Global Ecology and Biogeography, (Global Ecol. Biogeogr.) (2005) **14**, 241–248

RESEARCH PAPER

Microbial macroecology: highly structured prokaryotic soil assemblages in a tropical deciduous forest

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ABSTRACT

Aim To assess the hypothesis that free-living prokaryotes show a pattern of 'no biogeography' by examining the scaling of soil prokaryotic diversity and by comparing it with other groups' biogeographical patterns.

Location Two sites in the tropical deciduous forest of Chamela, Jalisco, on the western coast of Mexico.

Methods We examined the diversity and distribution of soil prokaryotes in two 8×8 m quadrats divided in such manner that we could sample at four spatial scales. Restriction fragment length polymorphisms of *16S rRNA* genes were used to define operational taxonomic units (OTUs) that we used in lieu of species to assess diversity.

Results We found highly structured species assemblages that allowed us to reject multiple predictions of the hypothesis that soil bacteria show 'no biogeography'. The frequency distribution of range size (measured as the occupancy of quadrats) of OTUs followed a hollow curve similar to that of vertebrates on continents. Assemblages showed high levels of beta diversity and a non-random nested pattern of diversity. OTU diversity scaled with area followed a power function with slopes $z = 0.42$ and 0.47.

Main conclusions We demonstrate a non-ubiquitous dispersal for soil prokaryotes, which suggests a complex biogeography similar to that found for terrestrial vertebrates.

Keywords
Bacterial diversity, beta diversity, biogeography, distribution, scales, soil prokaryotes, TRFLP.

INTRODUCTION

Prokaryotic species are essential components of the biosphere because they catalyse processes that are critical to sustaining life on Earth. In recent years, methods based on the phylogenetic analyses of the small subunit ribosomal RNA gene sequences have expanded dramatically our understanding of prokaryotic diversity (Hugenholtz *et al.*, 1998; Curtis *et al.*, 2002). Nevertheless, only 26 of the more than 50 major lineages (Phyla) of the domain *Bacteria* are represented in cultivated strains (Rappé & Giovannoni, 2003), and there are only about 4500 species that have been characterized. Considering that more than half a million bacterial species could occur in 30 g of soil, according to some estimates (Dukhuizen, 1998), it is clear that most of the diversity of prokaryotes remains unexplored.

A direct consequence of the insufficient knowledge on the diversity of prokaryotes is an almost total lack of information regarding their distribution and biogeography. A current debate is on whether microbial communities show patterns of distribution and diversity similar to those of macroscopic organisms (Godfray & Lawton, 2001; Finlay, 2002; Nee, 2003; Horner-Devine *et al.*, 2004). Recent research shows that free-living microbial eukaryotes (e.g. protozoa and microalgae) are cosmopolitan, so the same species are found in sites in any part of the world, implying a very low rate of species turnover (beta diversity) and a low global species diversity (Finlay & Clark, 1999; Finlay *et al.*, 1999; but see Foissner, 1999). This pattern of 'no biogeography', meaning a global homogeneous distribution, has been assumed to hold also for prokaryotes, arguing that their smaller size and higher abundance make them even less prone to be bounded by

 DOI: 10.1111/j.1466-822x.2005.00156.x

biogeographical barriers (Finlay, 2002) than microbial eukaryotes. Scant empirical evidence suggests that this generalization might be true for oceanic bacteria, but not for soil or sediment prokaryotic assemblages (Finlay & Clark, 1999; Finlay *et al.*, 1999; Torsvik *et al.*, 2002; Nee, 2003; Grundmann, 2004). However, no study has performed a sampling procedure designed specifically to address this issue, and the question of whether bacteria show biogeography or not remains unanswered (Curtis *et al.*, 2002; Nunan *et al.*, 2002; Fenchel, 2003).

We define a syndrome of ubiquity for species 'with no biogeography' to include the following traits: (1) a high local to global species ratio, meaning that a single site can contain a high percentage of the full global species set, which is comparatively small; (2) a very high dispersal rate, coupled with a very high abundance of individuals, providing a huge 'seedbank' of species; (3) extremely large distributional ranges, with very few or no species with restricted distribution; (4) a very low rate of species turnover (beta diversity), so samples tend to contain the same species regardless of the physical distance between them; (5) a flat species–area curve; and (6) unstructured local communities, which are random subsamples of the global species pool.

Available information seems to show that not all prokaryotes are cosmopolitan, and that at least some species do not show traits 1 and 2 of the syndrome of ubiquity (Massana *et al.*, 2000; Curtis *et al.*, 2002; Nunan *et al.*, 2002; Torsvik *et al.*, 2002; Fenchel, 2003; Whitaker *et al.*, 2003). Studies on the distribution of guild members, phylogenetically related populations (Cho & Tiedje, 2000) and particular species (Whitaker *et al.*, 2003) are consistent with the conclusion that prokaryotic species can be restricted to given locations, and their distribution probably reflects adaptive evolution to local conditions. In contrast, pathogenic bacterial species and *Bacillus* spore formers are reported to have global panmictic distributions (Massana *et al.*, 2000). Similarly, studies on other free-living prokaryotes have found apparently identical microorganisms in equivalent, but geographically separated environments, such as polar oceans (Hollibaugh *et al.*, 2002), ice (Staley & Gosink, 1999) and marine sediments (Bowman & McCuaig, 2003). Unfortunately, assertions concerning the biogeography of prokaryotes are largely based on fragmentary information, and the pattern of beta diversity, or how similar in species composition are the samples taken from different places, has not been examined (Nee, 2003). Also unexplored is the pattern in which the count of prokaryote species varies with the sampling scale (Grundmann, 2004). Knowing the pattern of beta diversity at different scales, researchers can make inferences regarding the distributional ranges of species, the species–area relationship, and the degree of randomness of local communities (Godfray & Lawton, 2001; Whittaker *et al.*, 2001; Arita & Rodríguez, 2002; Ricklefs, 2004). Here, we use such relationships to test the hypothesis that prokaryote assemblages show traits 3 to 6 of the syndrome of ubiquity.

MATERIALS AND METHODS

We examined the composition of prokaryotic soil assemblages at four spatial scales by systematically sampling sites within a fully nested system of quadrats (Fig. 1, Arita & Rodríguez, 2002). This sampling design allowed us to measure distribution, taxonomic diversity (see definition of our operational taxonomic units below) and beta diversity at four spatial scales (A_0, A_1, A_2, A_3). Starting with a quadrat of side $L_0 = 8$ m (and area $A_0 = 64$ m^2), containing S_0 taxa, we divided the sampling area into four smaller quadrats of side $L_1 = L_0/2 = 4$ m, area $A_1 = A_0/4 = 16$ m^2, and containing an average of S_1 taxa. By iterating the subdivision, we completed a series of increasingly smaller quadrats of side $L_2 = 2$ m and $L_3 = 1$ m, and area $A_2 = 4$ m^2 and $A_3 = 1$ m^2, containing S_2 and S_3 taxa, respectively. We used a checkerboard sampling design, including 32 of the 64 possible quadrats of size A_3, to optimize available resources without compromising the analytical power (Fig. 1). Using such design, we had at least two replicates for all samples at all scales, and this assured us against possible technical failures or sample losses. In fact, two of our samples yielded no DNA, but the robustness of the design allowed us to perform the comparisons without any loss of analytical power.

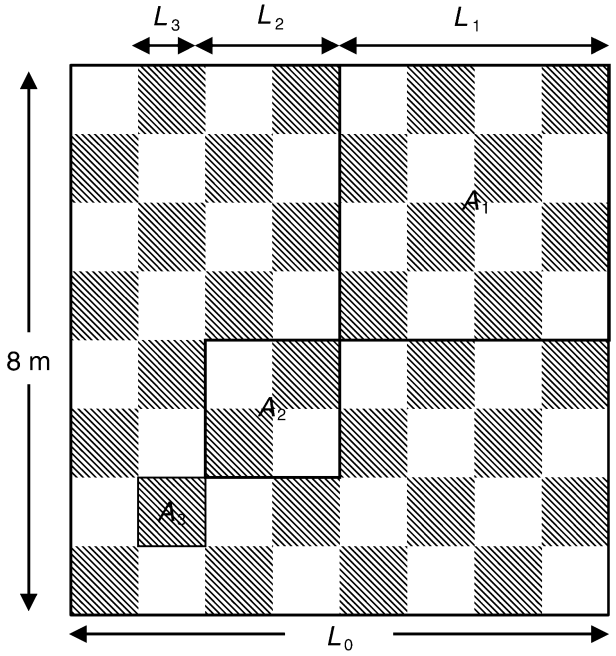

Figure 1 Fully nested system of quadrats designed to analyse the scaling of species diversity. An 8×8 m quadrat of area $A_0 = 64$ m^2 is divided into four quadrats of area $A_1 = 16$ m^2, 16 quadrats of area $A_2 = 4$ m^2, and 64 quadrats of area $A_3 = 1$ m^2. For clarity, only one quadrat of each size is marked. A soil sample was taken inside 32 of the smallest quadrats, following a checkerboard pattern. Operational taxonomic unit (OTU) diversity was measured at the four scales: S_0 is the total diversity found in the large quadrat; S_1, average cumulative diversity in the four quadrats of area A_1 (each including eight soil samples); S_2 is the average cumulative diversity in the 16 quadrats of area A_2 (each including two soil samples); and S_3 is the average diversity in the 32 sampling units.

This sampling scheme was deployed at two locations of the Chamela-Cuixmala Biosphere Reserve, on the western coast of Mexico (19°30′ N, 105°05′ W). One location was a flat hilltop,

and the second was a south-facing mid-slope (27°) of a small watershed that has been extensively studied for a long-term project on ecosystem function. Distance between the two locations was 300 m. Mean annual temperature is 24.9 °C and the mean annual precipitation is 763 mm, with the rainfall concentrated in a clearly marked wet season that lasts from June to October, showing a peak in September (García-Oliva *et al.*, 1991). The dominant vegetation is tropical deciduous forest, where most tree species are leafless during the dry season. Soils are sandy clay loams (Orthents in the United States Department of Agriculture [USDA] classification), poorly developed, with an organic matter content of < 5%, mainly concentrated in the top 5 cm, and with a pH of 6.9 (García-Oliva *et al.*, 2003).

On June 25, 2002, following the checkerboard sampling design (Fig. 1), we collected 5-cm^3 core soil samples from 32 of the 64 quadrats of size A_3 in each location, sieved them to remove gravel and other large (> 2 mm) material, and extracted genomic DNA from a 1-g aliquot of each sample. We assessed the diversity of prokaryotes based on restriction fragment length polymorphisms (RFLP) of *16S rRNA* genes that were used to define operational taxonomic units (OTUs). Genomic DNA extraction was performed on the same day of sampling from an aliquot of 1 g of sieved soil using the Ultra Clean Soil DNA kit (Mo Bio Laboratory, Inc.) and the products were stored at −20 °C. The *16S rRNA* genes in each sample were PCR(polymerase chain reaction)-amplified using fluorescently labelled domain-specific primers (forward 515 VIC 5′GCGGATCCTCTAGACTGCAGTGCCAG-CAG CCGCG GTAA-3′; reverse 1492 6FAM 5′-GGCTCGAGCG-GCCGCCCGGGTTACCTTG TTACGA CTT-3′, Applied Biosystems; Angert *et al.*, 1998). These are universal primers that target prokaryotic genes, so our results can be generalized to all groups of both Archaea and Eubacteria.

Three independent PCRs were performed for each sample, with each PCR containing 1X PCR buffer, 1.65 mM $MgCl_2$, 0.2 mM dNTP mixture, 0.6 μM of each primer, 1 unit *Taq* polymerase (ABI) and 5% BSA. All reactions were carried out in an MJ research thermocycler with the following program: 94 °C × 4 min; 35 cycles at 92 °C × 1.5 min, 50 °C 1.5 min, 72 °C × 2 min; and 72 °C × 10 min. To minimize PCR biases because of preferential amplification and reannealing, we standardized and set the optimum PCR conditions for our environmental samples as suggested by Osborn *et al.* (2000). We used the same DNA concentration and chose the number of cycles and the annealing temperature in order to obtain the best product, without compromising PCR quality. We also performed tests with different *Taq* polymerases until finding the most appropriate for our case. Tillmann and Friedrich (2003) found that there are no significant differences in terminal restriction fragment-length polymorphism (TRFLP) obtained between 28 and 45 PCR cycles and that temperature annealing should be set for the particular primer.

PCR products were combined and purified from a 2% agarose gel (Gel extraction kit Qiagen, Inc.). The amplicons were restricted using *Alu*I enzyme (Promega) in a 20 μL reaction during 3 h. Each reaction contained 10 units of *Alu*I enzyme and 50 ng of the PCR product, digestions were run in an MJ research thermocycler with the following program: 37 °C × 3 h and 65 °C × 30 min. Size and abundance of fluorescently labelled terminal restriction fragments (t-RFs) were determined using an ABI 3100 PRISM DNA analyser.

Each t-RF was considered an OTU and only those with heights of ≥ 50 fluorescent units (FU) were used for the analysis. Thresholds are chosen by assessing the noise in a region known to have no fragments, based on the particular background noise produced for each machine and on the appearance of peaks in samples run only with a control. Studies have shown that by cutting peaks at 100 FU or greater, there is an increase in the number of errors found (Blackwood *et al.*, 2003).

Characterization of microbial communities has been hindered in the past by traditional culture methods, because only a very small fraction of microorganisms found in environmental samples could be recovered. Recently, several molecular techniques have been developed to study phylogenetic relationships and diversity in microorganisms (Liu *et al.*, 1997; Tiedje *et al.*, 1999; Ranjard *et al.*, 2000; Norris *et al.*, 2002; Hill *et al.*, 2003). Among these, TRFLPs overcome most of the problems plaguing other fingerprinting approaches in terms of low resolution power, lack of replicability, differential electrophoretic mobility, and lack of capacity to quantify diversity. In particular, TRFLPs are very useful in comparing different communities because of their high level of sensitivity and replicability (Blackwood *et al.*, 2003).

For each location, we constructed presence–absence matrices describing the distribution of OTUs among 30 quadrats of size A_3 in the hilltop and 32 quadrats in the slope (we were unable to extract usable DNA from two of the hilltop samples). The purpose of the sampling procedure was not to measure the total OTU diversity of sites, a goal that is not feasible for prokaryotes with existing methods. Instead, the objective was to assess the spatial patterns of diversity by conducting a standardized sampling procedure that allowed us to carry out valid comparisons among quadrats. Thus, we assessed the adequacy of the sampling by its statistical representativeness (Gilbert, 1987) and not by a criterion of completeness, as in inventory-orientated studies (Gotelli & Colwell, 2001).

In environmental studies, a parameter is considered adequately sampled if the probability of a 20% variation around the mean value is < 0.1 (Gilbert, 1987). The probability can be estimated with the formula $Z_{1-\alpha/2} = \sqrt{nd_r}/\eta$, where α is the probability, $Z_{1-\alpha/2}$ is the value for the standardized normal distribution, n is the number of samples, d_r is the chosen acceptable relative error ($d_r = |\bar{x} - \mu|/\mu$; where $\bar{x}$ is the measured average and μ is the true, unknown population mean), and $\eta = \sigma/\mu$ (where σ is the true population standard deviation). Using this formula, we assessed the adequacy of our measurement of diversity at scale A_3, estimating σ with the observed standard deviation (s) and μ with the observed sampling mean ($\bar{x}$).

Rarefaction curves were built for the two sites by plotting the cumulative number of OTUs as a function of increasing numbers of samples. We used EstimateS version 7.0 (Colwell, 2004) to calculate the points of our rarefaction curves, using the procedures of Colwell *et al.* (2004) that allow the exact calculation of expected diversity values and associated variances for any number of samples (see also Ugland *et al.*, 2003).

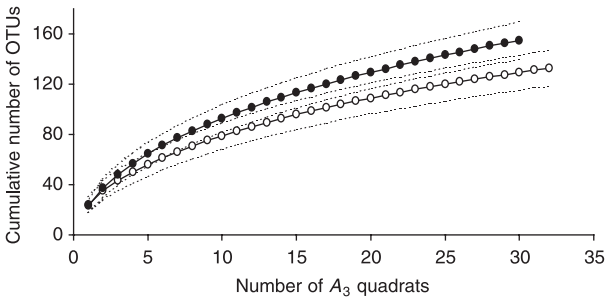

Figure 2 Rarefaction curves of OTU diversity for 30 soil samples in a hilltop (filled markers) and 32 samples in a slope (empty markers) in a tropical dry forest of western Mexico. Broken lines show the 95% confidence intervals for the means. The curves were built using the exact solution of Colwell *et al.* (in press) as implemented in EstimateS version 7.0.

We assessed β diversity at three scales using Whittaker's (1972) formulation $\beta_t = S_{t-1}/S_t$, where S_i is the average species diversity in quadrats of area A_i (Arita & Rodríguez, 2002). To determine the shape of the OTU–area relationship [equivalent to the species-area relationship (SAR)], we performed non-linear regressions of average OTU diversity against area for the two sampling sites. We examined the structure in the assemblages by measuring their degree of nestedness. In a perfectly nested assemblage, OTUs found in poor sites occur also in more diverse sites. Nestedness is a correlate of order or structure within communities, and can be measured with a temperature value (Atmar & Patterson, 1993). Low temperatures are characteristic of highly nested assemblages showing low degrees of disorder. The significance of the nestedness measure was assessed by assembling 1000 random sets of species using the temperature calculator of Atmar and Patterson (1995).

RESULTS AND DISCUSSION

OTU diversity

We documented the presence of 198 OTUs in the two sites. Of these, 155 occurred in the 30 samples from the hilltop and 133 in the 32 samples from the slope, with 56 (36.1%) and 34 (25.6%) taxa exclusively found in the hilltop and the slope, respectively. Thus, overall, only 108 of the 198 identified OTUs (54.5%) occurred in both sites. The hilltop was richer in OTUs than the slope, even after taking into account the differing sample sizes, as shown by rarefaction curves (Fig. 2).

Full inventories of prokaryotic taxa are not feasible with currently available techniques. To analyse patterns of diversity for this group, as for other highly diverse organisms, such as beetles, tropical butterflies or aquatic invertebrates, researchers rely on sampling to generate diversity estimates at different spatial or temporal scales (Gotelli & Colwell, 2001). Those estimates are comparable only if standardized field techniques are employed and if provisions are taken to consider the effect of differing sampling effort. The purpose of our study was not to measure the total prokaryotic diversity of sites, but to analyse spatial patterns in the distribution of diversity by comparing quadrats in which standard sampling and analytical procedures were performed. Because of the nature of our molecular techniques, we concentrated on the numerically dominant organisms, those with higher probabilities of being detected by our DNA analysis. Our molecular threshold (50 FU) established the 'veil line' (Preston, 1962a,b) that separated the detectable from the non-detectable taxa, in the same manner that sampling effort marks the veil line in diversity studies for other groups, such as moths and beetles. In those cases, valid comparisons can be made if standard field, laboratory, and statistical procedures are followed for all samples.

In assessing the representativeness of the sampling procedure, we found that the probabilities of sustaining a relative error of 20% or larger ($d_r \geq 0.2$) in measuring S_3 with $n = 30$ samples for the hilltop and $n = 32$ samples for the slope were $P = 0.064$ and $P = 0.0002$, respectively (in both cases, $P < 0.1$). Thus, the amount of variance (and thus, of potential bias) of our measures of S_3 OTU diversity at the two sites is low enough to make valid comparisons. Because of the fully nested design, estimations of S_0, S_1, and S_2 diversities, which are based on combinations of S_3 diversities put on a spatially explicit design, are also adequately sampled.

Occupancy

Within sites, the frequency distribution of occupancy of OTUs (occupancy defined as the number of quadrats in which a given OTU is present) followed a unimodal, right-skewed ('hollow') curve, which is the most common shape for a variety of organisms, from foraminifers to trees and vertebrates (McGeoch & Gaston, 2002). The curve is also very similar to that of the frequency distribution of range size for vertebrates in continental masses (Brown *et al.*, 1996; Gaston, 2003). However, the frequency distribution for occupancy differed from a log-normal distribution (test for normality using log-transformed data, $P < 0.001$), showing an overrepresentation of OTUs that occurred in very few quadrats. Sixty-eight (44%) of the 155 OTUs recorded in the hilltop and 56 (42%) of the 133 OTUs in the slope were detected in only one *A*3 sample. In contrast, only two OTUs in the hilltop and seven in the slope were detected in more than 25 samples in each location (Fig. 3a). There was a significant correlation between the occupancy in the two sites, that is, OTUs that were widespread in the hilltop were also widespread in the slope ($r = 0.875$, calculated as $n = 198$ occupancy pairs, $P < 0.001$).

In any study of species distribution, there is the potential problem of bogus patterns emerging from incomplete sampling. It is possible that the occurrence of some OTUs in some quadrats might have gone undetected because of our chosen molecular threshold. However, the effect of this potential problem, which is common to all studies based on sampling, is likely to be of minor importance. If we could lower the threshold to an imaginary level that allowed us to have a complete inventory of OTUs, it is likely that some of the OTUs would be detected in more quadrats than presently reported (that is, some OTUs would have a larger occupancy). However, by lowering the threshold, we would also

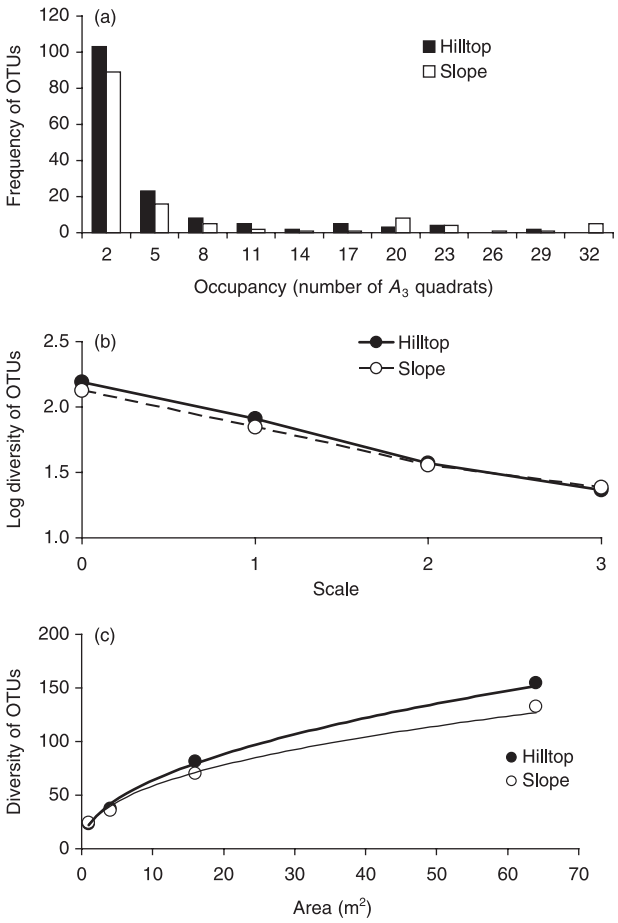

Figure 3 Diversity patterns of soil prokaryotes in two locations of a tropical dry forest in western Mexico. Black marks indicate hilltop samples and white mid-slope samples. (a) Frequency distribution of occupancy (number of occupied sampling quadrats) for prokaryotic OTUs in the two 64-m^2 squares. (b) OTU diversity-scale plots for the two 64-m^2 squares, showing OTU diversity as a function of scale as explained in Figure 1. (c) Species-area curve; data for OTUs from the two 64-m^2 squares were pooled to calculate the regression; the rightmost point is the total cumulative diversity in the two squares.

be able to detect many more of the rarest OTUs, those occurring at extremely low densities and, most likely, in fewer quadrats. We contend that by lowering the threshold, or by performing a more intense sampling, we would simply move Preston's (1962a,b) veil line, but that the shape of the histograms shown in Fig. 3(a) would not change significantly.

Thus, by documenting the presence of OTUs with extremely restricted distribution and by demonstrating that a large percentage of OTUs are found in only one of two locations, we rejected prediction 3 of the syndrome of species with no biogeography.

Scaling and OTU–area relationship

Assemblages of prokaryotic taxa followed similar scaling trends in both locations, as shown by the OTU scale plots (Fig. 3b). In these plots, log species diversity or log OTU diversity are functions of spatial scale, in this case, scales 0, 1, 2 and 3 corresponding to areas $A_0 = 64$ m^2, $A_1 = 16$ m^2, $A_2 = 4$ m^2, and $A_3 = 1$ m^2, respectively. The slope of the regression line is equal to $-\log \beta$, where β is Whittaker's (1972) beta diversity (Arita & Rodríguez, 2002). When β is small, there is very little species turnover and samples contain about the same OTUs regardless of their size; whereas large β values mean a high turnover rate that implies marked differences in the composition of OTUs among samples. Regression analysis for both of our locations fit a straight line with slopes –0.281 ($\beta = 1.91$, $r^2 = 0.99$) for the hilltop and –0.251 ($\beta = 1.78$, $r^2 = 0.99$) for the slope. Note that only the average values for each scale were used to perform the regressions to avoid pseudoreplication and reduce the effect of spatial autocorrelation. These results indicate that an increment in quadrat area by a factor of four represents an increase in the diversity of OTUs by a factor of $\beta = 1.91$ in the hilltop and by $\beta = 1.78$ in the slope. Arita and Rodríguez (2002) used the same sampling procedure as ours but with A_0 quadrats of 180,000 km^2 and found that β diversity for non-volant Mexican mammals ranged from 1.19 in a homogeneous (the Yucatán Peninsula) to 2.52 in a highly heterogeneous area (central México). Figures for β diversity of prokaryotes in our 64-m^2 locations correspond to high-end values for mammals in quadrats that are approximately 2.8×10^9 times larger in area. Hence, prediction 4 of the syndrome of ubiquity, a low rate of species turnover, can be unequivocally rejected for prokaryotes in our locations.

Linear OTU scale plots imply OTU–area relationships of the form $S = cA^z$, where c and z are constants (Rosenzweig, 1995; Harte *et al.*, 1999; Arita & Rodríguez, 2002). Performing nonlinear regressions, we estimated $z = 0.47$ for the hilltop and $z = 0.42$ for the slope ($r^2 = 0.98$ for both cases, Fig. 3c). These z values are higher than reported values for vertebrates in nested sampling units in continents (Rosenzweig, 1995), and are much higher than for invertebrates in the sea ($z = 0.161$, Azovski, 2002) and for ciliated protists ($z = 0.043$, Finlay, 2002). Prokaryotes in our locations clearly do not show a flat species–area curve; therefore prediction 5 of the syndrome of ubiquity can be safely rejected.

Our sampling design (quadrats arrayed in a contiguous grid) yielded type II OTU–area curves in the classification of Scheiner for species–area relationships (2003, 2004). A related sampling procedure uses strictly nested quadrats (type I in Scheiner, 2003), in which only one quadrat is sampled at each scale and smaller quadrats are nested within larger ones. The theoretical implications of such design has been explored by Harte *et al.* (1999), and similar sampling designs has been used for the analysis of the continental distribution of species diversity (e.g. Lyons & Willig, 2002). The design suffers, in our view, from the lack of replicates and from the fact that smaller scales cover only limited parts of the whole region, going to the extreme, where the smallest scale is represented by a single point (at the centre or at one extreme of the region). The sampling design used herein, in contrast, systematically arrays quadrats of every scale covering the whole region, providing true replicates and a better depiction of the spatial variation of diversity (Arita & Rodríguez, 2002).

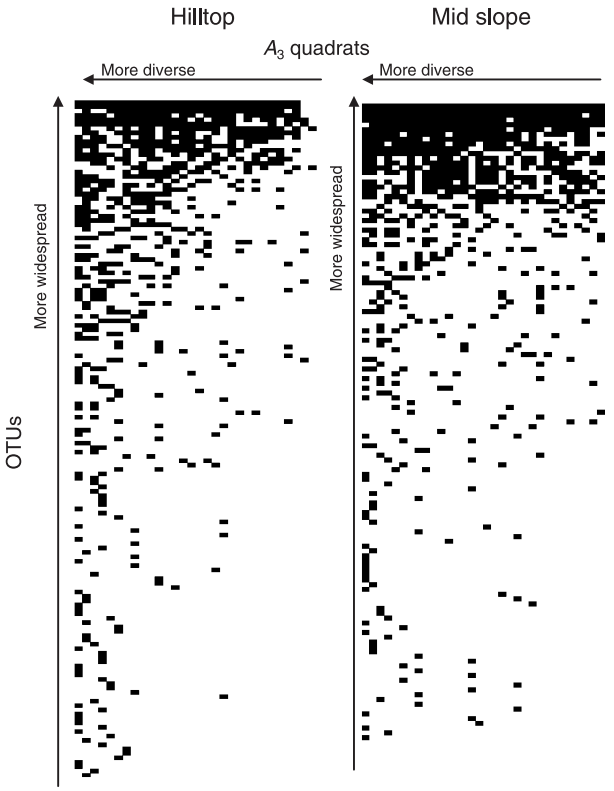

Figure 4 Highly nested pattern of distribution of soil prokaryotic diversity in two 64-m^2 locations in the tropical dry forest of western Mexico, hilltop (left), slope (right). OTUs ranked according to their occupancy (widespread taxa close to the top, restricted taxa close to the bottom). Samples are ranked by their OTU diversity (richer sites close to the right extreme, less diverse sites close to the left extreme). Each point represents the presence of a given OTU in a given sample. In a perfectly nested pattern, points would arrange in a triangular pattern in such a way that the lower left part of the figure would contain no points.

Nestedness

Both of our sampling sites showed a high degree of nestedness as measured using Atmar and Patterson's (1995) temperature calculator (Fig. 4). The hilltop location had a temperature value of $T = 12.55°$ [$P\,(T < 12.55) = 1.09 \times 10^{-78}$] and temperature at the mid-slope measured at $T = 25.05$ [$P\,(T < 25.05) = 7.56 \times 10^{-55}$]. P values are the probabilities of temperatures equal or lower than the one observed, based on the distribution of T values for randomly generated assemblages (Atmar & Patterson, 1995). In both locations, the nestedness values show that our prokaryotic assemblages are highly structured, clearly departing from values corresponding to random communities. In our locations, samples containing OTUs that occur in only one or very few samples are also the most diverse, thus generating the highly nested patterns. This result is consistent with the suggestion that microbial communities reflect adaptation to local environmental heterogeneity and are assemblages of generalist and specialist taxa (Balser *et al.*, 2002). Additionally, our results suggest that functions of microbial taxa are rarely interchangeable and are direct responses to environmental heterogeneity, as reported for macro-organisms. Moreover, these results demonstrate a non-random structure for prokaryotic assemblages, thus rejecting prediction 6 of the syndrome of species with no biogeography.

CONCLUSIONS

Our analyses of the prokaryotic communities of two locations have allowed us to reject multiple criteria exhibited by organisms with no biogeography. Still, it could be argued that our finding of highly structured assemblages is merely a local pattern, not necessarily rejecting the ubiquity hypothesis in a biogeographical scale. That is, the possibility could remain that soil bacteria had a global dispersal but occurred locally only in suitable microenvironments, thus showing structured local communities but no biogeography. This possibility is unlikely in our sites, however, as 45.5% of taxa were exclusive to one site or the other, suggesting a non-random arrangement at the between-sites scale. Because we only sampled two sites, a direct test of prediction 1 of the syndrome of ubiquity is not reasonable. However, our results clearly contrast with those used to document the ubiquity of microbial eukaryote species that have relied on similarly small sample sizes (Finlay & Clark, 1999; Finlay, 2002; Fenchel, 2003).

We contend that it is inappropriate to think of bacteria as organisms that have an exceptional ecology or biogeography. Prokaryotic species assemblages, both in laboratory and natural conditions, have proved to be adequate model systems for testing ecological questions (Bohannan *et al.*, 2002; Jessup *et al.*, 2004; Srivastava *et al.*, 2004). We argue that the same can be stated for biogeographical matters. Our data show that rules that determine the distribution of vertebrates at a continental scale can be applied to prokaryotes in a 64-m^2 quadrat. Thus, we contend that a biogeography for prokaryotes is possible at such small scales, and that we can talk about OTU ranges of only a few metres in size. As it is the case with vertebrates at the continental scale, the ecological and evolutionary processes that determine the patterns documented here are not yet clearly established. What is clear is that soil prokaryotes do not belong to the set of organisms with no biogeography, as suggested by previous studies (Finlay, 2002).

ACKNOWLEDGEMENTS

We thank Luis E. Eguiarte for ideas and review; Aldo Valera, Laura Espinosa, Maria Schneider, Stephen Bent and Mayee Wong, for technical assistance; and Salvador Araiza, Abel Verduzco, Manuel Montaño and Carlos Anaya for their help during the fieldwork. This project was supported by NASA NCC2-1051 and the Consejo Nacional de Ciencia y Tecnología, México CONACyT-G27674-N.

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BIOSKETCHES

Ana M. Noguez is conducting a project on biogeochemical processes, soil bacterial diversity, and community structure of the tropical deciduous forest of the west coast of Mexico.

Héctor Arita is interested in the scaling of biological diversity and its links with the structure and dynamics of geographical ranges.

Ana E. Escalante focuses her work on community ecology and population genetics of natural bacterial assemblages.

Larry Forney is an expert on the ecology of prokaryotic organisms, with emphasis on the adaptive evolution in various species to assess the role of competition in determining the structure and diversity of microbial communities.

Felipe García-Oliva conducts research on biogeochemical processes involving soils, focusing mainly on carbon and nitrogen cycles in tropical ecosystems.

Valeria Souza is interested in the ecology and evolution of prokaryotes, studying both laboratory and natural model systems to answer questions in evolutionary, population, and community dynamics.