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Spatial scaling of microbial eukaryote diversity

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Patterns in the spatial distribution of organisms provide important information about mechanisms that regulate the diversity of life and the complexity of ecosystems[1,2]. Although microorganisms may comprise much of the Earth's biodiversity[3,4] and have critical roles in biogeochemistry and ecosystem functioning[5–7], little is known about their spatial diversification. Here we present quantitative estimates of microbial community turnover at local and regional scales using the largest spatially explicit microbial diversity data set available (>10^6 sample pairs). Turnover rates were small across large geographical distances, of similar magnitude when measured within distinct habitats, and did not increase going from one vegetation type to another. The taxa–area relationship of these terrestrial microbial eukaryotes was relatively flat (slope $z = 0.074$) and consistent with those reported in aquatic habitats[8,9]. This suggests that despite high local diversity, microorganisms may have only moderate regional diversity. We show how turnover patterns can be used to project taxa–area relationships up to whole continents. Taxa dissimilarities across continents and between them would strengthen these projections. Such data do not yet exist, but would be feasible to collect.

Ecologists studying macroorganisms have long recognized that beta-diversity (how community composition changes across a landscape) is central to understanding the forces responsible for the magnitude and variability of biodiversity. Patterns of beta-diversity can offer valuable clues to the relative influence of dispersal limitation, environmental heterogeneity, and environmental and evolutionary change in shaping the structure of ecological communities[10–14]. Despite an increasing awareness that spatial patterning of soil microbiota can have important aboveground consequences in regard to plant community structure and ecosystem functioning[5,6,15,16], microbial beta-diversity patterns are largely unknown. Inadequate sampling has been a major limitation and scientists are only now beginning to explore emergent patterns and principles that may be common to microbes, plants and animals[17,18]. Thus, whereas it is widely accepted that the similarity in plant and animal community composition decays with increasing distance between samples[11,13,19], patterns of microbial turnover in terrestrial environments remain unstudied. Here, we test whether similarity in microbial eukaryote community composition decays with geographical distance as observed in macroorganisms. We also explore how these biodiversity turnover patterns are influenced by strong habitat-related environmental discontinuities. Finally, we apply spatial scaling theory to these turnover patterns to predict how microbial biodiversity might increase with sampling area from local to continental scales in Australia.

A total of 1,536 soil samples were collected in arid Australia using a spatially explicit nested design. The design resulted in 1,117,880 pairwise sample comparisons, with distances ranging from 1 m to ~100 km represented by multiple replicate sample pairs. Samples were taken from four distinct land systems that varied substantially in geology, topography and native vegetation (see Supplementary Information). We measured the similarity between any two samples using the Sørensen index, defined as the number of taxa in common divided by the average number of taxa in the two samples[20]. The rate at which Sørensen similarity decays with increasing distance between samples (the distance–decay relationship) can be directly related to the species–area relationship[21]. Other measures of similarity based on presence/absence of data yielded qualitatively similar results to those reported here.

We characterized the beta-diversity of ascomycete fungi by automated ribosomal RNA intergenic spacer analysis (ARISA), a commonly used DNA-based community fingerprinting method[22–24]. ARISA is a high-resolution, highly reproducible technique for detecting differences between complex fungal communities[22]. We chose ARISA over DNA sequencing because it allowed assessment of microbial community turnover at an unparalleled sample size and spatial scale. ARISA exploits variability in the length of the intervening transcribed spacer regions of rRNA genes (ITS) to sort samples rapidly into operational taxonomic units (OTUs). Members of different species may share the same ITS fragment size[22]. Although ARISA assays a different taxonomic resolution than species, it is a consistent measure of community composition

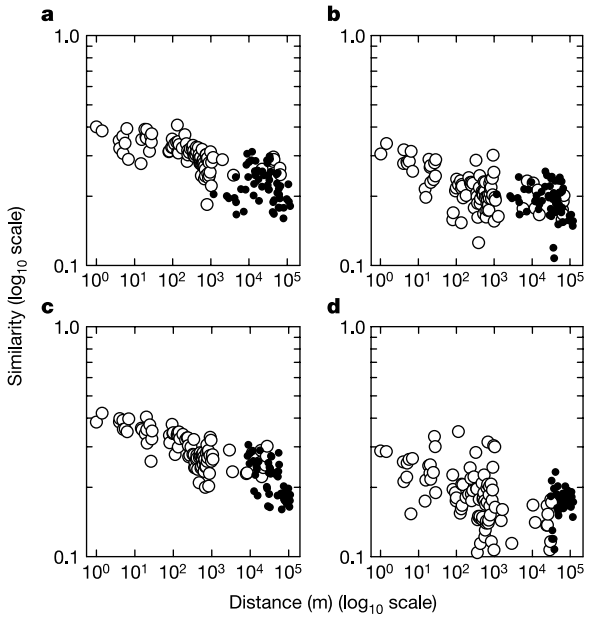

Figure 1 The distance–decay of similarity for microbial fungi OTUs. **a**–**d**, Shown are the average Sørensen similarity values for within land system data (open circles) and between land system data (filled circles). Averages were taken across similarity values within equidistant logarithmic intervals of 0.01. Data correspond to Pulgamurtie (**a**), Rodges (**b**), Olive Downs (**c**) and Corner (**d**). Summary statistics incorporating the replicate data within each distance class are listed in Table 1.

(that is, members of any one species consistently produce the same fragment size and different-sized fragments always derive from members of one or more different species). Consequently, differences between two OTU assemblages directly reflect changes in species composition.

Within each of the four distinct land systems, community OTU similarity decayed significantly with distance (Fig. 1). These data show that the sampled microbes were not randomly distributed, but rather exhibited spatially predictable, aggregated patterns over scales ranging from $1\,m^2$ to $10^{10}\,m^2$. The best fit and the most homoscedastic residuals were found in models that used the log transformation of similarity against the log transformation of distance, implying a power-law rate of distance decay. The average slope estimated from all pairwise replications within land systems was -0.043 (95% confidence intervals (CI): -0.049 to -0.037), and three of the four land systems had slopes that were statistically indistinguishable from one another (Table 1). A prevailing view[8] expects that microbial eukaryotes such as ascomycetes will prove to be nearly ubiquitous geographically, owing to their large population densities, producing abundant spores that can travel over long distances. The decline in similarity with distance found here demonstrates that there is at least some geographical differentiation.

Geographical distance was a more useful predictor of ascomycete community turnover than land system type. When the effect of land system was removed, we found a weak but highly significant negative correlation between similarity and geographical distance (Table 1). In contrast, after controlling for geographical distance, we did not consistently find lower similarity between compared to within land system types. For sample pairs with a member in the rocky land systems (Pulgamurtie and Olive Downs), similarity with other samples from the same land system averaged slightly higher than similarity with samples from other land systems, after adjusting for distance; but for sample pairs with a member in the sandy land systems (Corner and Rodges), similarity with other samples from the same land system actually averaged slightly lower than similarity with samples from other land systems, after adjusting for distance. The lack of correlation with land system suggests that ascomycetes respond to their environment at a scale that is poorly described by the geomorphic variables used to classify land systems, being more likely to respond to soil chemistry, water and resource concentrations at much smaller scales.

The slope of the distance–decay curve reflects the rate at which OTU richness increases with sampling area, or the taxa–area relationship. A greater rate of distance–decay between samples implies a faster turnover in OTU composition across a landscape and hence a steeper taxa–area relationship. Recent theory[21] suggests that if the distance–decay curve in a region is well approximated by a power law, then the taxa–area relationship will also be well approximated by a power law of the form

$$OTU_a = OTU_A\left(\frac{a}{A}\right)^z \quad (1)$$

where OTU_A is the number of OTUs in a region of area A, OTU_a is the number of OTUs in a smaller sampling area a within A, and z is a constant ranging between 0 and 1. The expression relating the distance–decay curve to the power-law OTU–area relationship exponent z takes the form

$$\chi_d = \chi_D\left(\frac{d}{D}\right)^{-2z} \quad (2)$$

where χ_d and χ_D are the expected Sørensen similarities between two samples of equal area separated by distances $d < D$, respectively. Hence the exponent $-2z$ of the power-law distance–decay curve (equation (2)) may be used to estimate the exponent z of the power-law taxa–area relationship (equation (1)) across the spatial scales $a = d^2$ to $A = D^2$. The theory underpinning equations (1)–(2) is equally valid for any biologically consistent taxonomic unit.

The distance–decay curve for the entire microbial data set at Sturt National Park is well characterized by a power-law distance–decay model across the spatial scales d ranging from 1 m to about 10^5 m. We may therefore estimate the slope z of the OTU–area relationship across these scales. A least squares regression of log-transformed similarity against log-transformed distance through all of the data gives a slope of -0.147 ($n = 907{,}878$, $r^2 = 0.0051$, $P < 0.001$, 95% CI: -0.152 to -0.143), predicting a power-law exponent $z = 0.074$ across the scales $1\,m^2 < a < 10^{10}\,m^2$. We can now extrapolate

Table 1 **Summary statistics for the fungal OTU distance–decay relationships at Sturt National Park**

Land system	Samples	Within land system regression statistics				Partial Mantel correlations		
		n	Slope	r^2	95% CI	*N*	*r*(*SD.L*)	*r*(*SL.D*)
Pulgamurtie	381	72,390	−0.047	0.0018	−0.056 to −0.039	442,165	−0.066	0.082
Olive Downs	379	71,631	−0.035	0.0005	−0.047 to −0.024	440,230	−0.097	0.023
Rodges	317	50,086	−0.076	0.0027	−0.089 to −0.064	378,261	−0.091	−0.054
Corner	271	36,585	−0.063	0.0014	−0.080 to −0.046	329,800	−0.087	−0.102

Within land system statistics result from weighted least squares regressions of log-transformed fungal OTU similarity against log-transformed geographical distance. All samples that gave >1 μg of PCR-amplifiable DNA were used in the analysis, and *n* is the corresponding number of similarity pairs. To account for zero similarity values, logarithmic transformations were of the form $\log(Y + 0.001)$[20]. All slopes were significantly different from zero ($P < 0.001$); *P*-values and 95% confidence intervals (CI) are based on 5,000 randomized pairings of OTU similarity and geographical distance[28]. The partial Mantel statistic *r*(*SD.L*) estimates the correlation between matrices *S* (OTU similarity) and *D* (geographical distance) while controlling for the effect of same versus different *L* (land system). Similarly, *r*(*SL.D*) estimates the correlation between matrices *S* (OTU similarity) and *L* (land system) while controlling for the effect of *D*. Land system matrices *L* contained ones where pairs of sites were from the same land system and zeros where sites were from different land systems. For any given land system, the number of similarity values *N* includes the within land system similarities and the similarities between that land system and the other three land systems. All partial regression coefficients were significantly different from zero ($P < 0.001$)[28].

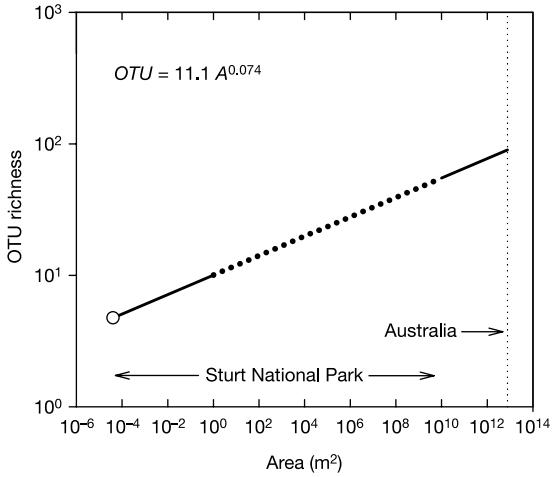

Figure 2 The projected taxa–area curve for microbial fungi. There was an average 4.74 OTUs per 4×10^{-5} m^2 per cm depth in the soil horizon (open circle). The dotted line shows the spatial scales over which the slope $z = 0.074$ is predicted from the observed distance–decay data (equation (2)) and the solid lines illustrate the taxa–area curve that follows from the simple assumption that the slope $z = 0.074$ extends to smaller and larger scales.

microbial biodiversity from the scale of an individual soil sample, where OTU richness is quantifiable, to regional scales using equation (1) (Fig. 2).

It is important to bear in mind that the estimated taxa–area relationship for any group of organisms may depend on the defined OTU. The ARISA-defined OTU used in this study will markedly underestimate the number of Ascomycota species in each soil sample[22]. The relationship between our estimated slope z and that derived using different taxonomic criteria, such as whole-genome relatedness or morphological similarity, is less clear. Our predicted taxa–area slope is remarkably consistent with those reported for microbial eukaryotic species in the Arctic benthos[9] (ciliates $z = 0.077$, diatoms $z = 0.066$) and in freshwater habitats[25] (ciliates $z = 0.043$). Thus, studies using different taxonomic criteria indicate that despite high local diversity, microbial eukaryotes may have only moderate spatial turnover and hence moderate regional diversity. It remains possible that z increases between regional and continental (or global) scales as new and distinct habitats are included or if dispersal barriers are crossed. Notably, no reliable data currently exist that would allow global-scale extrapolations of microbial diversity, and further large-scale quantification of community turnover is essential for improving microbial biodiversity estimates.

Many ecologists have treated the entire microbial community as a "black box" with no spatial structure[15]. Our data illustrate that like macroorganisms, microbial eukaryotes are not randomly distributed, but rather exhibit spatially predictable, aggregated patterns from local to regional scales. We have shown that by sampling localities spatially, in such a way that the decline in similarity with distance can be measured, the slope z of the taxa–area relationship can be estimated, leading on to estimates of the total diversity over wide areas. The relationship between biodiversity and area is central to ecology. Despite the ecological importance and ubiquity of microorganisms, little is known regarding microbial taxa–area relationships. Our findings offer exciting potential for a more synthesized view of micro- and macroorganism biodiversity, and ultimately a means to estimate global biodiversity much more accurately. □

Methods

Sampling methods and DNA extraction

The study area was within Sturt National Park, New South Wales, Australia. 'Land system' mapping undertaken by the New South Wales Soil Conservation Service made this region ideal for studying the influence of habitat type on microbial beta-diversity. Within the park, 23 land systems with distinct patterns of topography, soil and vegetation had been previously mapped. Four of these land systems, representing 42% of the area of the park, were included in the study. These were Olive Downs (stone-covered rolling downs), Pulgamurtie (stony uplands), Corner (sand dunes) and Rodges (sand plains). The soils, geology and vegetation in each land system, and the basis for choosing each land system type, are described in more detail by ref. 26.

Six locations within each of the four land systems were selected to provide a total of 24 study sites. Each study site comprised a 750 × 750 m plot with 64 sampling points, yielding a total of 1,536 sampling points and $1{,}536 \times 1{,}535/2 = 1{,}178{,}880$ sample pairs (see Supplementary Information). In September/October 1997, at each of the 1,536 sampling points a soil sample of approximately 10 g was taken at a depth of 8 cm below the ground surface and frozen on site.

Ascomycetes were sampled by collection of their DNA from soil. For DNA extraction, the total soil sample (10 g) was homogenized using a sterile mortar and pestle before a 400-mg subsample was taken for DNA extraction using a variant of the FastPrep bead beating method, as described previously[27]. The DNA yield was typically from 2.0 to 2.5 μg in a volume of 160 μl (see Supplementary Information).

ARISA

The primers SSU1758 (5′-GTCATTTAGAGGAAGTAAAAGTCG-3′, positions 1735–1758 of the SSU rRNA gene, *Sacharromyces cerevisiae* numbering) and 58S8 (5′-CAGAACCAA GAGATCCGTTGTTG-3′, positions 30–8 of the 5.8S rRNA gene, *S. cerevisiae* numbering) were used for amplification of the ITS1 region. This primer pair selectively targets ascomycete fungi in PCR (see Supplementary Information). Amplifications were performed in 50-μl volumes using 0.5-ml tubes in a Hybaid OmnE thermal cycler with the following thermal cycle: 94 °C for 3 min (1 cycle); 94 °C for 30 s, 62 °C for 15 s, 72 °C for 60 s (35 cycles); 72 °C for 5 min (1 cycle). The banding profile from individual soil samples was reproducible when either multiple DNA extractions were performed on subsamples from the same soil sample, or multiple PCRs were performed on the same DNA sample (see Supplementary Information). The size range of amplicons was ~170 to ~800 base pairs (bp), with approximately 80% being in the size range of 200–350 bp. For electrophoretic separation, a Corbett GS 2000 DNA fragment analyser (Corbett Research) was used with high-resolution, ultrathin 5% polyacrylamide gels and DNA fragments detected by laser. Data were analysed using the RFLPscan package (Scanalytics). Size of DNA fragments was determined using the desmile method in reference to a 50-bp ladder (Pharmacia) loaded as a standard every eighth lane.

Fragments differing by 1 bp were readily resolved in one gel, and the relative error of their measured length was 2%. For comparisons between gels, we accounted for this by sorting the electropherogram data into bins of size Y_{bin}, where $X < Y_1 < int(X + 0.02X)$, $int(X + 0.02X) < Y_2 < int(X + 0.04X)$, and so on. Here, X denotes fragment length and *int* denotes integer value. The observed DNA fragments were resolved from 130 bp to 568 bp, yielding a total of 69 OTU bins. The bin presence/absence data were then used for subsequent calculations of the Sørensen index, beta-diversity and taxa–area relationships.

Partial Mantel tests

Several extensions to the basic Mantel randomization test are sensitive to spatial autocorrelation[28]. Partial Mantel statistics were therefore estimated using the 'matrix permutation' method, which has been shown to perform the most reliably in the presence of spatial autocorrelation, being unlikely to reject the null hypothesis falsely when a conservative critical value is used[20,29,30].

Taxa–area relationship estimates

Numbers of OTUs per volume of soil were translated into numbers of OTUs per m^2 in a 1-cm-depth layer in the soil horizon by assuming 1 g cm^{-3} soil bulk density. Although each 400-mg sample was randomly drawn from the surrounding 10 g of soil, equation (2) holds assuming that equal numbers of individuals were drawn per sample.

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A taxa–area relationship for bacteria

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A positive power-law relationship between the number of species in an area and the size of that area has been observed repeatedly in plant and animal communities[1]. This species–area relationship, thought to be one of the few laws in ecology[2], is fundamental to our understanding of the distribution of global biodiversity. However, such a relationship has not been reported for bacteria, and little is known regarding the spatial distribution of bacteria, relative to what is known of plants and animals[3]. Here we describe a taxa–area relationship for bacteria over a scale of centimetres to hundreds of metres in salt marsh sediments. We found that bacterial communities located close together were more similar in composition than communities located farther apart, and we used the decay of community similarity with distance to show that bacteria can exhibit a taxa–area relationship. This relationship was driven primarily by environmental heterogeneity rather than geographic distance or plant composition.

In the 1920s, the empirical relationship between the number of species and area was generalized[4,5] as a power-law, $S = cA^z$, where S is the number of species, A is the area sampled and c is the intercept in log–log space. The species–area exponent, z, is a measure of the rate of change of the slope with increasing area, that is, the rate of turnover of species across space. Variation in the values for c, and especially for z, is of interest because it may indicate that different processes underlie the species–area relationship at different spatial scales[6,7]. Although not as well studied as species–area relationships, other taxa–area relationships (for example, genera–area and family–area) have been identified for plants and animals; such relationships conform to the same power-law as species–area relationships, although they differ in their values of c and z[8,9].

Bacteria are among the most abundant and diverse groups of organisms on earth[10] and mediate important ecosystem processes, including trace gas emissions, decomposition and nitrogen cycling. Whereas taxa–area relationships have been observed repeatedly for numerous plant and animal taxa regardless of ecosystem type[1], they have not been explicitly examined for bacteria. Unique aspects of bacterial biology may prevent bacteria from exhibiting taxa–area relationships. For example, if most bacteria are not dispersal limited (for example, owing to small size and environmental hardiness)[11] and if they exhibit a high degree of ecological redundancy (for example, if bacteria are flexible in habitat requirements and physiological abilities, or if they can easily obtain traits through horizontal gene transfer that are necessary for survival in a given habitat), then one would not expect to observe a taxa–area relationship[3].

Here we investigated whether bacteria exhibit a taxa–area relationship in a New England salt marsh. We conducted our work in a salt marsh because the spatial ecology of salt marshes is especially well understood[12]. There is an extensive literature regarding the main physical gradients in salt marshes, the spatial distribution of plant species and the ecological processes that underlie this distribution. This information provides an ideal reference point from which to investigate the spatial distribution of bacteria. We sampled 1-cm-diameter sediment cores in a nested manner over a scale of centimetres to hundreds of metres. With the possible exception of the most extreme and depauperate environments[13], the diversity of bacterial communities is too high to be exhaustively sampled. Therefore we used a previously refined distance decay approach[14], which uses data on the spatial turnover of taxa, to determine the taxa–area exponent, z. This approach uses comparisons of community composition rather than richness estimations to describe taxa–area relationships. For comparison, we also estimated the relationship between the number of plant species and area in this ecosystem, using the same distance decay approach.

Because a large proportion of microbes cannot be cultured with current laboratory techniques[15], bacterial taxa are often identified from the sequences of indicator genes extracted from environmental samples[16]. We determined the bacterial community composition of our salt marsh samples by amplifying via the polymerase chain reaction (PCR), cloning and sequencing a region of 16S ribosomal DNA (rDNA), the most commonly used indicator gene for bacterial biodiversity. Because the bacterial diversity of salt marshes is often very high, we used PCR primers targeting a subset of the bacterial