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Forest area and connectivity influence root-associated fungal communities in a fragmented landscape

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Abstract. Habitat fragmentation is well known to affect plant and animal diversity as a result of reduced habitat area and connectivity, but its effects on microorganisms are poorly understood. Using high-throughput sequencing of two regions of the rRNA gene, we studied the effects of forest area and connectivity on the diversity and composition of fungi associated with the roots of the dominant tree, *Metrosideros polymorpha*, in a lava-fragmented landscape on the Island of Hawaii. We found that local fungal diversity increased with forest area, whereas fungal species composition was correlated with fragment connectivity. Fragment size and connectivity were significant predictors even when we included environmental covariates, which were also associated with fungal diversity and composition. Fungal species composition was more similar among highly connected fragments than among poorly connected ones. We also identified individual taxa that varied in abundance with connectivity. Taken together, our results show that habitat fragmentation can alter microbial diversity and composition via differential response among fungal phyla and individual taxa to habitat connectivity.

Key words: dispersal; habitat fragmentation; island biogeography; root-associated fungi.

Introduction

Habitat fragmentation is considered a major threat to biodiversity through simultaneous effects on habitat area, connectivity, and conditions (Pereira et al. 2010). According to the theory of island biogeography (MacArthur and Wilson 1967), reduction in habitat area increases extinction rate, whereas reduction in habitat connectivity decreases immigration rate, both leading to a decline in local diversity. In addition, habitat conditions may be affected by fragmentation through changes in abiotic factors, e.g., soil nutrient availability and climate variability in forest fragments. These effects may be particularly pronounced at fragment edges owing to exposure to the matrix habitat, the phenomenon termed edge effects, which can also result in reduced diversity (Kapos 1989, Crockatt 2012). Although many studies have examined predictions of island biogeography theory and edge effects in the context of fragmented landscapes with plants and animals, fragmentation effects on microorganisms such as bacteria and fungi remain poorly understood (Penttilä et al. 2006, Rantalainen et al. 2008). Given the important roles that microbes play in ecosystems (van der Heijden et al. 2008, McGuire and Treseder 2010), the current dearth of knowledge of how microbes respond to habitat fragmentation hampers our effort to forecast anthropogenic changes to soil carbon storage, biogeochemical cycling, and other ecosystem processes (Balvanera et al. 2014).

To date, the limited number of microbial studies conducted have yielded mixed results. Some suggest that habitat fragmentation may have only a small effect, if any, on microbial diversity. For example, fungi and bacteria appear to exhibit shallower species-area relationships than those in plants and animals (Green and Bohannan 2006, Zhou et al. 2008), presumably because microorganisms disperse widely and have traits that allow for survival through adverse conditions (Finlay 2002, Queloz et al. 2011, Kivlin et al. 2014), consistent with the Bass-Becking hypothesis of microbial distributions (Becking 1934). Other studies suggest, however, that some fungi respond to habitat fragmentation in a manner more consistent with the island biogeographic theory developed for plants and animals (MacArthur and Wilson 1967). In Glomeromycetes, for example, species abundance, composition, and diversity have been shown to vary with habitat area (Mangan et al. 2004, Grilli et al. 2012). Similarly, the species richness and occurrence of wood-decay fungi (Penttilä et al. 2006) have been observed to increase rapidly with area. In ectomycorrhizal fungi on host-plant islands, not only a strong species-area relationship (Peay et al. 2007), but also a negative effect of decreased habitat connectivity on fungal diversity have been found (Peay et al. 2010).

These mixed results may, in part, reflect taxon-specific response to fragmentation due to differences in host specificity, dispersal mode, habitat affiliation, response to edge effects, and other ecological characteristics. For

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example, a recent study reported that ectomycorrhizal and saprotrophic fungi had contrasting geographic distributions (Sato et al. 2012), suggesting that life-history differences between taxonomic groups may generate variable biogeographic patterns. To our knowledge, however, few studies have simultaneously examined multiple groups (e.g., multiple phyla) of fungi for direct cross-taxon comparison. Furthermore, few microbial studies have sought to disentangle the effect of habitat connectivity from that of habitat area or various confounding factors that arise because of the non-random nature of anthropogenic fragmentation with respect to environmental conditions (Fahrig 2003, Ewers and Didham 2006).

In this paper, we seek to determine whether local species diversity and composition of fungi follow predictions based on island biogeography theory. We hypothesized that forest fragment size, connectivity, and edge effects would predict patterns within different taxonomic groups of fungi. To this end, we used high-throughput sequencing of two regions of the rRNA genes to examine the various fungal taxa associated with the roots of the dominant tree, ohia (*Metrosideros polymorpha*, Myrtaceae), in a fragmented landscape on the Island of Hawaii. We did not restrict our sampling of root-associated fungi to specific functional or taxonomic groups, and we simultaneously considered multiple aspects of fragmentation, including fragment size, connectivity and edge effects.

Methods

Study system

We sampled fungi from forest fragments, which are locally called "kipuka," located on the northeast slope of Mauna Loa Volcano on the Island of Hawaii (Flaspohler et al. 2010, Vaughn et al. 2014). Several characteristics of this system (Flaspohler et al. 2010) make it uniquely well-suited for our purpose. First, meandering lava flows from the 1855 and 1881 eruptions of Mauna Loa, rather than human activity, fragmented large contiguous tracts of forest on largely uniform soils that are about 3,000 to 5,000 yr old (Vitousek 2004). As a result, fragments were not subject to anthropogenic selection effects that often complicate fragmentation studies (Ewers and Didham 2006). Second, this landscape contains hundreds of lava-created forest fragments, which are all dominated by *M. polymorpha*, but vary in size and connectivity by several orders of magnitude. Consequently, it is possible to statistically decouple effects of fragment size from those of connectivity by sampling fungi from a set of fragments that minimize the correlation between these predictors. Third, although the lava matrix supports some plant growth (Fig. 1), environmental characteristics in the matrix differ greatly from forest fragments (Vandergast and Gillespie 2004), making the fragments ecologically distinct habitats to many groups of organisms, likely including soil fungi. Finally, this landscape was fragmented about a century and a half ago, which allowed for the loss of taxa that may persist for a long time but only transiently after fragmentation, the phenomenon called the extinction debt (Kuussaari et al. 2009). As such, long-term effects of fragmentation can be evaluated in a standardized fashion across many fragments.

Overall, plant species composition and soil characteristics are relatively constant across forest fragments (Raich et al. 1997), and the canopy is dominated by the tree *Metrosideros polymorpha*. We chose to focus on this single plant species (*M. polymorpha*) because fungal species composition on roots can vary among plant species (Davison et al. 2011). As a result, variation in fungal community composition across sites can be attributed to fragment size and connectivity rather than large changes in plant species composition or preexisting differences in soil characteristics. *M. polymorpha* trees are present in the more recent lava field, but are more sparse and lower-stature than those in fragments (Fig. 1).

Forest fragments ranging in size from 0.07 to 12.8 hectares (Fig. 1) were chosen for sampling. We estimated fragment area by digitizing forest edges using ArcGIS 10.1 (ESRI 2012). Two indices of connectivity among the forest fragments were calculated, including the distance from the focal fragment to its nearest-neighbor fragment and an area-weighted index of connectivity (Hanski 1994, Kindlmann and Burel 2008), calculated as $\Sigma\ a_i \exp(-d_i)$, where a_i is the area (in ha) of neighbor fragment i and d is the distance (in km) from fragment i to the focal fragment, and $\Sigma\ a_i \exp(-d_i)$ is the sum of $a_i \exp(-d_i)$ for all fragments, excluding the focal fragment, within 1 km from the edge of the focal fragment. Forest fragments varied in both fragment size and connectivity (Appendix S1: Fig. S1). Pearson correlations were calculated among all pairs of environmental variables (Appendix S1: Fig. S2) and indicated that fragment size and connectivity were not correlated.

Overview of sampling, preparation, and bioinformatics

A single *M. polymorpha* tree was chosen on the edge and interior of each of the 18 focal fragments (Appendix S2). In fall 2011, root fragments (~3–5 cm each) from each focal tree were positively identified as *M. polymorpha* (Appendices S2–S3), combined at the tree-level and molecular analyses of fungal communities were performed. Full details are contained in Appendix S2. Briefly, DNA was extracted, fungal ITS1 and 28S regions were amplified using primers ITS1f/ITS2 (White et al. 1990, Gardes and Bruns 1993) and glo454/NDL22 (Lekberg et al. 2012), respectively. Amplicon pools were sequenced using 454 (28S) and Illumina MiSeq (ITS). Raw sequence data were deposited in the NCBI short read archive under BioProject ID PRJNA316729. Sequence processing was carried out using QIIME (v1.7)

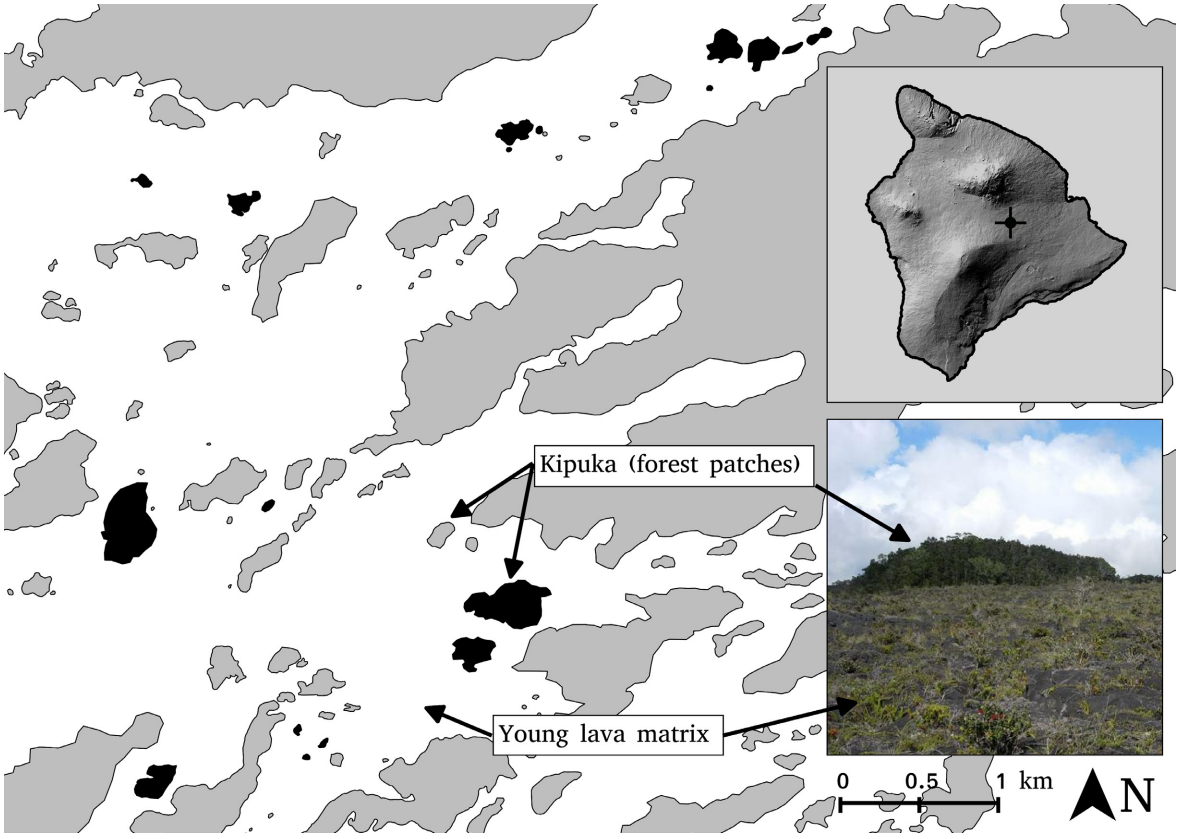

FIG. 1. Map of the forest fragments in the study landscape. Light area indicates lava flows from the 1855 and 1881 eruptions of Mauna Loa. Colored area indicates forested area, where the soil is thought to be approximately 3,000 to 5,000 yr old. Areas shaded in black indicate the 18 fragments from which we sampled fungi, and those in gray were not sampled in the current study. The upper inset map shows the location of the fragments on the Island of Hawaii and the lower inset photograph shows a representative fragment surrounded by the more recent lava field.

(Caporaso et al. 2010) pipeline for 454 reads and USEARCH/UPARSE pipeline for Illumina reads. Full bioinformatics details are contained in Appendix S2.

Oven-dried soil samples were also analyzed for total carbon (C) and nitrogen (N) using an elemental analyzer (Carlo Erba NA 1500). Percent soil carbon (C) and C:N ratio for each tree (Appendix S1: Fig. S1) were calculated using the mass of each sample and a standard curve generated using atropine standards. These measures reflect the combined effects of productivity and decomposition in young Hawaiian soils (Vitousek et al. 1992).

Fungi associated with *M. polymorpha* trees in the lava matrix were sampled in 2014, and ITS region was sequenced as above (see full details in Appendices S2–S3).

Statistical analysis of local diversity

Fungal diversity was estimated at the tree level (alpha diversity) for all samples. Because sequencing depth varied among samples, local diversity metrics, i.e., OTU (operational taxonomic unit) diversity metrics for each tree were estimated using Hill numbers (Chao et al. 2013) at a given sequencing depth using the iNEXT package (Hsieh et al. 2013; Appendix S4: Figs. S1–S2). We estimated species diversity ($q = 0$), Shannon entropy (exponent of Shannon entropy for $q = 1$), and inverse Simpson ($q = 2$) for each tree-level sample. We focus on Shannon entropy and inverse Simpson diversity, as these diversity metrics have been reported to be less sensitive to the detection of rare taxa than species richness and are suggested for the analysis of microbial communities (Bent and Forney 2008, Haegeman et al. 2013). Diversity metrics were estimated at a sequencing depth of 500 sequences/sample for 28S (454) data and 1,500 sequences/sample for the ITS (Illumina) data (Appendix S4: Figs. S1–S2). Analyses using nonparametric estimates of diversity were qualitatively similar to those reported here.

To examine the relationship between species diversity and fragment area and connectivity, $\log_{10}$-transformed local diversity estimates for all fungi at the tree level were regressed against $\log_{10}$-transformed fragment area (2011 data only). To assess edge effects, we included the sampling location (edge or center of the fragment). The full model included fragment area, sampling location,

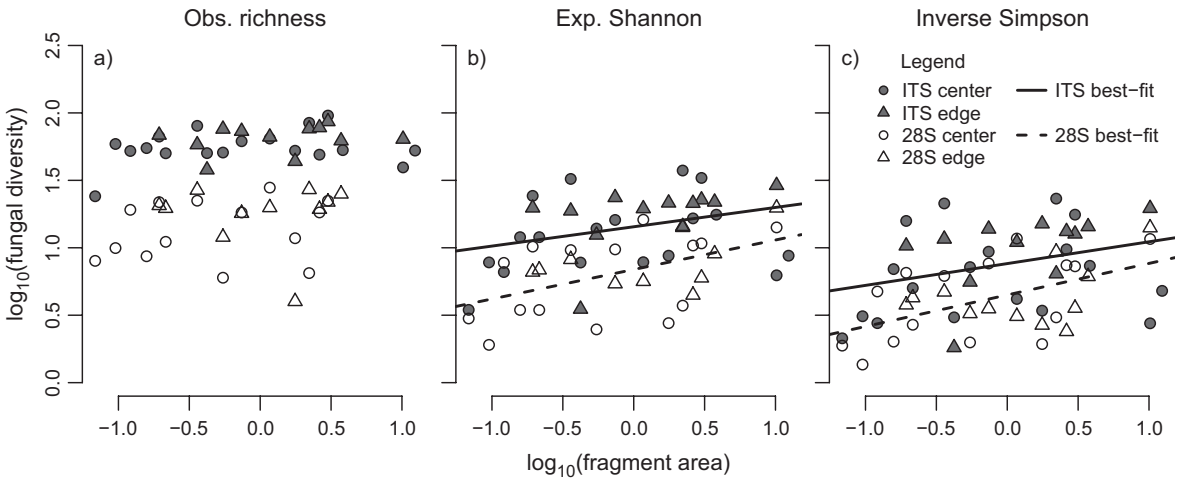

FIG. 2. Log_{10}-transformed local diversity metrics estimated at the tree level regressed against log_{10}-transformed area of forest fragments. Panels show species richness estimated using Hill numbers which correspond to (a) observed species richness ($q = 0$), (b) exponent of Shannon entropy ($q = 1$), and (c) inverse Simpson's diversity ($q = 2$), following Chao et al. (2013) using the iNEXT package (Hsieh et al. 2013). Filled points represent diversity estimated from the ITS region (Illumina sequencing) and open points represent diversity calculated from the 28S region (454 sequencing). Triangles represent samples from the edge of forest fragments and circular points represent samples from the interior of forest fragments. Lines represent best-fit regressions between fragment size and the diversity metric. Lines are shown where $P < 0.05$ for LSU and $P < 0.10$ for ITS. Regression lines for solid points (ITS region) are solid, while dashed lines represent best-fit lines for open points (28S region). Best-fit lines were estimated in the absence of the outlier fragments (see *Materials and methods* for details).

connectivity metrics, and two-way interactions among these three predictors, and environmental covariates, including percent carbon and C:N ratio. Model selection was performed using AIC, and the significance of predictors assessed using *t*- and *F*-tests. Models were fitted separately for each gene region and diversity metric. One sample was found to exert high leverage, so the analysis was run with and without this outlier sample.

Statistical analysis of species composition

We also tested whether species composition at the tree level was correlated with fragment properties using the 2011 data. Bray-Curtis distances were calculated among samples for each gene region separately. Dissimilarities were visualized using NMDS implemented in the vegan package (Oksanen et al. 2012). We performed permutational MANOVAs in R, using the adonis function. Predictors included log_{10}-transformed fragment area, sampling location, the interaction between the two, and nearest neighbor and area-weighted connectivity indices (Appendix S2c), and the soil parameters described previously. The adonis function is sensitive to the order in which variables are added, so multiple permutations of each model verified that the predictors that we identified were consistently the most important. Interactions among predictors were not significant, so were omitted from the models. To assess if connectivity was correlated with the variance in fungal community composition among sampling locations, we used a test of multivariate homogeneity of variance using the betadisper function in vegan, dividing fragments into three bins of connectivity (full details in Appendix S2).

We also assessed if the average abundance of particular OTUs in a sample varied with the area-weighted connectivity of its fragment, using glmFit and glmLRT in the package edgeR (Robinson et al. 2010) following McMurdie and Holmes (2014). We report those OTUs that differed between the high connectivity bin and the mid or low connectivity bins with a false discovery rate (FDR) cutoff of 0.05 (Appendix S2).

We also examined spatial autocorrelation in fungal species composition among forest fragments with Mantel tests, using the mantel function in the vegan package in R (Oksanen et al. 2012). The differences in diversity and species composition of matrix and edge communities were examined using linear ANOVA and PerMANOVA (adonis function in the vegan package), respectively. We used a Mantel test to examine the association between fungal species composition in the matrix and edge.

All statistical analyses were performed in the R environment (R Core Development Team 2012).

RESULTS

Local diversity

Fungal diversity varied among sampling locations, with species richness for pooled samples ranging from 24.1 to 95.7 for ITS and from 4.0 to 35.1 for 28S. Total species richness was not significantly correlated with fragment area (Fig. 2a, Table 1), but total Shannon diversity increased with fragment area (Fig. 2b, Table 1), as did inverse Simpson diversity (Fig. 2c, Table 1). This relationship was significant ($P < 0.01$) in the 28S dataset

TABLE 1. Best-fit models examining the effect of fragment size (log10), connectivity, tree location (edge or center) and environmental predictors (percent carbon and C:N ratio) on total fungal local diversity. Environmental predictors were calculated at the tree level. Richness estimates were calculated using Hill numbers (see *Materials and methods* for details). For each variable that was retained in the model, we report model β and indicate the significance of its *t*-value. Variables not retained are denoted by "NR" and variables not retained in any model are not shown.

Alpha diversity metric	Platform (Region)	Log_{10}area		Tree location		$Area_w$Conn		PercentC		NearestN		Model	
		Coef	*F*	Coef	*F*	Coef	*F*	Coef	*F*	Coef	*F*	*adj.* R^2	*P*-value
Observed species	Illumina (ITS)	NR	–	NR	–	0.00091+	2.94+	NR	–	NR	–	0.06	0.097
	454 (LSU)	NR	–	NR	–	NR	–	−0.84	2.13	−1.33	1.78	0.1	0.16
Exp (Shannon)	Illumina (ITS)	0.14+	3.37+	NR	–	NR	–	NR	–	NR	–	0.075	0.076
	454 (LSU)	0.25**	11.74**	NR	–	NR	–	1.41**	4.98*	0.28*	7.34*	0.48	0.0013
Inverse Simpson	Illumina (ITS)	0.13+	3.20+	0.16	2.03	NR	–	NR	–	NR	–	0.1	0.08
	454 (LSU)	0.23**	9.22**	NR	–	NR	–	NR	–	NR	–	0.24	0.0055

Note: In addition, we report the *F*-value for each predictor and indicate its significance, where $+P < 0.1$, $*P < 0.05$, $**P < 0.01$.

and marginally significant in the ITS dataset. In addition to fragment area, environmental covariates were also associated with fungal diversity. Percent soil carbon was negatively associated with fungal diversity, although this was only significant in the 28S dataset. Connectivity metrics had weak and variable effects on local fungal diversity (Table 1). When significant, increased connectivity was also associated with larger diversity estimates. Regression results for the 28S region were robust to the inclusion or exclusion of data from the outlier fragments, whereas their inclusion in the ITS dataset resulted in a unimodal relationship between area and fungal species diversity. Regression parameters and significance remained qualitatively the same when only trees from the center of each fragment were regressed against fragment area (ITS Shannon $r = 0.14$ $P = 0.07$; ITS Inv. Simpson $r = 0.16$, $P = 0.08$; 28S Shannon $r = 0.21$, $P = 0.01$; 28S Inv. Simpson $r = 0.23$, $P = 0.005$). The diversity of fungal communities associated with plants in the matrix tended to be more species-poor than those in the fragments (Edge 9.45 ± 1.10, Matrix 7.37 ± 0.75, mean Inverse Simpson ± SEM, $P = 0.08$).

Species composition

In most cases, fungal species composition was most closely associated with habitat connectivity and percent soil carbon (Table 2). Both Basidiomycete and Ascomycete composition in the ITS dataset responded to area-weighted connectivity, whereas the distance to the nearest-neighbor was a better predictor of Glomeromycete species composition (ITS and 28S) and Basidiomycetes captured by the 28S dataset (Table 2, Fig. 3). Percent soil carbon was also a significant predictor of overall fungal community composition (ITS and 28S) and Ascomycete species composition (Table 2), and had marginal significance in explaining variation in Basidiomycete composition.

Samples from highly connected fragments were more similar in species composition (lower variance) when examined for all fungi combined, Ascomycetes, Basidiomycetes, and for Glomeromycetes in the ITS dataset (Fig. 3; Appendix S5: Table S1).

The edgeR analysis indicated that 37 OTUs changed in relative abundance with area-weighted connectivity (FDR < 0.05). In the ITS dataset, approximately half of those OTUs (14/23) had a higher relative abundance in highly connected fragments, whereas the others were more abundant in poorly connected fragments (FDR < 0.05). Of those that could be identified at least to Order, three OTUs from the genus *Archaeorhizomyces* were more abundant in highly connected fragments, and one was more abundant in poorly connected fragments (Appendix S6: Fig. S1b). OTUs from the Hypocreales, Helotiales, and Trechisporales were also more abundant in highly connected fragments. In contrast, OTUs from the genera *Diaporthe* and *Microglossum* were more abundant in isolated fragments (Appendix S6: Fig. S1a). In the 28S dataset, single OTUs from the genera *Nidularia* (Agaricales), *Pachylepyrium* (Agaricales), *Auriculariales* (Auriculariales) and *Pholiotina* (Agaricales) were more abundant in highly connected fragments, whereas OTUs from the genera *Sparsitubus* (Polyporales), *Mycena, Catatrama,* and *Pholiota* (Agaricales), *Tremiscus* (Auriculariales), *Chaetotyphula* (Gomphales) and *Amaurodon* (Thelephorales) were more abundant in isolated fragments (Appendix S6: Fig. S1b).

Spatial autocorrelation was detected only in Basidiomycete communities at the scale of 500 m. (Mantel $r = 0.09$, $P = 0.037$). No spatial signature was detected in either the 28S dataset (all fungi: Mantel $r = 0.03$, $P = 0.23$; Basidiomycetes: Mantel $r = 0.047$, $P = 0.17$; Glomeromycetes: Mantel $r = 0.005$, $P = 0.41$) or in the ITS dataset (all fungi: Mantel $r = 0$, $P = 0.56$; Glomeromycetes: Mantel $r = 0.02$, $P = 0.28$; Ascomycetes: Mantel $r = -0.01$, $P = 0.60$).

The species composition of edge and matrix communities differed (PerMANOVA $F_{1,35} = 2.35$, $P < 0.001$), and the composition of edge and matrix communities sampled from the same fragment were only marginally related to each other (Mantel $r = 0.13$, $P = 0.067$).

TABLE 2. Results of adonis (permutational MANOVA) examining the relationships between tree location (edge/center), percent soil carbon, connectivity indices, and fragment area and the community composition of fungal groups. Predictors were included in the order indicated below. However, their relative importance remained unchanged among different order permutations. Values indicate the R^2 for each term in the model.

Dataset	Taxonomic Group	Total OTUs	Tree location	PercentC	$Area_wConn$	NearestN	$Log_{10}area$
Illumina	All fungi	529	0.023	**0.070****	**0.045***	0.037	0.038
Illumina	Basidiomycetes	79	0.021	0.025	**0.064****	0.03	0.04
Illumina	Glomeromycetes	32	0.032	0.054+	0.047	**0.056***	0.023
Illumina	Ascomycetes	190	0.019	**0.073****	**0.052***	0.036	0.04+
454	All fungi	136	0.24	**0.056***	0.036	0.046+	0.027
454	Basidiomycetes	118	0.027	0.046+	0.042	**0.051***	0.028
454	Glomeromycetes	14	0.071+	0.01	0.015	**0.12***	0.038

Note: Bold values are significant at the level of $P < 0.05$ and significance is indicated as follows $+P < 0.10$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

DISCUSSION

Our results indicate that, in this lava-fragmented landscape, the communities of root-associated fungi vary among forest fragments with decreasing habitat size and connectivity. We detected this pattern despite variation in the soil nutrient conditions among fragments. The lack of a significant concordance between fungal community composition in the lava matrix and nearby fragments suggests that spatial variation in matrix quality is unlikely to explain this pattern. In addition, we detected no strong edge effects: fungal communities sampled near fragment edges were not significantly different from those sampled in center plots in diversity or species composition. Fragment size was positively, albeit only loosely, related to local fungal diversity, whereas species composition varied with fragment connectivity. These patterns were largely consistent between two datasets that varied substantially in taxonomic breadth and species composition. This result, in combination with our taxonomic group analysis, points to the importance of taxon-dependent responses to habitat connectivity in understanding how habitat fragmentation affects fungal communities.

The paucity of ecological knowledge on tropical fungi, along with the polyphyletic nature of ecological and life-history traits in fungi (Berbee 2001), make it difficult to identify the characteristics of fungi associated with high or low habitat connectivity. However, changes in the relative abundance of sequences attributed to fungal taxa with well-characterized members across fragments can shed some light on potential mechanisms explaining the shift in fungal species composition with connectivity. Fungi commonly characterized by airborne spore dispersal, like Boletales, Russulales, and Polyporales (Kauserud et al. 2008), were more abundant in isolated fragments than in well-connected fragments (Appendix S6: Fig. S1). Studies on polypores and other wood-decaying fungi suggest that spores can disperse up to a few hundred meters to a few km (but see Norros et al. 2012, Nordén et al. 2013), which would allow spores to travel between nearby fragments in this landscape. Likewise, recent work has found that wind-dispersed Basidiomycete spores show spatial structuring on a similar scale to that uncovered here (Peay and Bruns 2014).

By contrast, fungi with poorly characterized or obligately biotrophic life histories, including OTUs from genera *Archaeorhizomyces*, *Nidularia*, and orders Hypocreales and Trechisporales, were more abundant in well-connected fragments (Appendix S6: Fig. S1). Characterized members of many of these groups have short-distance dispersal strategies, or are soil-dwelling with small or nonexistent fruiting bodies (Rosling et al. 2011), which may be less adept at long-distance dispersal (Porter et al. 2008). Other groups of tropical obligate biotrophic fungi exhibit spatial patterns on a scale similar to that of our study, suggesting that dispersal limitation at the spatial scale examined here may be a common phenomenon among such taxa (Higgins et al. 2014).

However, exceptions to this pattern are also present in the dataset. For example, OTUs within the order Agaricales, which are thought to be adept dispersers, were more abundant in highly connected patches, while some *Archaeorhizomyces* were more abundant in poorly connected fragments (Appendix S6: Fig. S1). Variation in dispersal ability within fungal taxonomic groups due to differences in phenology, spore characteristics, or fruiting body morphology could explain variable response to habitat connectivity (Norros et al. 2012, 2014), but experimental work is necessary to disentangle the relative role of dispersal from other potential factors such as local competition, host specificity, and stochastic extinction that could affect fungal distribution patterns (Hanson et al. 2012).

Connectivity also affected species composition among fragments, where communities were more similar among better-connected fragments. The specific connectivity index that predicted community similarity varied among fungal groups, which may reflect differential dispersal strategies. For example, many members of the Basidiomycota and Ascomycota have aboveground fruiting bodies and long-range dispersal strategies (Peay and Bruns 2014). In contrast, Glomeromycetes are characterized by

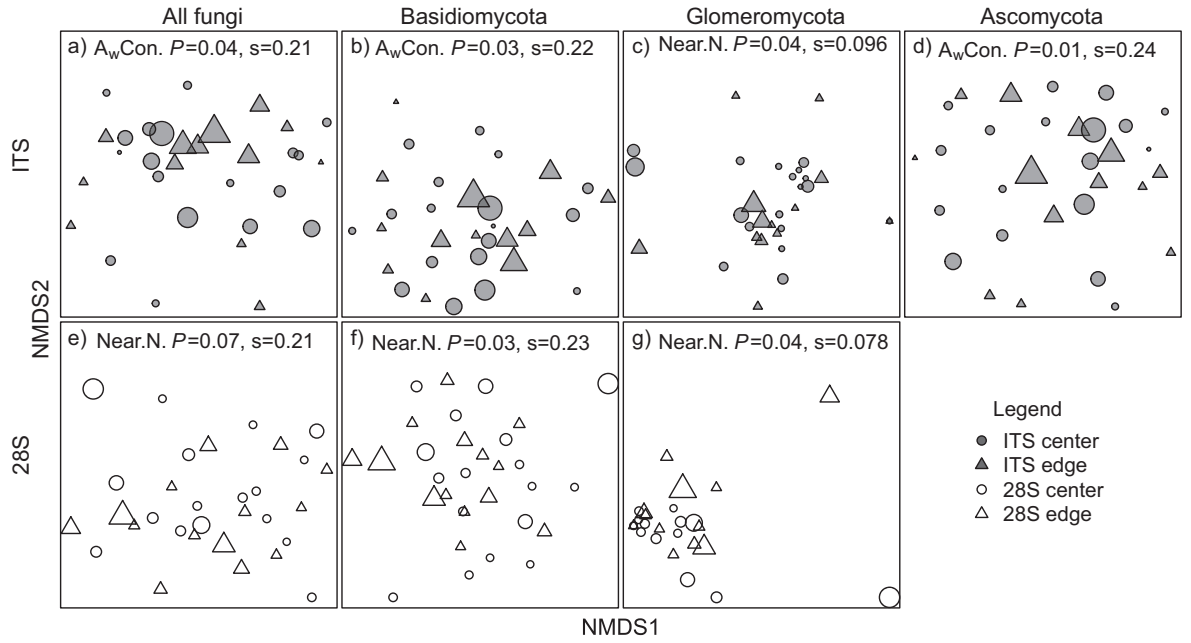

FIG. 3. Non-metric multidimensional scaling (NMDS) plots showing similarity among sites based on fungal species composition generated using Illumina sequencing of the ITS region (a–d) or 454 sequencing of the 28S region of ribosomal DNA (e–g), based on the Bray-Curtis distance metric. PerMANOVA statistics are listed in Table 2. Points represent sampling locations (pooled root fragments at the tree level). Triangles represent trees near the fragment edge, and circles represent trees near the center of fragment. The size of points represents connectivity index values with larger points corresponding to fragments with higher connectivity values. Area-weighted connectivity is used in a, b, d where larger points represent greater connectivity. Point size represents the minimum distance to the nearest forest fragment in c, e, f and g, where larger points represent a greater distance to the nearest fragment. Stress values (s) for NMDS plots are indicated on each panel.

belowground propagules. Wind dispersal of spores has been documented (Warner et al. 1987), but small-grained spatial patterns of the composition of Glomeromycetes suggest dispersal limitation at small scales (Wolfe et al. 2007). In either case, more highly connected fragments may receive more frequent immigration via dispersal from surrounding forested area. Theory suggests that, when dispersal from outside fragments is more frequent, processes operating at the local scale within fragments play a more important role in shaping the composition of these communities (Lockwood et al. 1997, Fukami 2005). A similar pattern has been found in plants, where more highly connected fragments were characterized by similar plant communities (Kemper et al. 1999).

Additional processes may interact with altered dispersal rate to moderate fungal response to habitat fragmentation. For example, small-scale experiments have suggested that habitat fragmentation may change predator-prey dynamics and soil food web structure (Holyoak and Lawler 1996, Rantalainen et al. 2005). In addition, fragmentation may also affect the distribution of hosts for obligatory parasitic or mutualistic fungal taxa (Cordeiro and Howe 2003), movement patterns of fungal vectors, and the availability of other abiotic or biotic factors, which may also contribute to disparate responses to habitat fragmentation among fungal groups that differ in life-history strategies (Sato et al. 2012) or environmental requirements (Nordén et al. 2013). Currently, we do not have the data needed to assess these possibilities in our study system.

Compared to other studies that also documented effects of habitat area on fungal diversity (Berglund and Jonsson 2001, Penttilä et al. 2006, Grilli et al. 2012), the relationship between landscape parameters and fungal diversity and community composition that we detected here are relatively weak. It could be that root-associated fungi respond only weakly to landscape factors at this scale (Grilli et al. 2015). However, methods used in this experiment may bias towards a weak relationship for a few reasons. First, we sampled only the fungi associated with a single tree species, *M. polymorpha*, on individual root tips. Although this allowed us to estimate local diversity at small scales, sampling across an entire fragment may increase the signal of fragment area because fungal diversity often changes in tandem with plant diversity (van der Heijden et al. 1998, Peay et al. 2013) and neighborhood diversity. Second, although our samples exhibited saturating curves for Shannon and inverse Simpson diversity measures (Appendix S4), a greater sampling depth (Smith and Peay 2014) or sample number could improve our estimates of fungal richness and, consequently, the power to detect the effects of fragment size. Including very large fragments or a contiguous forest tract could also strengthen this relationship. Finally, fungal community composition and diversity were also related to soil C (Table 2), which had

greater explanatory power than did habitat area or connectivity in some cases. It is possible that forest fragments vary in soil properties or local plant composition due to preexisting variation or plant-soil feedbacks generated by differential loss of taxa following disturbance.

In summary, our results demonstrate that habitat fragmentation can influence fungal species diversity and composition. Although the mechanisms responsible for the patterns we observed remain unclear, our analyses indicated a high abundance of fungi with presumed longer dispersal ranges and a low abundance of some cryptic and obligately biotrophic soil fungi in poorly connected fragments. We suggest that a key to understanding taxon-specific responses of fungi to habitat fragmentation may be differences in life history, particularly in the way different taxa disperse across fragmented landscapes.

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Literature Cited

Balvanera, P., I. Siddique, L. Dee, A. Paquette, F. Isbell, A. Gonzalez, J. Byrnes, M. I. O'Connor, B. A. Hungate, and J. N. Griffin. 2014. Linking biodiversity and ecosystem services: current uncertainties and the necessary next steps. BioScience 64:49–57.

Becking, L. G. M. B. 1934. Geobiologie, of Inleiding Tot de Milieukunde: Met Literatuurlijst en Ind. Van Stockum & Zoon, 1934.

Bent, S. J., and L. J. Forney. 2008. The tragedy of the uncommon: understanding limitations in the analysis of microbial diversity. ISME Journal 2:689–695.

Berbee, M. L. 2001. The phylogeny of plant and animal pathogens in the Ascomycota. Physiological and Molecular Plant Pathology 59:165–187.

Berglund, H., and B. G. Jonsson. 2001. Predictability of plant and fungal species richness of old-growth boreal forest islands. Journal of Vegetation Science 12:857–866.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich, and J. I. Gordon. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336.

Chao, A., N. Gotelli, T. Hsieh, E. Sander, K. Ma, R. K. Colwell, and A. M. Ellison. 2013. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. Ecological Monographs 84:45–67.

Cordeiro, N. J., and H. F. Howe. 2003. Forest fragmentation severs mutualism between seed dispersers and an endemic African tree. Proceedings of the National Academy of Sciences of the United States of America 100:14052–14056.

Crockatt, M. E. 2012. Are there edge effects on forest fungi and if so do they matter? Fungal Biology Reviews 26:94–101.

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

ESRI 2012. ArcGIS Desktop: Release 10.1 Redlands, CA: Environmental Systems Research Institute.

Ewers, R. M., and R. K. Didham. 2006. Confounding factors in the detection of species responses to habitat fragmentation. Biological Reviews 81:117–142.

Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics 34:487–515.

Finlay, B. J. 2002. Global dispersal of free-living microbial eukaryote species. Science 296:1061–1063.

Flaspohler, D. J., C. P. Giardina, G. P. Asner, P. Hart, J. Price, C. K. Lyons, and X. Castaneda. 2010. Long-term effects of fragmentation and fragment properties on bird species richness in Hawaiian forests. Biological Conservation 143:280–288.

Fukami, T. 2005. Integrating internal and external dispersal in metacommunity assembly: preliminary theoretical analyses. Ecological Research 20:623–631.

Gardes, M., and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113–118.

Green, J., and B. J. M. Bohannan. 2006. Spatial scaling of microbial biodiversity. Trends in Ecology & Evolution 21:501–507.

Grilli, G., C. Urcelay, and L. Galetto. 2012. Forest fragment size and nutrient availability: complex responses of mycorrhizal fungi in native–exotic hosts. Plant Ecology 213:155–165.

Grilli, G., C. Urcelay, L. Galetto, J. Davison, M. Vasar, Ü. Saks, T. Jairus, and M. Öpik. 2015. The composition of arbuscular mycorrhizal fungal communities in the roots of a ruderal forb is not related to the forest fragmentation process. Environmental Microbiology 17:2709–2720.

Haegeman, B., J. Hamelin, J. Moriarty, P. Neal, J. Dushoff, and J. S. Weitz. 2013. Robust estimation of microbial diversity in theory and in practice. ISME Journal 7:1092–1101.

Hanski, I. 1994. A practical model of metapopulation dynamics. Journal of Animal Ecology 63:151–162.

Hanson, C. A., J. A. Fuhrman, M. C. Horner-Devine, and J. B. H. Martiny. 2012. Beyond biogeographic patterns: processes shaping the microbial landscape. Nature Reviews Microbiology 10:497–506.

Higgins, K. L., A. E. Arnold, P. D. Coley, and T. A. Kursar. 2014. Communities of fungal endophytes in tropical forest grasses: highly diverse host- and habitat generalists characterized by strong spatial structure. Fungal Ecology 8:1–11.

Holyoak, M., and S. P. Lawler. 1996. Persistence of an extinction-prone predator-prey interaction through metapopulation dynamics. Ecology 77:1867–1879.

Hsieh, T., K. Ma, and A. Chao. 2013. iNEXT online: interpolation and extrapolation

Kapos, V. 1989. Effects of isolation on the water status of forest patches in the Brazilian Amazon. Journal of Tropical Ecology 5:173–185.

Kauserud, H., J. E. Colman, and L. Ryvarden. 2008. Relationship between basidiospore size, shape and life history characteristics: a comparison of polypores. Fungal Ecology 1:19–23.

Kemper, J., R. M. Cowling, and D. M. Richardson. 1999. Fragmentation of South African renosterveld shrublands: effects on plant community structure and conservation implications. Biological Conservation 90:103–111.

Kindlmann, P., and F. Burel. 2008. Connectivity measures: a review. Landscape Ecology 23:879–890.

Kivlin, S. N., G. C. Winston, M. L. Goulden, and K. K. Treseder. 2014. Environmental filtering affects soil fungal community composition more than dispersal limitation at regional scales. Fungal Ecology 12:14–25.

Kuussaari, M., et al. 2009. Extinction debt: a challenge for biodiversity conservation. Trends in Ecology & Evolution 24:564–571.

Lekberg, Y., T. Schnoor, R. Kjøller, S. M. Gibbons, L. H. Hansen, W. A. Al-Soud, S. J. Sørensen, and S. Rosendahl. 2012. 454-sequencing reveals stochastic local reassembly and high disturbance tolerance within arbuscular mycorrhizal fungal communities. Journal of Ecology 100:151–160.

Lockwood, J. L., R. D. Powell, M. P. Nott, and S. L. Pimm. 1997. Assembling ecological communities in time and space. Oikos 80:549–553.

MacArthur, R. H., and E. O. Wilson. 1967. The theory of island biogeography. Princeton University Press, Princeton, New Jersey, USA.

Mangan, S. A., A. H. Eom, G. H. Adler, J. B. Yavitt, and E. A. Herre. 2004. Diversity of arbuscular mycorrhizal fungi across a fragmented forest in Panama: insular spore communities differ from mainland communities. Oecologia 141:687–700.

McGuire, K. L., and K. K. Treseder. 2010. Microbial communities and their relevance for ecosystem models: decomposition as a case study. Soil Biology and Biochemistry 42:529–535.

McMurdie, P. J., and S. Holmes. 2014. Waste not, want not: why rarefying microbiome data is inadmissible. PLoS Computational Biology 10:e1003531.

Nordén, J., R. Penttilä, J. Siitonen, E. Tomppo, and O. Ovaskainen. 2013. Specialist species of wood-inhabiting fungi struggle while generalists thrive in fragmented boreal forests. Journal of Ecology 101:701–712.

Norros, V., R. Penttilä, M. Suominen, and O. Ovaskainen. 2012. Dispersal may limit the occurrence of specialist wood decay fungi already at small spatial scales. Oikos 121:961–974.

Norros, V., Ü. Rannik, T. Hussein, T. Petäjä, T. Vesala, and O. Ovaskainen. 2014. Do small spores disperse further than large spores? Ecology 95:1612–1621.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. Stevens, and H. Wagner. 2012. vegan: community ecology package.

Peay, K. G., and T. D. Bruns. 2014. Spore dispersal of basidiomycete fungi at the landscape scale is driven by stochastic and deterministic processes and generates variability in plant–fungal interactions. New Phytologist 204:180–191.

Peay, K. G., T. D. Bruns, P. G. Kennedy, S. E. Bergemann, and M. Garbelotto. 2007. A strong species-area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi. Ecology Letters 10:470–480.

Peay, K. G., M. Garbelotto, and T. D. Bruns. 2010. Evidence of dispersal limitation in soil microorganisms: isolation reduces species richness on mycorrhizal tree islands. Ecology 91:3631–3640.

Peay, K. G., C. Baraloto, and P. V. A. Fine. 2013. Strong coupling of plant and fungal community structure across western Amazonian rainforests. ISME Journal 7:1852–1861.

Penttilä, R., M. Lindgren, O. Miettinen, H. Rita, and I. Hanski. 2006. Consequences of forest fragmentation for polyporous fungi at two spatial scales. Oikos 114:225–240.

Pereira, H. M., P. W. Leadley, V. Proença, R. Alkemade, J. P. Scharlemann, J. F. Fernandez-Manjarrés, M. B. Araúo, P. Balvanera, R. Biggs, and W. W. Cheung. 2010. Scenarios for global biodiversity in the 21st century. Science 330: 1496–1501.

Porter, T. M., C. W. Schadt, L. Rizvi, A. P. Martin, S. K. Schmidt, L. Scott-Denton, R. Vilgalys, and J. M. Moncalvo. 2008. Widespread occurrence and phylogenetic placement of a soil clone group adds a prominent new branch to the fungal tree of life. Molecular Phylogenetics and Evolution 46:635–644.

Queloz, V., T. N. Sieber, O. Holdenrieder, B. A. McDonald, and C. R. Grunig. 2011. No biogeographical pattern for a root-associated fungal species complex. Global Ecology and Biogeography 20:160–169.

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

Raich, J. W., A. E. Russell, and P. M. Vitousek. 1997. Primary productivity and ecosystem development along an elevational gradient on Mauna Loa, Hawai'i. Ecology 78:707–721.

Rantalainen, M.-L., H. Fritze, J. Haimi, T. Pennanen, and H. Setälä. 2005. Species richness and food web structure of soil decomposer community as affected by the size of habitat fragment and habitat corridors. Global Change Biology 11:1614–1627.

Rantalainen, M.-L., J. Haimi, H. Fritze, T. Pennanen, and H. Setälä. 2008. Soil decomposer community as a model system in studying the effects of habitat fragmentation and habitat corridors. Soil Biology and Biochemistry 40: 853–863.

Robinson, M. D., D. J. McCarthy, and G. K. Smyth. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140.

Rosling, A., F. Cox, K. Cruz-Martinez, K. Ihrmark, G.-A. l. Grelet, B. r. D. Lindahl, A. Menkis, and T. Y. James. 2011. Archaeorhizomycetes: unearthing an ancient class of ubiquitous soil fungi. Science 333:876–879.

Sato, H., R. Tsujino, K. Kurita, K. Yokoyama, and K. Agata. 2012. Modelling the global distribution of fungal species: new insights into microbial cosmopolitanism. Molecular Ecology 21:5599–5612.

Smith, D. P., and K. G. Peay. 2014. Sequence depth, not PCR replication, improves ecological inference from next generation DNA sequencing. PLoS ONE 9:e90234.

Vandergast, A. G., and R. G. Gillespie. 2004. Effects of natural forest fragmentation on a Hawaiian spider community. Environmental Entomology 33:1296–1305.

van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72.

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

Vaughn, N. R., G. P. Asner, and C. P. Giardina. 2014. Centennial impacts of fragmentation on the canopy structure of tropical montane forest. Ecological Applications 24:1638–1650.

Vitousek, P. M. 2004. Nutrient cycling and limitation: Hawai'i as a model system. Princeton University Press, Princeton, New Jersey, USA.

Vitousek, P. M., G. Aplet, D. Turner, and J. J. Lockwood. 1992. The Mauna Loa environmental matrix: foliar and soil nutrients. Oecologia 89:372–382.

Warner, N. J., M. F. Allen, and J. A. MacMahon. 1987. Dispersal agents of vesicular-arbuscular mycorrhizal fungi in a disturbed arid ecosystem. Mycologia 79:721–730.

White, T. J., T. D. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322 *in* M. A. Innis, J. J. Gelfand, J. J. Sninsky, and T. J. White, editors. PCR protocols: a guide to methods and applications. Academic Press, New York, New York, USA.

Wolfe, B. E., D. L. Mummey, M. C. Rillig, and J. N. Klironomos. 2007. Small-scale spatial heterogeneity of arbuscular mycorrhizal fungal abundance and community composition in a wetland plant community. Mycorrhiza 17:175–183.

Zhou, J., S. Kang, C. W. Schadt, and C. T. Garten. 2008. Spatial scaling of functional gene diversity across various microbial taxa. Proceedings of the National Academy of Sciences of the United States of America 105: 7768–7773.

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Data Availability

Sequence data have been deposited at NCBI SRA under BioProject ID PRJNA316729.