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LETTER

A strong species–area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi

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Abstract

While the effects of habitat size and isolation have been successfully studied for macro-organisms, there is currently debate about their relative importance in explaining patterns of microbial species richness. In this study, we examine the species richness of a dominant group of eukaryotic soil microbes, ectomycorrhizal fungi, on 'tree islands' of constant age and host composition that range in size from < 10 to > 10 000 m^2. Our results show that ectomycorrhizal species richness is significantly reduced on smaller and more isolated tree islands, and the species–area slope that we observe (0.20–0.23) is similar to average slopes reported for macro-organisms. Additionally, species' occurrence patterns across tree islands and investment trends in fungal fruit bodies suggest that a trade-off between competition and dispersal could play an important role in structuring ectomycorrhizal assemblages.

Keywords

Baas-Becking, biogeography, competition, dispersal, ectomycorrhiza, fungi, microbe, species–area, trade-off.

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INTRODUCTION

Habitat size and isolation play a key role in our understanding of the processes that generate and maintain species richness. The predictable rise in species richness with increasing sample area was among the earliest quantitative patterns in ecology (Arrhenius 1921), and limited dispersal into isolated habitat patches played a central role in Gleason's conceptualization of non-equilibrium (i.e. individualistic) plant communities (Gleason 1927). The effects of both habitat size and isolation have since been elaborated and synthesized in theories such as island biogeography (MacArthur & Wilson 1967), metapopulations (Levins 1969; Gilpin & Hanski 1991), the spatial competition hypothesis of diversity (Tilman 1994) and the neutral theory of biodiversity and biogeography (Hubbell 2001). Increasing habitat size is thought to affect species richness primarily through increases in niche variety and population size, while isolation decreases species richness by reducing the number of potential colonists dispersing into an area (MacArthur & Wilson 1967; Rosenzweig 1995). Apart from its theoretical value, this body of work has proved important for conservation planning in the modern period of habitat destruction and fragmentation.

While the effects of habitat size and isolation have been successfully demonstrated for macro-organisms (e.g. Simberloff & Wilson 1969; Simberloff 1976; Lomolino 1984; Hanski *et al.* 1994; Wardle *et al.* 2003 and many others), there is currently strong debate about their usefulness in explaining richness patterns of microbial organisms (Finlay 2002; Whitaker *et al.* 2003; Green *et al.* 2004; Horner-Devine *et al.* 2004; Bell *et al.* 2005; Fenchel & Finlay 2005; Fierer & Jackson 2006), based primarily on the potential of microbes for ubiquitous dispersal and large populations (Green & Bohannan 2006). This view was famously articulated by Baas-Becking (1934) as, 'everything is everywhere, but, the environment selects', and has continued to receive support in a number of recent studies (Finlay 2002; Fenchel & Finlay 2004; Fierer & Jackson 2006).

Because the Baas-Becking view predicts that microbial species will be ubiquitous in suitable habitat, one approach to testing it is by examining the degree of spatial turnover

found in microbial species–area relationships (SARs). This has become truly possible only in recent years because of the advent of DNA-based molecular tools that provide the taxonomic resolution necessary for assessing the diversity of microbial organisms. While a number of recent, molecular-based studies have established microbial SARs, the degree of spatial turnover found has varied dramatically between studies and include some of the lowest SAR values thus reported (Woodcock *et al.* 2006). In addition, only a few studies have looked directly for evidence that isolation and dispersal limitation can affect microbial assemblages (Telford *et al.* 2006). Thus, it is still unclear at what scale habitat size, isolation and dispersal should be considered in microbial ecology (Green & Bohannan 2006).

Ectomycorrhizal (EM) fungi, a major group of soil microbial eukaryotes, are involved in an obligate symbiosis with the roots of many dominant tree families (such as the Pinaceae, Fagaceae and Dipterocarpaceae) in boreal, temperate and tropical ecosystems (Read 1991). They are important drivers of ecosystem function, receiving *c.* 15% of net primary productivity and can provide up to 86% of host nitrogen (Hobbie & Hobbie 2006; Hobbie 2006). Like most microbes, they produce microscopic propagules (*c.* 10 μm) that can disperse long distances and vegetative structures (hyphae, *c.* 5 μm diameter) that can obtain very high density in small areas (Taylor & Alexander 2005). However, relative to bacteria, EM fungi have better developed species concepts and less diverse assemblages, reducing the difficulty in obtaining adequate sample coverage. While no studies have used modern molecular techniques to produce a SAR for EM fungi, recent studies on arbuscular mycorrhizal fungi and soil ascomycetous fungi showed very low levels of spatial turnover (Green *et al.* 2004; Mangan *et al.* 2004).

In this study, we adopted a 'tree island' approach, first suggested by Janzen (1968), as a means to examine how both habitat size and isolation affect the richness of EM fungi. Because EM fungi are involved in an obligate symbiosis, we were able to clearly define patches of suitable habitat, something not easily performed for most terrestrial microbes. In addition, our study took place in a coastal ecosystem dominated by a single ectomycorrhizal tree species, *Pinus muricata* D. Don (Bishop Pine), which occurs in a matrix of scrub vegetation that is not ectomycorrhizal. Because *P. muricata* recruits almost exclusively after stand replacing fires (Sugnet 1985), we were able to identify and sample EM fungi exclusively from a single, even-aged cohort of *P. muricata*. From this study, we derive one of the first quantitative SARs for EM fungi, and demonstrate levels of spatial turnover comparable with most macro-organisms. In addition, we also provide evidence that dispersal limitation can have both direct and indirect effects on EM assemblage composition.

METHODS

Study site

The study was conducted at Point Reyes National Seashore, located in west Marin County, California (38 °04′N by 122 °50′W). The area has a Mediterranean climate, with cold wet winters and hot dry summers. Elevation of the study sites ranged from 150 m to approximately sea level. Coastal soils at Point Reyes are predominately in the Pablo-Bayview complex, an inceptisol formed from siliceous shale or sandstone.

The coastal pine forests at Point Reyes are dominated by a single EM host plant, *P. muricata*, a closed cone pine that requires high intensity fires for seed release. Near the coast, *P. muricata* intergrades with grasslands and scrub, which is dominated by *Baccharis pilularis*, *Toxicodendron diversiloba* and *Rubus ursinus*, all non-EM hosts. Because of the unique ecology of *P. muricata*, all of the trees that make up the habitat islands included in this study are the same age. All of these trees established in 1995, after the Vision fire burned approximately 5000 ha of coastal scrub and forest at Point Reyes. The fire burned through large stands of mature *P. muricata*, resulting in massive seed release and highly patchy recruitment of *P. muricata* into previously unforested areas of coastal scrub vegetation. Because *P. muricata* is the only EM host in the coastal scrub, these patches are essentially islands with respect to their EM colonists. In addition, because almost all mature *P. muricata* within the Vision fire perimeter were killed, we were able to situate our study in an area where all trees derive from the same cohort and are of the same age.

Island delineation and selection

To identify tree islands for inclusion in the study, we made maps of *P. muricata* based on manual classification of 2004 colour digital orthoquads of Marin County with submeter resolution using the program ArcGIS 8.1 (ESRI, Redlands, CA, USA). Island area for all newly mapped tree islands was calculated in ArcGIS as the area of contiguous *P. muricata* canopy as viewed from the aerial photos. To determine island isolation we used 1994 National Park Service GIS maps of pre-fire vegetation to calculate the distance of each new tree island from pre-existing patches of *P. muricata* (> 0.1 ha). These patches represent the closest possible source of EM fungal colonists on newly created islands and also correlate roughly with distance to mature *P. muricata* forest remaining outside the Vision fire perimeter. We also determined the number of soil types encompassed by each island using a digital copy of the Marin Soil Survey (USDA 1985). The Marin Soil Survey is a fine-scale soil classification which includes factors such as parent material, pH, soil texture and organic matter content. Using these characters,

the soil series at Point Reyes are further classified into 87 soil phases. To determine the number of soil types per island, we calculated zonal statistics in ArcGIS based on a 1-m^2 rasterized version of the Marin Soil Survey. We included all soil phases in our analyses except those distinguished only by slope.

To ensure that we sampled EM richness evenly across the range of potential island size and isolation, islands were divided into four size classes (1–10, 10–100, 100–1000 and > 1000 m^2) and three distances classes (0–100, 100–1000 and > 1000 m). One island from each size class was then randomly selected from the population of islands in each distance class ($4 \times 3 = 12$ islands). While we used the size and distance classes to devise a stratified random sampling scheme, actual distance and area of each island was used for all statistical analyses. This sampling scheme also assured that there was no cross-correlation between island area and isolation that might confound later statistical analyses ($R^2 = 0.17$, $P = 0.18$). Preliminary soil cores (see following section for dimensions) taken at 1, 2, 3 and 4 m along three transects moving away from patches of *P. muricata* into the surrounding scrub indicated that ectomycorrhizal roots were very rare after 1 m and were never detected at 4 m. While this may appear to be a small rooting zone compared with mature trees in a forest setting, the 10-year-old *P. muricata* are still relatively small (canopy diameter *c.* 1–2 m) and probably have reduced rooting zones compared with mature trees. In addition, they are surrounded by densely rooted scrub vegetation that likely limits extension of the rooting zone compared with forest settings. Although fungal hyphae and rhizomorphs may grow beyond the extent of colonized roots, fungal fruit bodies (which often appear at the advancing edge of the hyphal front) were never observed > 1 m from the canopy of study islands, even for the most rhizomorphic taxa (e.g. *Suillus pungens*; K.G. Peay, personal observation). For these reasons, islands were classified as insular (and included in the study) only if they were at least 8 m from any patch whose additional area would have changed its size class designation (i.e. 0–10, 10–100, 100–1000 and > 1000).

EM sampling design

To estimate the species richness of EM fungi on each island, we sampled the EM assemblage from both root tips and mushroom fruiting bodies. EM root tips were sampled by directly removing soil using PVC cores (2.5 cm internal diameter × 30.5 cm length). Because we expected more difficulty saturating sampling curves on larger islands, we took a variable number of cores depending on island size; six cores from 1 to 10 m^2 islands, eight cores from 10 to 100 m^2 islands and 12 cores from 100 to 1000 m^2 and > 1000 m^2 islands. As discussed below, the adequacy of these samples was assessed to determine if additional sampling was necessary. Cores were placed systematically within each island to maximize inter-core distance and cover as much of the island area as possible. After removal, cores ends were wrapped in Parafilm (Pechiney Plastic Packaging, Manasha, WI, USA), brought back to the laboratory, and stored at 4 °C until processing (no > 5 days after removal). To extract EM roots, soil from each core was washed through a series of soil sieves and the 500 μm fraction removed and spread evenly in a gridded, numbered Petri dish. Using the grid system and a random number generator, five individual root tips were randomly selected from each core for DNA extraction. Root tips were then flash frozen in liquid Nitrogen, lyophilized for 48 h, and stored at −40 °C until DNA extraction.

Mushroom fruit bodies were sampled 11 times on each island, in approximately 2 week intervals from November 11, 2005 to March 31, 2006. This time period spans the rainy season in coastal California when the vast majority of fruiting occurs, although some fruit bodies may be produced outside of these months. To maximize the number of fruit bodies observed, sampling during a given 10 week window was carried out within 2–5 days of measurable precipitation if possible. To standardize sampling effort across islands, each sample consisted of a timed 10 min search, during which the presence of all fruiting species at an island was noted. Each island was carefully checked for epigeous fruit bodies, as well as less conspicuous hypogeous and resupinate fruit bodies by using a rake to examine duff and coarse woody debris. Those fruit bodies that were not immediately identifiable or observed for the first time were transported to the laboratory for identification. Voucher specimens for each species were photographed and dried for long-term storage at the UC Herbarium. Fresh tissue samples from each collected fruit body were removed and stored at −20 °C in CTAB for DNA extraction.

Molecular protocols

To identify root tips and unknown fruit bodies we used the polymerase chain reaction (PCR) to amplify and then sequence DNA from the internally transcribed spacer regions (ITS) of the ribosomal RNA genes (rDNA). Because this is the most commonly sequenced DNA region for fungi there is a large database of sequences and we know that it correlates well with current morphological species concepts for these fungi (Horton & Bruns 2001).

To extract DNA for sequencing we used the DNeasy Tissue Kit (Qiagen Sciences, Valencia, CA, USA) with a slightly modified general protocol. Lyophilized root tips were placed in a 2-mL screw cap tube with a 0.5-mm glass beads and beaten for 30 s at max speed on a Mixer Mill MM 301 (Retsch, Haan, Germany), or until roots were suffi-

ciently pulverized. Pulverized roots were then placed in 1000 μL of 2× CTAB buffer and incubated at 65 °C for 1 h. Following incubation the samples were vortexed with a 24 : 1 chloroform:isoamyl alcohol mix, centrifuged for 10 min at 13 000 ***g***, and the aqueous phase mixed with 0.5 volumes 95% EtOH. From this point samples were extracted with the DNeasy kit following manufacturers' instructions.

PCR was carried out in 25 μL reactions using standard cycling parameters for the fungal specific primer combination ITS1f and ITS4 (White *et al.* 1990; Gardes & Bruns 1993). For mixed samples (and basidiomycete fruit bodies), we used the ITS1f and ITS4b primer combination to eliminate possible contamination from ascomycetous root endophytes (Gardes & Bruns 1993). PCR products were cleaned using 0.5 μL of ExoSAP IT (USB Corp, Cleveland, OH, USA) in 7–10 μL of DNA, and cycled at 37 °C for 15 min, followed by 80 °C for 15 min. Single pass sequencing was performed using an ABI3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were edited to remove priming sites and poor quality bases at the 5′ and 3′ sequence using the program Sequencher 4.2 (Gene Codes Corp., Ann Arbor, MI, USA).

Identification of EM taxa

EM taxa were identified using a combination of morphological and molecular techniques. Fruit bodies collected in the field were identified first using standard morphological keys based on both macroscopic and microscopic characters. Most fruit bodies were identifiable to either species or genus using these keys. To improve identification of difficult taxonomic groups (e.g. *Inocybe*, *Hebeloma*, *Cortinarius*), rDNA sequences from fruit bodies were also compared with sequences available on the National Center for Bioscience Informatics (NCBI) website using the BLAST tool and assigned to a nearest taxonomic rank according to the level of match. For root tips and unidentifiable fruit bodies, species were defined using a 95% DNA sequence similarity cutoff. This was performed by grouping individual sequences using the Contig feature of Sequencher with parameters set to a minimum of 20% overlap and 95% sequence similarity. The same 95% cutoff successfully differentiated EM fruit bodies in all but two instances. Root tips with ≥ 95% sequence similarity to an identified fruit body from our study site were named to the same taxonomic rank, while those that did not match identified fruit bodies were compared with sequences available on the NCBI website and assigned to a nearest taxonomic rank according to the level of match. To see if species delineation was sensitive to the degree of genetic similarity chosen, we also applied a 98% cutoff to our dataset and recorded the number of taxonomic changes. A randomly selected sequence from each of our identified species was submitted to GenBank (DQ822790–DQ822837, Table S1). Only unambiguously EM taxa were included in analyses.

Statistical analyses

Richness estimates and species accumulation curves for each island were made with the program EstimateS (Colwell 2005), using a total species list (both fruit bodies and root tips) as well as partial species lists based on either root tips or fruit bodies. Each estimate was based on 500 randomizations of sample order without replacement. Because the observed number of species is often the least accurate estimator of true species richness (Colwell & Coddington 1994), we chose *a priori* to use the non-parametric Jack1 estimator for our statistical analyses based on an anticipated sample coverage of > 74% (see Brose *et al.* 2003). Unless otherwise noted all reported results are based on this estimator. To analyze the relationship between species richness, island area, distance from pre-existing forest and soil type variability, we used both simple regressions and general linear models. Species richness and island area were $\log_{10}$ transformed and distance was $\log_{10}(x + 1)$ transformed to increase model fit and improve homoscedasticity of residuals. Because there was very little variation in soil type across islands (see Results), we pooled islands into those with 1 soil type and those with > 1 soil type and ran a one-way analysis of variance (ANOVA) to test for effects on species richness. (We chose to use an ANOVA rather than a regression because the lack of adequate variation in the explanatory variable made it inappropriate for the latter type of analysis). To see if results were sensitive to choice of richness estimator, we also ran selected analyses using a number of other common estimators (observed species richness, Jack2, Chao1, Chao2 and ACE).

Ectomycorrhizal assemblage patterns were analyzed using the program BINMATNEST (Rodríquez-Gironés & Santamaria 2006). This program was designed to measure the degree of 'order' in species occurrence patterns on fragmented habitat islands. In a perfectly ordered system, if islands are arranged from lowest to highest species richness, the least rich assemblages will always form proper subsets of the assemblages of all the islands above them. The degree of order in a species–island matrix is represented by Temperature (*T*), and the significance of a maximally ordered matrix (also called the packed matrix) is tested against the mean *T* value of randomly drawn matrices. The degree of nestedness of the packed EM species × tree island matrix was tested against 5000 randomly drawn matrices using the most conservative null model (Model 3) and the recommended parameter specifications of the genetic algorithm used by BINMATNEST (*PopSize* = 30, *TourSize* = 7, *nbGen* = 2000). Spearman rank correlation was

used to test for a relationship between island area and each island's nestedness rank. To test whether EM colonization patterns were correlated with dispersal ability, we performed a regression to see if relative fruit body abundance was a significant predictor of the number of islands a species colonized. We also performed a regression between relative root tip and fruit body abundance to see if fruit body production was related to overall abundance within the EM assemblage (Gardes & Bruns 1996). Relative abundance was calculated as the percentage of samples (fruit body surveys or soil cores) in which a taxon occurred, counting only those islands on which it had been found. To eliminate undue influence from rare taxa in these analyses, we chose *a priori* to include only fifteen taxa that were found on > 1 island and as both fruit bodies and on root tips (see Results). We checked the sensitivity of our results using this cutoff by running the same analysis including all taxa. Statistical analyses were run using the programs JMP v5.0.1 (SAS Institute Inc., Cary, NC, USA) and R v2.4.1 (R Development Core Team 2006), and were considered significant at the $P < 0.05$ level.

RESULTS

Across islands we observed 48 species of ectomycorrhizal fungi (see Table S1). Seventy-three percent (36) of the species were observed as fruit bodies and 57% (28) on root tips. While only 33% (16) of the species were observed both on roots and as fruit bodies, these species accounted for 89% of all observations. Application of a 98% sequence similarity cut off to our dataset resulted in the addition of four taxa. Of these four, two were represented by single sequences, while the other two were represented by three (*Tomentella sublilacina*) and seven (*Tricholoma imbricatum*) sequences, respectively. Because our dataset did not change appreciably, we retained the 95% cut off as the estimator of species level differences for all subsequent analyses.

Sampling effort was sufficient to saturate sampling curves for almost all islands (Fig. 1), suggesting that the variable number of samples taken per island gave us an accurate estimate of species richness. Total species richness (root tips and fruit bodies) increased significantly with island area ($R^2 = 0.74$, slope = 0.20, $P = 0.0003$; Fig. 2). The strong relationship between species richness and island area was not changed when we explored the use of richness estimators other than Jack1. Regressions using observed species richness, Jack2, Chao1, Chao2 and ACE were also highly significant ($P < 0.05$) and produced statistically overlapping slope estimates between 0.19 and 0.21 (data not shown). Similarly, the SAR estimated from root tips alone ($R^2 = 0.50$, slope = 0.18, $P = 0.01$) and fruit bodies alone ($R^2 = 0.81$, slope = 0.23, $P < 0.0001$) were not significantly different ($t_{20} = 0.75$, $P = 0.46$).

Distance alone was a poor predictor of species richness ($R^2 = 0.06$, $P = 0.58$), but a multiple regression including island area, distance and the interaction term significantly increased model fit ($R^2 = 0.92$, $P = 0.0001$) and all effects were highly significant (Table 1). While island size explained approximately 70% of the observed variation in species richness, the interaction term indicated that the smaller effects of distance were size-dependent, with distance reducing species richness only on the largest island sizes.

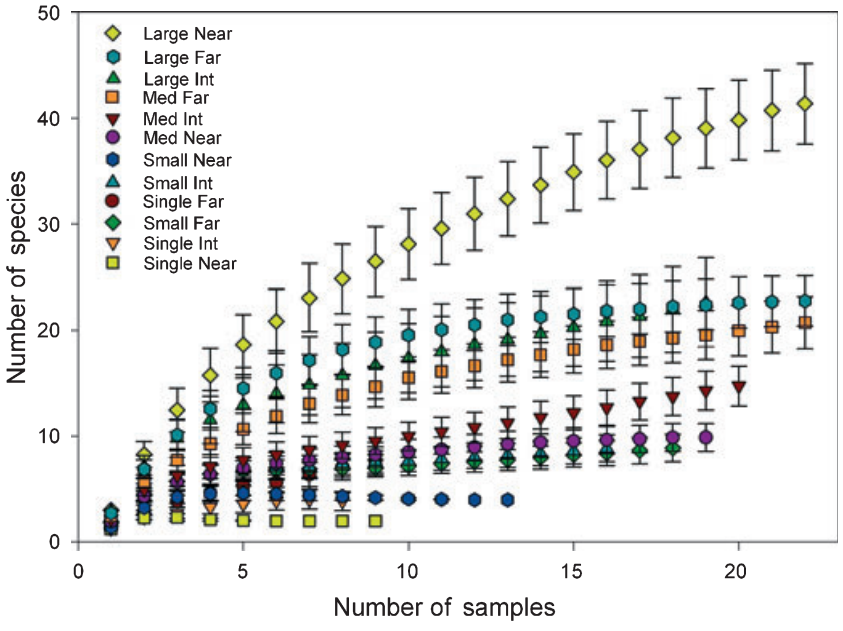

Figure 1 Sampling curves for individual tree islands. Each curve is a composite of samples taken from root tips and fruit bodies of ectomycorrhizal fungi. Size classes are Single trees (1–10 m^2), Small (10–100 m^2), Medium (100–1000 m^2) and Large (> 1000 m^2). Distance was measured from pre-fire patches of mature forest > 0.10 ha (see Methods), and are classified as near (0–100 m), intermediate (100– 1000 m) and far (> 1000 m). Species richness is based on Jack1 estimates and error bars represent 1 SD.

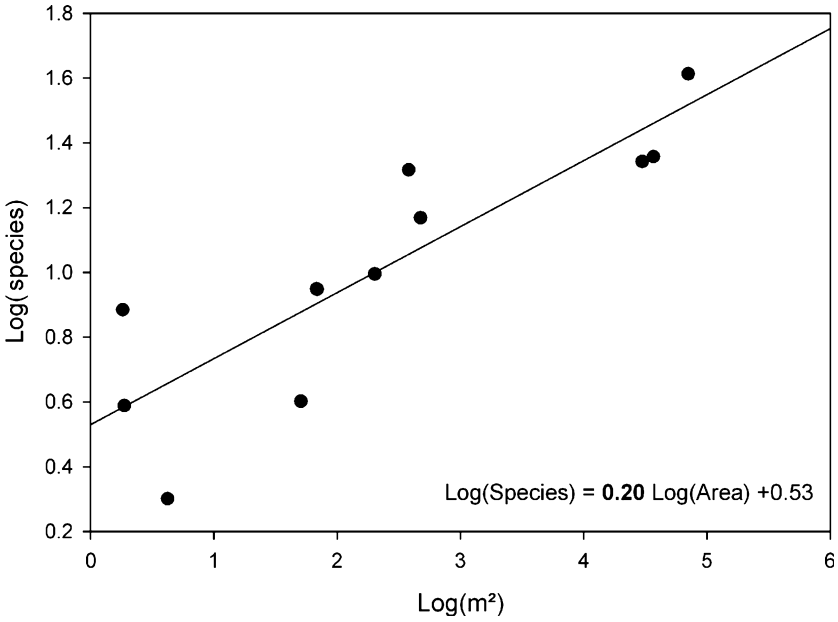

Figure 2 SAR for ectomycorrhizal fungi based on a simple regression of $\log_{10}$ species richness and $\log_{10}$ island area. The slope of the line is often referred to as the z-value, from the equation log (S) = log (c) + z log (A), where S is species richness, log (c) is the y-intercept, A is area and the slope z describes the rate of increase in richness with area. The relationship between species and area is highly significant ($R^2 = 0.74$, $P = 0.0003$).

Table 1 Model output for a multiple regression examining the effects of island area and distance from pre-existing patches of mature forest on ectomycorrhizal species richness. Richness is based on the Jack1 non-parametric estimator

Parameter	Estimate	Std error	Sum of squares	DF	F ratio	Prob > F
Log_{10} (area)	0.23	0.026	1.23	1	75.95	<0.0001
Log_{10} (distance + 1)	0.23	0.063	0.22	1	13.38	0.0064
Log_{10} (distance + 1)* Log_{10} (area)	−0.11	0.028	0.24	1	15.03	0.0047
Model	–	–	1.45	3	29.89	<0.0001
Error	–	–	0.13	8	–	–

Island size also had a strong effect on EM assemblage structure. The packed matrix was arranged almost perfectly with respect to island size (Fig. 3), so that species occurring on smaller islands were drawn from predictable subsets of the assemblages of larger islands. Five thousand randomizations of the island-species matrix showed that the level of nestedness was highly significant ($T = 5.05$, $P < 0.0001$), and the nestedness rank of each island was significantly correlated with island size (Spearman's rho = 0.91, $P < 0.001$). In addition, the number of islands that a species was observed on was positively correlated with its relative abundance in fruit body samples ($R^2 = 0.39$, $P = 0.01$) (Fig. 4). Although we chose *a priori* to include only common species in the analysis, the relationship was still significant ($P < 0.05$) when all taxa were included. Relative fruit body abundance was not, however, correlated with relative root tip abundance ($R^2 = 0.02$, $P = 0.55$), indicating that common fruiters were not necessarily dominant belowground (Gardes & Bruns 1996).

Soils were very homogenous across the study area. Of the 12 islands sampled, nine contained only a single soil type. The only islands with > 1 soil type were the three largest (> 1000 m^2). Using a one-way ANOVA, we found that islands with > 1 soil type had significantly higher species richness than those with only one ($F_{1,10} = 8.9$, $P = 0.01$). However, the number of soil types was a poor predictor of species richness compared with island area, and was rejected ($P > 0.10$) from statistical models that included both terms.

DISCUSSION

Our study found that habitat size played an important role in determining the richness and assemblage structure of ectomycorrhizal fungi. Our regressions show a significant relationship between species richness and area for habitat islands covering 5 orders of magnitude, from approximately 3 to 70 000 m^2. These results support the general finding of significant microbial SARs in a number of other recent molecular-based studies (see Green & Bohannan 2006 for review). However, the slope of 0.20–0.23 that we observed is relatively high compared with many microbial SARs and almost identical to average values reported for macro-organisms (Drakare *et al.* 2006). Unlike the studies of Mangan *et al.* (2004) and Green *et al.* (2004), our results indicate that spatial turnover of soil fungi can occur on a scale similar to that observed for most plants and animals.

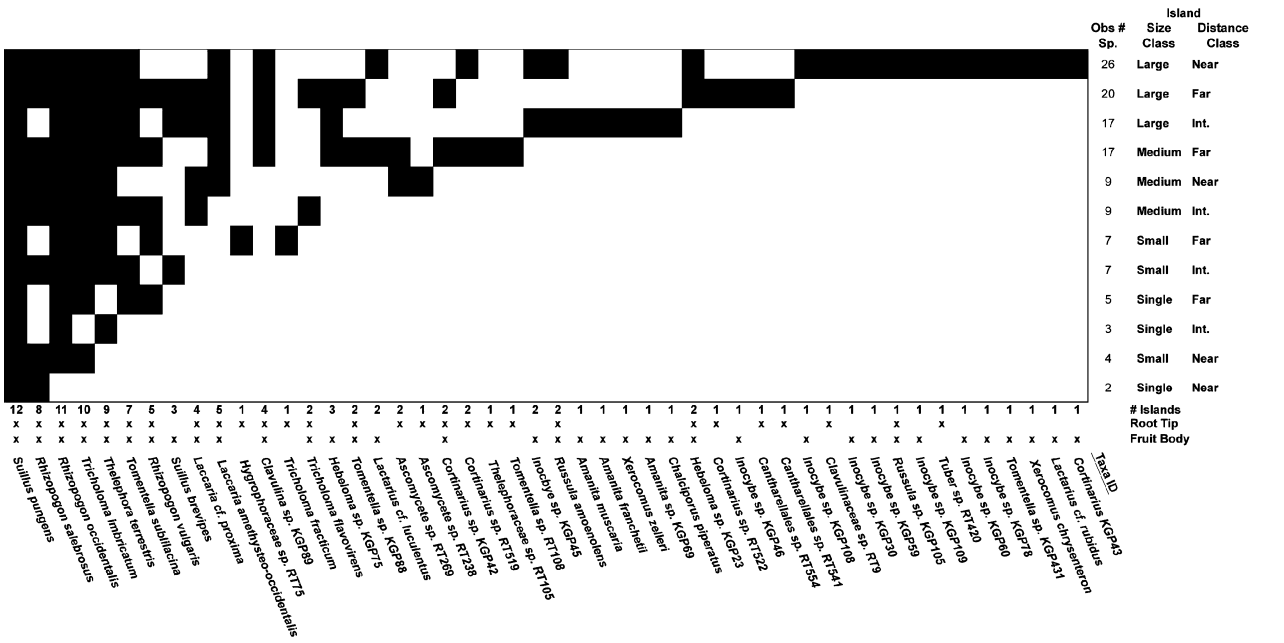

Figure 3 Nested pattern of ectomycorrhizal species occurrence on tree islands. Each column of the matrix represents an ectomycorrhizal species and each row represents an island. The number of islands on which a species occurred and the observed number of species for each island are listed at the end of each column and row respectively. Whether the species was observed as a fruit body or root tip is also indicated for each column. Species occupying the left side of the matrix are considered to be the most deeply nested. The nested pattern shows that assemblages on small islands tend to be drawn from a non-random subset of the large island species pool.

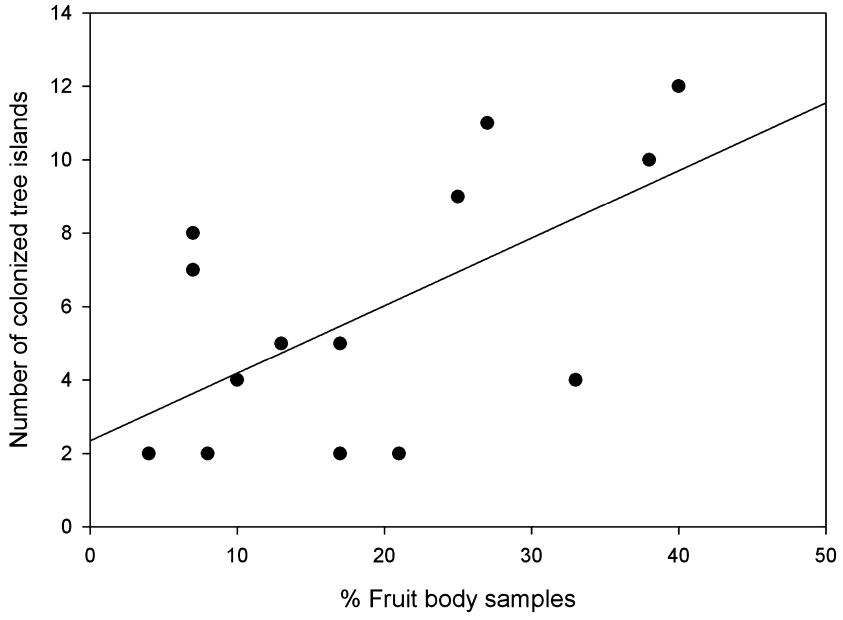

Figure 4 Regression between relative fruit body abundance and the number of tree islands a species was found colonizing. Relative abundance was calculated as the percent of fruit body samples a species was found in, counting only those islands on which it had been documented. $R^2 = 0.39$, $P = 0.01$.

We did not find good evidence linking the strong SAR for EM fungi in this system to increases in niche variety with habitat size. EM fungi are known to respond differentially to host carbon availability, nutrient source and abundance, soil moisture, pH, soil type, soil horizon and disturbance (Erland & Taylor 2002). However, our study took place in a relatively homogenous system, with a single species of even-aged host tree. While there was some evidence of an increased number of soil niches on the largest islands, this effect was insignificant when island area was also

considered, suggesting that area *per se* was the most important determinant of species richness in this system. In contrast to primarily niche-based theories, MacArthur & Wilson (1967) hypothesized that small population sizes and elevated extinction rates are responsible for low species richness with deceasing island size. The lack of strong evidence for a niche effect in our study is consistent with island biogeography and many of the recently proposed neutral community models, in which stochastic processes (e.g. birth, death, immigration) are the key determinants of species richness and abundance (MacArthur & Wilson 1967; Hubbell 2001). Direct validation and parameterization of such models for microbes is difficult because of practical problems, such as counting and delineating individuals (particularly for fungi) and estimating population sizes. However, recent studies have developed mathematical approaches that should allow estimation of key parameters necessary to construct neutral community models for microbes (Curtis *et al.* 2006; Sloan *et al.* 2006). Hopefully such techniques will facilitate further evaluation of the relative importance of stochastic vs. deterministic models of microbial community ecology.

The SAR that we derive is among the highest reported for microbial taxa (Green & Bohannan 2006; Woodcock *et al.* 2006). There are a number of factors that could explain the difference between this study and the lower values found in some other studies. One potential explanation is that the slope of the SAR is greater on islands vs. mainland (Rosenzweig 1995; Bell *et al.* 2005). However, a recent meta-analysis of 794 studies by Drakare *et al.* (2006) found no difference between island and mainland SARs. Alternatively, Woodcock *et al.* (2006) have proposed that the relatively flat SARs reported in some microbial studies may be due to inadequate sampling of rare taxa. In this relatively simple study system, we were able to show that sampling was adequate to capture most of the species richness. Because it took a greater number of samples to observe most species on the largest islands, rarefaction to a smaller number of samples would certainly have decreased our estimated slope. Bell *et al.* (2005) attributed the steep SAR they derive for bacterial tree holes to the ability of these insulated islands to reach a stable equilibrium, but it is possible that they were also working in a relatively simple system for which undersampling was not a problem. Third, as noted by Horner-Devine *et al.* (2004); defining microbial taxa at an ecologically relevant scale is difficult. Taxonomic definition of micro-organisms based on morphology or some molecular tools, such as fragment length polymorphisms, underestimates diversity in rich communities (Avis *et al.* 2006). Fortunately, reasonably well-defined species concepts for fungi (Taylor *et al.* 2006) and practicality of molecular techniques at species boundaries have made defining taxa easier in our study. As species concepts improve for other groups of micro-organisms, species–area patterns may also yield results more similar to those observed for macro-organisms.

We also found evidence that isolation affected microbial richness and assemblage structure. While our regression results suggest that island size set the primary constraint on species richness, on larger islands distance did reduce species richness. This indicates that at least some members of the EM assemblage were directly limited by dispersal ability and that habitat isolation may still play some role in microbial assemblages, even at the 0–2 km scale examined in this study. The finding that isolation has a weak effect relative to area in determining species richness has also been documented for mammals and plants (Lomolino 1984; Cody 2006). This is in part because island size may also influence colonization patterns by providing a larger 'target' for dispersal (MacArthur & Wilson 1967; Cody 2006).

Despite the weaker direct effect of isolation in determining species richness at this scale, we did find evidence that relative dispersal ability is an important factor in determining island colonization patterns. This was evidenced partly by the fact that the most deeply nested species in Fig. 3 came from genera of EM fungi that have been demonstrated to be effective spore colonizers (e.g. *Suillus*, *Rhizopogon*, *Thelephora* spp.), while the least nested (i.e. those present only on larger islands) rarely colonize seedlings from spore applications (e.g *Russula*, *Amanita*, *Inocybe* spp.; Dighton & Mason 1985; Miller *et al.* 1993; Lilleskov & Bruns 2003; Kennedy *et al.* 2007). This was evident as well from the positive correlation between the number of islands a species was found on and its relative abundance in fruit body samples. As in Gardes & Bruns (1996), we found that fruit body abundance did not correlate with below ground abundance on root tips. As such, the nested colonization pattern with respect to island size was most likely, driven by differences in dispersal ability rather than simply differences in abundance.

One way that differences in dispersal ability could drive the patterns in assemblage structure that we observe is through the existence of a dispersal-competition trade-off. Due to the strong target-effect mentioned above, only those species that invest heavily in dispersal are likely to find and colonize the smallest islands. However, investment in dispersal may come at a cost to competitive abilities, a trade-off that is thought to be important to the maintenance of diversity in assemblages of sessile organisms (Tilman 1994). When this trade-off does exist, Tilman *et al.* (1994) showed analytically that competitive dominants are unable to persist on small habitat patches. While we do not have direct measures of spore dispersal and competitive ability for most EM species, there are multiple lines of evidence suggesting that a competition-dispersal trade-off exists in

this system. For the species where we do have direct studies on dispersal rates and competitive interactions (*Rhizopogon occidentalis*, *R. salebrosus*, *R. vulgaris*) the nested pattern fits (PGK & TDB, unpublished data). Further, as mentioned above, those species that occurred widely and colonized small islands tended to be those that invested the most in dispersal structures relative to vegetative structures. For example, *Suillus pungens*, which occurred on every island surveyed, was found in 43% of fruit body samples but only 13% of root tip samples, while *Russula amoenolens*, which was found only on the largest islands, was present in only 23% of fruit body samples but 35% of root tip samples (on those islands). Species common on small islands, such as *S. pungens* and *R. occidentalis* were rarely found on root tips in large islands, indicating that they were not competitive dominants. Further research on spore dispersal and the competitive abilities of EM fungi (e.g. Kennedy & Bruns 2005) could verify the significance of these mechanisms in structuring EM assemblages. It is also important to note that species-specific differences in competitive abilities or dispersal are not incompatible with island biogeography and other stochastic or neutral community models (MacArthur & Wilson 1967; Sloan *et al.* 2006).

The results of this study fit well with our current understanding of fungal ecology. Despite the fact that fungal hyphae are microscopic (*c.* 5 μm diameter) and can obtain high densities in very small areas, fungal individuals are often large and can occupy discrete territories (Redecker *et al.* 2001) in which they compete for resources (Kennedy & Bruns 2005; Koide *et al.* 2005). Similarly, while fungal propagules are microscopic (often < 10 μm) and well-suited to long-distance dispersal, population and phylogenetic studies of EM fungi have revealed genetic structure consistent with dispersal limitation at both landscape and continental scales (Bergemann & Miller 2002; Kretzer *et al.* 2004; Taylor *et al.* 2006; Grubisha *et al.* 2007). This is probably due to the fact that even though an individual propagule can potentially travel large distances, its chances of successful establishment are very low.

Working in a relatively homogenous environment, we have demonstrated a strong species–area relationship at a scale relevant to most ecological studies. This result provides additional evidence against the Baas-Becking view of microbial ecology that 'everything is everywhere, but, the environment selects.' Our study also suggests that dispersal can have both direct (dispersal limitation) and indirect (competition-dispersal trade-off) effects on microbial species richness and assemblage structure. These results show that while microbial species can obtain high density in small areas and microbial propagules can potentially travel very long distances, the same ecological factors that affect the distribution and abundance of macro-organisms ensure that microbes are not ubiquitous, even in suitable habitats.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Table S1 Taxonomic information for EM species observed during the study.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/full/10.1111/j.1461-0248.2007.01035.x

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