

ORIGINAL ARTICLE

Biogeography of soil archaea and bacteria along a steep precipitation gradient

Roey Angel^{1,3}, M Ines M Soares¹, Eugene D Ungar² and Osnat Gillor¹

¹Zuckerberg Institute for Water Research, J. Blaustein Institutes for Desert Research, Ben-Gurion University, Sede Boqer Campus, Israel and ²Department of Agronomy and Natural Resources, Institute of Plant Sciences, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel

For centuries, biodiversity has spellbound biologists focusing mainly on macroorganism's diversity and almost neglecting the geographic mediated dynamics of microbial communities. We surveyed the diversity of soil bacteria and archaea along a steep precipitation gradient ranging from the Negev Desert in the south of Israel (<100 mm annual rain) to the Mediterranean forests in the north (>900 mm annual rain). Soil samples were retrieved from triplicate plots at five long-term ecological research stations, collected from two types of patches: plant interspaces and underneath the predominant perennial at each site. The molecular fingerprint of each soil sample was taken using terminal restriction length polymorphism of the 16S rRNA gene to evaluate the bacterial and archaeal community composition and diversity within and across sites. The difference in community compositions was not statistically significant within sites ($P=0.33$ and 0.77 for bacteria and archaea, respectively), but it differed profoundly by ecosystem type. These differences could largely be explained by the precipitation gradient combined with the vegetation cover: the archaeal and bacterial operational taxonomic units were unique to each climatic region, that is, arid, semiarid and Mediterranean ($P=0.0001$, for both domains), as well as patch type ($P=0.009$ and 0.02 for bacteria and archaea, respectively). Our results suggest that unlike macroorganisms that are more diverse in the Mediterranean ecosystems compared with the desert sites, archaeal and bacterial diversities are not constrained by precipitation. However, the community composition is unique to the climate and vegetation cover that delineates each ecosystem.

The ISME Journal (2010) 4, 553–563; doi:10.1038/ismej.2009.136; published online 24 December 2009

Subject Category: Microbial ecology and functional diversity of natural habitats

Keywords: arid; LTER; Mediterranean; precipitation; TRFLP

Introduction

For centuries, ecologists have observed the profound differences between dry and temperate regions with respect to vegetation cover and animal abundance. Studies on how communities respond to precipitation gradients led to the search for diversity patterns across a wide variety of taxonomic groups, including annuals, trees, mammals, birds, reptiles and insects (Hawkins *et al.*, 2003). Emerging patterns showed a general increase in the diversity of plants and animals with an increase in available water. The bulk of these studies established an ecological paradigm claiming that an increase in water availability is followed by elevated diversity and abundance of biological communities.

Although patterns of variation in species richness along precipitation gradients have been studied extensively, much less is known about the manner in which, and the extent to which, precipitation interacts with landscape diversity in determining patterns of species richness (van Rensburg *et al.*, 2002; Gardezi and Gonzalez, 2008). On a local scale, studies of community and landscape ecology of both macro- and microorganisms have indicated a relationship between species richness, internal structure of the habitat and landscape heterogeneity as the main controllers of biodiversity. In water-limited environments, one of the most widely accepted theories is the 'fertility' or 'resource island' hypothesis, which states that shrubs create heterogeneity in soils by localizing soil fertility under their canopies (Schlesinger *et al.*, 1996). Indeed, heterotrophic bacteria (Herman *et al.*, 1995) and protozoa (Robinson *et al.*, 2002) have been found to be more abundant under shrubs than in their interspaces. The links between microbial biogeography, local diversity of microorganisms and the factors that shape them represent largely unexplored territory. Here, we integrated a study of local-scale microbial diversity in bare soil

Correspondence: O Gillor, Zuckerberg Institute for Water Research, Ben Gurion University, J. Blaustein Institutes for Desert Research, Sede Boqer Campus, 84990, Israel.
E-mail: gilloro@bgu.ac.il

³Current address: Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse 8, Marburg, D-35043, Germany.
Received 12 August 2009; revised 9 November 2009; accepted 19 November 2009; published online 24 December 2009

and under a plant canopy with that of distinct climatic regions.

In the last three decades, microbial ecologists have experienced a quantum leap in the study of microbial ecosystems independent of their ability to culture the resident species. Microbiologists have gone to remote corners of the earth to analyze the microbial inhabitants of every environment. Of the microbial groups that are abundant in the soil, bacteria have been the most extensively studied. Nevertheless, our understanding of the spatial distribution patterns of bacterial diversity is limited, mainly because most studies are limited to local scales (Navarro-Gonzalez *et al.*, 2003; Zhou *et al.*, 2004). Recent large-scale surveys have revealed that different ecosystems support unique microbial populations (Zhou *et al.*, 2002; Fierer and Jackson, 2006; Green and Bohannan, 2006; Vishniac, 2006; Adler and Levine, 2007), giving rise to the notion that microbial populations can exhibit geographic distribution. These emerging microbial distribution patterns suggest that the ecological rules followed by macroorganisms do not necessarily apply to microorganisms (Fierer and Jackson, 2006; Green and Bohannan, 2006; Bryant *et al.*, 2008).

The majority of microbial biogeography studies have focused on the bacterial domain (Fierer, 2008). However, members of the archaea domain, once thought to be present only in extreme environments, have been found to be significant or even major components in mundane habitats such as ocean waters, freshwater sediments and soils (Kent and Triplett, 2002; Chaban *et al.*, 2006). Archaeal diversity has been relatively well documented in rice paddy soils (Grosskopf *et al.*, 1998) and peat bogs (Hoj *et al.*, 2008), and has also been reported in temperate, tropical and agricultural soils (Kent and Triplett, 2002). Those studies were confined mainly to local scales, but a handful of researchers have examined biodiversity patterns of archaea along spatial or temporal gradients (Ochsenreiter *et al.*, 2003; Nemergut *et al.*, 2005; Walsh *et al.*, 2005; Oline *et al.*, 2006; Hansel *et al.*, 2008). In this study, we compare and contrast the diversity patterns of soil archaea with those of bacteria. We explored the diversity of these domains on local and regional scales, addressing their richness and community composition. A prudent hypothesis would be that each domain is characterized by distinct patterns of diversity, with archaea having a unique distribution pattern, as they occupy specific soil niches, whereas bacteria are more widely distributed and thus are subjected to biogeographical patterns. Alternatively, niche occupation and abundance of archaea in the soil might not be a determinant, in which case the forces structuring biodiversity across the precipitation gradient would be the same for both domains, resulting in similar phylogenetic biogeographical patterns. To test these hypotheses, we adopted an approach that examines local and regional relationships with respect to microbial biodiversity.

As mentioned above, traditional precipitation diversity studies relied heavily on the sampling and identification of large number of plant and animal species, and were focused on how patterns of richness, abundance and phenotype change with water availability. As culturing techniques are currently limited for most bacteria and archaea, microbial ecologists use molecular techniques that are dependent on the universal marker gene encoding for the 16S rRNA to document microbial presence at every level, from division to strain. However, comprehensive sequencing of the 16S rRNA gene in soil samples is both labor intensive and expensive. Consequently, true replication and statistical characterization of microbial diversity in an environment, performed as demanded by plant ecologists, for instance, are rarely achieved. In this study, we addressed questions of local- and regional-scale distribution and diversity patterns by using a multiscale nested sampling approach. Five long-term ecological research (LTER) sites ranging from the Negev Desert in the south of Israel (with less than 100 mm annual rain) to the Mediterranean forests in the north (with over 900 mm annual rain) were examined and the diversity patterns of their soil bacteria and archaea were elucidated. Our nested sampling scheme consisted of the following: (i) triplicates of approximately 1000-m² plots in each site; (ii) two patches in each plot, one under the canopy of the predominant perennial (woody patch) and one in the perennial interspace (open patch) and (iii) a composite of eight soil samples taken from each patch type, at each plot. This scheme enabled us to compare patches within each plot, plots within each site and the different sites, answering, at least in part, the requirements for a comprehensive ecological survey.

Materials and methods

Site description

Sampling was performed in May and June of 2007 at five LTER stations in Israel (<http://lter.bgu.ac.il/>) located in areas with mean annual precipitation ranging from 100 to 900 mm per year (Supplementary Figure 1). At each station, sampling was performed in triplicate plots of 40 × 25 m, all fenced and thus protected from grazing livestock and undisturbed by human activity.

At each plot, eight randomly selected subsamples were taken from the bare soil in the interspaces between the dominant perennial plants (open patch) and under the perennial canopy (woody patch). The predominant perennial was singled out at each station (Supplementary Figure 1) and we sampled under its canopy alone. The eight subsamples of each patch type at each plot were composited to represent an average for that site, resulting in a total of six composite soil samples per station (Supplementary Figure 1).

Soil collection and physicochemical characterization

After crust and litter removal, the top 5 cm of the soil was collected into sterile Whirl-Pak sample bags (Nasco, Fort Atkinson, WI, USA) and placed in a cooler. The samples were transported to the laboratory and homogenized within 24 h of sampling. A 50 g subsample of each soil sample was stored at -80°C for molecular analysis, whereas the rest was used for physicochemical analysis.

Soil chemical analysis was performed according to standard methods for soil analyses (SSSA, 1996): soil water content by gravimetric method; percentage organic matter by dichromate oxidation method; pH and electrical conductivity in saturated soil extract (SSE); sodium, calcium and magnesium in SSE by inductively coupled plasma spectroscopy; sodium adsorption ratio by calculation from Na^+ and $\text{Ca}^{2+} + \text{Mg}^{2+}$ concentrations; total phosphate by the 'Olsen method' (sodium bicarbonate extract); K^+ in SSE by flame spectrophotometer; nitrogen as nitrate in aqueous extract; nitrogen as ammonium in KCl solution extract (including adsorbed nitrogen); percentage of calcium carbonate by hydrochloric acid digestion.

As different units were used to measure the various physicochemical parameters, they all had to be brought into an equal range before any analysis. In addition, the distribution in each factor had to approach normality to better meet the assumptions of the statistical models. Testing different transformation techniques showed standardization (Z-score) to yield the best results in terms of eliminating scale differences and achieving normality under Kolmogorov–Smirnov test.

DNA extraction, PCR amplification and TRFLP analysis

Bacterial and archaeal community fingerprints were obtained using terminal restriction fragment length polymorphism (TRFLP) (Liu *et al.*, 1997). DNA was extracted from triplicate soil subsamples, each consisting of 0.25 g (wet weight), using the Power-Soil DNA Isolation Kit (MoBio, West Carlsbad, CA, USA).

Polymerase chain reaction (PCR) amplification was performed with the primer pairs 341F (Ishii and Fukui, 2001): 5'-CCTACGGGAGGCAGCAI-3' and 908R (Lane *et al.*, 1985): 5'-CCGTCAATTCMTTGA GTTI-3' targeting bacteria, and 109F (Grosskopf *et al.*, 1998): 5'-ACKGCTCAGTAACACGI-3' and 934R (Stahl and Amann, 1991): 5'-GTGCTCCCCGC CAATTCCL-3' targeting archaea. All primers were modified by the addition of inosine at the 3' end in an attempt to broaden their target scope (Ben-Dov *et al.*, 2006). In both primer pairs, the forward primer was labeled with the fluorescent dye 6-FAM (6-carboxyfluorescein) (Metabion, Martinsried, Germany) at the 5' end.

PCRs were conducted in triplicates of 50 μl to minimize reaction bias. In addition, the following

steps were taken to minimize some of the artifact effects of PCR, such as the appearance of chimeras and pseudo-terminal restriction fragments (TRFs) (Egert and Friedrich, 2003): (i) the number of PCR cycles was reduced to 24 and 25 for bacteria and archaea, respectively, and elongation time was extended to 3 min; (ii) before cleanup and digestion with restriction enzymes, amplified DNA samples were treated with mung bean exonuclease (TaKara, Shiga, Japan) according to the manufacturer's instructions. Each PCR contained 2.5 μl 10 \times buffer (Bioneer, Daejeon, South Korea), 0.25 mM of each dNTP (Larova, Teltow, Germany), 2.5 mM MgCl_2 , 0.5 mM of each primer (Metabion), 1 $\mu\text{g}\mu\text{l}^{-1}$ BSA (New England Biolabs (NEB), Ipswich, MA, USA), 2.5 units *Taq* DNA polymerase (HyLabs, Rehovot, Israel) and 1 μl DNA template. The PCRs were carried out as follows: after an initial 5 min denaturation step at 95°C , 24 or 25 cycles (for bacteria and archaea, respectively) were run at 94°C for 45 s, 45°C for 1 min and 72°C for 3 min, followed by a final elongation step at 72°C for 10 min. After amplification, the triplicate PCRs were pooled, treated with mung bean exonuclease and purified using a PCR purification kit (Bioneer). The purified PCR products were digested with the restriction enzymes *TaqI*, *HhaI* (TaKara) and *HpyCH4IV* (NEB) for samples amplified using the bacterial primers (341F/908R). The restriction enzymes *TaqI*, *MseI* and *MboI* (TaKara) were used for the amplicons generated with the archaeal primers (109F/934R). For each enzyme, digestions were performed in reactions of 20 μl containing 2 μl of digestion buffer (TaKara), 20 units of restriction enzyme and approximately 200 ng of the purified PCR product. Digestion was followed by precipitation using standard ethanol precipitation with Pellet Paint (Novagen, Darmstadt, Germany), and resuspension in double-distilled water. These samples were analyzed with an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA). The peaks in each profile were related to specific fragment lengths based on a size marker (70–500 MapMarker, BioVentures, Murfreesboro, TN, USA). Data were retrieved using Peak Scanner software v1.0 (Applied Biosystems). Each sample was loaded at least twice and the profiles were treated as replicates.

Data manipulation and statistical analysis

Raw TRFLP data cannot be used directly for analysis, and therefore the following standardization and normalization procedures were applied prior to all statistical analyses. The size in base pairs of each peak (TRF) was used to indicate an operational taxonomic unit (OTU), whereas the area under the peak was used to determine its relative abundance in the profile. The TRFLP patterns of the replicates (method replicates) of each sample were standardized as described elsewhere (Dunbar *et al.*,

2001). Profiles were then aligned and a consensus profile was computed for each sample from its replicates by eliminating nonreproducible peaks and averaging shared peaks. The procedure was then applied again to standardize the consensus profiles and they were aligned to generate a sample-by-species matrix, which was used in subsequent analyses. The above procedure was repeated for each restriction enzyme separately. For a better fit of the data set to the assumptions of the statistical models, two additional transformations were applied. (i) To deal with possible skewness of the data set, a $\text{Log}(x+1)$ transformation was applied; this greatly improved the overall performance of the samples in a Kolmogorov–Smirnov test for normality (data not shown). (ii) To deal with the problem of null values in the matrix, it was transformed to give Hellinger distances between the samples when Euclidean distances were computed (Legendre and Gallagher, 2001). The matrix was tested under a detrended correspondence analysis (DCA) model and the length of the first gradient was found to be less than 4 s.d., and hence linear models were constructed (data not shown). A two-way cluster analysis was simultaneously performed on the TRFs and the samples, using Euclidean distances and Ward's linkage. A Scree plot showing the distance between the clusters as a function of clustering order was used to determine the relevant number of clusters. To test for the differences between species distributions, the standardized TRFLP data of each sample were ordered from the most abundant to the least abundant TRF (rank abundance) and a Kolmogorov–Smirnov test was performed on every pair of samples to test whether they were drawn from the same distribution.

All standardization and normalization procedures were performed using MATLAB 7 (<http://www.mathworks.com>) and the codes are available at <http://www.staff.uni-marburg.de/~angel>. Cluster and distribution analyses were computed using MATLAB. Hypothesis testing was performed using block-design redundancy analysis (RDA) and tested using Monte Carlo permutation tests (ter Braak and Smilauer, 1998). Correlations to physicochemical characteristics were performed using RDA with forward selection procedure (ter Braak and Smilauer, 1998). All RDA models were computed using Canoco 4.53 (<http://www.canoco.com>).

Results

We surveyed the diversity of soil bacteria and archaea along a steep precipitation gradient ranging from an arid area with less than 100 mm annual rain to a meso-Mediterranean forest receiving over 900 mm precipitation (Table 1 and Supplementary Figure 1). Thirty soil samples (each a composite of eight samples) were retrieved from five LTER stations, collected from open and woody patches at

Table 1 Characteristics of the sites and values of major physicochemical parameters of the sampled soils.

Climate	Location (coordinates, elevation)	Average precipitation (mm per year)	WC ^a (%)	pH	Na ⁺ (mg kg ⁻¹)	Ca ²⁺ (mg kg ⁻¹)	Mg ²⁺ (mg kg ⁻¹)	NO ₃ -N (mg kg ⁻¹)	NH ₄ -N (mg kg ⁻¹)	P _{tot} (mg kg ⁻¹)	K ⁺ (mg kg ⁻¹)	Calcium carbonate (%)	OM ^b (%)	Predominant perennials ^c
Arid	Avdat (30°47'N/ 34°46'E, 600–700 m)	100	1.9/1.8	7.9/8.0	110.5/40.2	55.7/35.4	10.5/8.4	4.0/9.4	3.4/6.3	0.03/0.04	9.4/19.1	33.0/33.7	0.6/0.8	<u>Hamada scoparia</u> and <u>Zygophyllum</u> dumosum
Semiarid	Lehavim (31°20'N/ 34°45'E, 350–500 m)	300	2.6/3.8	7.2/7.0	18.8/19.3	129/263	19.5/44.5	2.8/3.5	49.0/62.5	0.07/0.09	7.7/14.1	17.0/16.3	2.0/3.4	<u>Sarcopoterium spinosum</u> and <u>Thymelaea hirsuta</u>
Dry Mediterranean	Adulam (31°40'N/ 34°50'E, 200–300 m)	400	9.6/14.7	7.3/7.2	33.5/39.0	310/1051	30.2/162.6	5.9/3.7	21.0/27.5	0.09/0.57	6.9/63.7	14.0/6.7	6.1/19.9	<u>Quercus calliprino</u> and <u>Pistacia palaestina</u>
Mediterranean	Ramat Hanadiv (32°30'N/ 34°55'E, 100–200 m)	600	12.1/23.6	7.0/7.1	13.7/45.8	143/325	29.5/70.0	6.7/6.5	40.8/51.0	0.09/0.02	9.9/34.5	1.7/1.7	4.9/10.5	<u>Phillyrea latifolia</u> and <u>Quercus calliprinos</u>
Meso- Mediterranean	Mt. Meron (32°00'N/ 35°20'E, 700–900 m)	900	6.0/20.4	7.0/6.9	7.4/15.3	136/309	52.1/120.0	8.2/2.7	54.6/98.6	0.09/0.13	12.8/37.2	1.7/2.3	7.3/10.9	<u>Quercus calliprino</u> and <u>Quercus boissier</u>

For each site, values are presented as means for open/woody soil patches.

^aWater content.

^bOrganic matter.

^cThe woody patch samples were taken from under the canopy of the underlined plant species.

each site. Using TRFLP analysis of the 16S rRNA-encoding gene, the molecular fingerprint of each soil sample was taken to evaluate the diversity and composition of the microbial community within and across sites. Here, we present the patterns obtained when the bacterial and archaeal small subunit rRNA gene amplicons were digested with a single enzyme (*TaqI* for bacteria and *MseI* for archaea). Analyses of the 16S rRNA gene fragments digested with the additional enzymes used in this study (see Materials and methods) are presented in Supplementary Figure 2.

Bacterial and archaeal community distribution

To observe the differences in the overall distribution patterns of both the bacterial and archaeal communities in each sample, the rank and relative abundance of the TRFs were calculated and plotted (Figure 1). A Kolmogorov–Smirnov test was performed on all possible sample pairs (separately for each domain) to test whether they are derived from the same distribution. The test showed that in 83.7% and 85.5% of the cases, for bacteria and archaea, respectively, any two random samples were drawn from the same distribution at a 0.05 confidence level. Moreover, for both domains, only two samples showed distinctly different distributions from the rest of the samples (Supplementary Table 1).

Bacterial and archaeal diversity and community composition in woody and open patches

We tested the effect of patch type (using one categorical dummy variable) on the microbial community composition (Supplementary Table 2). The analysis showed that both bacteria and archaea are distributed according to the patch type ($P=0.0091$ and 0.0202 , respectively). This indicates that the perennial plant influences the soil's microbial communities and that this factor is responsible for a sizable portion of the variability in the community structure (7.3% and 9.8% of the variance in the data for bacteria and archaea, respectively). The analysis was block designed so that permutations were only

allowed within each station (reflecting the six composite soil samples). Both bacterial and archaeal scores of the first canonical axis were plotted versus the patch variable, showing two distinct groups clustered according to patch type (Figure 2).

Bacterial and archaeal community composition within and between stations

We tested the effects of all samples taken from the triplicate plots within each station (15 categorical dummy variables, three in each of the five stations) on community composition. The analysis was block designed so that permutations were only allowed within each station (corresponding to six samples). Neither bacteria nor archaea showed any significant differences in their distribution between the replica plots within stations ($P=0.33$ and 0.77 , respectively). These results indicated that the soil samples taken at a single station from three different plots are indeed replicates.

Our next step was to test the effect of stations (five categorical dummy variables) on community composition. The analysis was performed with unrestricted permutations between the stations. The community compositions of both bacteria and archaea were significantly different between different stations ($P<0.0001$, $R^2=0.174$ and $P<0.0001$, $R^2=0.386$, respectively), suggesting that the community composition is biogeographically structured.

Bacterial and archaeal diversity across the precipitation gradient

Figure 3 reflects the results of two-way cluster analysis of the samples (rows) and TRFs (columns). The consensus profiles of both bacteria and archaea were used in this cluster analysis, corresponding to the TRFs generated by two of the restriction enzymes used in this study (see Materials and methods): *TaqI* for 16S rRNA fragments amplified with the bacterial primers (Figure 3a) and *MseI* for fragments amplified using the archaeal primers (Figure 3b). Each individual square in the central colored matrix represents the relative abundance

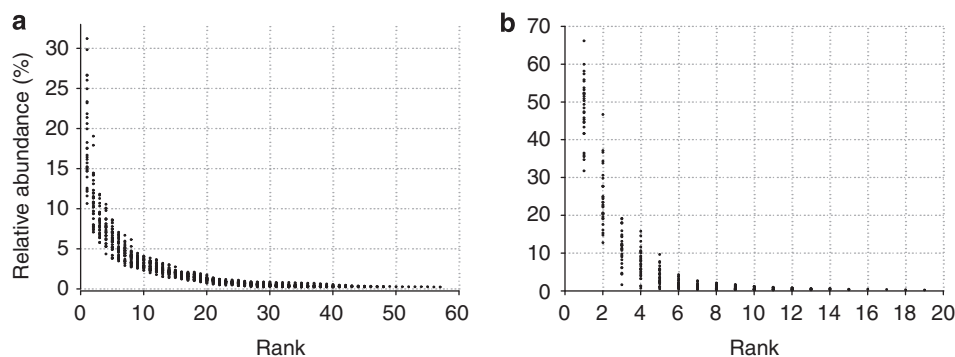


Figure 1 Rank-abundance plots of the TRFLP profiles of (a) bacteria and (b) archaea. The y axis shows the relative abundance of each TRF, whereas the x axis is the ordinal rank of the TRFs from most abundant (1) to least abundant (n).

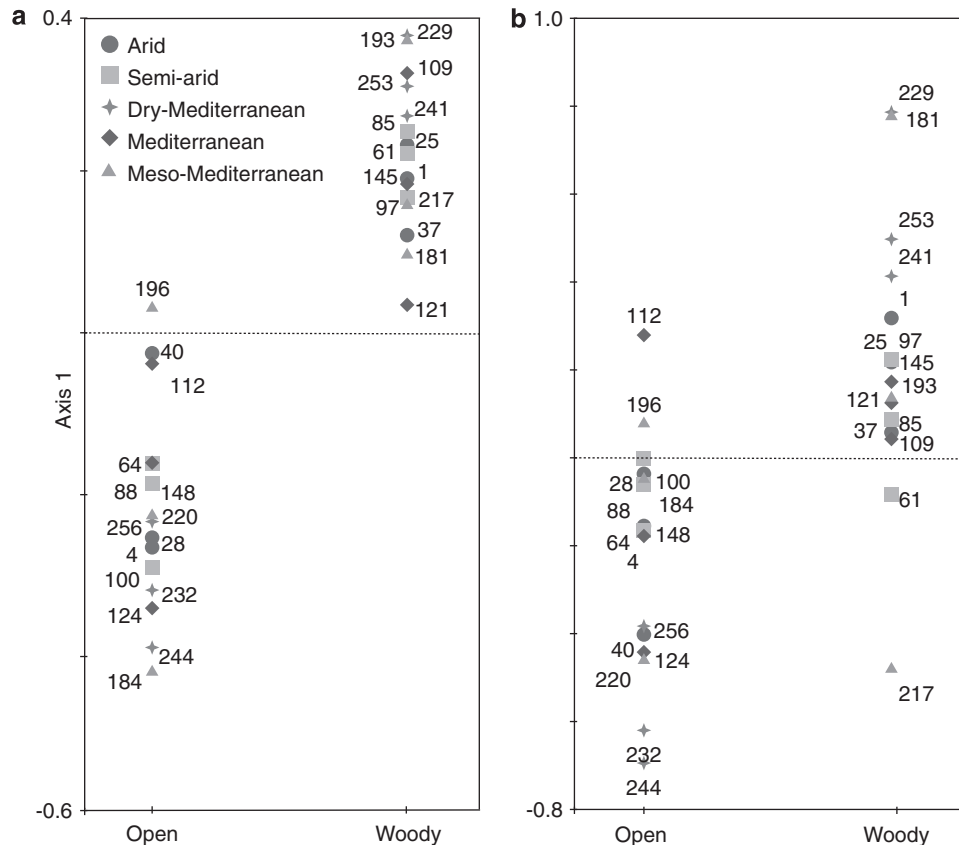


Figure 2 First noncanonical axis of the redundancy analysis (RDA) of (a) bacteria and (b) archaea TRFLP profiles versus patch type (open and woody) for each sample. The axes explain 7.3% and 9.8% of the variability in the data for bacteria and archaea, respectively, and the difference in the community between patches is significant at levels $P = 0.0091$ and 0.0202 for bacteria and archaea, respectively.

(indicated by color) of a single TRF. The top right Scree plot represents the distance between each of the two levels of clustering versus cluster number.

Analyses of both bacteria and archaea showed a clear clustering of the arid soil samples (marked dark brown) and, to a lesser extent, of the semiarid samples (marked light brown). Interestingly, the three Mediterranean stations (marked bronze green, dark green and light green) did not cluster: the bacterial heat map showed two major clusters at both ends, whereas the archaeal TRFs amplified from the Mediterranean soil samples clustered together.

Heat maps were constructed on the basis of the bacterial and archaeal 16S rRNA-encoding gene-amplified fragments digested with *HhaI*, *HpyCH4IV*, *TaqI* and *MboI* (Supplementary Figure 2). The bacterial TRFs (Supplementary Figures 2a and b) followed the pattern described above, unlike the archaeal TRFs (Supplementary Figures 2c and d) that clustered into dissimilar patterns.

Relationship between abiotic factors and bacterial and archaeal communities

Eleven physicochemical factors were measured in the soil samples used for the analyses of bacteria and archaea community composition (Table 1). The

standardized values of these parameters were correlated to the bacterial and archaeal TRF scores using a partial RDA model.

In a forward selection method, only water content, organic carbon and calcium carbonate correlated significantly with the community profiles of both domains ($P < 0.0001$). In addition, the bacterial distribution correlated to Mg^{2+} and nitrate ($P = 0.0051$ and 0.0152 , respectively). All the above parameters (except Mg^{2+} for the bacteria domain) were also found to be significant in a model that excluded the effect of the sites (i.e., allowing permutation testing only within sites). The analysis results indicated that the effect of these parameters is evident not only at a regional, but also at a local scale (data not shown). However, although showing significant correlations, all the above-mentioned parameters were also strongly auto-correlated (data not shown), making it difficult to determine which is the driving force of microbial soil diversity in this setting. Of these parameters, water content was found to have the strongest fit to the bacterial and archaeal community structures. Figure 4 shows how the communities of both domains are positioned along the water-content concentration axis. The bacteria and archaea communities amplified from arid environment soil samples clustered at the low end of the soil water content, whereas the

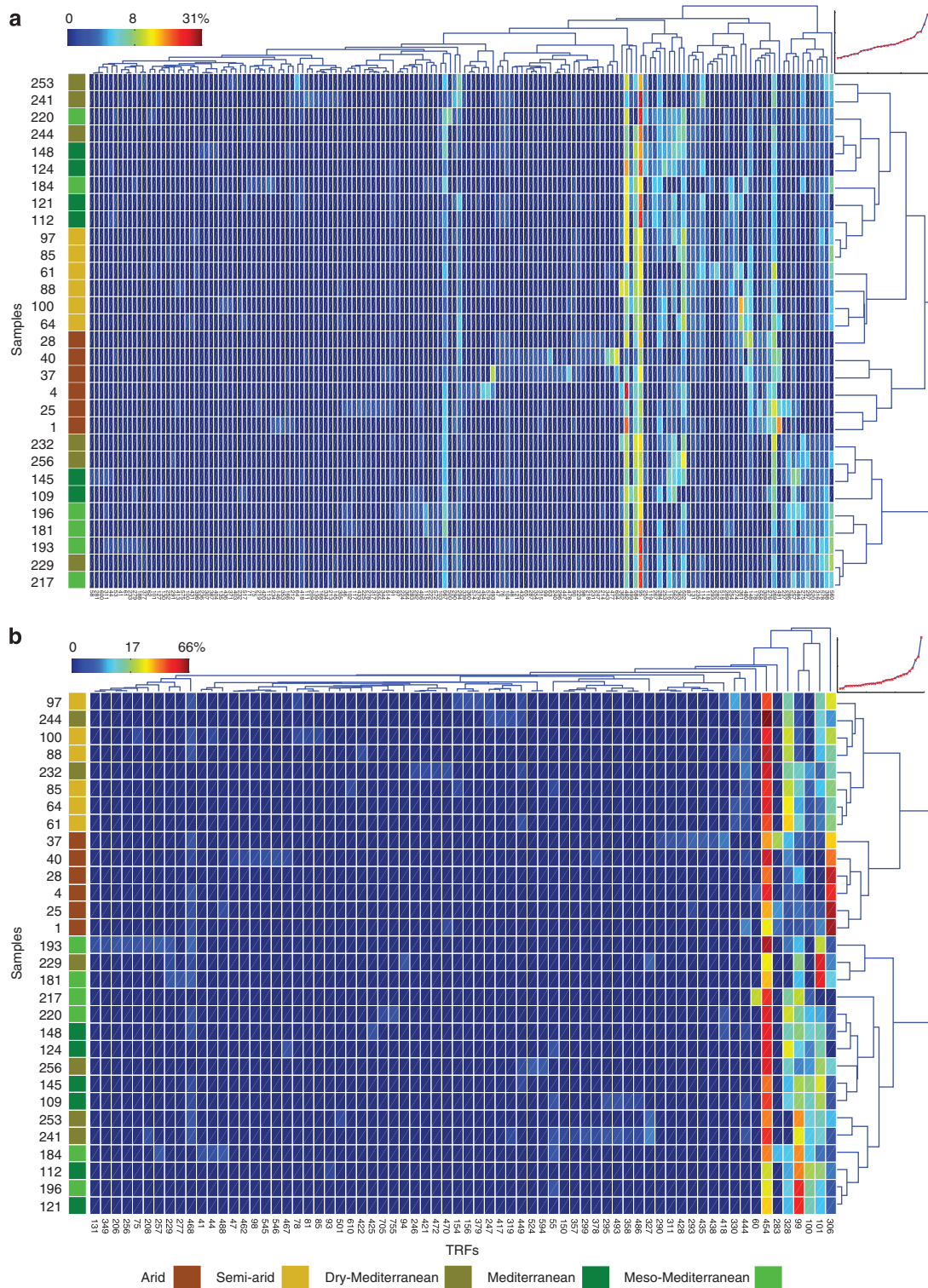


Figure 3 Two-way cluster analysis of consensus TRFLP profiles of (a) bacteria and (b) archaea. Each row in the heat map represents a sample, and each column represents a TRF. Columns are clustered according to samples, whereas the rows are clustered in accordance with the TRFs generated by restriction of each soil sample's amplified 16S rRNA fragment using the restriction enzymes *TaqI* for bacteria (a) and *MseI* for archaea (b). The color-coded column to the left of the heat map corresponds to the origin of each sample (see legend at the bottom for color coding). Heat map colors represent the relative abundance of the TRFs. Clustering was performed on an Euclidean distance matrix of the standardized and transformed TRFLP profiles (see Materials and methods). Scree plots (top right of each map) show the distance between each of the two hierarchical clusters versus cluster number.

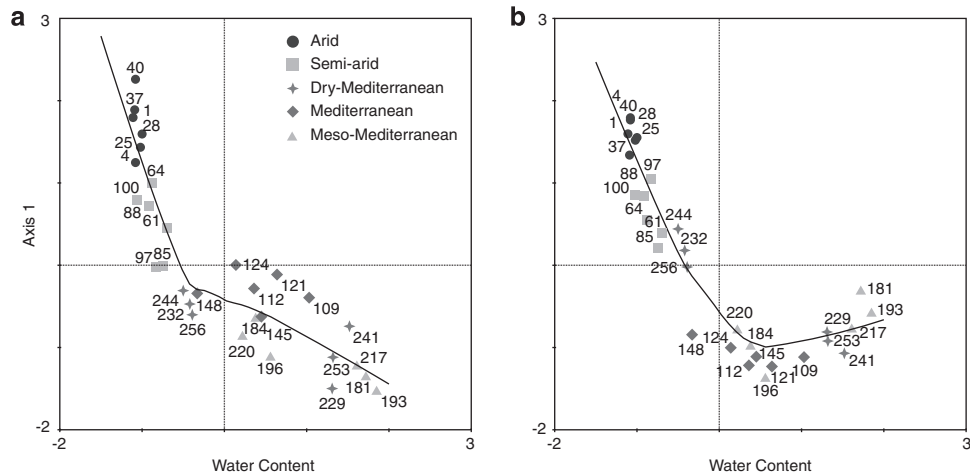


Figure 4 First noncanonical axis of the redundancy analysis (RDA) of (a) bacteria and (b) archaea TRFLP profiles versus the standardized (Z-score) values of the water content of each sample. Locally weighted scatterplot smoothing (LOESS) fit was performed with a local linear model and a span of $\alpha = 0.67$. The axes explain 15% and 29%, respectively, of the variability in the data for bacteria and archaea.

Mediterranean communities clustered along the higher end. Much like in the cluster analysis (Figure 3), the community compositions of bacteria and archaea did not strictly follow the water-content gradient, but rather formed three separate clusters of arid, semiarid and Mediterranean communities (supplementary Figure 3).

Discussion

A major goal in biogeography and ecology is to understand the causes of taxonomic diversity gradients. Such gradients occur on spatial scales ranging from a few centimeters (Carson *et al.*, 2009) to thousands of kilometers (Fierer and Jackson, 2006). For microorganisms, research has primarily focused on local scales (Fierer, 2008); however, the drivers of diversity and their relative influence may differ with scale, and understanding diversity gradients may require analyses of their variation relative to various spatial scales. To the best of our knowledge, this study is the first to link local and regional scales of bacterial and archaeal community diversities. We tested both domains within each of the five LTER sites, using a scheme that enabled us to examine a triplicate composite of eight soil samples in each patch at each site; this procedure ensured that the samples reflect the entire plot. Statistical analysis of bacterial and archaeal fingerprints in this sampling scheme revealed that the differences in diversity within sites are not statistically significant, unlike the diversity between sites across the precipitation gradient. This encompasses our most surprising result, that is, the spatial patterns of OTU diversity for archaea and bacteria are very similar in structure, despite the profound biological differences between these two domains.

Fingerprinting methods, such as TRFLP, are robust and can be applied to a large number of

samples; however, the TRFLP technique entails two major drawbacks. The first is inherent to all known fingerprinting techniques and concerns their detection limit; abundant species are well represented, whereas the rare species remain unseen. Consequently, the majority of species in a highly diversified environment, such as soil remains undetected and hence taxa–area relationships within microbial communities are difficult to decipher (Curtis *et al.*, 2006; Woodcock *et al.*, 2006). However, theoretical modeling has indicated that if significant shifts in microbial community are spatially correlated, as shown in this report, then the models will yield closer estimates reflecting the ‘true’ taxa–area relationship (Woodcock *et al.*, 2006). The other drawback concerns the choice of enzymes for restricting the amplified 16S rRNA fragment, which strongly influences the observed TRFs and thus the emerging diversity patterns (Schutte *et al.*, 2008). Although the enzymes used in our study were chosen in accordance with in-silico analysis of the RDP database (see Materials and methods), the outcome varied between the two domains: archaeal and bacterial TRFs were each analyzed using three distinct restriction enzymes, however, in contrast to bacteria, archaeal TRF clusters of the enzymes *TaqI* and *MboI* did not follow the same biogeographical structure across the precipitation gradient (compare Figure 3b with Supplementary Figures 1c and d). This observation could be attributed to the choice of the restriction enzymes.

Both domains followed similar biogeographical patterns (Figure 2), their diversity apparently unrelated to variables that typically govern plant and animal diversity. Diversity gradients of macroorganisms have been described on different scales in relation to latitude, climate, productivity and temperature, documenting the generality of the latitudinal diversity gradient, with stronger and steeper diversity gradients on regional as opposed to local scales (Hawkins *et al.*, 2003). Those studies

have shown a positive relationship between annual precipitation (an index of productivity in arid regions), species richness and phylogenetic composition. For instance, in grasslands, the number of species per square meter was shown to increase by one with each 100 mm increase in precipitation (Cornwell and Grubb, 2003; Adler and Levine, 2007); the diversity and community organization of North American ants (Keil *et al.*, 2008) and rodents (Bowers *et al.*, 1987) were shown to be tightly correlated to annual precipitation, and a survey across Western Europe and Northern Africa showed that water availability limits the richness of Odonata (dragonfly) species (Keil *et al.*, 2008). In contrast to macroorganisms, our results showed similar diversity and richness of the soil bacterial and archaeal communities across sites, whereas the taxonomic composition differed by ecosystem type. The species abundance distribution of the 30 soil samples showed a similar pattern for bacterial and archaeal communities: domination of a few of the more abundant OTUs, whereas most of the OTUs are relatively rare, exemplifying the classic 'long tail' phenomenon (Fuhrman, 2009).

The microbial communities in the arid, semiarid and Mediterranean sites were significantly different ($P < 0.0001$), whereas the microbial communities within the Mediterranean sites (although the annual precipitation differed markedly, at 400, 500 and 900 mm per year) shared key characteristics, with no significant differences among them ($P = 0.079$ and 0.244 for bacteria and archaea, respectively). The clustering of the microbial communities according to the ecosystem (arid, semiarid and Mediterranean) rather than strictly according to the precipitation gradient could be largely explained by a combination of precipitation, as reflected by the soil water content (Figure 4) and vegetation cover as reflected by the soil organic matter content (Table 1). It has been suggested that microbial biogeographical patterns are shaped by environmental factors (Fierer, 2008). For instance, pH has been found to be the best predictor of the continent-scale patterns exhibited by soil bacteria (Fierer and Jackson, 2006). Diversity of Antarctic soil bacteria changed along a temperature gradient, yet was comparable in locations with dense vegetation cover (Yergeau *et al.*, 2007), and the diversity of soil microbial community assemblages in the Chihuahuan Desert followed the precipitation patterns (Clark *et al.*, 2009). In this study, numerous factors were measured for each of the 30 soil samples (Table 1) including pH, salinity, calcium carbonate and nutrients (e.g., phosphorus, nitrogen, carbon, magnesium and potassium), yet the distribution pattern of both bacteria and archaea correlated mainly with soil water content (Figure 4), organic matter that is stored in the soil and calcium carbonate. We suggest that precipitation and vegetation cover are the major factors shaping the structure of the soil microbial community in the arid, semiarid and Mediterranean sites. Indeed, patch

types were found to vary in both bacterial and archaeal communities, with different OTUs found in the open areas and under the plant canopies (Figure 2). We speculate that the structures of the bacterial and archaeal communities were comparable among the three Mediterranean sites because of a combination of selection pressure exerted by plants and the protection from environmental fluctuations provided by the vegetation. However, in the exposed arid and semiarid sites, where vegetation is scarce and the open patches are devoid of plants, the resource islands support distinct microbial communities (Herman *et al.*, 1995).

Numerous studies have shown the strong correlation between precipitation and macroorganism richness and diversity, especially in water-limited regions (Hawkins *et al.*, 2003). Until recently, however, microorganisms' spatial diversity has received little attention, as the requisite sampling and analysis efforts were unrealistic considering the number of bacteria in a gram of soil (Schloss and Handelsman, 2006). The introduction of quick and reproducible fingerprinting techniques 12 years ago (Liu *et al.*, 1997; Fisher and Triplett, 1999) has enabled microbiologists to compare large number of soil samples and move beyond local-scale observations. Here, we examined local and regional diversity patterns of both bacteria and archaea and found that the two domains cluster in a similar manner. The fingerprint-based analysis suggests that separate evolutionary and ecological processes have directed the biogeography of micro- and macroorganisms, resulting in distinct patterns. Further work is needed to elucidate the following: (i) whether these biogeographical patterns are stable over time; (ii) the phylogenetic patterns in the three separate ecosystems delineated here and (iii) the functional groups within each community. Such comprehensive examination would improve our understanding of the spatial and temporal patterns of microbial life in different habitats and provide a link to the full breadth of the ecosystems.

Acknowledgements

We gratefully acknowledge Diethart Matthies, Itamar Giladi and Suzanna K Remold for their help with the statistical analyses. We thank Eduard Jurkevitch and Zohar Pasternak for their insightful comments on the manuscript. Finally, we thank Fred A Rainey for introducing us to the topic.

References

- Adler PB, Levine JM. (2007). Contrasting relationships between precipitation and species richness in space and time. *Oikos* **116**: 221–232.
- Ben-Dov E, Shapiro OH, Siboni N, Kushmaro A. (2006). Advantage of using inosine at the 3' termini of 16S

- rRNA gene universal primers for the study of microbial diversity. *Appl Environ Microbiol* **72**: 6902–6906.
- Bowers MA, Thompson DB, Brown JH. (1987). Spatial-organisation of a desert rodent community—food addition and species removal. *Oecologia* **72**: 77–82.
- Bryant JA, Lamanna C, Morlon H, Kerkhoff AJ, Enquist BJ, Green JL. (2008). Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. *Proc Natl Acad Sci USA* **105**: 11505–11511.
- Carson JK, Campbell L, Rooney D, Clipson N, Gleeson DB. (2009). Minerals in soil select distinct bacterial communities in their microhabitats. *FEMS Microbiol Ecol* **67**: 381–388.
- Chaban B, Ng SYM, Jarrell KF. (2006). Archaeal habitats—from the extreme to the ordinary. *Can J Microbiol* **52**: 73–116.
- Clark J, Campbell J, Grizzle H, Acosta-Martinez V, Zak J. (2009). Soil microbial community response to drought and precipitation variability in the Chihuahuan Desert. *Microb Ecol* **57**: 248–260.
- Cornwell WK, Grubb PJ. (2003). Regional and local patterns in plant species richness with respect to resource availability. *Oikos* **100**: 417–428.
- Curtis TP, Head IM, Lunn M, Woodcock S, Schloss PD, Sloan WT. (2006). What is the extent of prokaryotic diversity? *Philos Trans R Soc Lond B Biol Sci* **361**: 2023–2037.
- Dunbar J, Ticknor LO, Kuske CR. (2001). Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. *Appl Environ Microbiol* **67**: 190–197.
- Egert M, Friedrich MW. (2003). Formation of pseudo-terminal restriction fragments, a PCR-related bias affecting terminal restriction fragment length polymorphism analysis of microbial community structure. *Appl Environ Microbiol* **69**: 2555–2562.
- Fierer N. (2008). Microbial biogeography: patterns in the microbial diversity across space and time. In Zengler K (ed) *Accessing Uncultivated Microorganisms: from the Environment to Organisms and Genomes and Back*. ASM Press; Washington DC, pp 95–115.
- Fierer N, Jackson RB. (2006). The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci USA* **103**: 626–631.
- Fisher MM, Triplett EW. (1999). Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Appl Environ Microbiol* **65**: 4630–4636.
- Fuhrman JA. (2009). Microbial community structure and its functional implications. *Nature* **459**: 193–199.
- Gardezi T, Gonzalez A. (2008). Scale dependence of species–energy relationships: evidence from fishes in thousands of lakes. *Am Nat* **171**: 800–815.
- Green J, Bohannan BJM. (2006). Spatial scaling of microbial biodiversity. *Trends Ecol Evol* **21**: 501–507.
- Grosskopf R, Janssen PH, Liesack W. (1998). Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. *Appl Environ Microbiol* **64**: 960–969.
- Hansel CM, Fendorf S, Jardine PM, Francis CA. (2008). Changes in bacterial and archaeal community structure and functional diversity along a geochemically variable soil profile. *Appl Environ Microbiol* **74**: 1620–1633.
- Hawkins BA, Field R, Cornell HV, Currie DJ, Guegan JF, Kaufman DM *et al.* (2003). Energy, water, and broad-scale geographic patterns of species richness. *Ecology* **84**: 3105–3117.
- Herman RP, Provencio KR, Herreramatos J, Torrez RJ. (1995). Resource islands predict the distribution of heterotrophic bacteria in Chihuahuan desert soils. *Appl Environ Microbiol* **61**: 1816–1821.
- Hoj L, Olsen RA, Torsvik VL. (2008). Effects of temperature on the diversity and community structure of known methanogenic groups and other archaea in high Arctic peat. *ISME J* **2**: 37–48.
- Ishii K, Fukui M. (2001). Optimization of annealing temperature to reduce bias caused by a primer mismatch in multitemplate PCR. *Appl Environ Microbiol* **67**: 3753–3755.
- Keil P, Simova I, Hawkins BA. (2008). Water-energy and the geographical species richness pattern of European and North African dragonflies (Odonata). *Insect Conserv Divers* **1**: 142–150.
- Kent AD, Triplett EW. (2002). Microbial communities and their interactions in soil and rhizosphere ecosystems. *Annu Rev Microbiol* **56**: 211–236.
- Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML, Pace NR. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc Natl Acad Sci USA* **82**: 6955–6959.
- Legendre P, Gallagher ED. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia* **129**: 271–280.
- Liu WT, Marsh TL, Cheng H, Forney LJ. (1997). Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl Environ Microbiol* **63**: 4516–4522.
- Navarro-Gonzalez R, Rainey FA, Molina P, Bagaley DR, Hollen BJ, de la Rosa J *et al.* (2003). Mars-like soils in the Atacama Desert, Chile, and the dry limit of microbial life. *Science* **302**: 1018–1021.
- Nemergut DR, Costello EK, Meyer AF, Pescador MY, Weintraub MN, Schmidt SK. (2005). Structure and function of alpine and arctic soil microbial communities. *Res Microbiol* **156**: 775–784.
- Ochsenreiter T, Selezi D, Quaiser A, Bonch-Osmolovskaya L, Schleper C. (2003). Diversity and abundance of Crenarchaeota in terrestrial habitats studied by 16S RNA surveys and real time PCR. *Environ Microbiol* **5**: 787–797.
- Oline DK, Schmidt SK, Grant MC. (2006). Biogeography and landscape-scale diversity of the dominant crenarchaeota of soil. *Microb Ecol* **52**: 480–490.
- Robinson BS, Bamforth SS, Dobson PJ. (2002). Density and diversity of protozoa in some arid Australian soils. *J Eukaryot Microbiol* **49**: 449–453.
- Schlesinger WH, Raikes JA, Hartley AE, Cross AE. (1996). On the spatial pattern of soil nutrients in desert ecosystems. *Ecology* **77**: 364–374.
- Schloss PD, Handelsman J. (2006). Toward a census of bacteria in soil. *PLoS Comput Biol* **2**: e92.
- Schutte UME, Abdo Z, Bent SJ, Shyu C, Williams CJ, Pierson JD *et al.* (2008). Advances in the use of terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes to characterize microbial communities. *Appl Microbiol Biotechnol* **80**: 365–380.
- SSSA (1996). *Methods of Soil Analysis, Part 3*. Soil Science Society of America: Madison, WI, USA.

- Stahl DA, Amann R. (1991). Development and application of nucleic acid probes in bacterial systematics. In: Stackebrandt E and Goodfellow M (eds). *Nucleic Acid Techniques in Bacterial Systematics*. John Wiley & Sons Ltd Chichester: England, pp 205–248.
- ter Braak CJF, Smilauer P. (1998). *Canoco Reference Manual and User's Guide to Canoco for Windows*. Centre for Biometry Wageningen: Wageningen, The Netherlands.
- van Rensburg BJ, Chown SL, Gaston KJ. (2002). Species richness, environmental correlates, and spatial scale: a test using South African birds. *Am Nat* **159**: 566–577.
- Vishniac HS. (2006). A multivariate analysis of soil yeasts isolated from a latitudinal gradient. *Microb Ecol* **52**: 90–103.
- Walsh DA, Papke RT, Doolittle WF. (2005). Archaeal diversity along a soil salinity gradient prone to disturbance. *Environ Microbiol* **7**: 1655–1666.
- Woodcock S, Curtis TP, Head IM, Lunn M, Sloan WT. (2006). Taxa–area relationships for microbes: the unsampled and the unseen. *Ecol Lett* **9**: 805–812.
- Yergeau E, Newsham KK, Pearce DA, Kowalchuk GA. (2007). Patterns of bacterial diversity across a range of Antarctic terrestrial habitats. *Environ Microbiol* **9**: 2670–2682.
- Zhou J, Xia B, Treves DS, Wu LY, Marsh TL, O'Neill RV *et al*. (2002). Spatial and resource factors influencing high microbial diversity in soil. *Appl Environ Microbiol* **68**: 326–334.
- Zhou JZ, Xia BC, Huang H, Palumbo AV, Tiedje JM. (2004). Microbial diversity and heterogeneity in sandy subsurface soils. *Appl Environ Microbiol* **70**: 1723–1734.

Supplementary Information accompanies the paper on The ISME Journal website (<http://www.nature.com/ismej>)