we implemented generalized joint attribute modeling (GJAM; Clark 2016, Clark et al. 2017) approach to account for the underlying covariance structure, together with the heterogeneous data types that include composition (e.g., fungal OTUs composition), counts (e.g., seedling abundance), and continuous variables (e.g., community-weighted mean functional traits). This probabilistic approach allows us to conditionally predict seedling abundance, foliar damage, and community-weighted mean functional traits under different levels of AM fungal abundance.

Establishing clearer links between plant and fungal responses to environmental conditions is critical for advancing our understanding of forest dynamics. In this paper, we evaluated model 1, the abundance of seedlings and AM fungal abundance; model 2, the amount of foliar damage on seedlings and AM fungal abundance; and model 3, the joint responses of community-weighted mean functional traits and AM fungal abundance to environmental variation. We were interested in assessing how AM fungi might influence tree successional trajectories by differentially influencing the abundance of

seedlings associated with distinct successional stages, by influencing the amount of foliar damage to which trees are subjected, and by associating with tree species with different functional strategies.

In this study, we asked three questions using conditional predictions: (1) Are seedlings of early- or late-successional tree species more abundant when the 12 most common AM fungi are abundant? (2) Do seedlings of early- or late-successional tree species host less damage when the 12 most common AM fungi are abundant? (3) Is abundance of the 12 most common AM fungi associated with early successional traits of tree species- (low seed mass, large specific leaf area (SLA), and low wood-density) or with late-successional functional traits? We hypothesized that if early successional tree species are more AM responsive than late-successional species (e.g., Allen and Allen 1990, Zangaro et al. 2013) the abundance of the 12 most common AM fungi would be associated with high early-successional seedling abundance, low foliar damage on early-successional tree species, and with early-successional species traits. An alternative hypothesis is that, if late successional species are more AM responsive than early-successional species (Koziol and Bever 2015), we expect that the abundance of the 12 most common AM fungi would be associated with high late-successional seedling abundance, low foliar damage on late-successional species, and with late-successional species traits.

METHODS

We used seedling and fungal data, which were collected in and around long-term seedling plots in a subtropical montane forest in Puerto Rico. The seedling plots are strategically distributed across the Luquillo Forest Dynamics Plot (LFDP; see *Study site*; Zimmerman et al. 2007, Comita et al. 2009) to capture a broad range of environmental heterogeneity (Thompson et al. 2002). The fungal census was conducted during the same season as the 2012 seedling census, allowing us to test whether or not the local fungal community is associated with the community-weighted mean functional traits of adult traits (wood density, SLA, and seed mass), the abundance of tree seedlings of different successional status, and the amount of damage experienced by these seedlings.

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 with elevation ranging from 333 to 428 m above sea level (Thompson et al. 2002). This forest experiences a mean annual rainfall of 3,500 mm and temperature of 25.9°C (Thompson et al. 2004), classifying it as subtropical montane under the Holdridge system (Ewel and Whitmore 1973). Five volcanic soil types dominate the Luquillo plot (Thompson et al. 2002): a well-drained oxisol (Zarzal), a poorly drained ultisol (Cristal), and three additional soils that cover less than 11% of the

LFDP (Prieto, Fluvaquent, and Coloso). Here, we pooled these less common three soil types into a single category, "other." The 16-ha plot has a well-documented history of land use, which has had strong and long-lasting effects on tree (Thompson et al. 2002) and fungal communities (Bachelot et al. 2016b) and soil nutrients (Uriarte et al. 2015). The southern one-third of the plot, steeper and rockier than the northern part, experienced a low level of human activity whereas the northern part experienced farming and logging (Thompson et al. 2002).

Tree and seedling censuses

Trees.—Approximately every five years since 1990, all free-standing woody stems ≥1 cm dbh (diameter at 1.3 m height above the ground) in the LFDP have been mapped, identified to species, and measured (Zimmerman et al. 2010). Tree demography following natural disturbances (hurricanes Hugo in 1989 and Georges in 1998) enabled the classification of tree species into successional classes. To account for dispersal abilities and the fingerprint of the trees that can influence seedling counts, foliar damage, and fungal communities, we calculated the distance of each seedling plot to the closest early-, mid-, and late-successional tree species (Appendix S1: Table S1) for trees ≥1 cm dbh using the 2011 census.

Seedlings.—Starting in 1998, 213 1 × 2 m seedling plots were established throughout the LFDP (Comita et al. 2009). An additional 120 clusters of three 1-m^2 seedling plots were added in 2007 along a north-south-running trail, with each cluster approximately 10 m from the trail (Zimmerman et al. 2007). Seedlings in all of these plots have been mapped, identified to species, and measured annually since 2007. In 2012, we collected foliar damage data, using one of the three 1-m^2 seedling plots from each of the 120 clusters as well as 117 of the 213 1 × 2 m seedling plots (n = 237 plots total). From this 2012 census, seedling counts (excluding lianas), of early-, mid-, and late-successional tree species were assessed (42 seedling species were pooled into three successional categories; Appendix S1: Table S1). In total, early-, mid-, and late-successional tree species represented 19%, 45%, and 36% respectively of the 42 tree species.

Seedling foliar damage assessment.—In May–July 2012, we used a digital camera (Nikon D3100, Tokyo, Japan) with a macrolens (18–55 mm VR lens) to photograph all leaves, or a maximum of five leaves, of every seedling present in each of the 237 focal seedling plots. From these photos, we estimated the proportion of leaf area damaged by pathogens and herbivores, using a categorical 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 (see details in Bachelot et al. 2016a). In total, we obtained damage data for 1986 individual tree seedlings representing 42 tree species. We calculated mean foliar damage experienced by

early-, mid-, and late-successional species in each seedling plot. It is important to note that we did not separate the damage by types (such as leaf miner or herbivory).

Tree and seedling functional traits.—Several functional traits were measured on adults of all woody tree and shrub species present in the LFDP (Swenson et al. 2012, Swenson and Umaña 2015, Umaña et al. 2015) using standard procedures (Cornelissen et al. 2003). For this study, we focused on three functional traits associated with tree successional status: adult wood density, adult specific leaf area (SLA), and seed mass. For each plot and for each trait, we calculated a community-weighted mean functional trait value, as the average trait value across all species that are present in the seedling plot weighted by the number of seedling stems in the seedling plot. It is important to note that we do not assume that functional traits remain constant along ontogeny. However, interspecific trait variations remain consistent along ontogeny (Spasojevic et al. 2014), and adult traits capture differences in growth strategy and life history across species (Kraft et al. 2010). Therefore, we used adult traits to investigate how the abundance of the 12 most common AM fungi is associated with different growth strategies and life histories.

Fungal survey

During May–July 2012, we sampled soils (0–20 cm deep and 2.5 cm diameter cores) from four corners of each of the 237 focal seedling plots and combined the soil samples before using them to characterize the fungal community using DNA extraction and sequencing (see details in Bachelot et al. 2016b). Briefly, fungal DNA was extracted using the PowerSoil DNA isolation kit (MoBio, Carlsbad, California, USA) and was sequenced using barcoded high-throughput sequencing on the Illumina MiSeq platform at the University of Colorado, Boulder, Colorado, USA (see details in Bachelot et al. 2016b). We used two general fungal primers, targeting the first internal transcribed spacer region (ITS1) of the fungal rRNA gene, to enable full characterization of the fungal community: ITS1-F (CTTGGTCATTTAGAG-GAAGTAA) and ITS2 (GCTGCGTTCTTCATC-GATGC). All DNA reads were de-multiplexed, quality-filtered, and processed using the QIIME v. 1.5.0-dev pipeline with default settings except as noted (Caporaso et al. 2010). We used a 97% similarity threshold to cluster sequences into operational taxonomic units (OTUs) using the open reference-based procedure in QIIME (see details in Bachelot et al. 2016b). In this study, unlike previously done in Bachelot et al. (2016b), we did not rarify the data in order to keep as much information as possible to fit the joint-species model. DNA was successfully extracted and sequenced in soil from 214 seedling plots (86 from low land use history areas and 128 in high land use history areas). On average, there were 1,587 reads per sample (ranging from 340 to 3,596). We used

Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990) with the nucleotide database (GenBank), excluding sequences not associated with known organisms, to return the top 10 taxonomic identities for each OTU along with the coverage query, the maximum identity, the E value, and the taxon ID associated with each hit. In order to attribute a full taxonomy (to the species rank) of an OTU, the top hit had to have a query coverage of at least 95%, a maximum identity greater or equal to 90%, and an E value near 0. When the top 10 matches from BLAST belonged to different phyla and both the query coverage and the maximum identity were below 60%, we reported the OTU as being "unknown". Finally, when the 10 matches fell between the species and unknown criteria and were within the same phylum, we recorded solely the taxonomy to the family. A total of 8,997 distinct fungal OTUs were identified, including 716 AM fungal OTUs belonging to nine AM fungal families. Glomeraceae dominated the AM fungal OTUs (78%), followed by Acaulosporaceae (6%), Gigasporaceae (3%), and Diversisporaceae (2%). AM fungal OTUs were identified in all 214 plots. On average, 6% of fungal reads in each plot belonged to AM fungal OTUs (ranging from 0.01% to 23.5%). AM fungi extracted from the soil could have included inactive or unassociated fungi and no definite association with plant species or particular tree or seedling roots could be determined.

Environmental variables

We used eight environmental variables that were previously found to be associated with entire fungal community composition (saprotrophs, pathogens, parasites, and AM fungi) at our site, using the same data set as in this study (Bachelot et al. 2016b). For each seedling plot, we used intensity of land use history (low and high), soil type (Zarzal, Cristal, and other), light availability, water flow, and soil pH and element data (aluminum, iron, and carbon). Bachelot et al. (2016b) showed that these elements were strongly correlated with soil fungal communities. Soil pH and nutrient content (carbon, aluminum, and iron) were measured for each soil sample. Soil pH was measured after 30 min incubation in a 1:1 (weight fraction) soil:H_2O solution. Total soil carbon was measured by combustion analysis (Elementar Vario Macro CNS Analyzer, Auburn University). Plant available soil iron and aluminum concentrations were obtained using inductively coupled argon plasma (ICAP) spectroscopy. Plant available soil phosphorus was not significantly associated with these fungal communities and therefore removed from the analyses. A non-significant association between soil phosphorus and fungal communities could be because Mehlich 1 extraction solution followed by ICAP does not measure accurately the amount of phosphorus available for plants: Mehlich 1 extraction solution performs best at extracting phosphorus from Al-P, Fe-P, and Mn-P minerals but misses phosphorus in Ca-P and Mg-P minerals.

Alternatively, the lack of a significant association between soil phosphorus and fungal community at our site could be the result of low amount of variation in phosphorus concentration. Water flow, a proxy for soil drainage, was determined using ArcGIS from elevation maps of Luquillo (hydrology toolbox; ESRI 2011) and light (percent canopy openness) was obtained using the Sky package in R 3.1.1 (R Core Team 2013), from hemispherical canopy photographs taken in fall 2010 and spring 2011 (Bachelot 2016).

Models to determine associations among fungi, tree seedling communities, and environmental variables

To study seedling and fungal distributions, we used generalized joint attribute modeling (GJAM; Clark et al. 2017). This multivariate Bayesian model allowed us to analyze jointly the fungal and seedling community, accounting for direct and indirect responses to the environment (land use intensity, soil type, light availability, water flow, soil pH, soil iron, soil aluminum, soil carbon, and distances from early-, mid-, and late-successional trees). Use of GJAM for this application is motivated by the fact that data are (1) multivariate, (2) of different types (composition count OTUs, count seedlings, continuous foliar damage, and community-weighed mean trait values), and (3) dominated by zeros. GJAM accommodates all of these considerations in a fully coherent probabilistic model (Clark et al. 2017, Taylor-Rodríguez et al. 2017, see model details in Appendix S1).

GJAM presents four major advantages over alternative approaches by (1) combining data types (the OTU compositional structure of the fungal communities, the count structure of seedlings, the continuous structure of community-weighted traits); (2) accounting for the covariance among response variables; (3) accommodating the massive overrepresentation of zeros in environmental fungal DNA and seedling count data from the seedling plots; and (4) returning all parameters (coefficients and covariances) on the observation scale. We included in the analysis the 99 fungal OTUs that were observed in at least 10% of the plots and that showed strong dependence on each other; among these 99 OTUs there are 12 AM fungal OTUs (Appendix S1: Table S3) and 87 non-AM fungal OTUs. These 12 most common AM fungal OTUs were on average found in 60% of the 214 seedling plots (range 20–92%, Appendix S1: Table S3). The remaining (8,898) OTUs were pooled into an "other" category. These OTUs are too rare to enable estimation of their responses to the environment and pooling them enables us to maintain some of the codependence structure. The potentially high-dimensional response matrix resulted in three main products in GJAM: a predictor by species matrix of environmental covariates ($\mathbf{X}$), a species-by-species covariance matrix ($\mathbf{\Sigma}$), and predicted species responses to predictor variables ($\mathbf{\beta}$). The species-by-species covariance matrix ($\mathbf{\Sigma}$) captures the residual codependence among species after

removing the main structure explained by the model ($\mu = \beta X$). As a result, Σ plays a central role in predictions by allowing us to conditionally predict the responses of a set of species (y_{ss}) under different scenarios for the abundances of other species (y_o). For example, we can predict the responses of early-, mid-, and late-successional seedling species to different scenarios of AM fungal abundance.

Model fitting involved two response matrices, each a combination of more than one data type. This was necessary because seedlings, fungal communities, tree functional traits, and foliar damage might be interdependent. To determine if the environment and the fungal community were associated with the community-weighted mean functional trait values, the abundance of seedlings of early-, mid-, and late-successional species, and the amount of foliar damage experienced by seedlings of early-, mid-, and late-successional species, we fitted several models with a range of explanatory (Appendix S1: Table S2) and response matrices. The response matrices included combinations of fungal OTUs (100 OTUs), community-weighted mean functional traits (three traits), seedling abundances, and seedling foliar damage (42 species pooled into three successional groups, Appendix S1: Table S1). Model 1 was used to assess how the 12 most common AM fungi were associated with seedling abundances by incorporating a response matrix that combined the seedling abundances for the three successional groups with fungal OTU composition. Then, model 2 was developed to evaluate how the 12 most common AM fungi were associated with seedling foliar damage by using a response matrix that comprised seedling damage for the three successional groups and fungal OTU composition. Finally, model 3 was constructed to determine how the 12 most common AM fungi were associated with the three community-weighted mean functional adult and seed traits weighed by seedling abundance using a response matrix made of the three community-weighted mean functional traits and fungal OTU composition. In all cases, we tested 11 environmental covariates in the explanatory matrices and we used the deviance information criterion (DIC; Spiegelhalter et al. 2002) to select the best set of variables for each of the three models (that is the model with the lowest DIC, Appendix S1: Table S2).

Each model was fitted using a non-informative prior for the coefficients and covariance matrix, 20,000 iterations with a burn-in of 5,000 iterations. After fitting the three models, we conditionally predicted community-weighted mean trait values, seedling abundance, and foliar damage in the absence of AM fungal OTUs (columns of the response matrix set to 0 for the 12 AM fungal OTUs), and in the presence of 12 abundant AM fungal OTUs (columns of the response matrix set to their highest observed values for the 12 AM fungal OTUs). These conditional predictions allowed us to draw conclusions about associations of the 12 most common AM fungi with seedling abundance, seedling foliar damage,

and functional traits. We then used Kolmogorov-Smirnov (Sokal and Rohlf 1981) tests to compare the mean conditional predictions between the two AM fungal conditions (Abundant AM fungi or no AM fungi). Following a similar approach, we also investigated how the presence and absence of individual AM fungus OTUs altered the community-weighted mean functional traits, seedling abundances, and seedling foliar damage.

RESULTS

As hypothesized, late-successional species tended to have higher wood density and seed mass but lower SLA than early-successional species (Appendix S1: Fig. S1).

Early-successional species abundance was predicted to decrease in the presence of the 12 most common AM fungi (Model 1)

Model 1 converged well and was able to fit the fungal OTU and seedling abundance data (Fig. 1a). Land use history and soil types were strong predictors of seedling and fungal communities (Appendix S1: Figs. S2, S3). Abundance of many fungi (including AM fungi) increased with concentration of aluminum. Surprisingly, fungi were not strongly associated with the distance to the closest early-, mid-, and late-successional tree (Appendix S1: Fig. S2). Water flow, light, and soil carbon were not selected by the best form of model 1 (Appendix S1: Table S2). Early- and mid-successional tree seedling species responded in a similar way to the environment as a first group of AM fungi (group 1), late-successional species responded in a similar way to the environment as different of AM fungi (group 2, Fig. 2a).

Conditional predictions showed that early-successional tree seedlings were significantly more abundant when the 12 most common AM fungi were absent compared to when these AM fungi were abundant (Fig. 3a, $D = 0.95$, $P < 0.001$). In contrast, mid-successional species had significantly lower abundance when the 12 most common AM fungi were absent compared to when these AM fungi were abundant (Fig. 3a, $D = 0.61$ and $P < 0.001$). The individual presence of each of the 12 most common AM fungal OTUs was significantly associated with lower seedling abundance of early-successional species compared to in the absence of AM fungal OTUs (Appendix S1: Table S3, Fig. S4). The presence of two of the 12 most common AM fungal OTUs were significantly associated with higher seedling abundance of mid-successional species compared to in the absence of these two AM fungal OTUs (Appendix S1: Table S3, Fig. S4).

Both mid- and late-successional tree species were predicted to exhibit lower amounts of foliar damage in the presence of the 12 most common AM fungi (Model 2)

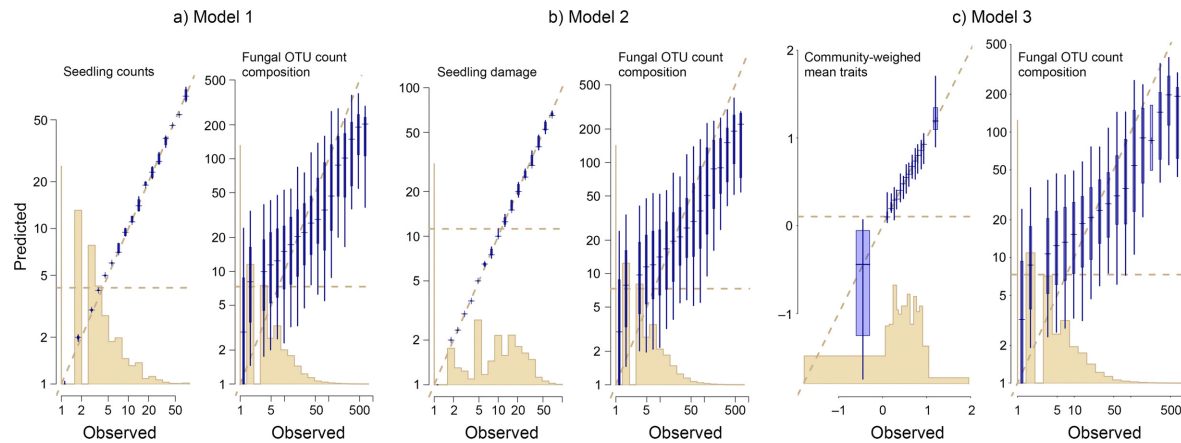Model 2 fitted fungal OTU abundances and seedling foliar damage (Fig. 1b). The best model did not include

Fig. 1. Fitted response variables of the three models. The response variables are pooled by response variable types (composition counts, discrete counts, or continuous): fungal operational taxonomic unit (OTU) abundance and seedling counts (model 1), fungal OTU abundance and foliar damage (model 2), and fungal OTU abundance and z-transformed community-weighted mean functional traits (model 3). The histograms represent the distribution of the observed response variables, the boxes show the 95% predicted response variables plotted in bins (width of the bin stands for the x-axis values in the bin, vertical lines show the full range of predicted values within the bin, middle line shows the mean of the observed variables), the red dash line is the 1:1 line, the horizontal dash line show the mean observed value.

soil type, water flow, or soil carbon (Appendix S1: Table S2). Fungal OTUs and foliar damage were similarly sensitive to the remaining variables (land use intensity, light availability, soil pH, soil aluminum, soil iron, distances from nearest early-, mid-, and late-successional trees; Appendix S1: Fig. S6). Foliar damage in early- and mid-successional species seemed to respond in the same ways to the environment as the group 1 of AM fungi (Fig. 2b). Foliar damage of early- and mid-successional species increased significantly with soil aluminum but decreased significantly with iron (Appendix S1: Fig. S5). Foliar damage on seedlings of early-successional species significantly decreased with distance from nearest mid-successional tree, but increased with distance from nearest late successional tree (Appendix S1: Fig. S5). Foliar damage on seedlings of mid-successional species significantly increased away from tree regardless of its successional status (Appendix S1: Fig. S5). Finally, foliar damage on seedlings of late-successional status significantly decreased with distance from nearest mid- or late-successional tree (Appendix S1: Fig. S5).

Conditional predictions showed that foliar damage in mid- and late-successional species were significantly higher when the 12 most common AM fungi were absent compared to when these AM fungi were abundant (Fig. 3b, $D = 0.24$ and 0.33, both $P < 0.001$, respectively). In contrast, foliar damage in early-successional species was very low and did not significantly change when the 12 most common AM fungi were absent compared to when these AM fungi were abundant (Fig. 3b, $P > 0.05$). The presence of each of the 12 most common AM fungal OTU were not significantly associated with changes in the amount of foliar damage of early-, mid-, or late-successional species (Appendix S1: Fig. S4).

The 12 most AM fungi were predicted to be associated with low SLA, low seed mass, and low wood density (Model 3)

Model 3, which investigated community-weighted mean functional traits and fungal responses to the environment, fitted the abundance of fungal OTUs and community-weighted mean functional traits (Fig. 1c). All environmental covariates were selected in the best form for model 1 (Appendix S1: Table S2). The community-weighted mean functional traits were strongly influenced by soil type, land use intensity, soil iron, aluminum, and pH (Appendix S1: Figs. S7, S8). In particular, late successional traits (high seed mass and low SLA) were associated with low land use intensity, and early successional traits (low seed mass and high SLA) were associated with high land use intensity (Appendix S1: Fig. S7). Overall, community-weighted mean SLA and wood density responded in a similar way to the environment as two-thirds of the AM fungal OTUs (Fig. 2c, Group 1). Seed mass responded similarly to the environment as the remaining one-third of the AM fungal OTUs (Fig. 2c, Group 2).

Conditional predictions showed that, in the absence of the 12 most common AM fungi, community-weighted mean wood density, SLA, and seed mass increased compared to when these AM fungi were abundant (Fig. 3c, $D = 0.24$, 0.55, and 0.25, respectively, $P = 0.001$, <0.001, and <0.001, respectively). Each of the 12 most common AM fungi was associated differently with community-weighted mean functional traits (Appendix S1: Table S3, Fig. S4).

DISCUSSION

Arbuscular mycorrhizal fungi might be important drivers of tropical tree succession by associating with

a) Model 1: Seedling abundance and fungal community

b) Model 2: Foliar damage and fungal community

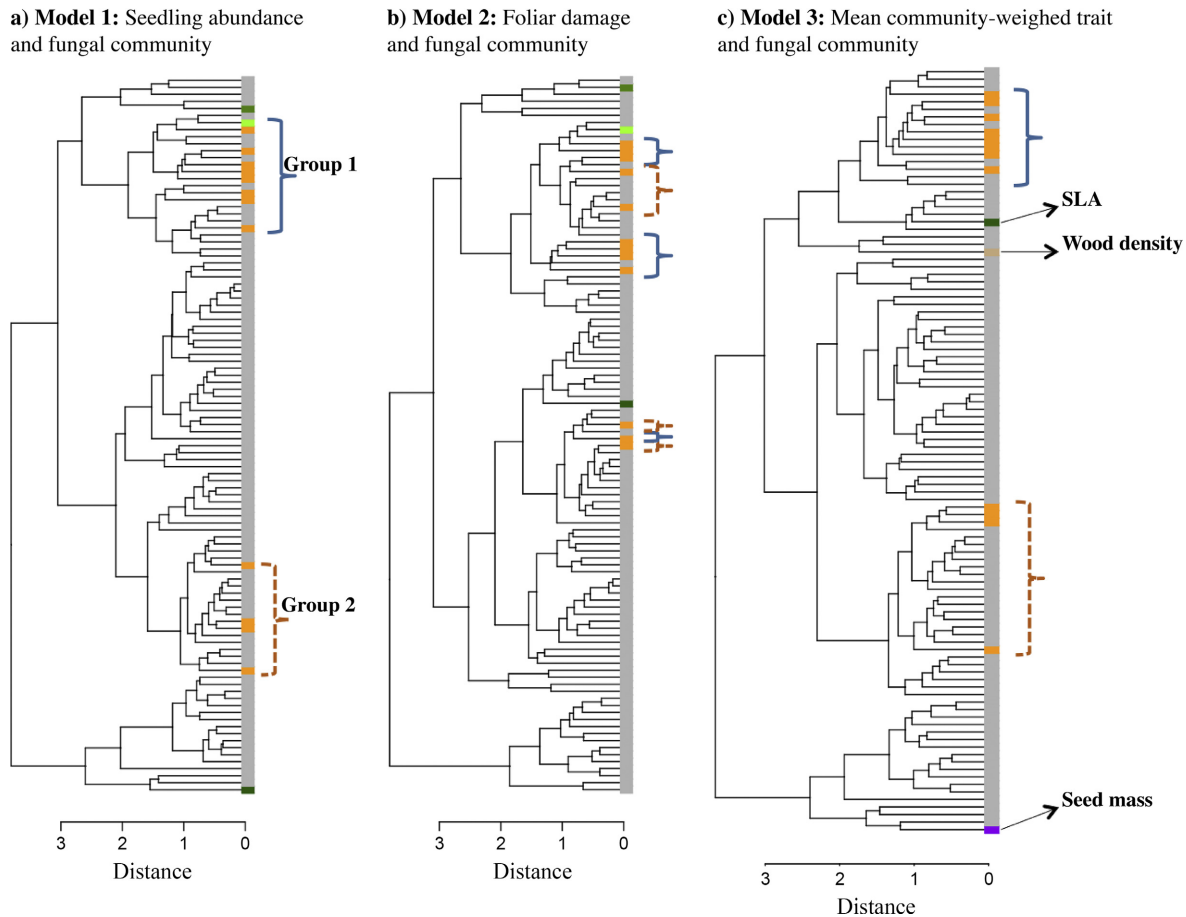**c) Model 3:** Mean community-weighed trait and fungal community

Fig. 2. Cluster analyses were performed for each of the three models based on distances of the mean responses of OTUs (in gray and orange) and (a) community-weighted mean functional traits for model 1, (b) seedling counts for model 2, and (c) foliar damage for model 3 to the covariates (matrices $\mathbf{E} = \mathbf{B'VB}$ where $\mathbf{B}$ is the matrix of coefficients by response variables and $\mathbf{V}$ captures the variation in the covariates $\mathbf{V} = \mathbf{cov(X)}$; Clark et al. 2017). To be similar, two response variables (for example a fungal OTU and early-successional seedlings) need to respond to the covariates in the same directions (both negative or both positive) and the covariates need to have substantial variation. Wood density is in light brown, specific leaf area (SLA) in dark green, and seed mass in purple. Early-successional species are in light green, mid-successional species are in intermediate green, and late-successional species are in dark green. The arbuscular mycorrhizal (AM) fungal OTUs (Appendix S1: Table S3) are indicated in orange. Two groups of AM fungal OTUs cluster with different functional traits: Group 1 clusters with SLA and wood density. Group 2 clusters with seed mass. Abundance of late successional species and their foliar damage respond differently to the environment than AM fungal OTUs (b, c).

seedling species belonging to trees of different successional status groups. Previous investigations into such associations (e.g., Zangaro et al. 2013) have been impeded by the lack of a probabilistic approach that could account for the codependence between seedling and fungi. Generalized joint attribute modeling (Clark et al. 2017), a multivariate Bayesian regression, enabled us to take into account the codependence of seedling and fungal communities to investigate how AM fungi might influence forest succession. We found that the presence of the 12 most common AM fungi was predicted to be correlated with low early-successional seedling abundance, high mid-successional seedling abundance, and a low amount of foliar damage on

seedlings of mid- and late-successional species (Fig. 3). These results suggest that AM fungi might be detrimental to early-successional species, that early-successional species might be less AM dependent than mid- and late-successional species, or that soil fertility might not be low enough (or too low) for early-successional species to benefit from AM fungi. However, we also found that different AM fungi were predicted to be associated with community-weighted mean functional trait characteristic of both early- and late-successional species, obscuring successional trends in changes of mycorrhizal responsiveness and likely contributing to differences in results across studies (e.g., Zangaro et al. 2013, Koziol and Bever 2015).
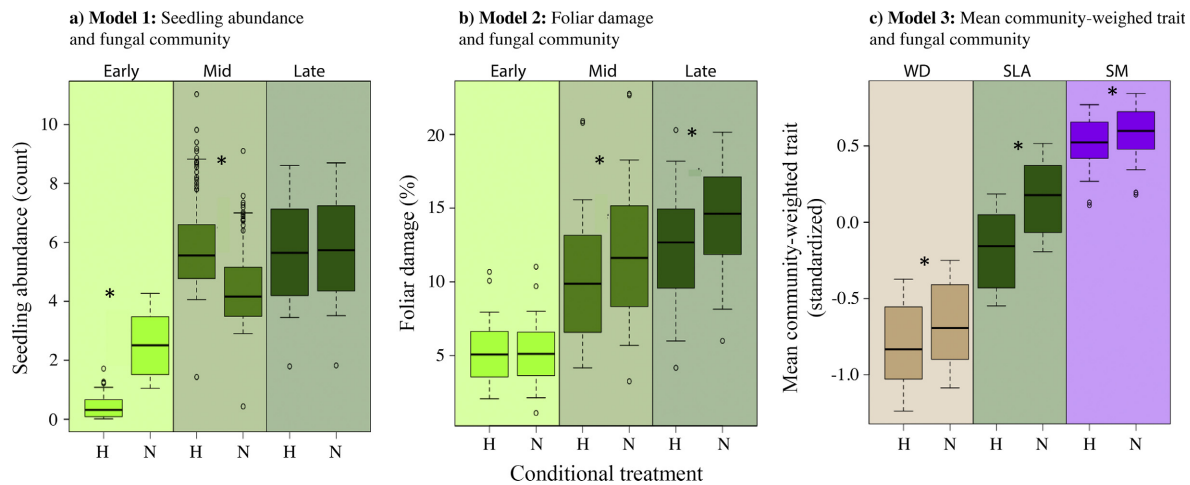
Fig. 3. Conditional predictions of three community-weighted mean functional traits (panels within a) and conditional predictions of early-, mid-, and late-successional species abundance (panel b) and average foliar damage (panel c) with high abundance (H) of the 12 most common AM fungi or in their absences (N). AM fungi are predicted to be associated with traits characteristic of early-successional species (low wood density in brown and low seed mass in purple) and late-successional species (small SLA in green) (a). Early-successional species are predicted to be more abundant (b) without the 12 most common AM fungi relative to with these 12 AM fungi. Mid-successional species are predicted to be more abundant (b) and host less damage (c) with AM fungi relative to without AM fungi. Late-successional species were predicted to host less damage (c) with the 12 most dominant AM fungi relative to without. Asterisks indicate significant difference (*P* < 0.05) in the response variable between abundant and absent AM fungi conditions. The bold horizontal lines indicate the median, the boxes expand from 25th to the 75th quantile, the whiskers expand from 2.5% the 97.5% quantiles, and the circles represent observations that fall outside of the 95% intervals.

Early-successional species abundance is predicted to be lower in the presence of the 12 most common AM fungi compared to in the absence of AM fungi

Contrary to previous work that found early-successional species perform better than late-successional species in the presence of AM fungi (Allen and Allen 1990, Zangaro et al. 2003, 2012, 2013), in our study, early-successional species abundance was predicted to be higher and mid-successional species abundance was lower when the 12 most common AM fungi were absent compared to when AM fungi were abundant (Fig. 3a). Furthermore, the absence of each of the 12 most common AM fungi was individually predicted to increase seedling abundance of early successional species (Appendix S1: Fig. S4). This result suggests that all AM fungi might have a negative effect (direct or indirect) on seedlings of early successional species. Therefore, our results support Janos' hypothesis (1980) that early-successional species might be less dependent on AM fungi than mid- or late-successional species. As a result of this low AM dependence, high seedling abundance of early-successional species might not be associated with high abundance of AM fungi in the soil, especially if phosphorus is limiting. Additionally, the presence of AM fungi in the soil might enhance competitive ability of seedlings of mid- and late-successional species if these species are strongly AM responsive leading to a low seedling abundance of early-successional species. Furthermore, the negative correlation between AM fungi and early-successional seedling

abundance might suggest that soil fertility at our site might not be low enough for early-successional seedlings to respond positively to AM fungi (e.g., Walker et al. 2010). Several studies have highlighted that the benefits conveyed by AM fungi might decrease with increasing soil fertility (Hoeksema et al. 2010). Phosphorus was, on average, 4.7 ppm at our site was slightly higher than in other tropical wet forests (3.0 ppm; Condit et al. 2013). Therefore, our site is, on average, slightly more fertile than other tropical forests.

Our result is in contrast with results from Atlantic rainforest and grasslands (e.g., Zangaro et al. 2003, 2012, 2013) and fragmented woodlands (Siqueira et al. 1998) in Brazil. The discrepancy among our result and these experimental studies could be due to the limitations of the experiments. As pointed out by Janos (2007), these experiments might have failed to detect responses from large-seeded late-successional species, if the experiments were too short, if the pots were too small, or if the quality of AM fungal inoculation was low. Our finding, however, is in agreement with a recent greenhouse study that manipulated AM fungal inoculum from a temperate prairie and measured a suite of responses on a set of tallgrass species differing in their successional status (Koziol and Bever 2015). Koziol and Bever (2015) found a trade-off between plant growth and mass and AM fungal responsiveness: late-successional species with lower growth and mass and fewer root tips were more responsive to AM fungal than early-successional species. Additionally, the authors found a

negative effect of AM fungi on some early-successional tallgrass species, suggesting a parasitic effect of AM fungi (Koziol and Bever 2015, 2016). Therefore, our result of predicted low seedling abundance of early-successional species in the presence of the 12 most common AM fungi might also arise from parasitic effects of AM fungi on seedlings that principally invest carbon towards fast seedling growth.

Seedlings of mid- and late-successional species are predicted to experience less foliar damage in the presence of the 12 most common AM fungi compared to soil without the 12 most common AM fungi

Besides influencing seedling abundance, AM fungi in the soil could have an impact on the amount of foliar damage from pathogens and herbivores experienced by seedlings. Indirectly, seedlings might be better defended and exhibit less damage because they benefit from improved nutrition, when associating with AM fungi (Smith and Read 2008). AM fungi can directly alter the jasmonate pathways (lipid-based hormonal signals that regulate plant defense) and boost tree tolerance against natural enemies such as pathogens and herbivores (Bi et al. 2007, Jung et al. 2012). Therefore, AM fungi might be associated with a decrease in seedling foliar damage. Consistent with this idea, we predicted that foliar damage in mid- and late-successional species was significantly reduced when the 12 most common AM fungi were abundant in the soil (Fig. 3b).

Early-successional species displayed similar predicted levels of foliar damage when the 12 most common AM fungi are abundant as when the 12 most common AM fungi are absent (Fig. 3b), suggesting that the effect of AM fungi on plant tolerance is highly variable (Koricheva et al. 2009). It is likely that not all AM fungi provide plants with an increase in tolerance to herbivory (Bennett and Bever 2007), and the diet breadth and feeding mode of the natural enemies can influence the effect of AM fungi on herbivory. In addition, early-successional species often have poorly defended and highly palatable leaves (Coley and Barone 1996, Bardgett and Wardle 2003). Therefore, AM fungal association might enhance the palatability of early-successional leaves by increasing nutrition, leading to foliar damage despite a potential protective effect. Alternatively if seedlings of early-successional species are not AM dependent, AM fungi might not associate with these seedlings explaining why the presence and absence of the 12 most common AM fungi in the soil had no predicted effect on the amount of foliar damage. Finally, early-successional species tend to be shade intolerant and to invest in short-lifespan leaves (Kitajima and Poorter 2010). These leaves might drop rapidly after being damaged. As a result, the little amount of damage observed on seedlings of early-successional species could be an artifact of unobserved damage on dropped leaves. This pattern is consistent with previous finding that shade-intolerant species (early-successional species) experience less damage than shade-tolerant species (late-successional species; Bachelot and Kobe 2013). Such pattern could also arise if natural enemies prefer shaded areas where early-successional species are less common.

AM fungal presence is predicted to be associated with early-successional functional trait (low wood density and low seed mass) and also with late-successional functional traits (small SLA)

The trait analysis predicts that AM fungi are associated with both early- and late-successional species (Fig. 2c). In particular, we found that the presence of AM fungi in the soil was predicted to be associated with small SLA, low wood density, and low seed mass (community-weighted mean functional traits, weighted by seedling abundance, Fig. 3c). We also found evidence, however, that different groups of AM fungi might be associated with seedling communities with traits representative of early- and late-successional species, a potentially important result in terms of the lack of consensus on whether AM fungal dependency increase or decrease along forest succession.

Specific leaf area (SLA) represents tree investment in structural components (Cornelissen et al. 2003). Early-successional species have large SLA that ensures high levels of photosynthetic activity to achieve fast growth (Poorter et al. 2004, Reich 2014). In contrast, late-successional species generally have smaller SLA because they invest in long-lasting leaves with more structural components than early-successional species (Poorter et al. 2004, Reich 2014). Therefore, some AM fungi appear to associate with only with late-successional species as predicted by Janos (1980) of increasing AM dependency during succession. In contrast, when the 12 most common AM fungi were abundant, predicted community-weighted mean wood density was lower than when these AM fungi were absent (Fig. 3c). Low wood density is a characteristic of early-successional tree species, suggesting that at least some AM fungi were predicted to be associated with early-successional species.

AM fungi were also predicted to be associated with high seed mass (community-weighted mean by seedling abundance). High seed mass is a characteristic of late-successional species (Grubb 1977, Wright et al. 2010). Early-successional species tend to produce many small seeds to facilitate wide dispersal, so they are readily available for early colonization of newly available areas (Westoby et al. 2002, Muller-Landau 2010). Seedlings emerging from these small seeds might be resource limited and require AM fungi to grow under nutrient limitation. Therefore, our result suggests that AM fungi might be more important for small seeded species (Walker et al. 2010), such as more often found in early-successional tree species.

By associating with traits that are characteristics of both early- and late- successional species, AM fungi

might not display a clear successional pattern following Janos' (1980) or Allen and Allen's hypotheses (1990). This would explain why there has been no consensus on whether AM fungi are more important in early or late succession. Interestingly, analysis of community-weighted mean functional traits and fungal OTU responses show two separate groups of AM fungi: the first group was associated with the late-successional trait of small SLA and the early-successional trait of low wood density (Fig. 2c, Group 1), and the second was also related to the early-successional trait of low seed mass (Fig. 2c, Group 2). However, analysis of individual AM fungal OTUs showed variation within and across these groups; the presence of individual OTUs predicted early- or late-succession traits (Appendix S1: Fig. S4). Therefore, early- and late-successional species appear to be associated with different AM fungi.

Our study site is in the path of hurricanes and our results might therefore not be applicable to succession in continental Neotropical forests where species do not experience such pressure. The effects of such infrequent but large disturbances on belowground communities are not well understood (Lugo 2008). One study found decreased sporulation and increased AM fungal colonization after hurricane Wilma hit the Yucatan Peninsula (Mexico) in 2005 (Vargas et al. 2010). However, the effects of hurricane on microbial communities are likely short-lived and soil microbiome was shown to recover a few months following a simulated hurricane treatment in Puerto Rico (Cantrell et al. 2014). Furthermore, at the time of our study in 2012, the latest hurricane to hit the forest was hurricane Georges 14 years earlier (September 1998). Therefore, our results are likely not specific to hurricane-disturbed forests.

To conclude, changes in seedling responsiveness to AM fungi during forest succession are complex due to successional-specific associations, as illustrated by the community-weighted mean functional trait analysis. Therefore, previous studies (e.g., Zangaro et al. 2013) might have missed important differences in AM fungal communities when inoculating plants of different successional status with a single AM fungal inoculum. A few empirical studies (Allen et al. 2003, 2005) have used AM fungal inoculum from stands at different successional status and found variable results, highlighting the complexity and context-dependency of the effects of AM fungi along succession. When considering seedling abundance and foliar damage, the presence of the 12 most common AM fungi is predicted to be associated with high seedling abundance and a low amount of foliar damage for mid-successional tree species, and with a low amount of foliar damage for late-successional species. In contrast, the presence of the 12 most common AM fungi is predicted to be associated with low seedling abundance of early-successional species, which supports the hypothesis of increasing AM responsiveness during forest succession.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.2122/suppinfo

DATA AVAILABILITY

DNA data are available at http://www.ncbi.nlm.nih.gov/sra with the following accession number: SRP052581. Functional trait data are available on Dryad (http://datadryad.org/resource/doi:10.5061/dryad.j2r53/1). We thank all anonymous reviewers and Dr. Hoeksema whose comments greatly improved our work.