Ecology, 91(12), 2010, pp. 3631–3640

Evidence of dispersal limitation in soil microorganisms: Isolation reduces species richness on mycorrhizal tree islands

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Abstract. Dispersal limitation plays an important role in a number of equilibrium and nonequilibrium theories about community ecology. In this study we use the framework of island biogeography to look for evidence of dispersal limitation in ectomycorrhizal fungal assemblages on "tree islands," patches of host trees located in a non-host vegetation matrix. Because of the potentially strong effects of island area on species richness and immigration, we chose to control island size by sampling tree islands consisting of a single host individual. Richness on tree islands was high, with estimates ranging up to 42 species of ectomycorrhizal fungi associating with a single host individual. Species richness decreased significantly with increasing isolation of tree islands, with our regression predicting a 50% decrease in species richness when tree islands are located distances of ~1 km from large patches of contiguous forests. Despite the fact that fungal fruit bodies produce large numbers of spores with high potential for long-distance travel, these results suggest that dispersal limitation is significant in ectomycorrhizal assemblages. There were no discernible effects of isolation or environment on the species identity of tree island fungal colonists. In contrast to the highly predictable patterns of tree island colonization we observed in a previous study on early successional forests, we suggest that over longer time periods the community assembly process becomes more dominated by stochastic immigration and local extinction events.

Key words: *bishop pine; colonization; community assembly; competition; diversity; ectomycorrhizal tree islands; functional redundancy; fungi; island biogeography; neutral; nonequilibrium;* Pinus muricata.

Introduction

Dispersal has been invoked in both nonequilibrium and equilibrium models of ecological communities. Early on, Gleason (1926) recognized the importance of chance dispersal events in his conceptualization of nonequilibrium (or individualistic) communities, and many studies have shown that variation in immigration history can have large impacts on richness, community structure, and ecosystem function (Sousa 1984, Chase 2003, Fukami and Morin 2003). Conversely, differences between species in dispersal ability may lead to predictable patterns of succession or equilibrium outcomes when there is a trade-off with competitive ability (Connell and Slatyer 1977, Nee and May 1992, Tilman 1994). Dispersal limitation has also been invoked as a major mechanism in neutral models in which community structure is the outcome of stochastic drift in the abundance of species with equal fitness (Hubbell 2001).

Understanding the degree to which assemblages are dispersal limited has important theoretical consequences for predicting levels of α- and β-diversity (MacArthur and Wilson 1967, Mouquet and Loreau 2003, Cadotte 2006), the match between organisms and their environment (Shmida and Wilson 1985), and coexistence of competing species (Tilman 1994), as well as important practical implications for predicting the effects of habitat fragmentation (Nee and May 1992) and the ability of species to persist in the face of disturbances (Spiller 1998).

For microbial organisms it has been postulated that dispersal limitation is not an important factor in determining assemblage structure (Berkeley 1863, Baas-Becking 1934, Finlay 2002). Small size and immense number of potential propagules suggests that microbes should have great capacity for dispersal. Indeed, large numbers of fungal spores and bacteria are recovered from the upper troposphere, and transoceanic dispersal of agricultural pathogens has been documented with some regularity (Ingold 1971, Brown and Hovmøller 2002). Such capacity for dispersal has led some to propose an extreme niche model in which microbes are globally dispersed and membership in an assembly is determined entirely by environmental conditions (Finlay 2002). Contrary to this view, microbial phylogeography and population genetic studies have repeatedly demonstrated barriers to gene flow at large spatial scales (Whitaker et al. 2003, Taylor et al. 2006). At the same time, small-scale laboratory studies have demonstrated that manipulating dispersal rates greatly affects microbial community structure (Andrews

Manuscript received 3 December 2009; revised 17 March 2010; accepted 31 March 2010. Corresponding Editor: J. N. Klironomos.

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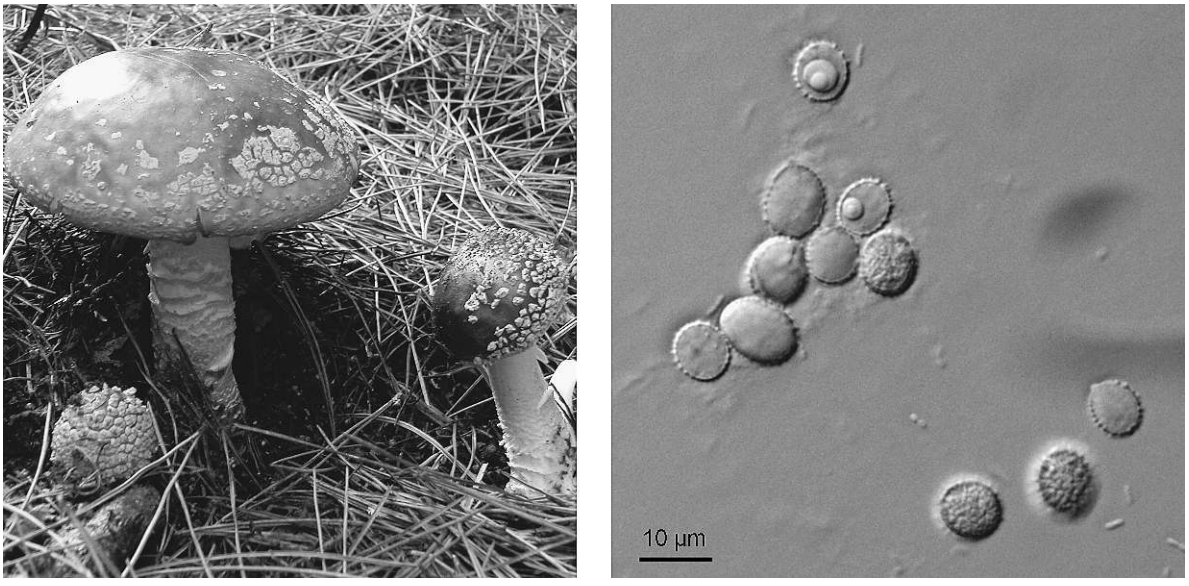

PLATE 1. Reproductive structures of ectomycorrhizal fungi. (Left) Fruiting bodies of *Amanita franchetii* and (right) 400× magnification of the spores of *Laccaria proxima*. Both species are common in the coastal pine forests at Point Reyes National Seashore, California, USA. A single ectomycorrhizal fruit body can produce billions of spores. Spores are generally wind dispersed, although some species rely on animal vectors. Photo credit: K. G. Peay.

et al. 1987, Kerr et al. 2002). However, there is still fairly limited evidence about the degree and manner in which dispersal limitation might shape diverse microbial communities at the landscape scales that most field ecological studies are conducted (Telford et al. 2006).

In this study, we used the predictions from island biogeography theory (MacArthur and Wilson 1967) to look for evidence that dispersal rates affect assemblage structure of a key group of soil microbial eukaryotes, ectomycorrhizal fungi, occurring on "tree islands" (patches of host trees located in a non-host matrix). The theory of island biogeography predicts that species richness is a function of immigration and extinction rates, and that increasing isolation (i.e., distance away from sources of potential colonists) will decrease species richness via reduction in immigration rates (MacArthur and Wilson 1967). If dispersal is unlimited, isolation will not reduce immigration rates and should have no effects on species richness (e.g., Diamond 1969). Thus, testing the relationship between isolation and species richness proposed by island biogeography theory provides one simple way to assess whether or not dispersal limitation is important in local microbial assemblages.

Ectomycorrhizal fungi form an obligate symbiosis with the roots of many dominant temperate and tropical tree families, such as the Pinaceae, Fagaceae, Myrtaceae, and Dipterocarpaceae. While fungi represent a hybrid of macro- and microscopic lifestyles (Peay et al. 2008), they fit the key characteristics of the unlimited microbial dispersal model. They produce immense numbers of microscopic propagules (~10 μm; see Plate 1) that can disperse long distances (Ingold 1971, Brown and Hovmøller 2002) and vegetative structures (hyphae, ~5 μm diameter) that can reach densities >1 km/g of soil (Bååth and Söderström 1979). Because they are involved in an obligate symbiosis with specific tree species, it is easy to delineate ectomycorrhizal habitat patches, something not easily done for many terrestrial microbes.

In a previous study using this approach in early successional forest we found that the size of tree islands strongly affected the richness of ectomycorrhizal fungi, but that the direct effects of isolation on species richness were obscured by the effect of island size (Peay et al. 2007). In this study we report results from a second set of tree islands where we hold island size constant in order to better estimate the effects of isolation on ectomycorrhizal assemblage structure. In addition to holding size constant, we sampled only mature trees to allow a longer period for dispersal to occur compared with the 10-year-old forest sampled in the previous study. In accordance with island biogeography theory, we predicted that species richness would decrease with increasing tree island isolation. We also expected that fugitive species common in early successional forests but rare in mature forests would be more common in isolated, mature tree islands through competition–colonization trade-offs.

METHODS

Study system

The study was conducted at Point Reyes National Seashore, located in west Marin County, California, USA (38°04′ N, 122°50′ W). Point Reyes belongs to the mediterranean climate of coastal California, with cold,

FIG. 1. Photograph of an individual tree island at Point Reyes National Seashore, California, USA. The intervening matrix of coastal scrub and grass is not receptive to ectomycorrhizal fungi. For this reason, patches of *P. muricata* are islands from the perspective of ectomycorrhizal fungal colonists. Photo credit: K. G. Peay.

wet winters and hot, dry summers. Mean annual temperature at the coast is ~11°C, with January averages ~10°C and September averages ~13.5°C. Mean annual precipitation is ~43 cm at the coast and falls almost exclusively in the winter months.

The coastal vegetation matrix at Point Reyes is ideal for studying the effects of habitat size and isolation on ectomycorrhizal fungi. *Pinus muricata* D. Don (bishop pine), an ectomycorrhizal host plant, is a closed cone pine that requires high-intensity fires for seed release. As a result of this autecology, it tends to form even-aged, monodominant stands where it occurs. Near the coast, stands of *P. muricata* intergrade with grasslands and scrub characterized by *Baccharis pilularis*, *Toxicodendron diversiloba*, and *Rubus ursinus*, all of which are non-ectomycorrhizal host plants. Because *P. muricata* is the only ectomycorrhizal host in many areas of the coastal scrub, these patches of *P. muricata* represent islands with respect to their ectomycorrhizal colonists. Our previous study (Peay et al. 2007) took place in early successional forests recruiting in the area burned by the 1995 Point Reyes Vision fire. For this study we chose to focus on mature trees growing outside the perimeter of the Vision fire.

Identification and selection of tree islands

Because island area has such a large effect on species richness and also possibly immigration (i.e., via the "target effect"; MacArthur and Wilson 1967, Cody 2006), we chose to hold island size relatively constant by sampling only tree islands consisting of a single individual of *P. muricata* (Fig. 1). Individual tree islands were defined simply as trees whose canopy were noncontiguous with neighboring trees and were located on or outside a perceived forest border. Trees were selected for the study using a stratified random sampling design. To do this, we used a 1994 National Park Service geographic information system (GIS) vegetation layer to create a map of distances from all large patches of *P. muricata* (>0.1 ha) using the ArcGIS 8.1 spatial analyst tool (ESRI, Redlands, California, USA). This corresponds to the "distance to mainland" measure of isolation in classic island biogeography. This was then broken into four distance classes: 1–10, 10–100, 100–1000, and >1000 m. We attempted to identify 20 potential tree islands in each distance class through the use of field surveys with a handheld GPS unit and visual inspection of 2004 color digital orthoquads of Marin County with submeter resolution. Four trees were then randomly selected from each distance class for inclusion in the study. On average individual trees sampled for the study were 2.5 km apart (minimum 66 m; maximum 7.8 km). This random selection protocol was feasible for the first three distance classes, but we were only able to identify four suitable tree islands in the >1000 m range. In addition, one island at that distance identified from

aerial photographs as a single tree canopy turned out to be two trees growing in close proximity. Because of the difficulty in finding trees at the >1000 m distance and because the canopy size was comparable to other single trees we chose to include this two-tree island in the study despite the fact that it was not technically a single tree island. In addition, we sampled the "mainland" ectomycorrhizal assembly by locating two plots inside large, contiguous patches of forest. In total we sampled 16 tree islands (four islands × four distance classes) and two mainland plots, for a total of 18 sites.

Island age and sampling design

All tree islands and one tree from each mainland plot were aged by sampling tree rings with an increment borer (Haglöf, Madison, Mississippi, USA). All tree cores used for this study were within a field estimated 10 years of the pith. Cores were brought back to the laboratory, mounted, sanded, and then ring number and width counted using a stereo microscope and a Velmex TA4030H1-S6 (Velmex, Bloomfield, New York, USA). For cores where we did not sample the pith, pith date was estimated using a transparent "pith finder." No attempt was made to cross-date or correct for core height, thus tree ages are minimum estimates.

To estimate species richness and assemblage structure of ectomycorrhizal fungi on each tree island, we sampled ectomycorrhizal root tips by directly removing soil using PVC cores (2.5 cm internal diameter × 30.5 cm length) and randomly selecting eight root tips following the protocol in Peay et al. (2007). At each tree island, eight soil cores were initially taken using a random compass bearing at a random distance up to 6 m (the approximate canopy radius of a mature *P. muricata*) from the center of the tree. For the two mainland sites, sampling was performed using a nested design, consisting of three transects separated by 45°. Samples were then taken at the shared zero point and at 1, 5, 20, 50, and 100 m along each transect, for a total of 16 samples per site. Thus our original sampling for tree islands and mainland plots consisted of 160 soil cores (8 cores × 16 islands + 16 cores × 2 mainland plots). Because some cores did not contain ectomycorrhizal root tips, we sampled an additional 29 soil cores to try to ensure even sampling across islands. In total, 1231 root tips were removed from 155 soil cores (four cores contained slightly fewer than eight ectomycorrhizal root tips).

Molecular protocols for identifying ectomycorrhizal fungi

We used molecular genetics techniques to sequence the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA genes to identify the ectomycorrhizal species colonizing *P. muricata* roots. ITS is a common barcode gene and provides high enough resolution to separate most ectomycorrhizal species (Hughes et al. 2009). For this study DNA was extracted using a modified protocol from the REDExtract-N-Amp Tissue PCR Kit (Sigma-Aldrich, Saint Louis, Missouri, USA) modified as in Peay et al. (2009) and fungal specific PCR and sequencing were done as in Peay et al. (2007). As in Peay et al. (2009), species were delimited based on a 97% sequence similarity cutoff and taxonomic designations assigned using the National Center for Biotechnology Information's Basic Local Alignment Search Tool (BLAST). A representative sequence from each operational taxonomic unit (OTU) has been deposited in GenBank (Appendix). A very small number of root tips colonized by well-known non-mycorrhizal pathogenic, saprophytic, or endophytic species (e.g., *Armillaria*, *Oidiodendron*) were excluded from analyses. All root tips colonized by taxa of ambiguous ectomycorrhizal status (e.g., *Trechispora*, *Helvella*) were retained.

Environmental characterization and quantifying isolation

At each site we sampled three additional soil cores in order to characterize the abiotic environment of each tree island. These cores (same dimensions as root tip cores) were combined to yield one composite sample for each site. A portion of each sample was sent to the Western Agricultural Laboratories (Modesto, California, USA) for measurement of pH, P (weak Bray), NO_3, K, Ca, Mg, cation exchange capacity (CEC), and percentage organic matter (OM). The remaining soil was used to determine percentage total carbon and nitrogen (CN) content on an NC2100 elemental analyzer (CE Elantech, Lakewood, New Jersey, USA).

In addition to measuring distance to mainland, we calculated a number of alternative metrics to characterize the degree of isolation experienced by each tree island. Tree islands were initially selected using rough isolation classes based on a 1994 GIS map of vegetation at Point Reyes (as mentioned previously). After this, we used the 2004 digital orthoquads to modify the 1994 GIS base layer to more finely map the vegetative matrix around focal tree islands, in particular to include smaller patches and adjacent single trees and to better map the boundary of large forest patches. Based on this map, we quantified the presence of adjacent *P. muricata* that could serve as sources of ectomycorrhizal propagules using several measures. As a model of stepping-stone or meta-community isolation, we measured the straight-line distance to the next nearest *P. muricata* individual, regardless of patch size. To quantify the spore rain potential of the landscape around a focal tree island, we quantified the area of all *P. muricata* (minus the area of the focal island) within radii of 200, 750, and 1500 m using the neighborhood statistics function in the ArcGIS spatial analyst. The same metrics were quantified for the mainland plots centered on the zero meter sampling point.

Statistical analyses

We had two primary goals in this study: (1) to see how species richness on tree islands changed with increasing isolation from sources of ectomycorrhizal propagules,

and (2) to see whether species composition on tree islands changed predictably with isolation.

To test whether species richness changed with degree of isolation, we performed univariate regression of species richness against the various isolation metrics. Observed richness is usually the least accurate estimate of true richness (Colwell and Coddington 1994, Brose et al. 2003). For this reason, we chose a priori to use the second order jackknife (Jack 2) estimator of species richness for our analyses. This choice was based on previous work in this system (Lilleskov et al. 2004, Peay et al. 2007) that led us to anticipate sample coverage of between 50% and 75% of species on our tree islands and following the recommendations in Brose et al. (2003). We used EstimateS (Colwell 2005) to calculate the Jack 2 estimator for species richness and rarefy to a common sample size for all tree islands (we were unable to obtain eight soil cores with colonized roots for all islands). To improve normality of residuals, distance to mainland and distance to nearest neighbor were $\log_{10}$ + 1-transformed and area of *P. muricata* within 200 and 750 m were square-root transformed.

We also performed univariate correlations to see whether environmental variables, island age or area changed predictably with isolation or affected patterns of species richness. To reduce the 12 measured soil environmental variables to a reasonable number of predictors we used a principal components analysis (PCA) on the *z*-scaled variables. After examining scree plots we chose to retain the first four principal components, which explained 84% (PC1 = 38%, PC2 = 22%, PC3 = 13%, PC4 = 11%) of the variation in our soil environmental variables. We then looked for univariate correlations between PCA scores, species richness, and our isolation metrics.

To see whether some combination of variables best predicted species richness we included the distance to mainland measure (the most predictive isolation metric; see *Results*), the first four principal components, island age, and island area in an additive multiple regression. We then used a step function with forward and backward selection to determine which parameters to retain in the final model based on Akaike's information criterion (AIC).

To analyze the factors affecting assemblage structure we calculated pairwise distances between all plots based on community similarity, degree of isolation, environmental similarity, and spatial proximity. Community distances were calculated using both β_{sim}, a presence–absence measure of community similarity that controls for differences in species richness (Koleff et al. 2003), as well as the quantitative Bray-Curtis metric based on root tip abundance. Environmental distance was calculated as multivaritate euclidean distance based on all 12 *z*-scaled soil environmental variables. Isolation distance was caclulated as the euclidean distance between samples for the single isolation metric distance to mainland. Spatial distance was simply the pairwise euclidean distance (in meters) between all tree islands and forest plots. In addition, because we had spatial locations and species lists for each individual soil core, we also calculated community and spatial distance matrices at this scale (this was not possible for environmental variables because soil cores were homogenized within each island). Mantel tests were then used to assess the relationships between community structure, environmental similarity, similarity in isolation from sources of ectomycorrihzal propagules, and spatial proximity. Because of the significant relationship between species composition and distance for individual soil cores (*Results*), we calculated a Mantel correlogram to test the strength of the correlation at different spatial scales (0–10, 10–50, 50–100, 100–250, 250–500, 500–750, 750–1000, 1000–5000, and 5000–7500 m), using a progressive Bonferroni correction to account for multiple tests (Legendre and Legendre 1998). To visualize assemblage structure of the single mature tree islands and mature forest plots we used nonmetric multidimensional scaling (NMDS).

All statistical tests and graphics were done using the program R version 2.7.2 (R Core Development Team 2008). Selection of the best multivariate predictors of species richness was done using the Step function in the R core package; distance matrices and NMDS ordination were performed with the package Vegan, and Mantel tests and Mantel correlogram with the package Ecodist. All statistical tests were considered significant at $P < 0.05$.

Results

Community overview

An identifiable sequence was produced on 77% (953) of the 1231 root tips from which DNA was extracted. Six root tips from four species of clearly non-mycorrhizal fungi were excluded from our analysis. In total we identified 107 species of ectomycorrhizal or potentially ectomycorrhizal fungi. The most species rich groups were the Thelephoraceae (24), Inocybeaceae (15), Russulaceae (13), and Cortinariaceae (12) (Appendix). The minimum number of cores containing root tips sampled per island was seven, and the minimum number of root tips recovered was 33. Observed species richness on tree islands ranged from 6 to 18 species (median = 10, SD = 3.8) and 1 to 5 species for individual soil cores (median = 2, SD = 1.1). Jack 2 estimates of species richness on islands ranged from 7 to 42 species (median = 16, SD = 9). Species accumulation curves did not plateau for most islands (*data not shown*). For this reason, unless indicated all measures of species richness used in further analyses are Jack 2 estimates rarefied to seven soil core samples.

Effects of isolation, area, and age on species richness

Species richness decreased with all measures of increasing isolation from potential sources of inoculum, i.e., with increasing distance or decreasing area of

TABLE 1. Univariate regression results predicting species richness from isolation, soil environment, age, and area for single tree islands at Point Reyes National Seashore, California, USA.

Predictor	R^2	Slope (SE)	t	P
Log_{10} distance to mainland	0.25	−5.04 (2.3)	−2.16	0.048*
Log_{10} distance to nearest neighbor	0.25	−6.13 (2.9)	−2.13	0.051
Area of *P. muricata* within 1500 m radius	0.16	2.4×10^{-6} (1.5×10^{-6})	1.63	0.125
Square root of area of *P. muricata* within 750 m radius	0.16	0.009 (0.005)	1.66	0.121
Square root of area of *P. muricata* within 200 m radius	0.16	0.031 (0.02)	1.63	0.124
Principal component 1	0.15	1.67 (1.1)	1.56	0.142
Principal component 2	0.00	0.15 (1.5)	0.10	0.921
Principal component 3	0.00	−0.16 (1.7)	−0.10	0.924
Principal component 4	0.01	−0.98 (3.1)	−0.32	0.755
Age of tree island	0.22	−0.23 (0.12)	−2.00	0.065
Log_{10} area of tree island	0.07	−13.2 (13)	−1.01	0.202

Note: An asterisk (*) indicates significant predictors at the $P < 0.05$ level.

neighboring trees (Table 1). However, distance to the mainland (i.e., patches of *P. muricata* > 0.1 ha; Fig. 2) was the only statistically significant predictor of species richness (Table 2). Average area for tree islands was 120 m^2, with a range of 64–234 m^2. There was no correlation between log island area and species richness (Table 1). Average tree age was 45 years (minimum = 29, maximum = 93, SD = 18). Species richness declined with tree age but this effect was only marginally significant (Table 1). The relationship between distance to the mainland and species richness was robust to estimator choice and remained similar when using observed species richness ($r^2 = 0.26$, slope = −2.19, $P = 0.044$) instead of the Jack 2 estimator.

Effects of environment on species richness

The first principal component was associated primarily with variation in base cation concentration (K, Mg, Ca, CEC), the second with nitrogen availability (NO_3 and percentage organic mater content), the third with pH and phosphorous, and the fourth with the ratio of C:N (*data not shown*). In univariate regression none of the PCA scores from PC1, PC2, PC3, or PC4 were significantly correlated with species richness (Table 1).

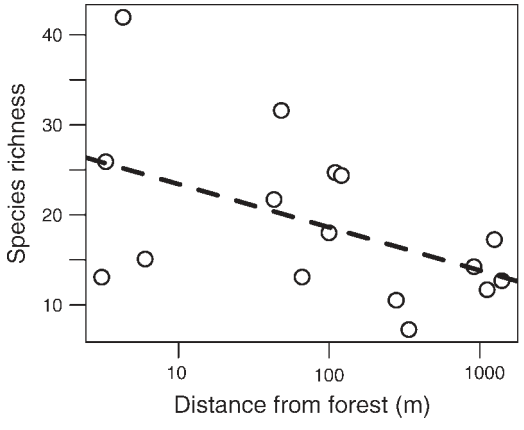

FIG. 2. Ectomycorrhizal species richness decreases with increasing island isolation. The plot shows estimated species richness for all tree islands plotted against log_{10}-transformed distance from the mainland, i.e., distance from forest patches of *Pinus muricata* >0.10 ha. The dashed line shows a significant univariate regression ($R^2 = 0.25$, $P = 0.048$). Species richness is the estimated number of species based on the nonparametric jackknife 2 estimator rarefied to a common sample number (see *Methods*).

Multivariate model of species richness

The full model used in the step function included $log_{10} + 1$ distance to mainland, principal components 1–4, island age, and island area in an additive regression model. The final model selected by the step function (based on AIC) explained 49% of variance in species richness and included three variables; $log_{10} + 1$ distance to mainland, island age, and island area (Table 2). The effects of age and isolation were both negative, while the effect of area was positive. All environmental principal components were rejected, indicating that isolation, age, and area were the best predictor of species richness and that other measured factors did not significantly add predictive value to the model.

Determinants of assemblage structure

There were no obvious patterns regarding species composition and isolation of mature tree islands. NMDS plots showed the two mainland sites sitting near the center of the ordination with no visible clustering of tree islands by distance class (Fig. 3). The pattern was nearly identical for both the β_{sim} and Bray-Curtis dissimilarity indices.

TABLE 2. Multivariate model selection results for predictors of ectomycorrhizal species richness on single tree islands.

Predictor	Slope (SE)	t	P
log_{10} distance to mainland	−8.32 (3.5)	−2.35	0.036*
log_{10} area of tree island	23.80 (17.8)	1.34	0.205
Age of tree island	−0.21 (0.1)	−2.07	0.060

Notes: Original variables included: isolation, soil environment principal components 1–4, age, and area for all tree islands. An asterisk (*) indicates significant predictors at the $P < 0.05$ level. R^2 for the final model was 0.49 ($P = 0.037$).

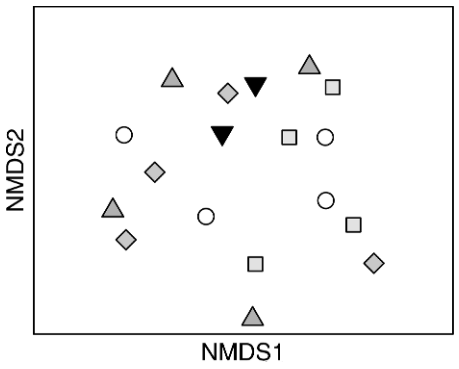

FIG. 3. Nonmetric multidimensional scaling (NMDS) plot of tree islands and mature forest plots from this study. Each point represents an individual tree island or mainland plot. Points closer together in space have more similar species composition. The ordination is based on the Bray-Curtis measure of community similarity. Symbols show the distance from mainland isolation classes used in the island sampling scheme: open circles, 0–10 m; gray squares, 10–100 m, gray diamonds, 100–1000 m; upright triangles, >1000 m; and inverted triangles, mainland plots. No obvious clusters appear with regard to isolation.

Mantel tests revealed no significant predictors of ectomycorrhizal species composition on mature tree islands. Tree islands with similar soil environments did not have similar communities (Mantel $r = 0.10$, one-tailed $P = 0.18$), nor did islands with similar degrees of isolation (Mantel $r = -0.09$, one-tailed $P = 0.61$) or in close spatial proximity (Mantel $r = 0.01$, one-tailed $P = 0.45$). There was also no significant correlation between environmental similarity and the distance between individual tree islands (Mantel $r = 0.26$, one-tailed $P = 0.075$). However, when examined at the level of individual soil cores, samples taken closer together did tend to have more similar species composition (Mantel $r = 0.07$, one-tailed $P = 0.001$). A Mantel correlogram showed that this pattern is driven by the fact that spatial correlation is highest at spatial scales of 1–10 m, i.e., soil samples taken within a single tree canopy (Fig. 4). Results were similar for β_{sim} (*data not shown*).

DISCUSSION

Our results show that isolation reduces species richness of ectomycorrhizal assemblages. Our univariate model predicts that richness around a single tree will decline from 29 species at the forest edge to 19 species at 100 m and 14 species at 1000 m. This is approximately a 50% decrease in species richness from forest edge to 1000 m. While the area effects on islands have been demonstrated repeatedly (Rosenzweig 1995), significant isolation effects have proved more elusive (Diamond 1969, Lomolino 1984). Despite the fact that isolation alone explained only 25% of variation in species richness, this is equivalent to the amount of variation in plant species richness explained on oceanic islands once the area effect was controlled for (Cody 2006:68–69). While a true test of island biogeography theory requires measurement of colonization and extinction (e.g., Simberloff and Wilson 1969), the patterns of species richness we observe in relation to isolation in this study and island area in a previous study (Peay et al. 2007), suggest that ectomycorrhizal tree islands fit some predictions from island biogeography theory. This is in contrast with a recent study of soil invertebrates that found little support for island biogeography theory (Jonsson et al. 2009), making it unclear if our results will generalize across the soil microbial community or other systems. While ectomycorrhizal fungi are not a common focus of conservation studies, our results do indicate that habitat destruction and fragmentation could lead to significant losses of ectomycorrhizal biodiversity. For instance, loss of habitat area and isolation likely explain the depauperate ectomycorrhizal assemblages in the heavily logged tropical forests of the Seychelle islands (Tedersoo et al. 2007).

Studies using bait seedlings have shown that colonization by mycorrhizal fungi decreases away from mature host trees (Dickie and Reich 2005, Weber et al. 2005). Dickie and Reich (2005) found that both species richness and colonization levels of ectomycorrhizal fungi decreased on oak seedlings as a function of distance from forest edge, with little colonization at the maximum studied distance of 20 m. However, most such studies take place over relatively small spatial and temporal scales (i.e., 10–100 m, 1–2 years), and thus it is difficult to extrapolate across the lifetime of a tree. Additionally, many species of ectomycorrhizal fungi will not initially

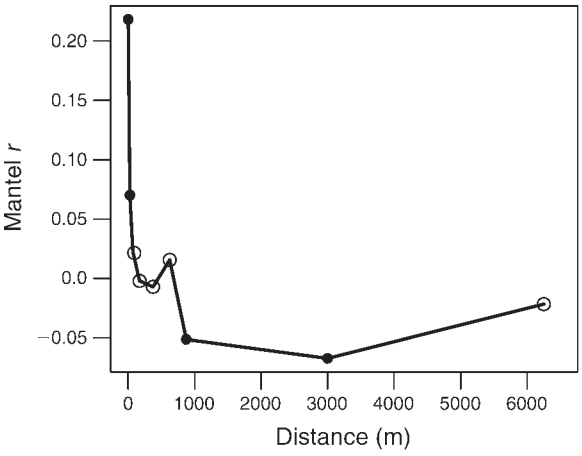

FIG. 4. Mantel correlogram showing the strength of the correlation between spatial proximity and ectomycorrhizal species composition for soil cores within different distance classes. Points are plotted at the midpoint of each distance class. Solid circles represent significant correlations, and open circles show correlations that are not significantly different from zero. Significant positive values for Mantel's r indicate that samples within a given distance class tend to share more species. The correlation decays rapidly beyond 10 m, the approximate diameter of a single tree canopy.

colonize seedlings from spore (Ishida et al. 2008), and much colonization on seedlings in these studies probably comes from mycelia on existing tree roots (e.g., vegetative dispersal), rather than spore dispersal. While our study lacks the strong causality associated with experimental manipulations, our results are important in scaling up to the landscape and demonstrate that the effects of isolation can persist for the lifetime of a tree. While seedling studies have shown reduced host fitness in the absence of mycorrhizal inoculum (Dickie et al. 2005, Weber et al. 2005), it is not clear that reduced diversity of ectomycorrhizal fungi has strong negative effects on plant performance (Baxter and Dighton 2001).

Our results imply that dispersal limitation is important in ectomycorrhizal communities. This may seem counterintuitive given the fact that a single ectomycorrhizal fruit body can produce on the order of 1×10^9 spores (Dahlberg and Stenlid 1994) and given the fact that there is well-documented potential for long-distance dispersal of fungal spores (Ingold 1971). However, as in plants (Nathan 2006), successful long-distance dispersal events are probably somewhat rare for fungi in nature (Brown and Hovmøller 2002). One of the few studies that measured dispersal distances for ectomycorrhizal fungi found that the vast majority of spores traveled only a few meters (Li 2005). In addition, many spores probably lose viability during dispersal. In a study of pathogenic fungi, Gonthier et al. (2001) found that two sites separated by a distance of 3 km had very different air spora composition and suggested that very few spores survive travel over such distances. Additionally, while small numbers of spores may be effective in highly susceptible agricultural monocrops, it is likely that spore quantity is important in natural systems. For example, even under optimal conditions 50–250 spores (1–5 spores/mL of soil) were necessary for ectomycorrhizal species with highly receptive spores to colonize 50% of inoculated pine seedlings (Bruns et al. 2009). Differences in the arrival time and germination speed of spores have also been shown to affect the outcome of ectomycorrhizal competition (Kennedy and Bruns 2005, Kennedy et al. 2009).

It is clear from recent studies that local competition (Kennedy and Bruns 2005), abiotic niche partitioning (Lilleskov et al. 2002), and biotic niche partitioning (Ishida et al. 2007) are important in structuring ectomycorrhizal fungal assemblages. However, in this study we found no strong relationships between environmental variables and ectomycorrhizal richness or assemblage structure. There are a number of potential explanations for this result. First, detecting abiotic habitat associations is very challenging for cryptic organisms in diverse communities (Peay et al. 2008). For this reason, it is likely abiotic factors play some role in ectomycorrhizal community assembly in this system but were not detectable with our sample size. Second, this study was specifically implemented in a simple system (similar soils, climate, host species, host ages) in order to maximize our ability to detect isolation effects. Thus, abiotic effects may be small in our system relative to other ectomycorrhizal communities.

Against this backdrop, our study provides some evidence for non-niche processes affecting ectomycorrhizal community assembly. The fact that composition of soil cores was strongly autocorrelated at small spatial scales (i.e., within individual tree islands) does suggest that we accurately characterized the dominant species occupying tree islands. Among tree islands, however, species composition showed no significant spatial autocorrelation, suggesting that assemblages on each island are the result of multiple independent colonization and extinction events. The fact that priority effects appear to be very strong in ectomycorrhizal competition (Kennedy et al. 2009) supports the idea that chance dispersal events and immigration history could be important in determining ectomycorrhizal assemblage structure. In concordance with this, experiments with wood decay fungi have found that varying immigration history strongly affects both species richness and community structure (Fukami et al. 2010). While this is not what we expected to find, it does correspond with island biogeography theory (MacArthur and Wilson 1967), which predicts an equilibrium species richness but nonequilibrium species composition due to local extinction and new immigration.

The unpredictable nature of ectomycorrhizal community assembly on tree islands in this study is in contrast with the clear patterns we observed in early successional tree islands. In that study, we found that ectomycorrhizal assemblages were significantly nested with island size (i.e., species on small islands were always drawn from a predictable subset of species occurring on large islands), and that the most widespread taxa produced a greater number of fruit bodies (uncorrelated with belowground abundance) (Peay et al. 2007). Other than copious fruit body production, these widespread taxa also share other characteristic traits, such as the ability to colonize seedlings easily from spores, ability to produce hyphae specialized for long-distance growth and transport (rhizomorphs), and the ability to grow saprophytically in culture. No nestedness was found on tree islands in the current study (*data not shown*). These successional patterns are in some ways reminiscent of those observed in diverse tropical tree communities, where a small number of species have distinctly ruderal life history strategies but the vast majority of dominant species target similar niches (Hubbell 2001). While there are certainly broad functional groups within mature ectomycorrhizal communities (Courty et al. 2005, Hobbie and Agerer 2010), the high diversity of ectomycorrhizal fungi that coexist in most assemblages makes it likely that many species are functionally similar. Thus, coexistence of ectomycorrhizal species

within a landscape could involve both niche and neutral processes (Cadotte 2007).

Most studies of ectomycorrhizal ecology have focused on small-scale, deterministic mechanisms that explain patterns of coexistence and species abundance. While these mechanisms certainly play an important role in ectomycorrhizal community ecology, large-scale dispersal-related processes and nonequilibrium dynamics are also likely important and deserve greater attention in future studies.

Acknowledgments

We thank Wayne Sousa for helpful comments on earlier versions of this manuscript, Alison Forrestel, Robbie Andrus, and John Sanders for help with dendrochronology, Andy Thomson for help with C:N analyses, and Point Reyes National Seashore for supporting our research efforts there. Financial support was provided by a NASA Earth Systems Science Fellowship, a Chang Tien Lin Environmental Scholarship, and a Pacific Coast Science and Learning Center grant to K. G. Peay, and National Science Foundation grants DEB 236096 and DEB 0742868 to T. D. Bruns.

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APPENDIX

Abundance of species observed in the study (*Ecological Archives* E091-255-A1).