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Title

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Permalink

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Journal

Functional Ecology, 30(11)

ISSN

0269-8463

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Publication Date

2016-11-01

DOI

10.1111/1365-2435.12651

Peer reviewed

Land use change in the Amazon rain forest favours generalist fungi

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Summary

1. Land use change is a significant threat to biodiversity, particularly within tropical ecosystems, but the responses of microbial communities remain poorly understood.
2. We used long-term plots established in multiple land use types in the Brazilian Amazon to examine the effect of land use change on soil fungal communities. We measured fungal richness and composition and identified factors associated with shifts in community composition across multiple land use types, including primary forest, two secondary forests and a chronosequence of differently aged pastures. Additionally, we used distribution patterns to estimate the niche breadth of fungal taxa in order to quantify changes in the relative abundance of generalists, or fungi with broad environmental tolerance, in response to land use change.
3. Conversion of primary forest to pasture resulted in large reductions in fungal richness coupled with substantial changes in community composition. Generalist fungi were strongly favoured in all pasture sites, regardless of time since conversion. Distance to primary forests was the strongest correlate of community composition in pastures, indicating that primary forests can act as reservoirs for recolonization by forest-associated fungi. The two secondary forests showed variable patterns of richness, composition and the overall abundance of generalist fungi, suggesting that community recovery is stochastic.
4. Fungal community response to land use change mirrors patterns observed in macroscopic organisms, which indicates that the increased prevalence of generalist taxa is a consistent response to disturbance across broad taxonomic groups.

Key-words: biotic homogenization, deforestation, fungal generalists, land use change, Tropical rain forest

Introduction

Land use change, such as deforestation, is one of the greatest threats to biodiversity world-wide (Dirzo & Raven 2003; Thomas *et al.* 2004), particularly within tropical ecosystems (Sala *et al.* 2000; Gibson *et al.* 2011). In terrestrial ecosystems, microbial communities drive key ecosystem processes (van der Heijden, Bardgett & van Straalen 2008), and disturbance has long-term effects on microbial community composition (Allison & Martiny 2008; Shade *et al.* 2012). As a result, understanding the long-term effects of land use change on ecosystem functions requires knowledge of microbial responses, and to that end, an increasing number

of studies have quantified the response of bacterial communities to land use change in tropical systems (e.g. Borneman & Triplett 1997; Rodrigues *et al.* 2013; Mendes *et al.* 2015). Comparably fewer studies have examined the effects of deforestation on soil fungal communities in tropical rain forests, but research conducted to date has documented variable responses to land use change, with land use change leading to significant shifts in fungal communities in some studies (e.g. Fracetto *et al.* 2013) but not in others (e.g. Alele *et al.* 2014). A better understanding of the drivers of observed shifts could provide insights into the factors that underlie variable fungal community responses to land use change in tropical rain forests.

Changes in community composition are driven both by the magnitude of environmental changes associated with disturbance, and by the specific traits of individual taxa that

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determine their sensitivity to environmental change. Fungal communities have shown to shift in response to changes in pH (Lauber *et al.* 2009; Rousk, Brookes & Baath 2009), physical soil properties (Crowther *et al.* 2014), nutrient availability (Waldrop *et al.* 2006) and plant community composition (Peay, Baraloto & Fine 2013). Factors related to recolonization potential, disturbance and historical factors, such as distance to intact forest (Pardini *et al.* 2010), intensity of land use change (Oehl *et al.* 2003) and patterns of community assembly that occurred prior to forest conversion (Peay, Garbelotto & Bruns 2010), can also contribute to differences in community composition following land use change. However, while shifts in richness and composition have been observed in these previous studies, not all taxa are negatively affected by disturbance, and as a result accurate predictions of the impact of land use change depend on identifying both edaphic factors and traits associated with significant shifts in fungal communities.

In studies of macro-organisms, a commonly observed response to environmental disturbance is the loss of specialist species (McKinney 1997), which can in turn lead to decreased functional diversity and functional homogenization (Clavel, Julliard & Devictor 2011; Nordén *et al.* 2013). Deforestation leads to numerous biotic and abiotic changes, including the loss of connectivity and specific resources, as well as the creation of novel habitats and environmental conditions. Such changes should favour generalists, which tend to be more successful in colonizing new ranges (McKinney & Lockwood 1999) and are less likely to be impacted by changes in environmental conditions than resource specialists (Vázquez & Simberloff 2002; Payne & Finnegan 2007). While shifts towards generalist taxa have not been extensively studied for fungi, Nordén *et al.* (2013) found that forest fragmentation negatively affected decomposer fungi that have restricted substrate utilization, with limited effects on generalist fungi. Although resource specialization is not fully understood in fungi (Treseder & Lennon 2015), there is evidence for variability among decomposer fungi in substrate utilization (Hanson *et al.* 2008; McGuire *et al.* 2010). Increased dominance by generalist fungi has been suggested as a driver of fungal community shifts in response to land use change (Alele *et al.* 2014), but this has not been measured directly. A recent survey of microbial communities found that a majority were classified as specialists (Mariadassou, Pichon & Ebert 2015) and so microbes may be particularly sensitive to disturbance. Within our study sites, we predicted that as a result of the large differences in both abiotic and biotic conditions among pasture, secondary forest and primary forest sites (Mueller *et al.* 2014), land use change would select for generalist fungi.

Our previous work at this site documented significant shifts in fungal richness and community composition at three of the sites (primary forest, secondary forest and pasture; Mueller *et al.* 2014), as well as significant changes in functional gene abundance, diversity and composition following pasture conversion (Paula *et al.* 2014). Our current

study expands on our previous findings by examining fungal community composition across multiple plots located in a primary forest, two secondary forests and pastures of various ages. Using high-throughput sequencing, we measured fungal richness and composition across multiple land use types, quantified the relationship between multiple biotic and abiotic factors and fungal community composition across pastures of various ages, and utilized operational taxonomic units (OTUs) distribution patterns and environmental data to estimate changes in generalist taxa in response to land use change.

Materials and methods

STUDY SITE

This study was conducted at the Amazon Rainforest Microbial Observatory (ARMO) site, which was designed to quantify microbial community responses to deforestation in the Amazon rainforest. ARMO was established in 2009 at a privately owned cattle ranch named Fazenda Nova Vida (10°10'18.71" S, 62°47'15.67" W), within the state of Rondônia, which has the highest percentage of forest loss (28.5%) of any state in the Brazilian Amazon (www.inpe.br). Additional site details can be found in Rodrigues *et al.* (2013) and in the Supporting information.

The ARMO site is made up of numerous single-hectare plots established within three land use types: primary forest, pastures of various ages (ranging from 6 to 99 years old) and secondary forests (ranging from 11 to 17 years old). Land use types and ages were assigned based on accounts from long-term land managers at the Fazenda Nova Vida. Within each hectare plot, a nested sampling scheme was established, with 10, 1, 0.1 and 0.01 m² quadrats nested within the 100 m² plot, for a total of 12 sampling points (Rodrigues *et al.* 2013; Paula *et al.* 2014). For the current study, we examined the primary forest, three pastures of varying ages (6, 38 and 99 years old at the time of sampling, designated P06, P38 and P99) and two secondary forests that developed naturally on pastures abandoned in 1998 and 1993 (S12 and S17). The soil sampling was conducted in April 2010, at the end of the rainy season. Soil was sampled to a depth of 10 cm using a 5-cm diameter corer, chilled immediately to 4 °C, frozen (within hours) at –20 °C and shipped on dry ice to the laboratory, where samples were sieved through 2-mm mesh and stored at –80 °C prior to molecular analysis.

SOIL ATTRIBUTES

Analyses of physical and chemical properties were conducted at the Universidade de São Paulo according to the methods described in van Raij *et al.* (2001). Due to limited material, soil analyses were not performed on soils collected at the smallest distances (0.01 and 0.1 m), resulting in a subset of nine samples analysed per hectare plot. Since one of the goals of the current study was to directly link fungal communities to soil factors, we included this same subset of soil samples in our analysis of fungal responses to land use change, for a total of 54 samples across six sites.

MOLECULAR ANALYSIS OF FUNGAL COMMUNITIES

Total soil DNA was extracted, and fungi were targeted for Illumina sequencing using methods described previously (Mueller *et al.* 2014). Briefly, the ITS1 region of the internal transcribed spacer (ITS) region, a universal barcode for fungi (Schoch *et al.* 2012) was targeted using the fungal-specific primers ITS1F and ITS2 (Gardes

& Bruns 1993), which were modified to include a unique six-nucleotide barcode and a partial Illumina adapter. The remaining Illumina-specific sequences were added in a second PCR. Amplicon samples were quantified using the Qubit HS dsDNA kit (Invitrogen, Carlsbad, CA, USA) and combined at equimolar concentrations. The final library was quantified using Illumina-specific qPCR and adjusted to a final concentration of 10 nM. Paired-end 150-bp sequencing was performed using the Illumina HiSeq at the University of Oregon's Genomics Core Facility.

Sequences were processed as described in Mueller *et al.* (2014). Briefly, quality filtering was performed using the fastx toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) and QIIME v. 1.6.0 (Caporaso *et al.* 2010). USEARCH (Edgar 2010) was used to identify putative chimeras and to delineate OTUs at 97% sequence similarity. Classification was performed against the fungal ITS 99_otus data base from the UNITE group (Kõljalg *et al.* 2013) using both the Bayesian classifier implemented through the Ribosomal Database Project (RDP; Cole *et al.* 2009) and BLAST (Altschul *et al.* 1990). To increase the resolution of classification, we removed any sequence in the reference data base that was unidentified at the class level prior to classification of environmental ITS1 sequences, which resulted in a data base of 61 826 sequences. For downstream analysis, only sequences classified as Fungi with both a high bootstrap value from the RDP (>50) and an e-score less than e-40 from BLAST were retained.

STATISTICAL ANALYSES OF FUNGAL COMMUNITIES

All statistical analyses were performed using the statistical platform R version 3.0.2 (R Core Team 2013; www.R-project.org) with the analytical packages APE (Paradis, Claude & Strimmer 2004), VEGAN (Oksanen *et al.* 2013) and ECODIST (Goslee & Urban 2007). Analyses were performed using community matrices rarefied to 3200 sequences across 99 rarefaction trials to limit the effects of unequal sampling. We compared fungal community metrics, including richness and composition, among the six sites (F, P06, P38, P99, S17 and S12), which represent three different land use categories (primary forest, pasture and secondary forest), estimated the environmental tolerances of individual OTUs using distribution patterns and used data from the three differently aged pastures (P06, P38 and P99) to examine specific factors associated with changes in the fungal communities across the different pastures, including time in pasture (e.g. pasture age), distance to primary forest, and soil nutrients and texture.

Responses to land use type

We compared richness among the six sites using one-way ANOVA. Community similarity was calculated using the abundance-based Bray–Curtis dissimilarity measure and compared using PERMANOVA (Anderson 2001). We chose PERMANOVA because with a balanced design, it is robust to differences in dispersion (Anderson & Walsh 2013), which often occurs when multiple groups are compared. Community composition was visualized using non-metric multidimensional scaling (NMS) ordination. To examine which factors were significantly associated with differences in fungal community composition across the six sites, we quantified the variance in NMS axis scores explained by individual values of soil chemistry and texture with vector analysis using the function *envfit* in the R package VEGAN. Significance for each factor was determined using nonparametric randomization tests.

Drivers of community shifts in pastures

Conversion to pastures is the most common form of land use change in much of the Amazon, but how biotic and abiotic

factors alter fungal communities within this single land use type is unclear. In particular, how time in pasture and proximity to intact forest will alter fungal composition could inform restoration and conservation efforts, especially within areas attempting to balance agricultural and conservation goals. To examine which factors were significantly associated with differences in fungal community composition in the three differently aged pastures, we quantified the variance in NMS axis scores explained by pasture age, soil parameters (separated into chemical and physical) and distance from remnant primary forest using vector analysis with the function *envfit*. Significance of the variance of each factor was determined using nonparametric randomization tests. Distance to nearest primary forest was measured using GPS coordinates for each soil core and satellite images viewed in Google Earth (earth.google.com). Primary forest delineations were based on ground surveys and verbal accounts from long-term land managers, and confirmed at the time of soil sampling.

To partition the variance explained by spatial distance and broadscale soil characteristics (calculated as distance matrices), we used partial Mantel tests, which examine correlations between two matrices while holding the third constant. Pairwise distances were calculated using GPS locations taken at each soil core. Similarity of soil chemistry and texture across the plots was calculated using Euclidean distances with Hellinger-transformed data (Legendre & Gallagher 2001).

Fungal niche breadth

To measure the environmental breadth of individual fungal OTUs, we used two approaches. First, we used the approach outlined by Levins (1968), which calculates niche breadth based on the uniformity of species' abundance distributions in relation to available resource states, which we defined as soil cores found across the different land use types. Secondly, to more directly link measured environmental parameters within environmental tolerance, we utilized the soil chemistry and texture measures described above. We applied this second approach because of the high environmental variation we observed both across and within the sites (Fig. S2, Supporting information), which could potentially impact an occupancy-based measure of niche breadth. For example, for two OTUs found at three sites, the OTU that occupies a primary, secondary and pasture site has a broader niche than one found only in pasture sites, but this would be obscured using a site-based metric. By utilizing the soil physical and chemical data to calculate environmental distances, the categorical variables of site can be treated as quasi-continuous, without directly referring to the individual sites. Distances were calculated using Hellinger-transformed data, and a dendrogram was constructed using hierarchical clustering with Ward's minimum variance method. The environmental breadth of each OTU was calculated as the sum of the branch lengths occupied in the full dendrogram. The mean value of Levin's niche breadth and the environmental breadth of OTUs found in each soil core were compared among sites and land use categories, using one-way ANOVA followed by Tukey HSD tests for pairwise comparisons. We used linear regression to compare niche breadth measures for each OTU generated from the two approaches.

To determine whether the survival of individual OTUs following conversion to pasture could be predicted from the environmental breadth of the OTUs, we conducted logistic regression using the presence or absence of a given OTU in each pasture as dependent variables. To directly compare communities in the primary forest and pastures, we included only OTUs found in the primary forest, with a minimum abundance of three. Individual analyses were run for each of the three pastures using general linear modelling.

Results

A total of 3 206 544 sequences were included in this analysis, representing 4810 OTUs classified as fungi with high confidence. Sequence coverage was variable across the 54 samples, ranging from 3271 to 256 785 sequences, with a mean of 47 898 sequences. A collectors curve constructed from all OTUs indicated that sampling approached an asymptote, but bootstrap analysis indicated that the actual richness was 5456 (SE \pm 152).

Analysis of the soil physical and chemical parameters indicated high variation across the six sites ($F = 15.4$, $R^2 = 0.62$, $P = 0.001$). Notably, many samples from the 12-year-old secondary forest (S12) were distinctive and clustered separately from the other five sites (Fig. S2). Soil parameters were also significantly different when compared among the three land use categories ($F = 15.4$, $R^2 = 0.38$, $P = 0.001$).

FUNGAL DIVERSITY AND COMMUNITY COMPOSITION

Averaged across the six sites, the majority of the OTUs observed (56%) were members of the phylum Ascomycota, 33% were identified as Basidiomycota and 11% as Glomeromycota. The remaining OTUs belonged to the Chytridiomycota and basal lineages and accounted for <1% of the community. At the class level, Sordariomycetes dominated both the primary forest and secondary forest (38% in both), but comprised 29% of the pasture community (Fig. 1). The class Agaricomycetes was also abundant and made up 23%, 24% and 20% of the primary forest, secondary forests and pastures, respectively.

Fungal OTU richness varied both across the six sites ($F_{5,48} = 11.7$, $P < 0.001$) and among the three categories of land use ($F_{2,51} = 16.4$, $P < 0.001$). Richness was highest in

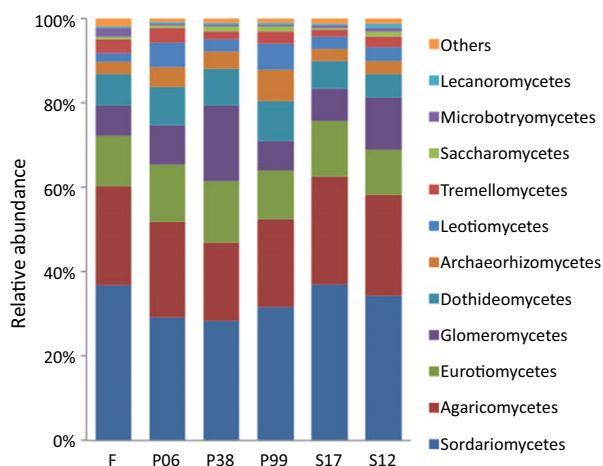


Fig. 1. Relative abundance of fungal classes in the Amazon soils identified in primary forest (F), three pastures (P) of different ages and two secondary forests (S) of different ages (ages are given in parentheses). Only classes with a relative abundance >1% across the sites are shown.

the 17-year-old secondary forest (S17), which was not significantly different from the primary forest. Richness in the 12-year-old secondary forest (S12) was significantly lower than either primary forest or the 17-year-old secondary forest (S17) and did not differ from two of the pasture sites (Fig. 2).

We also found significant differences in fungal community composition across the six sites (Pseudo $F = 5.20$, $R^2 = 0.35$, $P = 0.001$) and across the three land use classes (Pseudo $F = 6.06$, $R^2 = 0.39$, $P = 0.001$). The NMS ordination indicated that the secondary forests clustered more closely with the primary forests, while the three differently aged pastures were distinct (Fig. 3). Mean Bray–Curtis similarity to fungal communities found in the primary forests was 0.16 (± 0.02 SE) for the secondary forest, and 0.07 (± 0.01 SE) in the pastures. These differences remained when the communities were compared at class level both across the sites (Pseudo $F = 3.75$, $R^2 = 0.28$, $P = 0.001$)

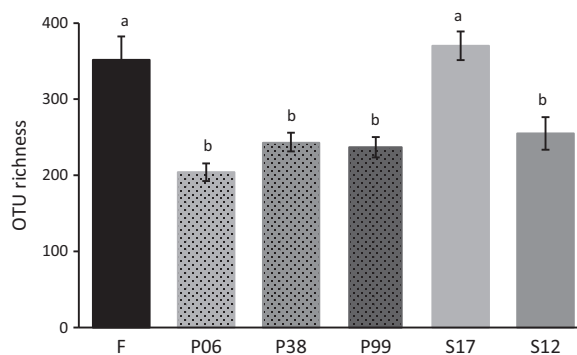


Fig. 2. Fungal operational taxonomic units richness (97% similarity) among primary forest (F), pastures (P) and secondary forests (S). Different letters indicate significant differences based on Tukey's Honestly Significant Difference test at $\alpha = 0.05$. Error bars represent one standard error calculated across the nine samples taken at each site.

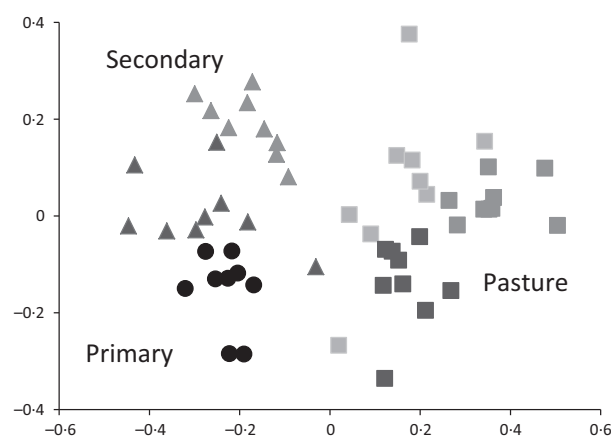


Fig. 3. Non-metric multidimensional scaling ordination (Bray–Curtis dissimilarity) of fungal communities in primary forest (circle), in pastures P06 (light grey square), P38 (medium grey square) and P99 (dark grey square), and in secondary forests S17 (light triangle) and S12 (dark triangle).

Table 1. Results of vector analysis linking environmental factors to fungal community composition across all six sites and across the three pastures. Nonsignificant factors included pH, clay, sand, magnesium, potassium, phosphorus, zinc, base saturation index, aluminium saturation index and sum of bases

	All sites		Pastures	
	R^2	P	R^2	P
F distance	–	–	0.73	0.001
Age	–	–	0.58	0.001
C : N	0.51	0.001	0.30	0.02
B	0.39	0.001	0.17	0.12
Mn	0.35	0.001	0.51	0.001
N	0.31	0.001	0.66	0.001
T	0.31	0.001	0.72	0.001
S	0.27	0.001	0.28	0.009
Ca	0.26	0.001	0.51	0.001
C	0.23	0.002	0.58	0.001
Cu	0.17	0.01	0.44	0.001
Fe	0.15	0.007	0.20	0.57
MO	0.14	0.02	0.72	0.001
Silt	0.00	0.93	0.41	0.003
Al	0.06	0.17	0.48	0.001

F distance, distance to nearest primary forest; age, time since conversion from primary forest. Mo is organic matter, T is ion exchange capacity

and across the three land use categories (Pseudo $F = 4.26$, $R^2 = 0.14$, $P = 0.001$).

DRIVERS OF PASTURE FUNGAL COMMUNITY COMPOSITION

Based on vector analysis, distance to primary forest edge was the highest correlate to fungal community composition across the three pastures, followed by the amount of soil organic matter and ion exchange capacity (T) (Table 1). Pasture age was also significant in the overall analysis. We found only a trend for pH ($R^2 = 0.19$, $P = 0.07$), and a relatively minor correlation with C : N ($R^2 = 0.25$, $P = 0.03$). The factors found to be strongest within the pastures differed from those observed across all six sites. For example, C : N ratio had the highest correlation to fungal community composition across the six sites, but explained a comparably smaller amount of variance in the pasture-focused analysis (Table 1).

When environmental variables were aggregated into a single distance value, partial Mantel tests indicated that pairwise geographic distances explained the majority of the variance in fungal communities (Mantel $R = 0.44$, $P = 0.001$). After correcting for spatial effects, neither heterogeneity in soil chemistry (Mantel $R = 0.10$, $P = 0.13$) nor soil texture (Mantel $R = 0.13$, $P = 0.07$) was significantly associated with fungal composition.

FUNGAL NICHE BREADTH

Values generated for OTUs using Levin's niche breadth and direct comparison of environmental tolerance using

soil chemistry measures were highly correlated ($R^2 = 0.74$, $F = 1386$, $P < 0.001$), and comparisons across the six sites using the two metrics yielded nearly identical outcomes. As a result, only the findings from Levin's niche breadth are presented. Analysis of the environmental conditions tolerated by the fungal OTUs indicated that on average, the fungal taxa found in the pasture were able to colonize a broader range of environments than the communities present in the primary forest, with intermediate levels in the secondary forests ($F_{2,51} = 14.3$, $P < 0.001$). When examined at the site level, the lower environmental breadth in the secondary forest category was driven by S12 ($F_{5,48} = 11.4$, $P < 0.001$), which was not significantly different from any of the pasture sites based on Tukey's Honestly Significant Difference tests (Fig. 4).

In addition to differences in mean niche breadth among the sites and land use types, we also found a negative correlation between mean OTU richness and mean niche breadth across the 54 samples analysed across the six sites (Fig. 5). When we examined potential sampling biases that could have contributed to this pattern, we found that the relationship between OTU evenness and niche breadth was very weak ($R^2 = 0.06$, $F_{1,1971} = 6.56$, $P = 0.04$) and there was no relationship to the number of total (un-rarefied) sequences generated per sample ($R^2 = 0.02$, $F_{1,1971} = 0.97$, $P = 0.33$).

Different levels of environmental breadth were driven at least in part by differences among taxonomic groups ($F_{10,4716} = 3.75$, $P < 0.001$). When comparing the two most abundant fungal classes, we found that on average, the environmental breadth of OTUs in the *Sordariomycetes* was higher than that of those classified as *Agaricomycetes* (Fig. S3). Although the relationship was statistically significant, niche breadth was only weakly correlated with relative abundance ($R^2 = 0.04$, $P < 0.001$).

Based on logistic regression analyses, we found significant correlations between presence or absence of individual OTUs in each of the three pastures and the environmental breadth calculated for each OTU. Based on the Akaike

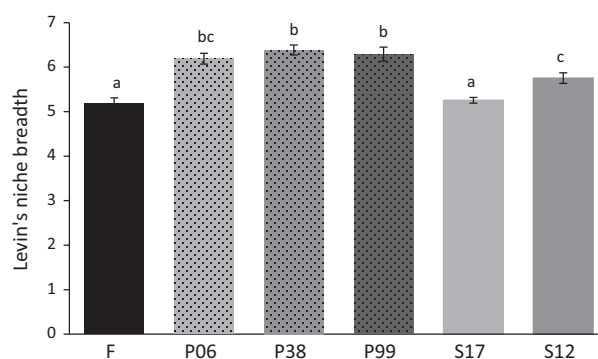


Fig. 4. Mean niche breadth of fungal operational taxonomic units found in the primary forest (F), pastures (P) and secondary forests (S) calculated using Levin's niche width. Different letters indicate significant differences based on Tukey's Honestly Significant Difference test at $\alpha = 0.05$. Error bars represent one standard error.

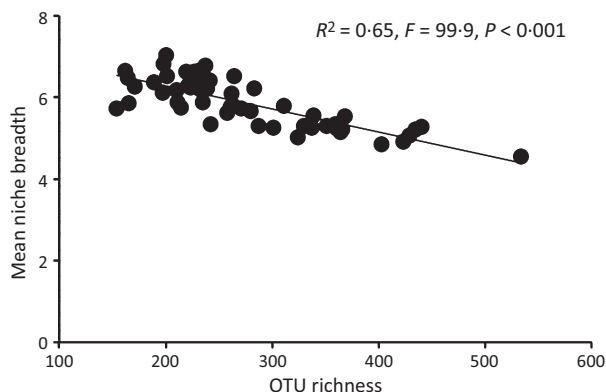


Fig. 5. The relationship between operational taxonomic units richness and mean niche breadth (Levin's) within individual soil cores across the six sites. Error bars represent one standard error.

Information Criterion (AIC), the model was strongest for the youngest pasture site (P06, $Z = 22.3$, $P < 0.001$, AIC = 1614), but was also significant for the other two pastures (P38: $Z = 22.9$, $P < 0.001$, AIC = 1767, P99: $Z = 22.3$, $P < 0.001$, AIC = 1754).

Discussion

The Amazon rain forest is the largest equatorial forest in the world and represents the largest reservoir of plant and animal species (Moreira, Siqueira & Brussaard 2006), hosting an estimated one-quarter of all terrestrial species (Dirzo & Raven 2003). Numerous studies have shown that the diversity of macroscopic organisms responds negatively to land use change in the Amazon region (Gardner *et al.* 2009). Here we show, using deep sequencing across multiple land use types, that soil fungal richness declined following the conversion of primary forest to pasture, with associated increases in the relative abundance of generalist fungi.

FUNGAL COMMUNITY AND TAXONOMIC SHIFTS

The majority of the fungal community belonged to two phyla, the Ascomycota and Basidiomycota. This is consistent with previous studies of soil fungi in tropical forests in Peruvian Amazonia and Panama (McGuire *et al.* 2011; Peay, Baraloto & Fine 2013). In our sites, the Sordariomycetes was the most abundant class, followed by the Agaricomycetes, as seen in soil fungi in *terre firme* rain forests in Peru (Peay, Baraloto & Fine 2013) and fungal communities colonizing leaf litter in Panama (Kerekes *et al.* 2013).

Fungal communities showed significant compositional shifts even at the class level ($P < 0.001$). How these compositional shifts translate into ecosystem functions remains to be determined. Deforestation has been linked to strong shifts in biogeochemical cycling (e.g. Neill *et al.* 1997; Cramer *et al.* 2004), including the carbon and nitrogen cycles,

which are driven largely by microbial communities (van der Heijden, Bardgett & van Straalen 2008). Shifts in microbial community composition following land use change have also been associated with changes in catabolic profiles (Crowther *et al.* 2014) and exoenzyme profiles (McGuire *et al.* 2014). Similarly, a microarray study of a subset of our sites found significant shifts in the abundance, richness and composition of functional genes in response to deforestation (Paula *et al.* 2014), although most genes were linked with bacterial, rather than eukaryote, taxa. Additional studies that quantify both community and functional shifts, through either enzyme analyses (e.g. Talbot *et al.* 2014) or biogeochemical flux measurements, would provide further insights into the functional consequences of these changes.

VARIABILITY WITHIN LAND USE TYPES

At the scale of the three land use types, we found large changes in community composition, particularly between the primary forest and the pasture, as well as significant variability within a single land use type (Fig. 3). One striking pattern from this study was community differences between the two secondary forests. The role of land use type vs. shifts in soil characteristics has been shown to vary. For example, in Australia, Kasel, Bennett & Tibbits (2008) found that fungal communities grouped by land use type, even when they were separated by up to 215 km, and land use type consistently explained more variation in fungal communities than geographic location. In contrast, across a gradient from temperate forest to agriculture, Lauber *et al.* (2008) found that soil texture was the main driver of fungal community composition, independent of land use type.

The variability we observed in the secondary forests suggests that there is some level of stochasticity in recovery of fungal communities when plant communities re-establish in pasture sites, such that fungal communities become more similar to those of primary forests in some sites, while recovery is limited in other locations. Similar patterns have been observed in tree communities, where reassembly in secondary forests is affected by external factors, such as changes in soil characteristics (Guariguata & Ostertag 2001) and interactions between dispersal limitation and environmental conditions (Grman *et al.* 2014) that alter patterns of species' establishment. Compositionally, both secondary forests were more similar to the primary forest than the pasture sites (Fig. 3), but the trajectory of recovery differed between the two sites, as has been seen in plant communities (Norden *et al.* 2009). This suggests that over time, fungal communities in secondary forests may become more similar to those in primary forests, but not necessarily in predictable ways. In temperate ecosystems, there is evidence for irreversible community shifts following land use changes (Dupouey *et al.* 2002), and this remains a possibility for our system.

DRIVERS OF FUNGAL COMMUNITY SHIFTS

Primary forests have been described as irreplaceable reservoirs for diversity (Gardner *et al.* 2009, 2010; Gibson *et al.* 2011), and our findings provide evidence for similar effects on the fungal community. In our analysis of the three pasture sites, we found that distance to primary forest was the strongest correlate to fungal community composition, which suggests that remnant forests serve as reservoirs for recolonization of pastures by forest-associated fungi (Rantalainen *et al.* 2006). This is further supported by the magnitude of community shifts in pastures, and the observation that communities in secondary forests become more similar to communities in primary forests (Fig. 3).

Land use intensity has been linked to sharp declines in the richness of arthropods (Philpott *et al.* 2008) and loss of functional diversity in mammals and birds (Flynn *et al.* 2009). Across the three land use types, we found larger community differences between primary forests and pastures than between pastures and secondary forests (Fig. 3), suggesting that the effects of land use intensity on fungi are similar to those seen in other taxonomic groups. When fungal communities inhabiting pastures were examined more closely, the age of pasture, which was used as a surrogate for land use intensity, was significantly correlated to fungal composition, but the effects were not linear; for example, richness was not significantly lower in the pasture that had been converted in 1911 (P99) than more recently converted pastures (Fig. 1). Across the three pastures, factors related to soil chemistry showed stronger correlations to the composition of the fungal communities than pasture age (Table 1). This suggests that the cumulative effects of cattle grazing and associated disturbances are outweighed by factors shared across the pastures, such as the presence of similar plant communities, leading to small differences among pastures, but large differences across the three land use types.

Variation in community composition was associated with multiple environmental parameters in the pasture sites (Table 1), but when compared at the broadest scale of overall chemical and physical soil heterogeneity, we found no relationship once the effects of spatial distance were removed. Overall, this indicates that although individual environmental factors do play a role in determining the fungal composition in pastures, effects linked to spatial distances, such as dispersal limitation, have a larger impact.

LAND USE CHANGE AND GENERALIST FUNGI

Short-term shifts towards generalist fungi as a result of disturbance might be expected immediately after pasture conversion, as fungi must be able to both survive environmental shifts and utilize dissimilar resources, but the shift towards generalist communities persisted in the 99-year-old pasture (Fig. 4). Generalists are thought to be favoured in heterogeneous environments (Kassen 2002),

and in terms of resources, forests are generally more heterogeneous than pastures (Crowther & Grossart 2015). Higher specialization in primary forests could also be due to plant–fungal linkages, which have been observed for both soil (Peay, Baraloto & Fine 2013) and leaf-associated (Kembel & Mueller 2014) fungal communities in tropical forests.

We found a strong relationship between mean niche breadth and OTU richness (Fig. 5), but weak or non-existent relationships with richness and sequencing depth, suggesting that the observed relationship between niche breadth and richness was likely not due to biases arising from sampling effects. The positive relationship between richness and degree of specialization has been observed in previous empirical and theoretical studies. For example, a simulation study designed to examine coexistence of specialists and generalist found that as the number of competing species increased, the average niche breadth decreased due to the loss of generalist species (Büchi & Vuilleumier 2014). Whether ecosystems with higher abundance of generalists tend to support fewer species is an area of study that deserves further testing, particularly in the context of global changes.

The effects of disturbance on generalist and specialist taxa have primarily been examined in macroscopic organisms, such as plants and animals. Here, we show that deforestation, particularly conversion of forest to pasture, resulted in increased dominance by generalist fungi. Similarly, Nordén *et al.* (2013) found that forest fragmentation negatively affected decomposer fungi that specialized on particular carbon substrates, with limited effects on generalist fungi. We also found that niche breadth was significantly correlated with fungal persistence in pastures. Comparable responses have been shown for plants, where niche breadth was a significant predictor of susceptibility to climate change (Broennimann *et al.* 2006). Numerous studies have shown that generalist plant and animal taxa are favoured by environmental perturbations (McKinney & Lockwood 1999), and our findings indicate that the increased prevalence of generalist taxa is a consistent response to disturbance across broad taxonomic groups.

Acknowledgements

We thank Wagner Piccinini, Fabiana da Silva Paula, Kyunghwa Baek and Babur Mirza for field and laboratory support, the owners of the Fazenda Nova Vida for site access and lodging support and two anonymous reviewers for helpful comments. Funding was provided by a Lewis and Clark Fund for Exploration and Research from the American Philosophical Society and a Agriculture and Food Research Competitive Grant 2009-35319-05186 from the US Department of Agriculture. The authors state no conflict of interest.

Data accessibility

Raw sequence data can be accessed in the MG-RAST data base under the ID 4536676.3.

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Received 15 July 2015; accepted 10 February 2016

Handling Editor: Kathleen Treseder

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Methods.

Fig. S1. Sampling design of hectare plots within the ARMO site.

Fig. S2. Hierarchical clustering of samples based on soil chemistry and texture data from soil cores taken within the primary forest (F), pastures (P), and secondary forests (S).

Fig. S3. Mean niche breadth of fungal Classes. The Classes are sorted by relative abundance from high (Sordariomycetes) to low (Saccharomycetes). Only Classes that comprised >1% relative abundance are shown.