Spore dispersal of basidiomycete fungi at the landscape scale is driven by stochastic and deterministic processes and generates variability in plant–fungal interactions

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Summary

- Fungi play an important role in plant communities and ecosystem function. As a result, variation in fungal community composition can have important consequences for plant fitness. However, there are relatively few empirical data on how dispersal might affect fungal communities and the ecological processes they mediate.
- We established sampling stations across a large area of coastal landscape varying in their spatial proximity to each other and contrasting vegetation types. We measured dispersal of spores from a key group of fungi, the Basidiomycota, across this landscape using qPCR and 454 pyrosequencing. We also measured the colonization of ectomycorrhizal fungi at each station using sterile bait seedlings.
- We found a high degree of spatial and temporal variability in the composition of Basidiomycota spores. This variability was in part stochastic and in part explained by spatial proximity to other vegetation types and time of year. Variation in spore community also affected colonization by ectomycorrhizal fungi and seedling growth.
- Our results demonstrate that fungal host and habitat specificity coupled with dispersal limitation can lead to local variation in fungal community structure and plant–fungal interactions. Understanding fungal communities also requires explicit knowledge of landscape context in addition to local environmental conditions.

Introduction

Spatial and temporal heterogeneity in environmental conditions and resource availability is at the heart of most conceptual models of ecology (Hutchinson, 1961). Latitudinal and elevational gradients in temperature and precipitation drive generalizable patterns of species turnover (Whittaker, 1956; Gaston, 2000; but see Fierer et al., 2011) and decomposition (Aerts, 1997; Silver & Miya, 2001), while local-scale heterogeneity in environment and resource abundance underlies a rich body of literature on niche partitioning and species coexistence (Hutchinson, 1959; Wiens, 1976; Chesson, 2000). Variability in the strength of biotic interactions in space and time also has the potential to be a powerful ecological and evolutionary driver of both community structure and ecosystem function (Thompson, 1988). For example, spatial variability in the strength of plant interactions with herbivores and pathogens may control habitat associations (Fine et al., 2000) and local abundance (Mangan et al., 2007). Temporal variability in species arrival times has also been shown to influence rates of wood decomposition (Fukami et al., 2010). While the processes generating variability in the abiotic environment are well studied (Lomolino et al., 2006), less is known about the processes generating variability in biotic interactions, particularly in mutualisms (Agrawal et al., 2007).

Variability in the timing or quantity of propagules that disperse to a given location appears to be one important mechanism for generating variability in biotic interactions (Fukami et al., 2007; Chase, 2010). As a process, dispersal figures prominently in the generation of stochastic variability in community assembly for ecological paradigms such as neutral theory (Hubbell, 2001), meta-community theory (Leibold et al., 2004), and historical contingency (Fukami, 2010). At the species level, dispersal-related traits are highly variable between species (Nathan & Muller-Landau, 2000; Moles et al., 2005) and can lead to predictable patterns of community assembly (Tilman, 1994; Levine & Murrell, 2003). In addition, dispersal limitation of host-specific pathogens is an important assumption of the predictable spatial variability in pathogen loads that drives the Janzen–Connell effect (Janzen, 1970; Connell, 1971; Terborgh, 2012). Thus, dispersal can generate biotic variability through both random and predictable processes. Few studies have actually measured dispersal of whole communities across real landscapes, so the scale of dispersal and the extent to which it is predictable versus random are not known for most communities.

Fungi are the primary plant symbionts (both pathogens and mutualists) and decomposers in most terrestrial ecosystems (Peay et al., 2008). An increasing number of studies show that variability in fungal community structure may control patterns of plant
community assembly (Terwilliger & Pastor, 1999; Packer & Clay, 2000; Weber et al., 2005) and ecosystem function (Fukami et al., 2010). There is good reason to expect that chance events and dispersal limitation are underlying determinants of fungal community variability (Peay et al., 2010a; Nortos et al., 2012) and variation in plant–fungal interactions (Gilbert et al., 1994). Spatial and temporal variation in the abundance of mushroom fruiting bodies is well established from field surveys (Egli et al., 2006; Gange et al., 2007). However, because of the effort and taxonomic expertise required, such surveys are often limited to infrequent visits to relatively restricted areas. Additionally, because spores are aerially dispersed and mixed, the presence of mushrooms cannot be used directly to quantify the airspora encountered at a particular landscape location. Volumetric air samplers have been used for direct sampling of fungal spores, but these studies are generally restricted to coarse morphological identification techniques (Robertson & Brandys, 2011) and single sampling locations because of instrument costs and power requirements (DeSantis et al., 2005; Bowers et al., 2009). Thus, there are few data sets that directly measure variation in fungal community dispersal across real landscapes, leaving open the extent to which dispersal might generate variability in plant–fungal interactions.

In order to characterize the magnitude of spatial and temporal variability in spore dispersal and relate this to variability in biotic interactions, we designed a spore sampling method that is cheap to deploy and requires minimal effort to maintain. Paired with high-throughput next-generation sequencing (NGS), this method allows the replication necessary for ecological studies of fungal dispersal. Using taxon-specific primers, we targeted a key group of fungi, the Basidiomycota, that are the dominant decomposers and mycorrhizal mutualists in many forested ecosystems. We deployed this method at multiple sites across a well-defined vegetation matrix and sampled continuously across the season when spores of most mushroom-forming Basidiomycota are likely to be produced. To test the ecological potential of heterogeneity in fungal spore dispersal, we also characterized aerial colonization of pine seedlings by ectomycorrhizal fungi at the same sites. In a previous publication based on the same experiment (Peay et al., 2012), we demonstrated that the richness and quantity of ectomycorrhizal spores in a nonhost vegetation matrix decrease with increasing spatial distance from host vegetation and that this reduces ectomycorrhizal colonization of seedlings. In this study, we used the same data set to examine spore dispersal of the entire Basidiomycota community (ectomycorrhizal fungi are only 6% of the total sequence data set). We expanded the focus of our analysis by including species composition (not just richness), examining temporal variability in the spore community as well as spatial variability, incorporating climatological predictors into our statistical models, and comparing temporal patterns seen in our spore trap results with collections of fruiting bodies from the same site.

While the scale of ‘microbial’ dispersal is a matter of some debate (Green & Bohannan, 2006; Peay et al., 2010b), our work in this system has led us to expect high spatial and temporal heterogeneity of dispersal leading to variability in Basidiomycota spore composition across sites and sampling periods. We expected that some of this variability would be predictable based on seasonal variables and spatial proximity to landscape features. However, we also expected that the majority of variance would remain unexplained as a result of the highly stochastic nature of spore dispersal. By demonstrating that there is both predictable and unpredictable variation in fungal spore dispersal and linking this to biotic interactions, this work sets the stage for quantification of how variation in the biotic landscape affects the development of plant communities and the rate of ecosystem processes.

Materials and Methods

Study site

The study was conducted at Point Reyes National Seashore (PRNS) in the Mediterranean scrub vegetation characteristic of coastal California (USA). PRNS is found in Marin County, California (38°04′N, 122°50′W). Typical of Mediterranean systems, the climate is mild and wet in the winter, with cool and dry summers. Mean annual temperature at the coast is c. 11°C, with January averages c. 10°C and September averages c. 13.5°C. Mean annual precipitation is c. 43 cm at the coast, most of which falls during the winter months (approx. November–February).

The coastal vegetation matrix at PRNS creates an ideal backdrop for studying landscape-scale dispersal patterns in fungal communities. This is because the landscape is dominated by a patchwork of plant species with contrasting mycorrhizal associations, tissue quality and physiognomic structure that lead to different microclimates. On one hand, Pinus muricata D. Don (Bishop Pine) is one of the few abundant ectomycorrhizal hosts in the coastal scrub at PRNS. As a result of its fire-dependent autecology, P. muricata tends to form even-aged, monodominant forest stands (Forrestel et al., 2011). The dense growth form creates a dark, wet understory with an abundant carpet of pine needles. On the other hand, stands of P. muricata form sharp boundaries with a coastal scrub vegetation matrix that is characterized by Baccharis pilularis, Toxicodendron diversiloba and Rubus urinatus, all of which host only arbuscular mycorrhizal fungi. In contrast to the pine forest, the coastal scrub is an open, low-stature vegetation with a relatively dry understory and little accumulation of dead plant material. Because P. muricata forms monodominant stands, this creates a matrix of discrete vegetation features that host contrasting fungal communities.

Study design

To characterize patterns of dispersal across space and time, we sampled fungal spores at multiple time-points and sampling locations across the primary fungal fruiting season in both 2008 and 2009. Fungi may disperse small distances through expansion of the vegetative mycelium but here we focus on aerial spore dispersal. While taxa probably vary in the relative importance of mycelial versus aerial spore dispersal, spores are the primary mechanism for movement beyond the centimeter scale over short...
time-periods. Fungal propagules were sampled using spore traps installed at experimental stations (16 in 2008; 17 in 2009). To characterize spatial variability in spore composition, the experimental stations were established within the dominant coastal scrub vegetation at locations chosen to represent a gradient of distances away from stands of *P. muricata*. This allowed us to look at the effect of spatial proximity to a vegetation feature known to host a contrasting fungal community – ectomycorrhizal pine forests versus arbuscular mycorrhizal scrub – on fungal spore composition. Distances to bishop pine stands ranged from 0.5 m to 5.4 km and pairwise distances between sites ranged from 0.34 to 12.8 km (median = 4.2 km). Peay et al. (2012b) include more details on the construction of sampling stations and a map of the study site.

Spore trap design and harvest

We designed a simple spore trap that would allow us to integrate across ecologically relevant temporal scales and that could be deployed across a natural landscape where there is no easy access to electricity. To do this, we collected rainwater and any aerial deposition using a 16-oz plastic funnel (12 cm diameter) inserted into the lid of a 1-l glass jar with a screw-top lid. Jars were sterilized, covered in duct tape, and contained 20 g of Chelex resin (Bio-Rad Laboratories, Hercules, CA, USA) sealed in a 50-μm mesh pouch to prevent any in situ growth and curtail the activity of DNases. Water samples were collected approximately once a month (median deployment time = 29 d; range = 13–44 d) or when full. At collection, all liquids in the spore trap were mixed well and then filtered through cheesecloth to remove large particulate matter and debris. The filtered liquids were centrifuged at 12 800 g for 10 min and the supernatant was decanted. Any remaining liquids and pellet were further centrifuged at 15 000 g for 5 min in a 2.0-ml microcentrifuge tube, the supernatant was decanted, and the resulting pellet was resuspended in 500 μl of 2× CTAB buffer and stored at −20°C until used. DNA was then extracted from 100 μl using the protocol described by DeSantis et al. (2005) with modifications found in Amend et al. (2010a). This protocol was designed specifically for extraction and amplification of bacteria and fungal propagules from filters used with high-volume air samplers (DeSantis et al., 2005).

Molecular characterization of spore samples

We used 454 pyrosequencing and quantitative PCR to characterize the fungal composition of our spore samples. For these analyses, we chose to focus on an important phylogenetic subset of the fungal community, the Basidiomycota. These fungi play an important ecological role as major pathogens, saprotrophs and ectomycorrhizal fungi. In addition, by excluding the hyper-diverse Ascomycota we hoped to increase sampling power, reduce the possibility of secondary growth (there are many aquatic Ascomycota) and take advantage of better taxonomy. In addition, because most mushroom-forming fungi belong to the Basidiomycota, we can also compare molecular results with patterns based on fruit body surveys.

To characterize diversity of the Basidiomycota, extracted DNA from each spore trap was amplified using modified versions of ITS1F (fungal-specific) and ITS4B (Basidiomycota-specific) primers (Gardes & Bruns, 1993) that bracket the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA genes. These primers were modified by adding the standard Roche A & B adapter tags for Lib-L sequencing and the Roche recommended 12-basepair barcodes (Roche Applied Science, Branford, CT, USA). Primers were thus A-Barcode-ITS1F and B-ITS4B. Amplification, quantification and pooling were carried out using the methods of Amend et al. (2010b) and sequenced at the Duke University Genomics Facility in two 1/2 plate runs. Sequences were cleaned using the qiime pipeline (Caporaso et al., 2010) to remove all sequences with ambiguous bases, primer mismatches, homopolymers >10 bp, and length <300 or >700 bp, reducing the total number of sequences from 324 545 to 203 002. To account for sequencing error that can arise as part of the PCR and 454 sequencing process, we used flowgram clustering (Knight & Reeder, 2010) as implemented in the qiime v1.3.0 software package with a Titanium error profile. Sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using the USEARCH algorithm (Edgar, 2010). Chimeric sequences were removed using a combination of de novo and reference-based chimera checking using the program UCHIME (Edgar et al., 2011), implemented during the USEARCH clustering process. Sequences flagged as chimeric by both methods were removed before clustering. Taxonomic affiliation of OTUs was assigned for representative sequences from each OTU using the BLAST option in the assign_taxonomy.py script. Our database consisted of a previously published sequence database (Tedersoo et al., 2010b) to which we added a number of ITS sequences derived from fruiting bodies collected in an ongoing effort to catalogue the mycobionta at PRNS. The qiime formatted taxonomy file was initially based on downloaded GenBank taxonomy using a script provided by Jason Staigh (http://bit.ly/ RlZNV5) that was then manually edited so that all sequences included the same subset of ranks (phylum, class, order, family and genus). Phylogenetically uninformative designations (e.g. mitosporic Ascomycete) were coded as Unknown. To see how mycorrhizal associations of vegetation affected different components of the fungal community, we identified all potentially ectomycorrhizal OTUs based on genus-level taxonomic assignments and a recent literature review and classification (Tedersoo et al., 2010a).

To quantify seasonal changes in absolute spore abundances, we used quantitative real-time PCR (qPCR) for two common ectomycorrhizal taxa: *Suillus pungens* Thiers & A.H. Sm. 1964 and *Thelephora terrestris* Ehrh. 1787. Full methods are described in Peay et al. (2012b), but in brief specific ITS primers were designed for each species and the number of spores in each sample estimated based on Sybrogreen fluorescence (iTAQ SYBR Green Supermix, Bio-Rad, Hercules, CA, USA) calibrated against a standard curve from DNA extractions of spores from each species. As a previous publication examined spatial patterns in absolute spore abundance (Peay et al., 2012b), we focused here on seasonal patterns.

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Bait seedlings

To see how spore dispersal could lead to variability in plant–fungal interactions across the landscape, we included *P. muricata* bait seedlings at each station. Each station consisted of 12 initially uncolonized seedlings placed in a rack on top of a plastic tray to prevent contact with the ground. Seedlings were harvested destructively at the end of each sampling season to assess biomass and ectomycorrhizal community composition. Ectomycorrhizal colonists were identified by direct sequencing of colonized root tips for all morphotypes on each seedling using the primers ITS1F and ITS4.

Statistical analyses

In order to examine seasonal changes in spore abundance, we downloaded major climatic variables (precipitation, temperature, solar radiation, relative humidity, and wind speed) as daily data from the Olema Valley weather station (http://www.wrcc.dri.edu) located within PRNS (38°02’33”N, 122°47’45”W) (Supporting Information Fig. S1). From the daily data, we calculated total rainfall in the 30 d before harvesting of each spore trap and the 30 d mean for average temperature, minimum temperature, maximum temperature, solar irradiation, relative humidity and wind speed.

To see which variables best predicted spore richness, we used the lme4 package (Bates et al., 2014) to estimate a linear mixed effects model. We included random effects for *site* and *sampling date* in order to account for the nonindependence of repeated samples taken at the same site and the fact that weather variables at each sample date came from a single weather station. In addition to weather variables, we also included spatial distance to the nearest pine stands, the amount of water collected from each spore trap, sampling year and the number of sampling days (e.g. how long the traps had been out for) as covariates. To quantify seasonality with respect to the Mediterranean winter, we used the number of days since 1 October as a measure of progression through the wet season. We used the Step function in the lmerTest package (Kuznetsova et al., 2014) to select the best set of predictor variables. We also compared model results to a linear mixed effects model estimated using the nlme package (Pinheiro et al., 2013) and a generalized additive mixed model (GAMM) in the mgcv package.

To visualize changes in fungal spore composition over space and time, we used nonmetric multidimensional scaling (NMDS). To test for significant predictors of spore composition, we used spatial distance to bishop pine forest, seasonal progression (the number of days from 1 October) and year of sampling as predictors in permutation multivariate analysis of variance (perMANOVA) (Anderson, 2001). The effects of proximity to pine forest and season on ordination patterns were visualized using vectors from the envfit algorithm in the R package Vegan (Oksanen et al., 2008). Because we added an additional site in year 2 and found small but significant differences between years, we also analyzed data for each year separately. To see if physical proximity led to increased similarity in spore rain among sampling sites, we conducted separate Mantel tests on pairwise community similarity and spatial distance for all sets of samples collected on the same date (*n* = 12 sampling dates). Because there was some variation in the strength of spatial autocorrelation across harvest dates, we tested for a correlation between strength of the Mantel r statistic and wind speed during the sampling period. Because pairwise distance between sampling stations was correlated with relative distance to pine forest, we conducted partial Mantel tests to see if the effect of vegetation features on spore composition was independently significant.

To control for possible artifacts from variation in sequencing, we dropped all samples with fewer than 500 sequences and all OTUs represented by one sequence from our analyses. This removed 12 out of 190 sequenced samples and 303 out of 1493 OTUs. We used two approaches to ensure that remaining differences in sequencing depth did not affect our results. First, we used a presence/absence metric of similarity that controls for differences between samples in species richness (*β*<sub>sim</sub>). Secondly, to incorporate species relative abundances, we used the Bray–Curtis metric based on proportional transformed data (Legendre & Gallagher, 2001). While there are some issues with quantitative use of sequence count data, recent studies have also shown that including relative sequence abundance in the analysis is more likely to capture known ecological patterns (McMurdie & Holmes, 2013) and less likely to produce inflated estimates of community dissimilarity (i.e. pseudo-β-diversity sensu Smith & Peay, 2014).

To see how well the NGS data from our spore traps compared with other ways of measuring fungal dispersal, we also plotted seasonal abundance of spores based on qPCR and seasonal abundance of fruit body collections for two of the most common ectomycorrhizal species at PRNS (Peay et al., 2007). Fruit body collections were made during the 2005–2006 fruiting season in bimonthly visits to 12 separate *P. muricata* patches. Each patch was searched carefully for a fixed amount of time (10 min) and the presence of all ectomycorrhizal species recorded, including hypogeous and resupinate fruiting bodies. More details are available in Peay et al. (2007).

To see how spore variability might translate to variability in the biotic interactions between plants and fungi, we used NMDS to visualize changes in the community of ectomycorrhizal fungi colonizing bait seedlings at each site. To see what factors explained changes in the seedling ectomycorrhizal fungus community between years and sites, we used permANOVA to test for the effects of sample year and distance from bishop pine forest. For this analysis, we also explored a range of dissimilarity metrics, including Bray–Curtis (abundance based), β-sim (presence/absence; controls for richness difference) and Euclidean distance (abundance-based and treats joint absences as informative).

To see how variability in dispersal might affect seedling health, we used linear regression to relate ectomycorrhizal colonizer richness to seedling biomass. We also used analysis of variance to investigate whether there were differences in seedling biomass effects between the two most common ectomycorrhizal colonizers (*S. pungens* and *T. terrestris*), which demonstrated different spore phenology.
Supplementary data
Root tip DNA sequences are available under GenBank accession numbers JN858071–JN858083. Site metadata and NGS sequence data are available at DRYAD entry doi:10.5061/dryad.b70pn.

Results
The number of Basidiomycota species detected per spore trap sample ranged between c. 50 and 100 species at a given time-point. This variation loosely followed seasonal trends in precipitation (Fig. 1). However, our linear mixed effects model showed that average solar radiation and average wind speed over the 30 d before sampling were the best predictors of spore diversity (Table 1). Mixed effects models built in the lme and mgcv packages showed similar parameter estimates and significance. The mgcv GAMM estimated that 37% of total variance in spore trap richness was explained by the best model. Parameter estimates indicated that spore richness was greatest during windy periods with low solar radiation. Richness also increased with spore trap sample volume (i.e. total precipitation during the sample period) and was generally higher in year 2. Precipitation over the previous 30 d measured at the weather station was found to have a slight negative effect on spore trap richness after all other factors had been taken into account.

The composition of spores was highly variable across space and time. All community dissimilarity indices varied predictably with increasing distance from pine forest and temporarily with time of sampling. Because the results were qualitatively the same, we present here only the results based on the presence/absence βsim metric. After eliminating insignificant interactions, the final model explained 43% of variation in spore composition and included distance to pine forest ($\hat{\tau}^2 = 0.074; P < 0.001$), time of sampling ($\hat{\tau}^2 = 0.054; P < 0.001$), year ($\hat{\tau}^2 = 0.049; P < 0.001$), site ($\hat{\tau}^2 = 0.22; P < 0.001$) and interactions between distance to pine forest and year ($\hat{\tau}^2 = 0.009; P < 0.009$), and time of sampling and year ($\hat{\tau}^2 = 0.017; P < 0.001$). Analysis of each year separately showed that the effects of distance to pine forest and time of sampling were both highly significant for each year, although they were perhaps slightly stronger in year 2. (Fig. 2) While the predictable proportion of variation explained by the season and distance to bishop pine forest was overall small (~15%) total when analyzed independently for both year 1 and year 2 – these effects were generally orthogonal and explained large fractions of variation within the ordinations for both year 1 (distance, $\hat{\tau}^2 = 0.6066; P < 0.001$; season, $\hat{\tau}^2 = 0.5395; P < 0.001$) and year 2 (distance, $\hat{\tau}^2 = 0.7382; P < 0.001$; season, $\hat{\tau}^2 = 0.8378; P < 0.001$).

Mantel tests showed that sampling stations closer together in the landscape experienced more similar spore rain of Basidiomycota (Fig. 3). Spatial autocorrelation of the spore community held despite the fact that sampling stations were widely spaced with median pairwise distance between sites of 4.2 km (range 0.34–12 km). Mantel tests were significant ($P < 0.05$) for 10 of 12 sampling periods. The Mantel r-statistic was stronger during periods of high wind gustiness, although the correlation

Table 1 Parameter estimates for linear mixed effects model predicting richness of basidiomycete fungi detected in spore trap samples

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>SE</th>
<th>df</th>
<th>t value</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>59.838</td>
<td>9.41</td>
<td>7.05</td>
<td>6.359</td>
<td>0.0004*</td>
</tr>
<tr>
<td>Precipitation (mm)</td>
<td>−0.067</td>
<td>0.02</td>
<td>5.20</td>
<td>−2.696</td>
<td>0.0413*</td>
</tr>
<tr>
<td>Solar radiation (W h m$^{-2}$)</td>
<td>−25.703</td>
<td>3.59</td>
<td>4.24</td>
<td>−7.153</td>
<td>0.0016*</td>
</tr>
<tr>
<td>Sample volume (ml)</td>
<td>0.022</td>
<td>0.01</td>
<td>10.11</td>
<td>2.163</td>
<td>0.0555</td>
</tr>
<tr>
<td>Wind speed (m s$^{-1}$)</td>
<td>49.882</td>
<td>9.86</td>
<td>4.86</td>
<td>5.057</td>
<td>0.0042*</td>
</tr>
<tr>
<td>Year</td>
<td>15.736</td>
<td>5.33</td>
<td>5.88</td>
<td>2.954</td>
<td>0.0261*</td>
</tr>
</tbody>
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* , $P < 0.05$.  

Fig. 1 Seasonal trends in precipitation and the richness and quantity of fungal spore production. (a) Thirty-day running total of precipitation measured at the Bear Valley meteorological station during the 2-yr period of the study. (b) Diversity of Basidiomycota spores collected in spore sampling stations and determined through 454 pyrosequencing. Each point is the average of 16 (year 1) or 17 (year 2) sampling stations ± 1 SD. (c, d) Average number of spores detected ± 1 SD using quantitative PCR for (c) Suillus pungens and (d) Thelephora terrestris, two of the most common ectomycorrhizal fungi detected in the study. For all panels the x-axis is shown in ‘month/year’ format.
Fig. 2 Spatial and temporal variability in the species composition of fungal spores visualized using nonmetric multidimensional scaling (NMDS). Each point represents the Basidiomycota fungal community detected in a single spore trap sample using 454 pyrosequencing. Symbols increase in size with distance away from pine forest and are colored in a gradient from yellow (autumn) to red (spring) with progression through the sampling season. Vectors show the direction of strongest correlation with these factors in the ordinations. For clarity, year 1 (a) and year 2 (b) are shown in separate panels.

was marginally insignificant ($r^2 = 0.24; P = 0.06$). Physical distance between sampling sites was positively correlated with our other measure of spatial location – distance to bishop pine (Mantel $r = 0.43; P = 0.01$). This was in part unavoidable because it is physically impossible to locate sampling stations close to each other and yet at different distances from the pine forest. Despite this, partial Mantel tests showed that the effects of pairwise physical distance and distance to bishop pine had significant independent effects on spore community composition ($P < 0.05$ in eight of 12 sample periods for the effect of distance to bishop pine controlling for pairwise physical distance; $P < 0.05$ in six of 12 for effect of pairwise physical distance while controlling for distance to pine forest).

These patterns of spore composition are explained by differences between Basidiomycota fungi in phenology and dispersal limitation. For example, ectomycorrhizal fungi were observed less frequently at sites located large distances from their pine host and later in the season (Fig. 4a,b). The dominant basidiospore producers were almost exclusively wood-decaying Basidiomycota that make resupinate fruiting bodies (Fig. S2), such as *Peniophora*, *Steccherinum*, *Diplomitoporus*, and *Exidiopsis*. Most of these common taxa also showed evidence of strong spatial or temporal niches for spore production (Fig. 4c). We observed a number of taxa with agaricoid fruiting bodies, such as *Suillus*, *Armillaria*, *Clitocybe* and *Mycena*, but they were less common. In total, OTUs were assigned to 381 genera and 128 families. A few common taxa dominated the sequence pool, with the 20 most abundant genera accounting for 72% of all sequences, the 20 most abundant families accounting for 91% of all sequences, and the 20 most abundant orders accounting for 99% of all sequences (Fig. S2). Ectomycorrhizal fungi made up a small proportion of the total species pool (6% of total sequences, 7% of total observations, and 11% of species). While we know that ectomycorrhizal fungi are restricted to the bishop pine forest because of their trophic status, the patterns we identify for ectomycorrhizal fungi likely also hold for a greater range of saprotrophic and pathogenic taxa that are specialized on the habitat created by *P. muricata*. For two common ectomycorrhizal fungi, *S. pungens* and *T. terrestris*, quantitative PCR (Fig. 1) and fruit body surveys (Fig. S3) indicated strong differences in temporal fruiting window. *Suillus pungens* fruiting peaks after the first rains and *T. terrestris* fruiting peaks toward the end of the wet season.

The community composition of ectomycorrhizal fungi that colonized bait seedlings through aerial spore dispersal was also related to landscape context (Fig. 5a). The full perMAANOVA models were able to explain $c. 20\%$ of variation in species composition. Regardless of the metric chosen, spatial proximity to the pine forest was significant in all models and explained a large fraction of variance (Table S1). Bray–Curtis and Euclidean distance measures attributed more variation in seedling composition to distance from pine forest, consistent with the fact that abundance and richness of ectomycorrhizal fungi also decreased with distance away from the pine forest (Peay et al., 2012b). The effect of year was generally weak and nonsignificant except when the $\beta_{	ext{sim}}$ metric was used (Table S1).

At the individual seedling scale, we also saw evidence of even greater variability in community composition and richness of ectomycorrhizal colonizers. Ordination of ectomycorrhizal fungal communities at the individual seedling level showed frequent within-site variability in species composition (Fig. 5b) and richness (data not shown). To see whether this variability might affect plant performance, we compared growth of seedlings colonized by a single species of ectomycorrhizal fungus, either *S. pungens* or *T. terrestris*. Despite large differences in hyphal growth form and relatedness between these two fungi, there was no difference in biomass between plants colonized by the two fungi ($P > 0.05$). We found that there was a significant effect of ectomycorrhizal richness on plant biomass that was described by a negative quadratic function (coefficients: number of species $= 0.69; P < 0.001$; number of species$^2 = -0.174; P < 0.001$; model $R^2 = 0.18$; Fig. 6).

**Discussion**

Our analyses showed that there was significant variability in the spatial and temporal composition of fungal spores reaching a particular place in the landscape and that this could have consequences for the fungal community and for plant–fungal interactions. We found that a portion of this variation was predictable based on contextual knowledge of the surrounding...
vegetation and season; that is, dispersal was not a totally random process in real landscapes. However, more than half of the variation remained unexplained or was explained by unique site features (i.e. site identity), demonstrating a large degree of stochasticity in the deposition of fungal spores in otherwise similar places or times. In interpreting this result, it is important to distinguish between variation that is unexplained for lack of good predictor variables and truly random variation. While it is certain that more predictor variables could help explain some additional variation in our data set, in our estimation the patchiness of fungal communities may drive very interesting ecological dynamics. For example, limited dispersal of fungal pathogens may help maintain plant diversity through Janzen–Connell dynamics (Gilbert, 2002) and variation in the arrival time of species may lead to alternate pathways of community assembly and function (Fukami et al., 2010). Our study provides empirical support for one of the general assumptions underlying these theories.

The effects of spatial context are likely to be generally important in structuring fungal communities. Spatial changes in fungal community structure may be attributable to underlying environmental variables, soil or substrate (Dickie et al., 2002; Taylor et al., 2010; Baldrian et al., 2012), biotic interactions with plant hosts (Ishida et al., 2007; Bogar & Kennedy, 2012; Gao et al., 2013), succession stage of vegetation (Last et al., 1987; Visser, 1995; Nara et al., 2003), or simply random variation in community assembly (Fukami et al., 2010; Dickie et al., 2012). In many cases the focus of this work has been on the direct effect of conditions measured at the sample scale on the fungal community. Here we show that the fungal community arriving at a particular point in space is also the product of the larger biotic and abiotic landscape (Figs 2–4) and that this directly affects realized diversity (Peay et al., 2012b) and community structure (Fig. 5). Similarly, Bogar & Kennedy (2012) found that spatial proximity to other host plants can cause spillover of normally host-specific ectomycorrhizal fungi. In both cases, the fungal community

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**Fig. 3** Spatial autocorrelation of Basidiomycota spore composition across a California landscape. Each panel shows the relationship between dissimilarity in spore composition between two samples and physical distance between the samples. Panels are arranged in chronological order starting from the top left corner. Y indicates the year (1 or 2) and the number following the ‘.’ indicates the order of samples taken in that year (e.g. Y1.1, first sample taken in year 1). The strength of the Mantel correlation and associated P-value are given for each sample period.
Fig. 4 Abundance of fungal spores across space and time. (a, b) Heatmap and isoclines showing the percentage of (a) observations and (b) sequences assigned to ectomycorrhizal lineages plotted against progression of the fruiting season (measured as the number of days past 1 October) and distance in meters from pine patches (log$_{10}$ transformed). Warm colors represent higher values. Data were interpolated based on individual spore traps from both sampling years. The representation of ectomycorrhizal fungi in the total spore pool decreased strongly with distance from propagule sources and with progression of the fruiting season. (c) Individual patterns of basidiospore abundance for the 20 most common taxa.

Fig. 5 Variability in species composition of ectomycorrhizal fungi colonizing seedlings depicted using NMDS ordination on Bray–Curtis distances. (a) Ectomycorrhizal community similarity at the scale of sampling sites. The size of symbols increases with increasing distance from pine patches and the vector shows the direction of correlation with distance in the ordination. (b) Ectomycorrhizal community similarity at the scale of individual seedlings. As a result of the simplicity of the community, a small amount of jitter was added to separate tight clusters. The topo color scale progresses from blue to yellow with increasing distance from pine patches. Site ID (1–17) is plotted on top of each point, with text for year 1 samples in black and year 2 in red.
responds to factors that could not be measured directly within the actual samples collected.

In our study, spatial patterns of spore dispersal could be observed on the meter to kilometer scale and were driven by a complex mixture of vegetation features and physical proximity between sites. Partial Mantel tests and permANOVA showed that spore composition was affected by proximity to contrasting vegetation features (in this case pine forest) with a unique resident fungal community. Despite the fact that sampling stations in our study were separated by a minimum of 300 m, we still detected a signature of spatial autocorrelation in Basidiomycota spore composition (Fig. 3) that was not totally related to proximity to pine forest. This spatial autocorrelation is probably driven by the general patchiness of fungal communities and fruiting body production even within similar habitats (Peay et al., 2010a; Talbot et al., 2014). For example, because many of the wood decay taxa shown in Fig. 4(c) are not restricted to the pine forest, it is likely that the spatial patterns depicted are driven in part by localized spore production around one or a few sampling stations rather than distance to pine forest per se as depicted on the x-axis. Our finding of localized dispersal, in tandem with the importance of priority effects in fungal communities (Kennedy et al., 2009; Fukami et al., 2010), helps to explain the establishment and subsequent maintenance of divergent fungal communities across habitat patches. We found some evidence that the strength of spatial autocorrelation in spore composition increased with wind speed over the sampling period, probably as a result of steeper dispersal gradients into the predominately offshore winds. Although the mechanism is not totally clear, this finding suggests that the degree of dispersal linkage between locations fluctuates temporally. Overall, the finding that dispersal creates significant local differences in composition of incoming spores suggests that a complete understanding of microbial communities must integrate mass effects and other meta-community paradigms (Leibold et al., 2004).

Seasonal variation in the production of fungal fruiting bodies is well known (Corner, 1935; Straatsma et al., 2001; Kauserud et al., 2010b; Sato et al., 2012). Fruiting peaks often correspond with the onset of spring and autumn in temperate habitats and with winter precipitation in Mediterranean climates. While these patterns are well known from fruiting bodies, our study demonstrates that a molecular approach can be effective in monitoring patterns of fruiting diversity and abundance throughout the year even without large-scale, time-intensive field surveys. Although the data were collected in a different year, our fruit body collections match well with the molecular data in terms of peaks in diversity and timing of fruiting for ectomycorrhizal fungi (Figs 1, S2). One other advantage of this molecular approach is that it is not likely to be biased toward species with large, easy-to-locate fruiting bodies. Indeed, the most abundant taxa we observe in our spore traps have resupinate fruiting morphologies (Fig. S2) that are less often collected in mushroom surveys. Timing of fruiting is probably driven by a combination of temperature and precipitation, although individual species vary in their factors (Straatsma et al., 2001; Kauserud et al., 2010a; Sato et al., 2012). Interestingly, in this study the amount of solar radiation over the spore sampling season was the best predictor of fruiting richness. Although the reasons for this are unclear, the amount of incoming solar radiation changes both on a daily basis with local weather systems (i.e. it decreases when it is cloudy) and seasonally with changes in day length. Further investigation of the role of solar radiation and incorporation of other important local variables, such as wind direction with respect to spore sources, will be important in future studies on fungal spore production. While these results are intriguing, it is important to note that this study was focused primarily on variation within a season and that seasonal climate variables are always correlated to some extent (Fig. S1), making it difficult to pick out single drivers of spore production from the overall climate syndrome. Most previous studies that examined correlations between fruiting patterns and climate were based on data sets spanning many years (Straatsma et al., 2001; Gange et al., 2007; Kauserud et al., 2008). As a result, mushroom surveys require many years to capture the majority of species (Straatsma et al., 2001) and predictable variation in the relationship between fruiting and climate is probably most obvious at the inter-annual scale. While many temperate and tropical regions show a bimodal fruiting peak (spring and autumn), in this Mediterranean climate we found that fruiting could be either bimodal (year 1) or unimodal (year 2) depending on precipitation patterns. In addition, we found that there were relatively poor correlations between recent precipitation and total spore diversity, probably because of the nonlinear response of fruit body production to precipitation within a given year. In addition, because our sampling strategy relied on precipitation as a means of sampling spores, there was some conflation between precipitation as a driver of fruiting and the effects of sample volume in capturing more spores. While ballistosporic basidiospores are hydrophilic, spore dispersal studies incorporating a broader range of taxa (e.g. anamorphic Ascomycota) may need to take into account how variation in spore hydrophobicity could affect the likelihood with which particular species are washed out in rainwater. Thus, it would be worthwhile to compare results obtained using this method with those obtained using more traditional adhesive or volumetric air samplers. Despite these considerations, overall this method shows great promise and future
research could focus on deploying this type of approach as a low-cost method to monitor fungal fruiting, including both micro- and macrofungi.

Many ecological models treat the dispersal and colonization process as purely random (Hubbell, 2001), despite the well-known differences in species dispersal ability (Howe & Smallwood, 1982) and the potential trade-offs with competitive ability (Tilman, 1994; Kennedy et al., 2011). Our study of fungal dispersal shows evidence of stochasticity within similar parts of the landscape (e.g. variation within a site or across sites near to the forest edge) but also predictable changes across the landscape based on differences in species dispersal abilities and habitat affinities. The ecological realization of this is evident in both the emergence of clear patterns in ectomycorrhizal fungal colonization with respect to distance at the site level (Fig. 5a) and large variation in ectomycorrhizal fungal colonization across individual seedlings within a site (Fig. 5b). Within-site variability in fungal colonists of seedlings could be attributable to centimeter-scale variation in the identity of spores that arrived at different seedlings (most seedlings at a site were within 10–20 cm of each other), priority effects from differences in arrival time (Kennedy et al., 2009) and historical contingency from variation in arrival order (Fukami et al., 2010). While most ectomycorrhizal seedling colonists tend to come from a limited number of early successional taxa at Point Reyes, Peay et al. (2010a) found evidence that these kinds of stochastic effects continue to influence mature tree ectomycorrhizal fungal communities over decades. Despite the fact that we did not see strong species-level effects on seedling growth in the two dominant seedling colonists (T. terrestris and S. pungens), there is significant evidence for functional differentiation among ectomycorrhizal fungi (Courty et al., 2010) and thus it is possible that such effects could arise, particularly as communities become more complex with time. We did find evidence that variability in the richness of ectomycorrhizal fungi colonizing seedlings caused by the dispersal process has significant effects on plant growth (Fig. 6). While there was an increase in seedling biomass with ectomycorrhizal colonization, there was only a marginal increase in seedling growth when colonized by two species of ectomycorrhizal fungi and some decrease when colonized by three species. Given that seedling roots were dominated by two species (S. pungens and T. terrestris), it is not possible to totally separate our results from the specific biology of these species. However, similar richness–function relationships have been seen in other work on ectomycorrhizal and saprotrophic fungi. Baxter & Dighton (2001) found that increasing ectomycorrhizal richness decreased shoot biomass but had no effect on total seedling biomass. Kennedy et al. (2007) found that two- and three-species ectomycorrhizal treatments often had lower biomass than single-species treatments. Similarly, studies on saprotrophic fungi have found that increased diversity leads to reduced decomposition rates, probably as a consequence of an increase in energy spent on competitive interactions (Fukami et al., 2010; Peay et al., 2012a). Thus, our results show that realistic stochasticity in dispersal outcomes can lead to variation in the nature of biotic interactions between plants and their mycorrhizal fungi and that this in turn can have significant effects on plant health.

Conclusions

There are few studies of whole community dispersal in the ecological literature and uncertainty about the importance of the dispersal process in structuring microbial communities. By measuring dispersal across a heterogenous landscape, we show that even at this scale there are limits to microbial dispersal and that this can have significant consequences for assembly of microbial communities and the functions that they mediate. Because of the potential for strong variability in community composition driven by meso-scale dispersal limitation, we conclude that some of the most interesting ecological dynamics of microbial communities will occur at the landscape scale.

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References


Galante TE, Horton TR, Swaney DP. 2011. 95% of basidiospores fall within one meter of the cap - a field and modeling based study. *MycoLogia* 103: 1175–1183.


### Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Daily weather data from the Bear Valley Weather station.

**Fig. S2** Taxonomic distribution of OTUs recovered from 454 pyrosequencing of the basidiomycete spore community.

**Fig. S3** Patterns of precipitation, fruit body species richness and fruit body production for *Suillus pungens* and *Thelephora terrestris*.

**Table S1** Significant predictors of seedling ectomycorrhizal community composition using permANOVA

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