MICROBIAL ECOLOGY

Microb Ecol (2003) 46:312–321
DOI: 10.1007/s00248-003-1004-0

Small-Scale Vertical Distribution of Bacterial Biomass and Diversity in Biological Soil Crusts from Arid Lands in the Colorado Plateau

F. Garcia-Pichel,[1] S.L. Johnson,[1] D. Youngkin,[1] J. Belnap[2]

[1] Microbiology Department, Arizona State University, Tempe, AZ 85287, USA
[2] US Geological Survey, 2290 West Resource Blvd., Moab, UT 84532, USA

Received: 13 December 2003; Accepted: 3 March 2003; Online publication: 17 September 2003

ABSTRACT

We characterized, at millimeter resolution, bacterial biomass, diversity, and vertical stratification of biological soil crusts in arid lands from the Colorado Plateau. Microscopic counts, extractable DNA, and plate counts of viable aerobic copiotrophs (VAC) revealed that the top centimeter of crusted soils contained atypically large bacterial populations, tenfold larger than those in uncrusted, deeper soils. The plate counts were not always consistent with more direct estimates of microbial biomass. Bacterial populations peaked at the immediate subsurface (1–2 mm) in light-appearing, young crusts, and at the surface (0–1 mm) in well-developed, dark crusts, which corresponds to the location of cyanobacterial populations. Bacterial abundance decreased with depth below these horizons. Spatially resolved DGGE fingerprints of Bacterial 16S rRNA genes demonstrated the presence of highly diverse natural communities, but we could detect neither trends with depth in bacterial richness or diversity, nor a difference in diversity indices between crust types. Fingerprints, however, revealed the presence of marked stratification in the structure of the microbial communities, probably a result of vertical gradients in physicochemical parameters. Sequencing and phylogenetic analyses indicated that most of the naturally occurring bacteria are novel types, with low sequence similarity (83–93%) to those available in public databases. DGGE analyses of the VAC populations indicated communities of lower diversity, with most types having sequences more than 94% similar to those in public databases. Our study indicates that soil crusts represent small-scale mantles of fertility in arid ecosystems, harboring vertically structured, little-known bacterial populations that are not well represented by standard cultivation methods.

Introduction

Surface-bound assemblages of microorganisms that consolidate soils into millimeter- to centimeter-thick crusts

Correspondence to: F. Garcia-Pichel; E-mail: ferran@asu.edu

occur on arid lands where the lack of water restricts the development of higher plant cover. These microbial communities, variously known as cryptogamic, cryptobiotic, cyanobacterial, or simply biological soil crusts, are dependent on the primary production of cyanobacteria and eukaryotic microalgae. The primary producers there are either free-living or within-lichen symbioses, although mosses may also be present in well-developed crusts [24, 11, 12]. Crusting is initiated by filamentous oscillatorian cyanobacteria during episodic events of available moisture (rain, snow, dew), with the subsequent entrapment of mineral particles by the network of cyanobacterial filaments or by a matrix of extracellular slime [3, 14]. Biological soil crusts cover large portions of undisturbed soils in the arid Western United States, in Australian and African rangelands and deserts, in the Middle East, in continental Asia, and in Southern Europe [24, 3]. Our recent biomass estimates indicate that the populations of microbial primary producers in desert crusts amount to some 54×10^{12} g C globally [17]. Biological desert crusts seem to play important roles in the biogeochemistry and geomorphology of arid regions (see [11, 12]). They have been reported to affect the overall hydrology of arid lands [40] and to decrease soil erodibility dramatically by consolidating soil surfaces [7, 2, 35]. In addition, biological desert crusts may exert a passive or indirect filter effect on exchanges of gas or particulate matter between the atmosphere and the bulk soil beneath them, since any such mass transfer must check through this cryptic live mantle. In spite of their geographic extent and ecological importance, many aspects of soil crust biology remain understudied, especially those related to their microbial populations [14]. Although biotic surveys on crust lichen and microalgae exist, there has been essentially no single, sustained effort to study the bacterial biota of what could easily be regarded as the Earth's most extensive biofilm. Microsensor measurements have shown that microenvironments with strong vertical components develop within a short time after crust wetting, because of the alleged presence of large numbers of microorganisms operating at high metabolic rates [15, 9]. But the diversity of microorganisms that form and thrive in soil crusts has yet be gauged and described.

The distribution of bacteria in desert soils is thought to mirror largely the distribution of higher plants, according to the "resource island" hypothesis [13]. But supporting evidence is based on sampling scales (centimeter to meter) that are not necessarily appropriate to study microbial systems such as soil crusts, which are constrained below the centimeter scale in the vertical dimension, and characterizing soils requires sampling at a temporal and spatial scale appropriate for the questions being studied [6]. Bacterial segregation along vertical gradients is a basic feature in analogous communities such as aquatic biofilms [34], microbial mats [19, 37], and endolithic communities [39]. We hypothesized that vertical stratification may be a characteristic of soil crusts as well. We adapted standard methods of microbial biomass assessment and microbial community analysis to the study of bacteria in soil crusts at a spatial vertical resolution (millimeter) appropriate to their vertical extent: a scale that allows distinction between contrasting microhabitats as observed in biogeochemical studies.

Materials and Methods

Materials, Sampling, and Sample Sectioning

Two types of crusts were used in this study: light and dark crusts. They were selected to match crust types studied previously with respect to microenvironment formation [15] and were collected around the town of Moab, Utah, in the Colorado Plateau. Light crusts were from a site known as "Slick Rock" (N38°34′ W109°31′). Dark crusts were from a site known as "Sunday Churt" (N38°38′ W109°39′). Both sites contain shallow, sandy soils. The two distinct crust types represent, respectively, early and later stages in crust formation, with conspicuous surface populations of dark-pigmented cyanobacteria (*Nostoc* spp. and *Scytonema* spp.) being absent in light crusts and present in dark crusts. We selected samples that were devoid of pronounced surface microtopography and that would lend themselves to simplified studies on vertical stratification. Samples were taken with PVC coring tubes (2.5 cm internal diameter) by wetting the soil, gently inserting the tube, and capping both sides with rubber stoppers. This procedure maintained the apparent architecture of the crust, which is otherwise very brittle in the dried state. After coring, samples were allowed to dry for storage and transport to the laboratory. All samples were sectioned into millimeter-scale horizons before analysis. Prior to sectioning, approximately 20 mL of a fluid (40–45°C), 1.5% (w/v) aqueous agar solution (Bacto-Agar in Milli-Q H_2O) was poured over the cores and injected along the core walls. It was then allowed to embed the soil and solidify. This served the purpose of stabilizing loose mineral particles and preserving the integrity of the sections upon handling. A subcore of the agar-immobilized core was taken with a 1.5-cm diameter plastic corer and extruded onto the metal stage of a table microtome using a piston. The core was sliced using the table microtome. In all cores, the following six sections were obtained from top to bottom: horizons 1 through 3 were 1 mm thick each, horizons 4 and 5 were 2 mm thick, and the sixth horizon was 3 mm thick. Sections were allowed to dry

overnight and the mass of each sample measured gravimetrically. For further handling in protocols requiring dispersion of the sample, each section was placed into a separate tube containing 3.2 mL of a 25% Ringer's solution [29] and 16 units of β-agarase (Sigma). They were then incubated at 35°C for 20 min to hydrolyze the agar. Tubes were amended with 6 mg of sodium pyrophosphate and vortexed [29] to disperse cells and mineral particles. Pyrophosphate addition was omitted for DNA extraction, cell counting, and pigment analyses.

Cell Counting

Each core section was placed in 100 mL (final volume) of 25% Ringer's solution and blended for 2 min in a Waring blender. Because we were interested in nonphototrophic bacterial biomass, the blends were allowed to settled for 48 h at 4°C to remove mineral particles and large cells (cyanobacteria, fungal hyphae). Microscopic slides were prepared with 10 mL of the suspension, 5 mL of Milli-Q H_2O, and 5 drops (approx 0.2 mL) of a 1% DAPI (4′,6-diamidino-2-phenylindole, Sigma) stain solution. This was filtered under vacuum onto a black polycarbonate filter (0.22 μm pore size, Osmonics) with a white cellulose acetate backup filter (0.8 μm pore size, Micron Sep.). Filters were allowed to air-dry and placed onto a glass slide, sandwiched between two drops of immersion oil, and covered with a coverslip. Slides were frozen until analysis. We used a Nikon epifluorescence microscope under UV illumination equipped with a CCD camera to visualize and photograph the cells. Ten digitized images of different microscopic fields were counted per sample. Cell numbers in the original soil were calculated according to dilution factors used and gravimetric determinations of original soil mass in the sample.

Plate Counts

Serial dilutions from sectioned samples were done in 25% Ringer's solution. Plate counts for viable aerobic copiotrophs (VAC) were done on 1.5% agar-PGY medium containing only peptone, glucose, and yeast extract [29], with 2 weeks of incubation at room temperature. VAC spores present in the soil were counted by dilution plating on PGY medium after pasteurization of the initial dilution at 70°C for 20 min to inactivate vegetative cells.

DNA Extraction, Isolation, and Quantification

Extractable DNA was determined after grinding with mortar and pestle, followed by eight freeze–thaw cycles and phenol–chloroform–isopropanol extraction according to Garcia-Pichel et al. [18]. While DNA yields from soils can vary significantly as a function of the method used, they still provide a quantitative picture of relative distributions if used in a single soil type. For soil community analysis, DNA was extracted and purified using UltraClean Soil DNA Isolation Kit from Mo Bio Laboratories (Solana Beach, CA, USA) following the protocol provided, including cell disruption by bead beating and separation through a spin filter. Isolated DNA was quantified using standard agarose gel electrophoresis followed by ethidium bromide staining. Gels were analyzed in a Bio-Rad Fluor-S MultiImager system using a Bio-Rad EZ Load precision molecular mass ruler.

Chlorophyll a Extraction and Measurement

Chlorophyll was extracted in 90% acetone and quantified after HPLC separation and on-line spectrophotometric determination according to Karsten and Garcia-Pichel [25].

PCR Amplification of 16S rRNA Gene Segments, DGGE Fingerprinting, Band Reamplification, and Tentative Phylogenetic Assignments

Primers GM5F and 907R [30], universal for the domain Bacteria, were used for amplification of ca. 590 bp-long 16S rRNA gene segments from the DNA extracts. This was repeated for 21 cycles (denaturations at 94°C for 1 min, extensions at 75°C for 3 min). An additional three cycles with denaturations at 94°C for 1 min, extensions at 72°C for 3 min, and an annealing temperature at 55°C were used, as well as a final extension at 72°C for 7 min. The reaction was carried out utilizing "touchdown" PCR [10] where the annealing temperature decreases from 65 to 55°C (1 min each) in 1°C increments. All reactions were done in a Bio-Rad iCycler thermal cycler and began with denaturation at 95°C for 5 min (hot-start) followed by the addition of 2.5 units of Takara Ex Taq DNA polymerase (PanVera Corporation, Madison, WI, USA) to each reaction at 80°C. Each 100 μL PCR contained the following: 10 μL of 10× Takara Ex Taq PCR buffer, 8 μL of Takara dNTP mixture (2.5 mM each), 50 pmol of each primer (synthesized by Operon Technologies, Inc., Alameda, CA, USA), 200 μg of bovine serum albumin (BSA, PanVera), 20 μL of 5× Eppendorf TaqMaster PCR-enhancer (Brinkmann Instruments, Westbury, NY, USA), and 10 ng of template. Quantification of PCR product was done as described above for isolated DNA. Denaturing gradient gel electrophoresis (DGGE) fingerprinting was used to separate PCR-amplified 16S rRNA alleles. DGGE uses a gradient of chemical denaturants (urea and formamide) in a polyacrylamide gel to separate DNA segments of equal length but different sequence. PCR product (350 ng) was electrophoresed through a 30–50% denaturing gradient for 4 h at 200 V in a Bio-Rad Dcode universal mutation detection system and analyzed as described above for agarose gels. Conspicuous bands in the gels were excised and its DNA allowed to diffuse out for at least 2 days in Tris buffer; this was used as template for a PCR reaction and the amplificate sequenced commercially. Sequences were compared to existing database entries using a BLAST analyses for tentative phylogenetic assignment. A total of 13 sequences were obtained. These have been submitted to GenBank under accession numbers AF538333 to AF538346.

Replication, Data Processing and Calculation of Richness and Diversity

We analyzed at least three independent vertical profiles for each parameter and crust type. For presentation, averages and standard deviations of each horizon in independent profiles are provided. However, for statistical analyses of vertical trends, each profile was treated separately, since the horizons in a profile are not independent of each other. Space-averaged contents within crusts were obtained by vertical integration of the measured values in each profile with weighing according to horizon thickness. For statistical comparisons, *t*-tests were used using space-averaged values of each profile as single determinations.

For the calculation of richness and diversity indices in the DGGE [31], we quantified the number of bands in each lane and each band's intensity relative to the total in its lane using automated image analysis procedures contained in Bio-Rad's "Quantity One" software. Equal sensitivity was used for all analyses. The number of bands gave directly the Richness index; Shannon-Weaver diversity indices were calculated as the negative sum of the products of each band's relative abundance by the natural logarithm of its relative abundance, for all bands in a lane. A detailed discussion on the theoretical basis for this procedure, as well as on its limitations and possible drawbacks, can be found in Nübel et al. [31].

Results

Bacterial Counts

DAPI microscopic counts revealed the presence of large bacterial numbers within the top centimeter of crusted soils. In dark crusts, the space-integrated densities reached $2.13 \pm 0.9 \times 10^9$ stainable cells per gram of soil (with $n = 3$ independent profiles), whereas in soils deep beneath the crusts (3–5 cm) only $1.42 \pm 0.1 \times 10^8$ bacteria per gram of soil could be detected. The two values were significantly different ($P = 0.023$). In light crusts (with $n = 3$ independent profiles) densities were of similar magnitude: $1.37 \pm 0.2 \times 10^9$ g^{-1} in average for the top 1 cm, and $1.15 \pm 0.2 \times 10^8$ g^{-1} deep under the crusted soil. These two values were also significantly different ($P = 0.006$). The difference between dark crust and light crust space-integrated counts, though large, was not statistically significant ($P = 0.24$). Within the crusts themselves (Fig. 1), we found maximal densities in the immediate subsurface (1–3 mm deep), as high as 6×10^9 g^{-1} in some horizons. Below this maximum, numbers showed decreasing trends with depth in all profiles and crusts, as well as in the average data presented in Fig. 1 (negative correlation coefficients between cell number and depth varied between 0.61 and 0.94).

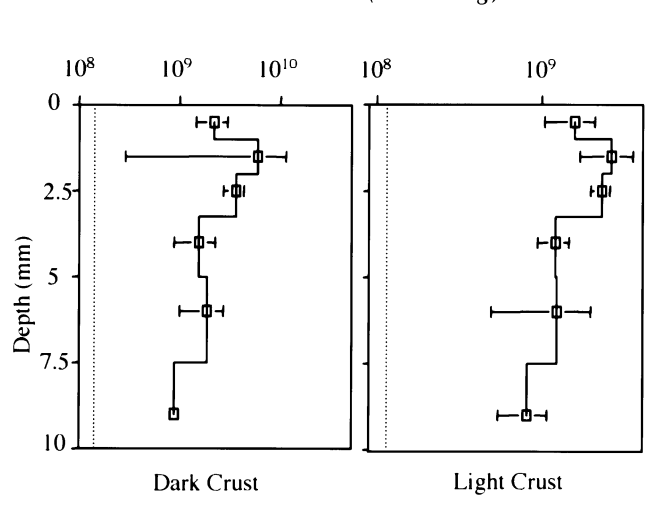

Fig. 1. Vertical distribution of bacterial microscopic counts within soil crusts. Values are means of three independent profiles. Error bars are 2 standard deviations. The broken line indicates average counts obtained from bulk, uncrusted soil below the crusts.

Extractable DNA

Extractable DNA concentrations showed patterns similar to those observed with microscopic counts. The space-integrated extractable DNA in the top 1 cm of light crusts varied among samples ($n = 3$ independent profiles) between 1.1 and 2.3 μg g^{-1}, with a mean (± standard deviation) of 1.6 ± 0.6 μg g^{-1}. Maximal values were found in the subsurface (1–3 mm) where DNA could reach as much as 6.6 μg g^{-1} (Fig. 2). All profiles, however, were characterized by a clear maximum in DNA content between 1 and 3 mm below the surface, and by a significant decreasing trend with depth below this maximum (Fig. 2). Dark crusts ($n = 5$ independent profiles) had higher space-integrated (top 1 cm) extractable DNA content than light crusts, ranging from 2.7 to 7.5 μg g^{-1}, with an average of 4.7 ± 2 μg g^{-1}. In dark crusts, maximal values were always found in the top horizon (0–1 mm) and reached as much as 16.7 μg g^{-1}. The differences in DNA content between dark and light crusts were highly significant ($P = 0.034$). Significant trends of decrease with depth were detected in all dark crusts as well. In all crust samples, extractable DNA was about tenfold higher that that found deep below the crusted soil (0.33 ± 0.09 and 0.45 ± 0.05 μg g^{-1}, for soil beneath light and dark crusts, respectively).

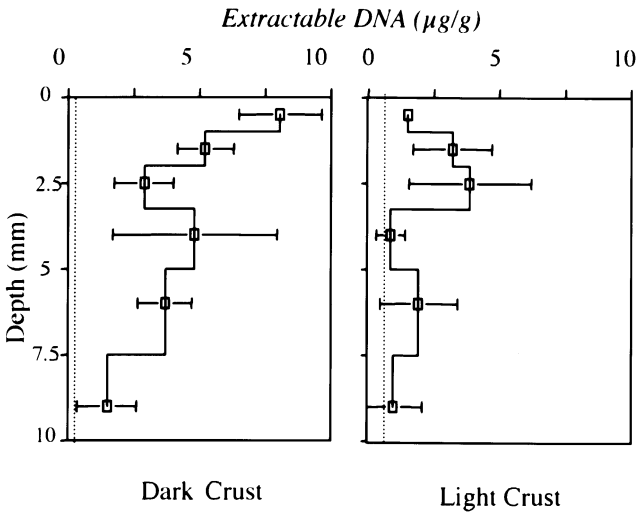

Fig. 2. Vertical distribution of extractable DNA within crusts (μg of DNA per g of soil). Values are averages of at least three independent profiles. Broken lines indicate backgroundlevels determined in bulk, uncrusted soil. Error bars indicate 2 standard deviations.

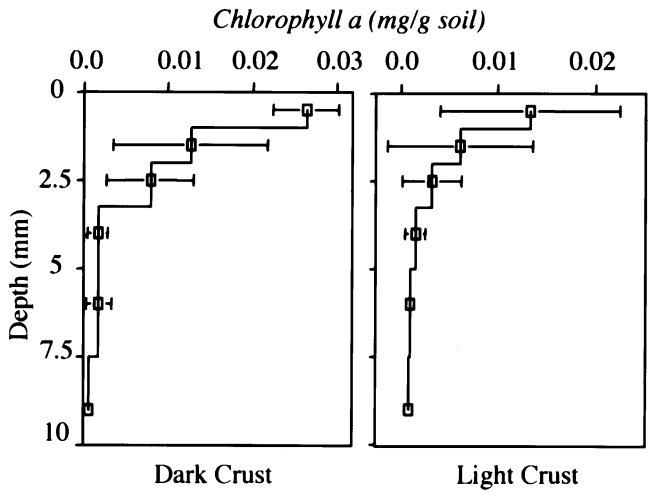

Fig. 3. Vertical distribution of oxygenic phototrophic biomass (cyanobacteria) estimated though the concentration of chlorophyll *a*. Each value is the mean of three samples obtained in three independent profiles; error bars indicate 2 standard deviations.

Chlorophyll a

The distribution of Chl *a* within both crusts clearly indicates that oxygenic phototrophs were concentrated in the top 2 mm, which contained on average more than 75% of the total Chl *a* in the crusts (Fig. 3). This is consistent with previously presented data and with measurements of light penetration into these soils [15]. Chl *a* concentration in both dark and light crusts decreased steeply and exponentially (dark crusts, $r^2 = 0.9802$; light crusts, $r^2 = 0.9857$) with depth. There was at least a 100- to 300-fold difference in Chl *a* levels between the top and the deepest layers in the crusts. The Chl *a* values in the top 2 mm of soil were significantly different between dark and light crusted soils ($P = 0.023$).

Viable Counts

The space-integrated number of viable aerobic copiotrophs (VAC) within the top 1 cm of crusts, as judged by PGY plate counts (Fig. 4), was $7 \pm 3 \times 10^6$ cfu g^{-1} in light crusts ($n = 3$ independent profiles) and $8 \pm 6 \times 10^5$ cfu g^{-1} ($n = 3$ independent profiles) in dark crusts. The differences in space-integrated VAC numbers between light and dark crusts was statistically significant (*t*-test; $P = 0.04$), despite the large variability between profiles and microhorizons. Maximal values found peaked at 3.6×10^7 cfu g^{-1}, and minimal values were around 6×10^4 cfu g^{-1}. In dark crusts there was a clear trend of VAC decrease with depth, but this trend, if present, was not as conspicuous in light crusts. In both types of crusts, however, VAC counts were two orders of magnitude higher (statistically very significant, with $P < 10^{-4}$) than those found in the uncrusted soils below them: $2.6 \pm 3.2 \times 10^4$ and $5 \pm 3 \times 10^3$ for light and dark crusts, respectively.

The fraction of VAC originating from bacterial spores was also very low, on average accounting for 1.4% in light crusts and 1.8% in dark crusts. No conspicuous trends with depth were detected in the VAC-spore counts (data not shown).

Molecular Fingerprints of Natural and VAC Communities

DGGE fingerprints of the natural samples (Fig. 5) revealed the presence of a complex community of bacteria. Automated fluorescence detection of the number of bands in each lane yielded between 18 and 27 individual alleles per horizon in light crusts and between 19 and 28 bands in dark crusts. Shannon-Weaver diversity indices, calculated from band number and relative intensity for each horizon, varied between 2.7 and 3.1 in light crusts, and between 2.7 and 3.3 in dark crusts, indicating that the two types of crust did not differ significantly in terms of bacterial diversity indicators. No significant trends with depth in either richness or diversity indices could be detected in this analysis. The absence of similarly positioned bands in two

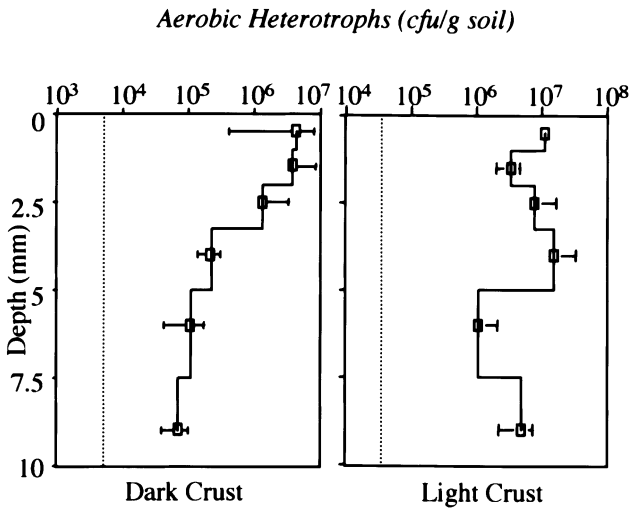

Fig. 4. Vertical distribution of viable aerobic copiotrophs (VAC) estimated through plate counts. Each value represents the mean of three counts obtained from independent profiles; error bars are standard deviations. In cases where the standard deviation was greater than the mean, only the positive standard deviation is shown. Broken lines indicate average VAC numbers in bulk, uncrusted soil.

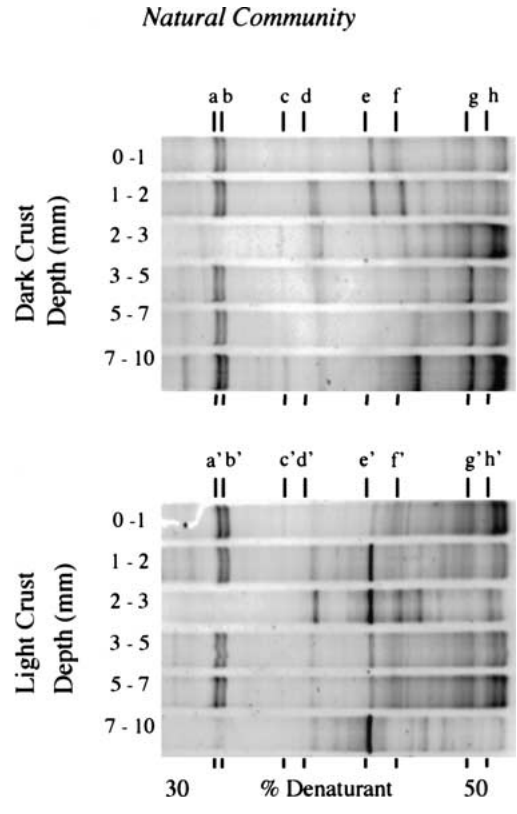

Fig. 5. DGGE image (bacterial fingerprint) of 16S R genes PCR amplified from DNA extracts of the microbial communities in millimeter-sized depth horizons. Bands labeled a through g and a' through g' are discussed in the text in further detail.

different lanes offers proof of a difference between samples, while the presence of two similarly positioned bands in two different lanes provides only tentative evidence for the presence of the same allele. Keeping this in mind, marked differences (i.e., band d is present in dark crusts but not in light crusts) and apparent similarities (bands a, b, and a', b') were evident between dark and light crusts. The fingerprints also revealed an apparent stratification along the vertical dimension in bacterial community composition in both crusts types. While some major bands (bands a, b, a', b') were found through the profile with no obvious pattern of distribution, others displayed marked vertical distribution trends: bands c and g increased in proportional abundance with increasing depth, while band d had a clear maximum between 0 and 2 mm, decreasing with depth below those horizons. Bands e and f were preferentially found in the top 2 mm. Although an exhaustive study of the phylogenetic affiliation of community members was not a central part of this study, several bands were excised, eluted, PCR-amplified, and sequenced. Most of those bands yielded sequences of low similarity (83–93%) to those in the public databases, either public sequences from characterized bacteria or from uncharacterized field samples. Many of them could be tentatively assigned to the diverse *Holophaga/Acidobacterium* group (bands d, g,) or the *Cytophaga/Flexibacter/Bacteroides* group (bands a', c). Band b' matched best with a group of sequences from uncultivated but apparently widespread arid soil bacteria [26]. Band f' matched with 85% similarity sequences from *Angiococcus* and several other unidentified Myxococccales. In a few cases, similarities better than 95% were obtained: bands g' and h' matched with 100% and 98% similarity sequences from cultivated cyanobacteria that were isolated previously from desert crusts: *Microcoleus vaginatus* [18] and *M. steenstrupii* [5].

In order to obtain a rapid determination of the overall diversity in the cultured populations, we obtained DGGE fingerprinting of the VAC community obtained from pooled colony biomass (Fig. 6). This clearly revealed a community of lower diversity and quite different from that obtained in the fingerprints from the natural community. In light crusts the VAC community had richness (number of detectable bands per lane) values between 5 and 20, with the Shannon-Weaver diversity indices ranging between 0.9 and 2.2. In dark crusts, richness varied between 8 and 19 with Shannon-Weaver indices varying between 1.3 and 2.2.

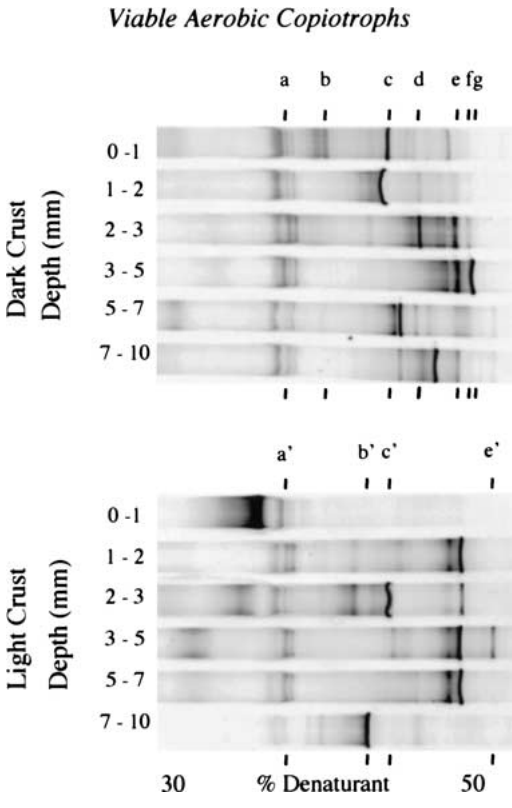

Fig. 6. DGGE fingerprint of the VAC community obtained after cultivation of different depth horizons in the crusts. Bands labeled a through g and a' through e' are further discussed in the text.

Sequences from reamplified bands in this fingerprint found much better matches in the databases (>94% similarity) than those obtained from the natural community. While some bands were distributed through the profile, and even between crust types (for example, bands a and a'), other bands were preferentially enriched only in some horizons. For example, band e was found as a dominant member of VAC between 3 and 5 mm horizons of dark crusts. This band could be assigned through phylogenetic analyses to a member of the genus *Paenibacillus* (96% similarity). Band f, found only between 2 and 3 mm, matched *Methylobacterium* with 94% similarity. Band g, also constrained to VAC in 3–5 mm, matched members of *Arthrobacter* with 98% similarity; band b' was obtained exclusively in plates from the deepest horizons of light crusts and was found to be phylogenetically allied to *Brevundimonas* (100% similarity) in the *Caulobacter* group. Band c' matched (95%) sequences from *Massilia timonae* (β-Proteobacteria), and band d' matched best those of *Microbacterium* (98%).

Discussion

Microbial biomass

Clearly, bacterial populations were significantly higher in the crusts than below the crusted soil and exceeded by at least one order of magnitude those typically measured in (bulk) desert soils with comparable methods, even those measured in plant litter fractions [8, 1]. Bacterial population numbers within the crusted soil were more in line with, or slightly below, those typical of organic-rich soils [32, 38]. They are similar to the $0.4–2.2 \times 10^9$ cells g^{-1} obtained from the top 5 mm in Antarctic soil crusts by Wynn-Williams [41]. In light of the vertical attenuation of bacterial numbers with depth, previously reported bacterial abundances in macroscale samples of crusted soil, though lower, are not inconsistent with our own determinations. Bamforth [1], for example, found $4–8 \times 10^8$ cells g^{-1} in lichen and moss soil crusts in the Lower Sonoran Desert, but this averaged the top 3 cm of soil. Because detrital, plant-derived biomass is a very minor component of desert crusts in general, extractable DNA is most likely of microbial origin and should also reveal microbial abundance. The concentrations found in our crusts are similar to those found for temperate agricultural soils, which cluster around 1.2–1.3 μg g^{-1} [38]. Using space-integrated values of cell counts and extracted DNA, we can calculate roughly a DNA content of 2.2 fg $cell^{-1}$ for dark crust and 1.2 fg $cell^{-1}$ in light crusts. Even though the extractable DNA concentrations include components from both bacteria and fungi, whereas the counts were exclusively bacteria (excluding cyanobacteria), these calculated DNA contents are not too deviant from a typical bacterial ratio of 1 fg $cell^{-1}$. This indicates that the bacterial biomass in the crust is significant and of the same order of magnitude as that of cyanobacteria and fungi. Regardless of the specific biases, both microscopic counts and extractable DNA profiles yielded a similar picture of the magnitude and distribution of microbial biomass in desert crusts. This picture can be summarized in three apparent trends. First, crust microbial biomass is much higher than that found in uncrusted soil; second, within the crusts themselves, microbial biomass tends to decrease with depth; and third, there is a trend of increasing biomass with crust maturity. These trends are consistent with the view that crusts represent "mantles of fertility" in arid soils, in a manner similar to plant rhizospheres, with bacterial numbers reflecting the availability of organics, and perhaps also nitrogen, provided by leakage and

excretion of cyanobacteria. In this way, bacterial numbers decline away from the 2 mm thick photic zone and eventually reach levels in the undercrust typical of bulk arid soils. As cyanobacterial biomass increases with crust maturity (i.e., light vs dark crust Chl *a* levels in Fig. 3), so does the overall microbial biomass especially as measured by extractable DNA.

Interestingly, light crusts consistently displayed a subsurface maximum of microbial biomass, apparently decoupled from the Chl *a* maximum in the top mm. This top millimeter of desert soils is known to be a very harsh environment subject to intense solar radiation, erosional abrasion, and extreme temperatures. In light crusts, cyanobacteria are found in the subsurface when dry, but rapidly migrate to the surface when wet, and back down to 1–3 mm depths upon subsequent drying [20]. The presence of sessile cyanobacteria with abundant scytonemin (*Nostoc*, *Scytonema*), a dark-colored secondary metabolite providing protection from UV exposure, may allow heterotrophic populations to colonize this top millimeter in dark crusts.

The significant differences between in-crust and below-crust VAC plate counts are consistent with microscopic counts and extractable DNA concentrations in revealing that soil crusts represent areas of concentration of bacterial populations: in a sense, areas of bacterial fertility that are small in the vertical scale, but vast in the horizontal scale. But the consistency between VAC plate counts and other biomass estimates ends here. In fact a simple calculation of the ratio of space-integrated VAC counts to microscopic bacterial counts (3.7×10^{-4} cfu cell^{-1} in dark crusts vs 51×10^{-4} cfu cell^{-1} in light crusts) clearly indicates this. The discrepancy is largely driven by the relatively high abundance of VAC in light crusts, which is in principle not easy to explain. Our values are, however, not discordant with a few values reported from similar environments. Kuske et al. [28], for example, found 8–105 $\times$ 10^5 cfu g^{-1} in the top 10 cm of crusted desert soil from a nearby location. Bolton et al. [4], using plate counts of aerobic heterotrophs, reported values around 3×10^6 cfu g^{-1} for semiarid shrub-steppe grasslands where soil crusts covered most of the interplant spaces, which are similar to our space-integrated values. They found little evidence of vertical stratification between the top 5 cm and the underlying 10 cm. At a smaller scale, we could detect very significant differences between the top 1 cm and the undercrust (5–10 cm deep). Our in-crust VAC numbers were lower than the 4×10^7 cfu g^{-1} found for temperate sandy agricultural soils by Taylor et al. [38]. Aerobic heterotrophs estimated through MPN counts [22] in the interspaces of mesquite (*Propopsis* sp.) shrub and creosote (*Larrea tridentata*) bush in the Chihuahuan Desert yielded 10^5–10^6 cells per gram, again values similar to those found in our Colorado crusts.

Microbial Diversity in Desert Crusts

The fact that most environmental sequences obtained from crusts presented low similarity to those in public databases indicates that desert crusts contain a large number of undescribed bacterial types. This parallels the results of a recent study focusing on cyanobacteria where at least four of the five phylogenetic clusters constructed on the basis of both environmental sequences and novel isolates represented undescribed, deeply branching entities at the generic or suprageneric level, with no known members from other environments [18]. This is perhaps not too surprising, given the particular, and often extreme, environmental conditions typical of desert crusts: Lengthy evolutionary histories in such environment must have been the rule for at least the "specialists" among the local microbes. This is likely to have resulted in an evolutionary distinct biota. Cultivation efforts other than standard plate counts are obviously needed to characterize such bacteria, since analyses of the VAC community yielded a very different set of sequences, which, for the most part were of high similarity to known bacterial types. In fact, this observation may extend to arid soils at large. Traditional enrichment and cultivation techniques have repeatedly yielded novel bacterial species of well-known genera: *Rhizobium yangliense* among the legume-nodulating symbionts, *Bacillus mojavensis* among the spore-formers, as well as *Geodermatophilus* spp. and *Streptomyces* spp. [36, 33, 21]. This has been the case even when molecular studies were carried out on a large number of isolates of *Pseudomonas* spp. [27]. Recent molecular studies, however, have detected the presence of novel, deeply branching environmental sequences in desert soils, belonging, in one case, to novel uncultivated genera of the Rubrobacter subdivision in the Actinobacteria (Actinomycetes, [23]). In another case [26], fully undescribed "candidate" supergroups at the level of division were detected. These "phylotypes" have yet to be cultured or characterized.

Small-Scale Vertical Segregation of Microbial Types

The DGGE fingerprints offered evidence for the presence of small-scale segregation in bacterial community mem-

bers within the bounds of desert crust themselves. The fingerprints also revealed that the vertical stratification of microbial types was maintained even in a highly selective subsample of the natural bacterial community (the VAC community). This is likely the result of environmental gradients, biotic or abiotic, present within the habitat. Light gradients due to strong absorption and scattering of incident irradiance as well as chemical gradients in the soil solution of wet crusts (oxygen, pH, nutrients) [15, 16] have been demonstrated within the small-scale bounds of crusted soils. Additionally, and because carbon inputs into the system are generated within the top 2 mm, where cyanobacteria reside, diffusion of leaked organics is likely to create a gradient in trophic types (tending toward increased oligotrophy with depth). It should prove interesting to probe with more detail some of these possible relationships.

Acknowledgements

We gratefully acknowledge the technical help of Keith Varty, Christopher Eagle-Hawk, and Sasha Reed. This research was supported by grants from the US Department of Agriculture (NRI 35107-10054) and the National Science Foundation (BSI-0206711) to F.G.-P.

References

1. Bamforth SS (1984) Microbial distributions in Arizona deserts and woodlands. Soil Biol Biochem 6:133–137
2. Belnap J, Gardner JS (1993) Soil microstructure in soils of the Colorado Plateau: the role of the cyanobacterium *Microcoleus vaginatus*. Great Basin Naturalist 53:40–47
3. Belnap J, Lange OL (2001) Biological Soil Crusts: Structure, Function and Management, vol 1. Springer-Verlag, Berlin, p 503
4. Bolton Jr H, Smith JL, Link SO (1993) Soil microbial biomass and activity of a disturbed and undisturbed shrub-steppe ecosystem. Soil Biol Biochem 25:545–552
5. Boyer SL, Johansen JR, Flechtner VR (2002) Phylogeny and genetic variance in terrestrial *Microcoleus* (Cyanophyceae) species based on sequence analysis of the 16S rRNA gene and associated 16S–23S ITS region. J Phycol 38:1222–1235
6. Campbell CG, Ghodrati M, Garrido F (1999) Comparison of time domain reflectometry, fiber optic mini-probes, and solution samplers for real time measurement of solute transport in soil. Soil Sci 164:156–170
7. Campbell SE (1979) Soil stabilization by a prokaryotic desert crust: implications for Precambrian land biota. Orig Life 9:335–348
8. Colwell FS (1989) Microbiological comparison of surface soil and unsaturated subsurface soil from a semiarid high desert. Appl Environ Microbiol 55:2420–2423
9. de Winder B, Pluis J, Reus LD, Mur LR (1989) Characterization of a cyanobacterial, algal dune crust in the coastal dunes in the Netherlands. In: Rosenberg E (Ed.) Microbial Mats: Physiological Ecology of Benthic Microbial Communitites, American Society for Microbiology, Washington, DC, pp 77–83
10. Don RH, Cox PT, Wainwright B, Baker K, Mattick JS (1991) "Touchdown" PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res 19:4008
11. Eldridge DL, Greene RSB (1994) Microbiotic soil crusts: a review of their roles in soil and ecological processes in the rangelands of Australia. Aust J Soil Res 32:389–415
12. Evans RD, Johansen JR (1999) Microbiotic crusts and ecosystem processes. Crit Rev Plant Sci 18:183–225
13. Garcia-Moya E, Kell CMM (1970) Contribution of shrubs to the nitrogen economy of a desert-wash plant community. Ecology 51:81–88
14. Garcia-Pichel F (2002) Desert Environments: Biological Soil Crusts. In: Bitton G (Ed.) Encyclopedia of Environmental Microbiology, John Wiley, New York, pp 1019–1023
15. Garcia-Pichel F, Belnap J (1996) Microenvironments and microscale productivity of cyanobacterial desert crusts. J Phycol 32:774–782
16. Garcia-Pichel F, Belnap J (2001) Small-scale environments and distribution of biological soils crusts. In: Lange OL (Ed.) Biological Soil Crusts: Structure, Function, and Management, Springer-Verlag, Berlin, pp 193–201
17. Garcia-Pichel F, Belnap J, Neuer S, Schanz F (2002) Estimates of global cyanobacterial biomass and its distribution. Arch Hydrobiol Suppl Alg Studies 119:213–228
18. Garcia-Pichel F, Lopez-Cortez A, Nübel U (2001) Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. Appl Environ Microbiol 67:1902–1910
19. Garcia-Pichel F, Mechling M, Castenholtz RW (1994) Diel migrations of microorganisms in a hypersaline microbial mat. Appl Environ Microbiol 60:1500–1511
20. Garcia-Pichel F, Pringault O (2001) Cyanobacteria track water in desert soils. Nature 413:380–381
21. Garrity GM, Heimbuch BK, Gagliardi M (1996) Isolation of zoosporogenous actinomycetes from desert soils. J Indust Microbiol 17:260–267
22. Herman RP, Provencio KR, Herrera-Matos J, Torrez RJ (1995) Resource islands predict the distribution of heterotrophic bacteria in Chihuahuan desert soils. Apply Environ Microbiol 61:1816–1821
23. Holmes AJ, Bowyer J, Holley MP, O'Donoghue M, Montgomery M, Gillings MR (2000) Diverse, yet-to-be-cultured members of the Rubrobacter subdivision of the Actinobac-

teria are widespread in Australian arid soils. FEMS Microbiol Ecol 33:111–120
24. Johansen JR (1993) Cryptogamic crusts of semiarid and arid lands of North America. J Phycol 29:140–147
25. Karsten U, Garcia-Pichel F (1996) Carotenoids and mycosporine-like amino acid compounds in members of the genus *Microcoleus* (Cyanobacteria): a chemosystematic study. System Appl Microbiol 19:285–294
26. Kuske CR, Barns SM, Busch JD (1997) Diverse uncultivated bacterial groups from soils of the arid southwestern United States that are present in many geographic regions. Appl Environ Microbiol 63:3614–3621
27. Kuske CR, Busch JD, Adorada DL, Dunbar JM, Barns SM (1999) Phylogeny, ribosomal RNA gene typing and relative abundance of new *Pseudomonas* species (sensu stricto) isolated from two pinyon-juniper woodland soils of the arid southwest U.S. system. Appl Microbiol 22:300–311
28. Kuske CR, Ticknor LO, Miller ME, Dunbar JM, Davis JA, Barns SM, Belnap J (2002) Comparison of soil bacterial communitites in rhizospheres of three plant species and the interspaces in an arid grassland. Appl Environ Microbiol 68:1854–1863
29. Lorch HJ, Beckiser G, Ottow JCG (1995) Basic methods for counting microorganisms in soil and water. In: Nannipieri KAAP (Ed.) Methods in Applied Soil Microbiology and Biochemistry, Academic Press, San Diego, pp 162–170
30. Muyzer G, Teske A, Wirsen CO, Jannasch HW (1995) Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. Arch Microbiol 164:165–172
31. Nübel U, Garcia-Pichel F, Kuhl M, Muyzer G (1999) Quantifying microbial diversity: morphotypes, 16S rRNA genes, carotenoids of oxygenic phototrophs in microbial mats. Appl Environ Microbiol 65:422–430
32. Ranjard L, Poly F, Combrisson J, Richaume A, Gourbiere F, Thioulouse J, Nazaret S (2000) Heterogenous cell density and genetic structure of bacterial pools associated with various soil microenvironments as determined by enumeration and DNA fingerprinting approach (RISA). Microb Ecol 39:263–272
33. Roberts MS, Nakamura LK, Cohan FM (1994) *Bacillus mojavensis* sp. nov., distinguishable from *Bacillus subtillis* by sexual isolation, divergence in DNA sequence, and differences in fatty acid composition. Int J Syst Evol Microbiol 44:256–264
34. Schramm A, DeBeer D, Gieseke A, Amann R (2000) Microenvironments and distribution of nitrifying bacteria in a membrane-bound biofilm. Environ Microbiol 2:680–686
35. Schulten JA (1985) Soil aggregation by cryptogams of a sand prairie. Am J Bot 72:1657–1661
36. Tan ZY, Kan FL, Peng GX, Wang ET, Reinhold-Hurek B, Chen WX (2001) *Rhizobium yanglingense* sp. nov., isolated from arid and semi-arid regions in China. Int J Syst Evol Microbiol 51:909–914
37. Tankéré SPC, Bourne DG, Muller FLL, Torsvik V (2002) Microenvironments and microbial community structure in sediments. Environ Microbiol 4:97–106
38. Taylor JP, Wilson B, Mills MS, Burns RG (2002) Comparison of microbial numbers and enzymatic activities in suface soils and subsoils using various techniques. Soil Biol Biochem 34:387–401
39. Wade B, Garcia-Pichel F (2002) Evaluation of DNA extraction methods for molecular analysis of microbial communities in modern microbialites. Geomicrobiology (in press)
40. West NE (1990) Structure and function of soil microphytic crusts in wildland ecosystems of arid and semi-arid regions. Adv Ecol Res 20:179–223
41. Wynn-Williams DD (1985) Photofading retardant for epiflourescence microscopy in soil micro-ecological studies. Soil Biol Biochem 17:739–746