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LONG-LASTING EFFECTS OF LAND USE HISTORY ON SOIL FUNGAL COMMUNITIES IN SECOND-GROWTH TROPICAL RAIN FORESTS

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ABSTRACT

Our understanding of the long-lasting effects of human land use on soil fungal communities in tropical forests is limited. Yet, over 70% of all remaining tropical forests are growing in former agricultural or logged areas. We investigated the relationship among land use history, biotic and abiotic factors, and soil fungal community composition and diversity in a second-growth tropical forest in Puerto Rico. We coupled high-throughput DNA sequencing with tree community and environmental data to determine whether land use history had an effect on soil fungal community descriptors. We also investigated the biotic and abiotic factors that underlie such differences and asked whether the relative importance of biotic (tree diversity, basal tree area, and litterfall biomass) and abiotic (soil type, pH, iron, and total carbon, water flow, and canopy openness) factors in structuring soil fungal communities differed according to land use history.

We demonstrated long-lasting effects of land use history on soil fungal communities. At our research site, most of the explained variation in soil fungal composition (R^2 = 18.6%), richness (R^2 = 11.4%) and evenness (R^2 = 10%) was associated with edaphic factors. Areas previously subject to both logging and farming had a soil fungal community with lower beta diversity and greater evenness of fungal OTUs than areas subject to light logging. Yet, fungal richness was similar between the two areas of historical land use. Together, these results suggest that fungal communities in disturbed areas are more homogeneous and diverse than in areas subject to light logging. Edaphic factors were the most strongly correlated with soil fungal composition, especially in areas subject to light logging, where soils are more heterogenous. High functional tree diversity in areas subject to both logging and farming led to stronger correlations between biotic factors and fungal composition than in areas subject to light logging. In contrast fungal

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richness and evenness were more strongly correlated with biotic factors in areas of light logging, suggesting that these metrics might reflect long-term associations in old-growth forests. The large amount of unexplained variance in fungal composition suggests that these communities are structured by both stochastic and niche assemblage processes.

Keywords: Forest soil fungi, High-throughput sequencing, Land use history, Tropical forest

INTRODUCTION

Soil fungi are critical to the functioning of forests, playing an important role in carbon sequestration (Coleman and Crossley 1996, Clemmensen *et al*. 2015) and in structuring plant communities (Bagchi *et al*. 2014, Liang *et al*. 2015). Despite the recognized importance of fungi for these ecosystems, the factors that structure soil fungal communities are poorly understood in tropical forests compared to temperate forests (Frey *et al*. 2004, Fierer and Jackson 2006, Lauber *et al.* 2008, Öpik *et al.* 2010). This gap in knowledge reflects the difficulties associated with characterizing soil fungal communities given their inconspicuousness and high diversity in tropical rainforests and the challenge of accounting for the myriad factors that can influence variation in soil fungal communities.

Abiotic factors can shape soil fungal community composition directly by altering biochemical and physical conditions, or indirectly by affecting the plant communities on which fungi depend. Soil physical, chemical, and nutritional properties have been recognized as the main drivers of soil microbial community structure (Garbeva *et al.* 2004, Waldrop *et al.* 2006, Lauber *et al.* 2008). Soil carbon affects soil fungal decomposers (de Graaff *et al.* 2010, Chapin *et*

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al. 2011) and arbuscular mycorrhizal (AM) fungi by enhancing their mineral nutrient uptake (Leigh *et al.* 2009) and by influencing plant growth (Zink and Allen 1998). Other soil nutrients, such as iron, shape soil fungal communities by influencing wood decomposition rates (Hall and Silver 2013) and by potentially toxic effects on plants and fungi (Li and Christie 2001). Additionally, light availability and soil pH and moisture can influence community composition of fungal decomposers (DeAngelis *et al*. 2013), pathogens (Prusky and Yakoby 2003), and mycorrhizal fungi (Zheng *et al*. 2015).

Soil fungal communities are also shaped by the local plant community, which provides them with resources and acts as hosts for some fungal groups (Ferrer and Gilbert 2003, Wardle *et al.* 2011, McGuire *et al.* 2011, Mueller *et al.* 2014, Urbanová *et al.* 2015). The quantity and nutritional quality of litter produced by the plant community may favor specific soil fungi (Gholz *et al.* 2000), thereby structuring fungal community composition (Prescott and Grayston 2013). Plant community characteristics might also affect fungal symbionts that exhibit some degree of host-plant preferences (Husband *et al.* 2002a, 2002b, Toju *et al.* 2013, Peay *et al.* 2013, Tedersoo *et al*. 2014, Martinéz-García *et al.* 2015). Overall, the importance of abiotic and biotic factors in shaping soil fungal communities vary across environment (Garbeva *et al.* 2004). The reasons underlining this variation remain unclear but could be associated with variation in environmental conditions and heterogeneity.

In the absence of long-term replicated experiments, successional tropical forests represent a useful system to study the biotic and abiotic factors that underlie land use impacts on the composition and structure of soil fungal communities. Human land use can alter soil carbon (Marín-Spiotta *et al.* 2008) and soil pH (Guariguata and Ostertag 2001), key drivers of soil fungi. For example, conversion of forest to crops can reduce soil carbon whereas conversion of forest to

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pasture can increase soil carbon (Marín-Spiotta *et al.* 2008). In addition, land use might homogenize soil properties via soil mixing or altered vegetation-mediated soil feedbacks (Fraterrigo and Turner 2005). Several studies in temperate forests have shown that intensive land use may reduce within-stand spatial heterogeneity in abiotic factors such as soil water content, pH, and nutrients (Fraterrigo and Turner 2005, Flinn and Marks 2007,Vellend *et al.* 2007). Soil fungi are sensitive to small-scale heterogeneity in abiotic factors (Kliromonos *et al.* 1999, Martiny *et al.* 2006, Barberán *et al.* 2015, Waring *et al.* 2016). This small-scale sensitivity could be explained if higher heterogeneity in abiotic factors results in greater availability of fungal niches and/or greater fungal resource limitation. Therefore, loss of environmental heterogeneity might result in a slight decrease in the importance of abiotic factors in structuring soil fungal communities, resulting from a decrease in fungal niches and/or limiting conditions.

The hypothesis of homogenization has also been proposed for the biotic community (Vellend *et al.* 2007) but has received mixed support (Grman *et al.* 2014). However, tropical rain forests in areas subject to human activities contain a mixture of tree species of various successional stages (Chazdon 2003). As a result, tree species in second-growth forests can span a greater range of leaf and wood traits than those growing in old-growth forests (Swenson *et al.* 2012). Plant species of distinct successional strategies and with different functional traits can favor distinct soil fungal communities (Ohsowski *et al. 2*014). Thus, greater plant trait functional diversity in forests recovering from more intense human land use could result in greater spatial heterogeneity of biotic factors shaping soil fungal communities in these areas relative to less disturbed areas. In summary, previously logged and farmed forests should exhibit more homogeneous abiotic conditions but a more heterogeneous biotic environment compared to old-growth forests.

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In general, human land use has been shown to result in a decrease in soil fungal richness or diversity (Lodge 1997, Glinka et al. 2014,-Mueller *et al.* 2014). However, some studies have found greater soil fungal richness in areas with more intensive land use (e.g. Glinka *et al.* 2014, Moora *et al.* 2014) or no effect of land use on fungal richness (e.g. Violi *et al.* 2008). Discrepancies may arise because of variation is sampling methodology or because land use effects on soil fungal communities are likely ecosystem-specific (Öpik *et al.* 2006) and non-linear depending on the type of land use, its intensity, and time since disturbance (Lodge 1997, Carpenter *et al.* 2001, Glinka et al. 2014,-Mueller *et al.* 2014, Vályi *et al.* 2015, Dickie *et al.* 2015). For instance, Carpenter *et al.* (2001) found that AM fungal richness in pastures with low grazing intensity is higher than in second-growth tropical forest. However, change in sporulation and spore longevity could be responsible for change in the effects of land use across studies (Violi *et al*. 2008). Few studies have assessed long-lasting effects of land use on soil fungal communities (Hartmann *et al.* 2012) and fewer still have been conducted in the tropical forest biome (McGuire *et al*. 2015), which has undergone drastic deforestation followed by an expansion of second-growth forests in recent decades (FAO 2010).

Here, we investigate the relationship among land use history, biotic and abiotic factors, and three components of soil fungal biodiversity --community composition, richness, and evenness-- in the 16-ha Luquillo Forest Dynamics Plot (LFDP). We ask:

1. Do composition, richness, and evenness of soil fungal communities in a tropical forest differ between two areas with different intensities of historical human land use? We hypothesized that soil fungal community composition would vary with intensity of land use (ILU). Given conflicting results from past studies, we did not have an expectation about how soil fungal richness would vary in low between ILU. Fungal evenness,

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however, should be higher in a heterogeneous compared to a homogeneous environment. Since high ILU areas have a more homogeneous environment but more heterogeneous tree communities than low ILU areas, we did not have an expectation about how fungal evenness would vary between ILU.

2. Are the impacts of land use on soil fungal community composition, richness, and evenness explained by present-day variation in biotic or abiotic factors? We hypothesized that land-use driven differences in soil characteristics, light availability, and tree community composition would be associated with variation in soil fungal community metrics.
3. Does the relative importance of abiotic and biotic factors in structuring soil fungal communities differ among areas with high and low ILU? We expected that the relative importance of abiotic factors in structuring soil fungal communities would be higher in areas with low relative to high ILU due to high heterogeneity in soil characteristics, which could result in high fungal niche availability and/or high limiting conditions associated with edaphic factors. In contrast, we expected that greater functional tree diversity in areas of high ILU would lead to a greater importance of biotic factors in structuring soil fungal communities in these areas compared to areas of low ILU. This would arise if high biotic heterogeneity results in more available fungal niches or greater carbon source limitation than in areas with low biotic heterogeneity.

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MATERIALS AND METHODS

Study site and field sampling

The study site is the 16-ha LFDP (1820'N, 6549'W) in northeast Puerto Rico with elevation ranging between 333 m above sea level (a.s.l.) and 428 m a.s.l. (Thompson *et al.* 2002). Mean annual rainfall is 3500 mm, classifying the forest as subtropical montane (Walsh 1996). All free-standing woody stems > 1 cm dbh (diameter at 1.3 m height) have been mapped, identified to species, and measured every five years (Thompson *et al.* 2002). Human activities in the Luquillo forest ceased in 1934. We divided the plot into two categories: high (canopy cover < 80%) and low (canopy cover ≥ 80%) ILU (Fig. 1) determined from aerial canopy photos taken in 1936 (see Thompson *et al.* 2002). The high ILU was intensely logged and used for small-scale coffee and large fruit (e.g. mango) plantations. The low ILU was selectively logged but never cleared. The high ILU encompasses the northern two thirds of the plots and has less rocky soils than the southern low ILU area that has a more rugged topography. These differences between ILU classes in the abiotic environment can be confounded with any effects of ILU so we included them in the analyses.

From May to July 2012, we sampled soil adjacent to 237 plots, in which naturally - occurring seedlings, distributed systematically throughout the LFDP were monitored (Fig. 1). This sampling enabled us to encompass the two areas of ILU and the different types of soil (Fig. S1). Prior to soil sampling we removed loose leaf litter (O_{i-e} horizons) and decaying logs. To prevent cross-sample contamination, the soil corer was cleaned with 70% ethanol, and new gloves were used at each seedling plot. Soil cores (0-20 cm deep and 2.5 cm diameter) were pooled from the four corners of 120 1 x 1 m seedling plots and from two corners and 1 m along opposite sides of 117 1 x 2 m seedling plots, so that each sample represented the corners of a 1

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m^2 area. Pooling all soil samples within each plot minimized small-scale heterogeneity (van der Gast *et al.* 2011). Pooled samples were located at least 20 m apart and distributed across the whole LFDP (Fig. 1). Samples were stored at -20°C within 8 hours of collection, shipped overnight to the laboratory at Barnard College where they were stored at -20°C prior to molecular analysis.

We quantified a number of abiotic factors that can affect soil fungal communities: light availability (canopy openness), soil iron and carbon, soil pH, potential water flow, and soil type (Table 1, Fig. S2). Other abiotic variables (e.g. soil nitrogen) were not included to avoid collinearity among covariates. Specifically, total soil nitrogen and total soil carbon were highly correlated (Pearson correlation 0.93). Because soil types and soil pH captured variation in soil fertility, we decided to remove soil nitrogen from the analysis and keep soil carbon. Soil types were derived from the USDA Soil Survey, which classified the soil into five volcaniclastic types: Zarzal has low fertility, Cristal and Coloso low to moderate, Prieto moderate, and Fluvaquents moderate to high fertility (1995 U.S. Department of Agriculture). The dominant soil types are Zarzal (148 samples), a deep and well-drained oxisol, and Cristal (50 samples), a deep but poorly drained ultisol. Prieto soils are moderately deep and poorly drained. Coloso and Fluvaquents soils are formed from alluvium in stream channels. Here, we considered Zarzal and Cristal soil types separately and created a third category by combining the three other soil types (16 soil samples: Coloso (2), Prieto (9) and Fluvaquents (5)), which had similar pH (Fig. S3). In Fall 2010 and Spring 2011, we used a camera with a fisheye lens (Nikkor, Nikon Inc., Tokyo, Japan) positioned at 1 m in height at the center of each seedling plot under uniform light conditions. The pictures were analyzed following the Ridler and Calvard (1978 algorithm) and we calculated Gap Light Index (GLI), a metric of canopy openness and light availability. To quantify

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differences in soil water drainage, we estimated potential water flow using an elevation grid of the LFDP plot (5 x 5 m) and the hydrology toolbox of ArcGIS (ESRI 2011). Soil pH was determined after 30 minutes incubation in a 1:1 (weight fraction) soil:H_20 solution. Total soil carbon was measured by combustion analysis (Elementar Vario Macro CNS Analyzer, Auburn University). Soil iron concentration was measured using inductively coupled argon plasma (ICAP) spectroscopy. Since physical attributes of the forest might have influenced where land use occurred thereby confounding the effects of human activities on soil biota (Foster 1992), we included the percentage of rock present in each plot (reported as a categorical value binned by increments of 10 from 0 to 100) and the slope calculated at the 20x20m scale in the analyses.

We considered a number of biotic factors that could influence soil fungal community structure, including adult tree diversity, sum of basal tree area as a measure of aboveground plant biomass, and litterfall biomass (Table 1). Using the 2011 tree census, we calculated tree species diversity (Shannon index, Shannon and Weaver 1948), and the sum of basal tree area in a circle of 15-meter radius around each seedling plot. Previous analyses in the LFDP (Uriarte *et al*. 2005) showed that a 15-meter radius captured neighborhood interactions between individual trees, and exploratory analyses of the fungal data also showed that the strongest associations occur at this scale (*data not shown*). We also estimated total annual litterfall biomass for each soil sample using models of leaf litter dispersal derived from tree plots data and litter falling into baskets for the 12 dominant tree species of the LFDP (Uriarte *et al*. 2015, Table S1).

Soil fungal community analyses

Soil samples were prepared for DNA sequencing, following Caporaso *et al.* (2012). Soil samples were thawed at room temperature, sieved, and extracted using the PowerSoil® DNA isolation kit

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(MoBio, Carlsbad, CA, USA). We extracted and then pooled three DNA extracts, using 3x0.25g of soil per sample, to determine the fungal community present in each sample. Fungi in the DNA extracts were sequenced using barcoded high-throughput sequencing on the Illumina MiSeq platform at the University of Colorado, Boulder, CO, USA following McGuire *et al.* (2013). The first internal transcribed spacer region (ITS1) of the fungal rRNA gene was amplified using the modified primer pair ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC; Bellemain *et al.*, 2010). 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. 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 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. Each sample was rarified to 500 sequences prior to downstream analyses. For taxonomic assignments, we used Basic Local Alignment Search Tool with the nucleotide database, excluding sequences not associated with known organisms, and the R package 'taxize' (Chamberlain and Szocs 2013) to extract the best taxonomy for each OTU from the GenBank taxonomic database (See supporting information for details). We obtained fungal composition data for 214 soil samples (86 in low ILU and 128 in high ILU). For the analyses, we used the relative abundance of distinct OTUs defined as 97% similar. Because the Illumina sequencing platform limits but does not fully eliminate PCR bias, relative OTU abundance should be interpreted as real abundance with caution (Lindhal *et al.* 2013). Fungal richness was

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calculated as the number of OTUs present in each plot, and fungal evenness was assessed using Pielou's index (Pielou 1966) which is calculated as Shannon diversity index / ln (species richness)). A value close to 1 indicates that all fungal species have equal abundance and a value near 0 means that some fungal species have very high abundance whereas other species are rare.

Statistical analysis

1. Do composition, richness, and evenness of soil fungal communities in a tropical forest differ between two areas with different intensities of historical human land use?

All composition analyses were performed using standardized Hellinger distance matrices to give less weight to rare phylotypes, which are often problematic in microbial community data (Legendre and Gallagher 2001). Due to this advantage, Hellinger transformation followed by Euclidean distances is commonly used in DNA type composition data analysis in microbiology (a few examples are Ramette *et al.* 2007, Lekberg *et al.* 2012, Vasileiadis *et al.* 2013). To investigate how soil fungal composition differed with ILU, we first used PERMDISP analysis (Anderson 2006) to test for homogeneous dispersion in fungal community composition between ILU areas. This test enabled us to assess whether or not beta diversity was significantly different between high and low ILU, with higher dispersion indicating greater beta diversity. Preliminary analyses showed that fungal beta diversity was reduced in high relative to low ILU ($F_{1,213}$= 12.1, p value < 0.001). For this reason, we used permutational multivariate analysis of variance (PERMANOVA) that is more robust to dispersion effects (Anderson and Walsh 2013) to determine if soil fungal community composition differs with ILU. Significant results from PERMANOVA would suggest that soil fungal composition was different in high and low ILU due to both location and dispersion effects. In order to correct for differences in topography

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between ILU, we also incorporated the slope and the amount of rock as covariates. We then used non-metric multidimensional scaling (MDS, Kruskal 1964) for visual representation of the differences in fungal composition between soil samples.

Neither amount of rock nor slope had an effect on fungal richness or evenness (*data not shown)*; therefore we did not incorporate these covariates in any richness or evenness models but they were incorporated in analyses of composition. Wilcoxon rank-sum tests were used to compare how fungal phylum, class, order and family richness and evenness differed between ILU. To account for the greater number of samples in high (128) relative to low (86) ILU, we bootstrapped the high ILU samples by randomly selecting 86 samples without replacement 100 times. The significance of the Wilcoxon test was corrected to account for multiple tests using the False Discovery Rate correction (FDR, Benjamini and Hochberg 1995). We used an analysis of variance (ANOVA) combined with bootstrapping as described above to assess the effect of land-use history on total fungal richness and evenness.

2. Are the impacts of high and low ILU on soil fungal community composition, richness, and evenness explained by present-day variation in biotic or abiotic factors?

We used multivariate redundancy analyses (RDA; Legendre and Gallagher 2001) to investigate the importance of abiotic and biotic factors for fungal community composition (Table 1), while partitioning out the effects of rock and slope on fungal composition from the analyses to account for differences in topography between the two areas of ILU. We first checked for collinearity among covariates using Pearson correlation tests and found that only tree diversity and sum of basal tree area 15 m around seedling recruitment plots were slightly correlated ($r = 0.38$). We used backward and forward approaches for variable selection and variance inflation factors to investigate spurious correlation among explanatory variables (Legendre and Gallagher 2001).

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To assess how abiotic and biotic factors affected soil fungal richness and evenness, we used linear regression followed by backward and forward approaches for variable selection. Covariates of the full model included the same nine variables used in the RDA. However, rock and topography were not included because these factors were not significantly correlated with fungal richness and evenness. Differences in sampling effort across ILU were addressed using bootstrapping followed by FDR correction as described above.

To correct for differences in sampling effort across soil types, we used 100 permutations of soil types across the 214 samples and the previously described RDA and linear regression to create distributions of *p* values under random soil type associations. We compared the *p* value of soil type obtained in the final RDA and linear models with these distributions. Soil type effect was considered significant if the *p* value was lower than 0.05 and the 5% quantile of the random *p* value distributions (see Table S2).

3. Does the relative importance of abiotic and biotic factors in structuring soil fungal communities differ among areas with high and low ILU*?*

Prior to analyses, we tested for significant differences in abiotic and biotic factors between high and low ILU. To do so we bootstrapped data from high ILU by randomly sampling 86 plots a thousand times, and used *t*-tests to assess significant differences in average values and coefficients of variations of the selected abiotic and biotic factors. The significance of the *t-tests* was corrected to account for multiple tests using the FDR correction. Differences in abiotic and biotic factor values and coefficients of variation were then used to interpret differences in the relative importance of abiotic and biotic factors in structuring soil fungal communities among areas with high and low ILU. To assess how ILU altered the relative importance of abiotic and biotic factors in structuring the soil fungal communities, redundancy analyses were performed

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for each ILU dataset, using the variables retained in question 2. Significance of each variable was assessed using 1000 permutations. We used variance partitioning to determine the proportion of variance in the soil fungal community composition, richness, and evenness that was explained independently by abiotic factors (A), biotic factors (B), jointly by A and B, and unexplained in either ILU (Legendre 1993). The amount of variance jointly explained by abiotic and biotic factors can be interpreted as the variation explained by their indirect effects. Similarly, we separated soil fungal richness and evenness according to ILU and used a linear regression of fungal richness and evenness against the variables previously retained in question 2, followed by variance partitioning. Finally, we used Chi-square test with 1000 Monte Carlo simulations to assess the significance of variance partitioning standardized by the total amount of explained variance between ILU for fungal composition, richness, and evenness.

Each covariate was *z* transformed in order to allow comparison between effect sizes (Gelman and Hill 2007). Leaf litterfall biomass, soil carbon, and iron were first log transformed to correct for skewness. In all analyses, variance inflation factors were lower than 1.3, indicating lack of collinearity among selected variables (Gross 2003). All analyses were performed in R 3.1.1. (R Core Team 2013) using the 'vegan' package (Oksanen *et al.* 2013).

RESULTS

We obtained fungal composition data for 214 soil samples. On average, there were 1,791 successful reads per sample (min = 606, max = 3737, SD = 551). After taxonomic identification, we found 7,379 distinct OTUs, comprising seven phyla, 25 classes, 89 orders, and 231 families. There were 4,957 OTUs found in high ILU and 3,826 detected in low ILU. The most common fungal phyla were Ascomycota (4,878 OTUs), Basidiomycota (1,477 OTUs), and

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Glomeromycota (553 OTUs). The most abundant orders were Pezizales (1,178 OTUs), Sordariales (1,191 OTUs), Agaricales (769 OTUs), Glomerales (454 OTUs), Capnodiales (441 OTUs), and Polyporales (164 OTUs).

1. Long lasting effects of land use on soil fungal communities

Fungal community composition. Comparison of dispersion in community composition between high and low ILU indicated that fungal beta diversity was significantly higher in low relative to high ILU ($F_{3,211}$= 12.1, $p < 0.001$). Consistent with our expectation, past land use intensity was a significant predictor of soil fungal community composition, explaining a small but significant difference in the composition of soil fungal communities (PERMANOVA, $F_{1,213}$ = 4.49, p = 0.001, R^2 = 2.1%, Fig. 3, Table 2). At the phylum level, Basidiomycota and Glomeromycota were more abundant in high relative to low ILU, while Ascomycota and Chytridiomycota were more abundant in low compared to high ILU. At the class level, Agaricomyctes, Glomeromycetes, Tremellomycetes, Geoglossomycetes, Archaeorhizomycetes, Lecanoromycetes and Schizosaccharomycetes were more abundant in high relative to low ILU. In contrast, Saccharomycetes, Chytridiomycetes, Dothideomycetes, Leotiomycetes, Agaricostilbomycetes and Archaeosporomycetes were more abundant in low compared to high ILU.

Fungal richness. ILU was marginally negatively correlated with soil fungal richness (p = 0.07, R^2 =2.3%, Table 2). Average OTU richness was highly variable among soil samples for all fungi (108 ± 25 SD in high ILU, 97± 30 SD in low ILU. At lower taxonomic resolution, we detected significant effects of ILU: Thirty out of 90 identified orders of fungi showed a significant difference in their OTU richness between high and low ILU and of these, all but five

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orders (Archaeosporales, Boletales, Peltigerales, Rhizophydiales, and Saccharomycetales) had greater fungal richness in high relative to low ILU (Fig. 2, Table S3).

Fungal evenness. Soil fungal evenness was high across the plot (mean = 0.73, sd = 0.11) and significantly higher in high ILU. ILU explained about 7% of the variance in the evenness of soil fungal communities ($p < 0.001$, Table 2). Thirteen out of 90 identified orders showed a difference in fungal evenness between high and low ILU: for all but two (Rhizophydiales and Saccharomycetales) orders, fungal abundance was more even in high relative to low ILU (Fig. 2, Table S3).

2. Correlation between fungal communities and abiotic and biotic factors

Fungal community composition. Soil fungal community composition was influenced by both biotic and abiotic factors. The final model retained seven variables, in order of importance: pH, soil type, soil iron, canopy openness, total soil C, adult tree diversity, and total leaf litterfall biomass (Table 2). Altogether, these seven variables accounted for 18% of the variation in soil fungal community composition (RDA, $pseudo\text{-}F_{11,203} = 5.81$, $p = 0.001$, Table S4), with two variables, soil type and pH, explaining most of the explained variation (12%).

Fungal richness. Biotic and abiotic factors, including soil type, tree diversity, soil pH and carbon, and potential water flow also influenced richness of soil fungal communities. These variables captured 12% of the variance in soil fungal richness (Table 2). Soil fungal richness was marginally higher in Cristal clay soils (intermediate fertility), positively associated with tree diversity and total soil carbon, and negatively associated with potential water flow and soil pH (Table S5).

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Fungal evenness. Biotic and abiotic factors, namely soil type, tree diversity and canopy openness, captured 10% of the variance in soil fungal evenness (Table 2). Soil fungal evenness was higher in Cristal clay soils (intermediate fertility) and was positively correlated with tree diversity and canopy openness (Table S5).

3. *Relative importance of abiotic and biotic factors in structuring soil fungal communities in the two land use areas*

Abiotic and biotic factors differed between areas of low and high ILU. Areas with high intensity of land use had lower average soil iron and carbon but higher pH than areas subject to low intensity of land use (Table 1, Fig. S2). Additionally, soil carbon and iron were more homogeneous in high relative to low ILU (Table 1). Tree diversity, basal area and canopy openness were also higher in areas of high ILU (Table 1, Fig. S2). In contrast to soil metrics, adult tree diversity and canopy openness were more homogeneous in low relative to high ILU (Table 1).

Fungal community composition. As expected because of reduced heterogeneity in soil factors and greater functional tree diversity in high relative to low ILU, abiotic factors were more strongly correlated with soil fungal community composition in low ILU whereas biotic variables were more important in high ILU (Chi-square test, $p = 0.01$). In high ILU, soil iron, soil type, total carbon C, and pH, canopy openness, tree diversity, and leaf litter explained 20% of the observed variation in composition of the soil fungal community (RDA, $pseudo\text{-}F_{10,204} = 3.73$, $p = 0.005$, Table 2, Table 3). Variance partitioning showed that 15%of the variation of soil fungal composition in high ILU was explained by abiotic factors with biotic factors accounting for only

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3% of the variation (Fig. S4A). The remaining variance (2%) was jointly explained by abiotic and biotic factors. In low ILU, iron, soil type and pH, soil carbon, and leaf litter accounted for 26% of the variation in soil fungal community composition (RDA, *pseudo-*$F_{10,204}$ = 3.30, p = 0.005, Table 2, Table 3). As with high ILU, variance partitioning showed that the majority of observed variation (22%) was captured by abiotic factors and only 3% by biotic factors (Fig. S4A). The remaining variance (1%) was jointly explained by abiotic and biotic factors.

Fungal richness. Contrary to our expectation, abiotic factors were more strongly associated with soil fungal richness in high ILU, whereas biotic variables were more important in low ILU (Chi-square test, $p < 0.001$, Fig. 4). In high ILU, 18% of the observed variation in soil fungal richness was explained by soil type and soil carbon (Table 2, Table 4). Specifically, fungal richness increased with soil carbon and peaked in Cristal soil clays (intermediate fertility). Tree diversity captured 1% of the variation in fungal richness and the remaining 1% was jointly captured by abiotic and biotic factors. In low ILU, 11% of the observed variation in soil fungal richness was explained by soil pH and carbon, soil type, and potential water flow (Table 2, Table 4). Tree diversity captured 3.7% of the variance in soil fungal richness (Table 2, Table 4).

Fungal evenness. Contrary to our expectation, abiotic factors were more strongly associated with soil fungal richness in high ILU, whereas biotic variables were more important in low ILU (Chi-square test, $p < 0.001$, Fig. 4). In high ILU, fungal evenness peaked in Cristal soil and this effect explained 3% of the variance in fungal evenness (Table 2, Table 4). In low ILU, tree diversity was the only variable significantly correlated with fungal evenness and it explained 5% of the variance in evenness of soil fungal communities (Table 2, Table 4).

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DISCUSSION

Land use is an important global driver of change in biotic communities (Sala *et al.* 2000), including communities of belowground organisms (Anderson 2005). Intense land use is often associated with changes in soil fungal communities (Bardgett 2005), and these effects can persist long after land use has ceased (Brussaard 1997). The distribution of land use, however, is not random on landscapes and areas of the LFDP that were steeper and rockier only experienced light logging. After accounting for these non-random differences, our results show long-lasting effects of land use history on the composition, richness, and evenness of soil fungal communities. Soil characteristics, canopy openness, and tree diversity were the main factors associated with variation in soil fungal community composition, richness, and evenness. Furthermore, we detected differences in the relative importance of abiotic and biotic variables in structuring soil fungal richness, evenness, and composition between areas subject to different intensities of historical land use. Across the study site, abiotic factors were the major drivers of soil fungal community composition, richness, and evenness, particularly in areas with low intensity land use. In contrast, biotic factors had greater explanatory power for fungal richness and evenness in low than in high ILU.

1. Long lasting effects of land use on soil fungal communities

Fungal community composition revealed a significant land use signature 80 years after abandonment of farming and logging. This substantiates the hypothesis that land use legacies may affect ecosystems for decades (Foster *et al.* 2003, Martiny *et al.* 2006), including soil fungi (Hartmann *et al.* 2012, McGuire *et al.* 2015). Specifically, this result supports other studies demonstrating effects of land use on arbuscular mycorrhizal fungi (Öpik et al. 2006, Tachbi *et al.* 2008) and soil decomposers (Carlile *et al.* 2001) in agroforestry and savannah tropical

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ecosystems. Our data indicated that soil fungal beta diversity is reduced in areas that were previously farmed and logged, suggesting that fungal composition in these areas exhibits lower turnover between plots relative to areas that underwent light logging (Whittaker 1960). Lower turnover in high relative to low ILU could arise from different processes (Legendre *et al.* 2005). First, areas of high ILU might be dominated by a suite of competitive fungal species that have been favored by farming and logging. Second, fungal species in high ILU might be less dispersal limited than in low ILU. This might be the case if ruderal species have been favored in areas of high ILU. Finally, beta diversity might be lower in high ILU due to more homogeneous environmental conditions.

We found that the richness of soil fungal communities was similar among areas with different land use legacies but that evenness was highest in areas with historical farming and logging. Previous studies of the effects of land use on soil fungi typically compared forests and pastures or fields and often detected reduction in fungal richness in disturbed areas (Carpenter *et al.* 2001, Kulmatiski and Beard 2008). Discrepancies between our results and previous studies could arise because our site contains old-growth and well-developed second-growth forests (80 yrs old), where differences in vegetation are less marked, explaining similar fungal richness in low and high ILU. Furthermore, results from other studies on the effects of ongoing land use on indices of soil fungal richness are mixed (Guadarrama and Alvarez-Sanchez 1999, Carpenter *et al.* 2001, Kulmatiski and Beard 2008, Violi *et al.* 2008, Vályi *et al.* 2015). This disparity could arise from variation in the index of diversity used. Our results show that species evenness responds to previous land use intensity more than species richness, as previously suggested by Chapin *et al.* (2000). Although we found no association between historical land use and fungal richness, there was greater community evenness in high ILU areas. This pattern could reflect

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differences in heterogeneity between ILU. Greater evenness in soil fungal communities in high ILU could arise from high heterogeneity in tree communities. In contrast, there is lower tree diversity in low ILU, which could favor dominance of a few fungal types, thereby reducing fungal evenness.

2. Association between fungal communities and abiotic and biotic factors

Several abiotic and biotic factors were associated with soil fungal community composition. Overall, we found that soil properties (soil types, soil nutrients, and soil pH) were correlated with fungal community composition, which confirms recent findings at the global scale (Fierer *et al.* 2009, Tedersoo *et al.* 2014). Consistent with previous studies (Garbeva *et al.* 2004, Lauber *et al.* 2008, Berg and smalla 2009, Peay *et al.* 2013), we found that soil type was correlated with soil fungal community composition, richness, and evenness. Soil type represents variation in soil characteristics at broad scales, encompassing a range of soil fertility across which different fungal species might be favored. Soil fungal community composition and evenness also responded to variation in pH. This is consistent with the idea that pH has species-specific effects on fungal metabolism (Tedersoo *et al.* 2014, Oehl *et al.* 2010). Our findings support another study showing strong positive correlation between indexes of fungal diversity and pH ranging from 3.2 to 7 (Bååth and Anderson 2003 but see Rousk *et al.* 2010). The discrepancies among our results and those from other studies could arise from methodological differences, or from non-linear effects of pH on soil fungal communities (Shi *et al.* 2013). We also found an association between soil fungal community composition and soil iron, which could be due to the biochemical uses of iron in fungi. Many saprotrophic fungi require iron to decompose wood because it stimulates decomposition via formation of hydroxyl radicals (Hall and Silver 2013). Soil fungal community composition was also associated with total soil C and amount of leaf

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litterfall, which might occur when soil fungal decomposers are carbon limited (Chapin *et al.* 2011). Finally, canopy openness and potential water flow were associated with soil fungal community composition consistent with the fact that both canopy openness and potential water flow alter litter decomposition rates (Gholz *et al.* 2000, DeAngelis *et al.* 2013), and enhance fungal development and spread because water is important for spore dispersal (Agrios 2005).

Our study supports the existence of linkages between plant and belowground fungal communities (Peay *et al.* 2013, Mueller *et al*. 2014, Martínez-García *et al.* 2015, Urbanová *et al.* 2015, Barberán *et al.* 2015). Although the correlation between soil fungal richness, evenness, and composition and biotic factors was not as strong as the associations with abiotic factors, consistent with previous research (Wagg *et al.* 2015), soil fungal richness and evenness increased with tree diversity, suggesting some degree of host-preference between fungi and plants (Toju *et al.* 2013). For instance, fungal decomposer communities may be associated with particular tree species (Prescott and Grayston 2013), just as pathogenic and mycorrhizal fungi exhibit host-preferences (Toju *et al.* 2013, Tedersoo *et al.* 2014).

3. Shifts in the importance of abiotic and biotic factors in structuring soil fungal community composition, richness, and evenness across areas with differing land use intensities

Although abiotic factors were the main driver of variation in soil fungal community composition across the study plot, the importance of biotic factors differed between areas with differing land use history. Biotic factors shaped the composition of soil fungal communities more strongly in areas of high relative to low ILU, suggesting that biotic heterogeneity drives fungal community composition more strongly in areas of high than low ILU. In contrast, biotic factors shaped the richness and evenness of soil fungal communities more strongly in areas of low relative to high

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ILU, suggesting that biotic factors are more important in areas of low than high ILU.

The greater importance of biotic factors in shaping fungal composition in high compared to low ILU was associated with tree diversity. This might arise because of greater functional trait diversity in the high ILU area of the LFDP (Swenson *et al.* 2012), and possibly, the higher functional diversity of tree hosts. The lower contribution of abiotic factors to soil fungi composition in these areas may arise from a more homogeneous soil abiotic environment in high ILU. The facts that several soil factors, such as soil iron and soil carbon, showed reduced heterogeneity in high relative to low ILU areas (Table 1) support this notion.

Biotic factors captured more of the explained variation in both richness and evenness in areas subject to low historical land use that have low functional tree diversity than in high ILU areas. In contrast, edaphic factors were more important in areas subject to farming and logging where soils are more homogeneous but canopy openness is more heterogeneous than in low ILU areas. Richness and evenness metrics, which reduce a very heterogeneous fungal community of pathogens, saprotrophs, lichens, and mutualists to a single number, might represent a mean response of the fungal communities to abiotic and biotic factors. Therefore, as a factor become more heterogeneous, the strength of is association with fungal biodiversity metrics might decrease.

Tree diversity was positively correlated with fungal evenness and richness. However, in area of high ILU where tree diversity is more heterogeneous, fungal evenness and richness were not significantly correlated with tree diversity. Therefore, this might explain why the importance of biotic factor was stronger in low relative to high ILU. Alternatively, biotic linkages might be tighter in old-growth forests where the lack of human disturbances might have enabled tree and

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fungal species to interact for longer ecological times and to develop strong above and belowground linkages (Peay *et al.* 2013). The main driver of fungal richness in high ILU was soil total carbon. However, in areas of low ILU, this factor was not significantly correlated with fungal richness. As previously discussed, this loss of significant correlation could be the results of greater heterogeneity in soil total carbon in low ILU. Alternatively, soil total carbon was significantly lower in areas of high ILU and therefore could be a limiting factor for soil fungi. Therefore, the tight correlation between fungal richness and soil total carbon might suggest carbon limitation in areas of high ILU.

Our study demonstrates long-lasting effects of historical land use on soil fungal community composition, richness and evenness, which might influence tropical forest functioning, highlighting the need for additional studies in second growth tropical forests. We found evidence that areas subject to farming and logging hosted a more even soil fungal community than areas subject to light logging, and that these differences might reflect changes in environmental factors and limiting conditions, or spatial homogeneity in biotic and abiotic factors. Such differences in soil fungal communities could result in changes in their functional roles. Fungi are key actors in nutrient cycling by decomposing organic matter, retaining, and providing nutrients to plants (Lodge 1991, Waring *et al.* 2016). Change in these functions could lead to less or more fertile tropical forest floors. Fungi are also important plant pathogens and mutualists that are key actors to maintain high tropical tree diversity (Bagchi *et al.* 2014, Liang *et al.* 2015, Bachelot 2015). Therefore, change in fungal communities could not only impact forest floors but also the overall diversity of tropical forests. Future studies should develop more tools to measure changes in functional diversity and investigate these changes are associated with variation in fungal community composition. Most of the variation in fungal communities was

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unexplained by our factor, suggesting that fungi might by randomly structured (Lekberg *et al.* 2012). However, our study emphasizes the important role of soil and biotic heterogeneity in structuring soil fungal communities, indicating that fungal assemblages might also reflect environmental niches.

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Supporting information: Description of taxonomic identification and additional tables and figures summarizing statistical analyses.

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Table 1. Distribution of the selected variables across land use history with the mean values and coefficient of variation in parentheses. * and ¥ indicate that means and that coefficients of variation, respectively, were significantly different across land use intensity (see Fig. S2 and methods). N indicates the number of samples collected from a given soil type in high and low intensity of land use history.

Variable	Intensity of land use		Hypothesized effect
	High	Low	
Adult tree diversity within 15 m radius (unitless)*, ¥	2.57 (0.11)	2.46 (0.13)	Host specificity, litter diversity
Basal tree area (BA) within 15 m radius*	1,809.53 (0.14)	1,708 (0.15)	Root biomass and carbon allocation belowground
Leaf litterfall biomass (g/m^2)	519.30 (0.59)	548.7 (0.44)	Aboveground nutrient inputs
Canopy openness (%)*,¥	3.1 (0.30)	2.6 (0.29)	Temperature, moisture and plant tissue quality, tree community
Soil iron (ppm)* ,¥	53.02 (0.95)	121.62 (0.99)	Wood decomposition; toxicity to plants and fungi

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Soil total carbon (%)*,,¥		4.23 (0.23)	4.89 (0.38)	Indicator of soil nutrient availability and buffering capacity
Soil pH (log(mol/L))*		4.77 (0.10)	4.58 (0.10)	Fungal metabolism and functioning, tree community
Water flow (m^2)		0.62 (1.08)	0.67 (0.87)	Affects soil erosion and fertility, fungal metabolism
Soil type*	Zarzal	N = 91	N = 57	
	Cristal	N = 29	N = 21	Nutrient and water availability, tree community
	Others	N = 8	N = 8	

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Table 2. Summary of the amount of variation in soil fungal community composition (R^2 in %), richness and evenness captured by land use history as a categorical variable or by heterogeneity in biotic and abiotic factors. We also show differences in the explanatory values of biotic and abiotic factors between the two land use types.

Metric	Land use	Abiotic/biotic factors	High ILU	Low ILU
Composition	2.1%	18.6%	24.4%	25.6%
Richness	2.3%	11.4%	20.1%	17.4%
Evenness	6.7%	10%	3.5%	10.7%

Table 3. Results of the redundancy analysis for the soil fungal community composition across intensity of LU. Empty cells indicate that tree diversity was not retained in the high ILU model.

	High ILU		Low ILU	
Factor	***p* value**	**R^2 (%)**	***p* value**	**R^2 (%)**
Canopy openness	0.03	1.5	0.25	1.2
Leaf litterfall	0.01	2.2	0.03	1.9
Soil C	0.03	1.0	0.02	2.1
Soil pH	0.01	4.0	0.01	6.5
Soil type	0.01	4.5	0.01	7.5
Soil iron	0.01	5.6	0.01	5.4
Tree diversity			0.44	1.0

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Table 4. Mean and standard error for standardized parameters of the regression models for soil fungal richness and evenness in high and low ILU. Intercepts for Cristal and other soil type represent the average differences in soil fungal richness in Cristal and other soils relative to Zarzal soils.

		High ILU				Low ILU			
Model	Factor	Estimate	Std. error	*p* value	R^2 (%)	Estimate	Std. error	*p* value	R^2 (%)
Richness	Soil C	0.37	0.1	<0.001	8.8	0.1	0.1	0.27	1.3
	Soil pH	-0.1	0.08	0.22	1	-0.29	0.12	0.02	6.4
	Soil type: Cristal	0.95	0.4	0.02	9	0.46	0.42	0.27	1.4
	Soil type: Other	0.59	0.2	0.004		0.14	0.25	0.58	
	Tree diversity	0.09	0.09	0.32	0.7	0.21	0.11	0.06	3.7
	Water flow	-0.08	0.09	0.33	0.6	-0.21	0.13	0.1	2.9
Evenness	Canopy openness	0.05	0.09	0.57	0.2	0.23	0.13	0.08	3.3
	Soil	0.22	0.37	0.56	3	0.34	0.38	0.37	2.4

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type: Cristal								
Soil type: Other	0.42	0.22	0.06		0.33	0.25	0.19	
Tree diversity	0.05	0.1	0.62	0.2	0.22	0.1	0.03	5

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FIGURE LEGENDS

Figure 1. Map of the Luquillo plot and the locations of all of the 2 m^2 (triangle) and 1 m^2 (circle) seedling plots. Filled symbols indicate those seedling plots from which we collected soil samples. The dark area represents low intensity of past land use and the light section represents high intensity of past land use.

Figure 2. Taxonomic composition of the low and high intensity of land use areas of the 16-ha LTER plot. Size of the bars represent the relative abundance of each of the dominant 16 orders recovered through Illumina sequencing using ITS-1F and ITS2 primers expressed as the relative number of OTUs. Together, these 16 orders capture about 90% of the total fungal OTU abundance. Letters in parentheses indicate the phylum: A: Ascomycota, B: Basidiomycota, G: Glomeromycota, Z: Zygomycota.

Figure 3. Two-dimensional non-metric multidimensional scaling showing the difference in soil fungal composition (A, ANOSIM, global R = 0.17, $p = 0.001$) between low (circles) and high (triangles) intensity land use history.

Figure 4. Variance partitioning among abiotic (filled bars), biotic (hashed) factors, and their intersection (lightly hashed) that structure the community composition, richness, and evenness of soil fungi in low (Low) and high (High) ILU. Total R^2 is indicated above each bar (%).

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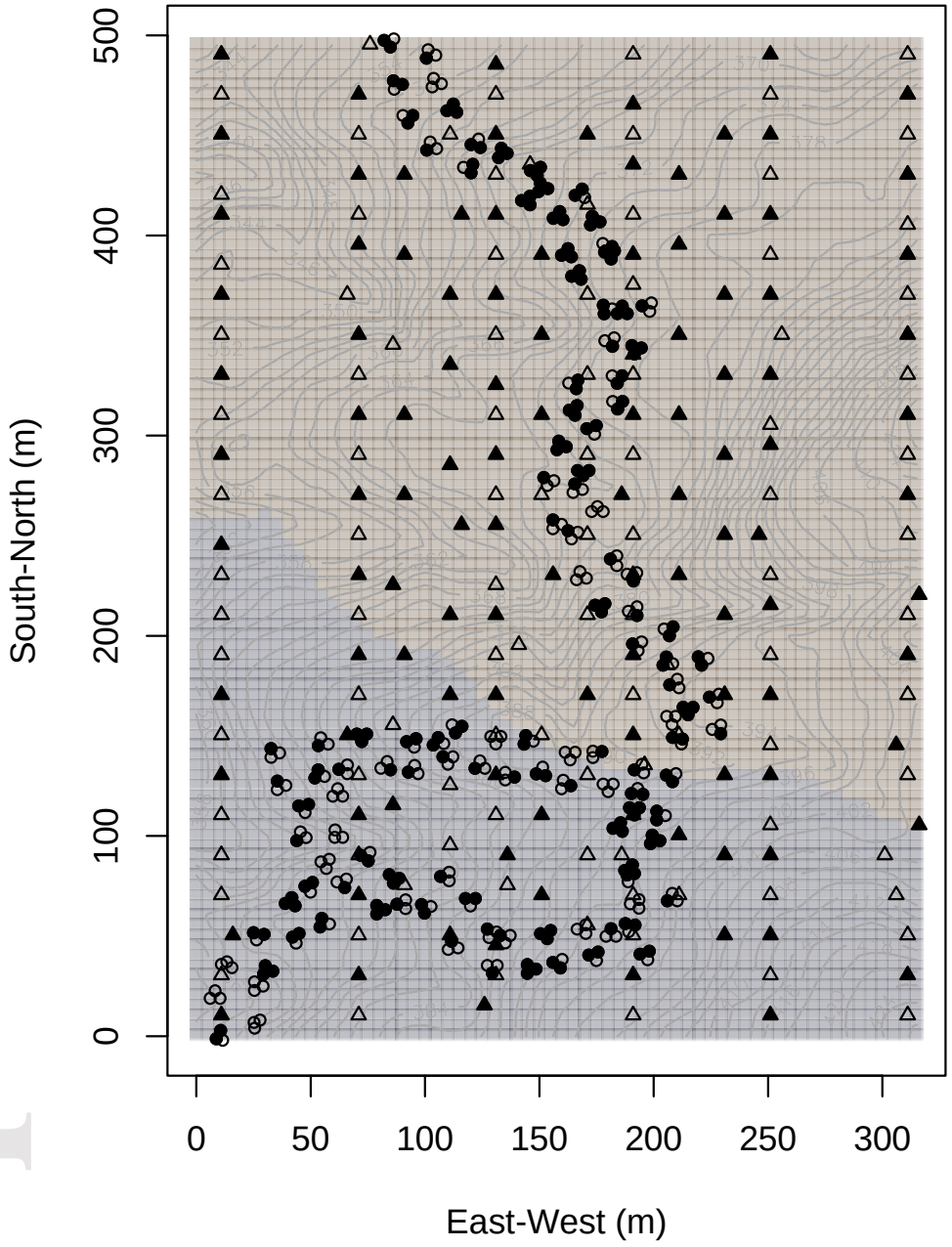

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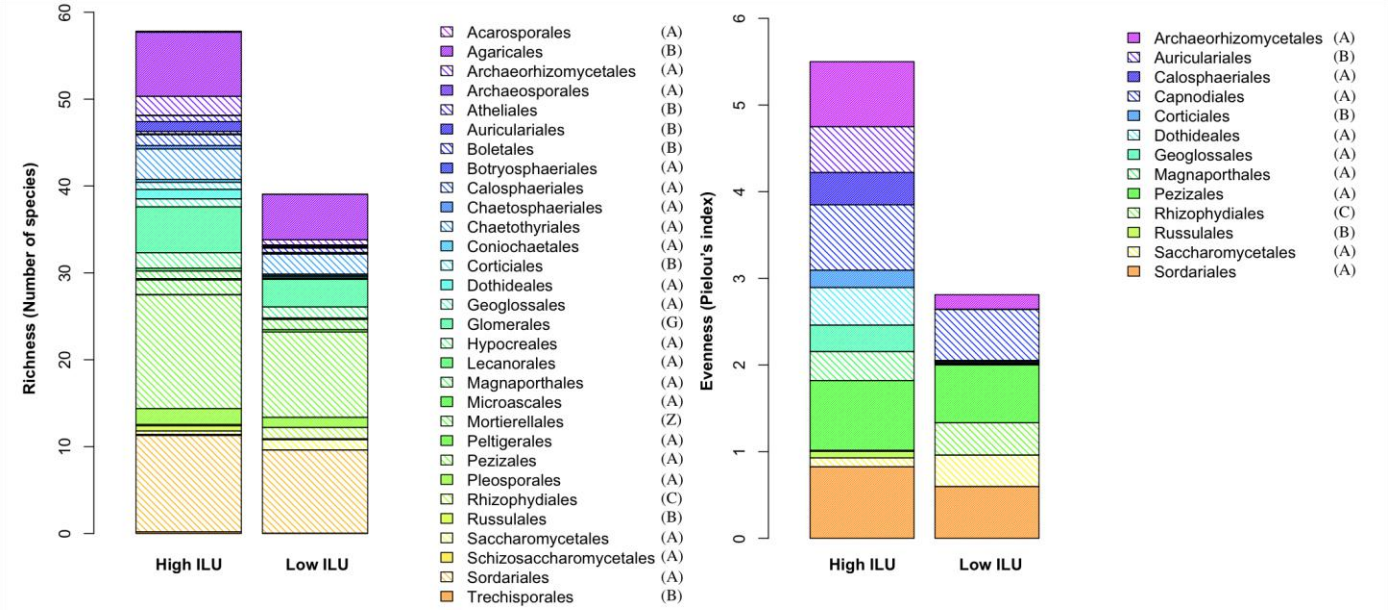

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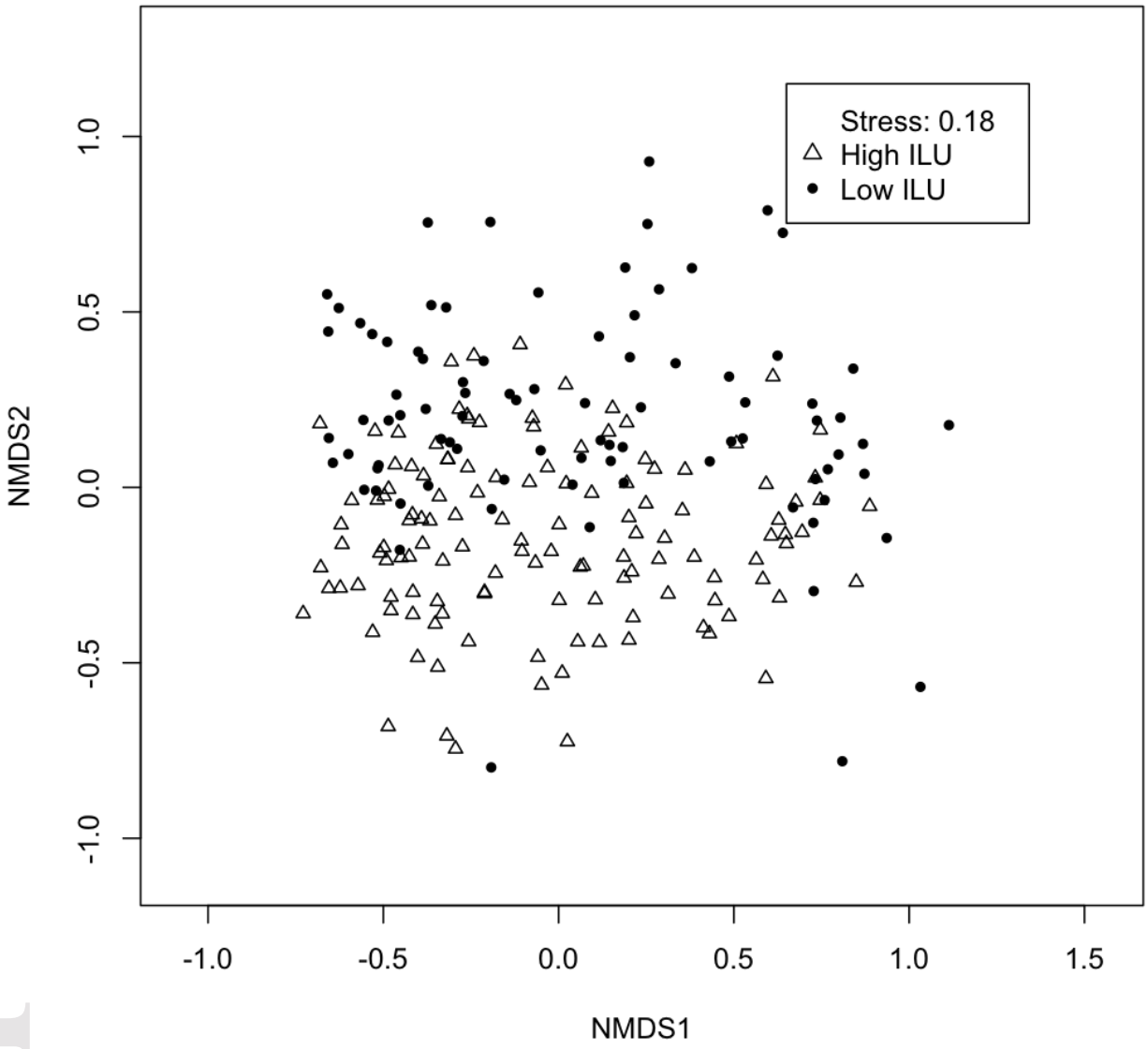

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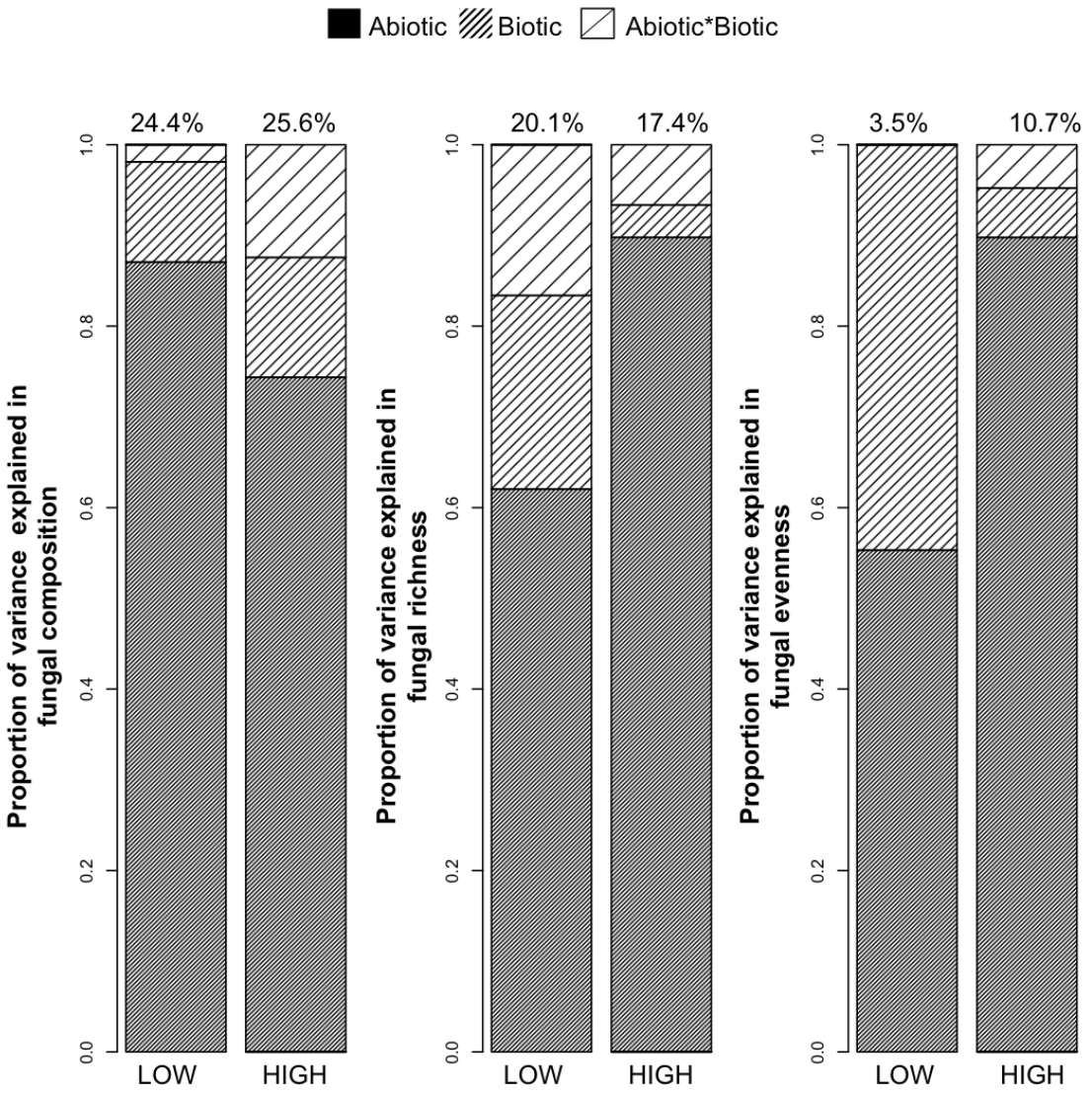

Figure 4.

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