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Archaeal populations in biological soil crusts from arid lands in North America

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ABSTRACT

Archaea are common and abundant members of biological soil crust communities across large-scale biogeographic provinces of arid North America. Regardless of microbial community development, archaeal populations averaged 2×10^7 16S rRNA gene copies per gram of soil, representing around 5% of the prokaryotic (total calculated bacterial and archaeal) numbers assessed by quantitative-PCR. In contrast, archaeal diversity, determined by denaturing gradient gel electrophoresis fingerprinting and clone libraries of 16S rRNA genes, was very restricted. Only six different phylotypes (all Crenarchaea) were detected, three of which were very dominant. Some phylotypes were widespread, while others were typical of Southern desert areas.

1. Introduction

Biological soil crusts (BSCs) are assemblages of microorganisms that develop on plant interspaces in a variety of habitats, and are common in arid lands of the Western US. They are normally found as thin mantles (millimeters to a centimeter in depth) that encompass microbial and mineral components, and cover otherwise bare soils. BSCs are important for the fertility of arid ecosystems, their effect on soil hydrology, and their role in providing resistance to erosion have all been well documented [see (Evans and Johansen, 1999; Belnap and Lange, 2001; Garcia-Pichel, 2002) for reviews]. Floristic surveys of algal/cyanobacterial components of these communities have a long history (Killian and Fehér, 1935; Vogel, 1955; Shields, 1957; Shields and Durrell, 1964; Friedmann and Galun, 1974; Garcia-Pichel et al., 2001). In recent years, modern molecular approaches have been applied to examine the microbial diversity of phototrophic and non-phototrophic Bacteria (Kuske et al., 2002; Yeager et al., 2004, 2007; Nagy et al., 2005; Gundlapally and Garcia-Pichel, 2006), as well as fungi (Bates and Garcia-Pichel, 2009) present in BSCs. Archaea, particularly the Crenarchaeota, are common in a variety of soil habitats (Ochsenreiter et al., 2003; Nicol and Schleper, 2006), including aridisols (Rutz and Kieft, 2004; Fierer et al., 2005; Chanal et al., 2006); however, reports of Crenarchaeota in BSCs are only anecdotal (Nagy et al., 2005). Archaea are also well represented in other cyanobacteria-driven, desiccation-prone microbial systems, such as upper intertidal microbial mats (Rothrock and Garcia Pichel, 2005).

In this contribution we present a molecular study of the populations of Archaea in BSCs from a variety of sites in several biogeographically distinct arid regions of western North America. We examine the size, diversity, and composition of archaeal populations, relative to those of the Bacteria, based on 16S rRNA gene analyses.

2. Materials and methods

2.1. Sampling and DNA extraction

Sampling sites were visited between 2003 and 2005, and were classified into crust types (e.g., light, dark, lichen, moss) in the field (see Belnap and Lange, 2001). Details of the samples used, can be found in Table 1. The soil was lightly wetted with sterilized, ultrapure laboratory grade (Milli-Q) water using a spray bottle to ease sampling, and the bottom of 55 mm Petri plates were used to excise the topsoil to a depth of approximately 1 cm. Each sample was then air-dried, covered, given a unique identification number, and sealed in Zip-lock plastic bags for transportation. Drying times were between one to several hours, which are unlikely to artificially alter the composition of these microbial communities as this amount of

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Table 1
Origin and type of biological soil crust samples used in this study.

Biogeographical province site	ID	Type of crust[a]	Latitude N	Longitude W
Sonoran				
Alamo Wash	2	Lichen	32° 06′ 220″	112° 46′ 150″
Kuakatch Wash	11	Lichen	32° 10′ 610″	112° 46′ 587″
Bates Well Road	23	Light	32° 11′ 930″	112° 54′ 151″
	43	Light	32° 12′ 003″	112° 54′ 133″
	51	Light	32° 11′ 898″	112° 54′ 133″
Camino de Dos Republicas	63	Light	31° 53′ 305″	112° 48′ 437″
	67[b,c]	Dark	31° 53′ 267″	112° 48′ 400″
	75	Light	31° 53′ 239″	112° 48′ 322″
Quitobaquito Spring	87[b]	Lichen	31° 56′ 519″	113° 01′ 133″
	99	Lichen	31° 56′ 492″	113° 00′ 963″
Puerto Blanco Drive	103[b]	Dark	31° 56′ 359″	112° 59′ 602″
Colorado Plateau				
Slick Rock	125	Light	38° 34′ 822″	109° 31′ 631″
	129	Light	38° 34′ 861″	109° 31′ 584″
	133[b]	Light	38° 34′ 902″	109° 31′ 541″
	135[b]	Light	38° 34′ 922″	109° 31′ 518″
	141[b,c]	Light	38° 34′ 984″	109° 31′ 451″
Sunday Churt	153	Dark	38° 38′ 557″	109° 38′ 910″
	155	Dark	38° 38′ 541″	109° 38′ 883″
	161	Dark	38° 38′ 491″	109° 38′ 799″
	163	Dark	38° 38′ 480″	109° 38′ 771″
	165	Dark	38° 38′ 457″	109° 38′ 753″
Canyonlands NP, Needles District	173	Dark	38° 09′ 945″	109° 44′ 458″
	187	Dark	38° 09′ 806″	109° 44′ 614″
Acoma Tribal Land	568	Dark	35° 00′ 22.8″	107° 29′ 14.4″
NW Great Basin				
Culver Road	302	Mossy	44° 29′ 603″	121° 04′ 730″
	304	Mossy	44° 29′ 623″	121° 04′ 706″
	306	Mossy	44° 29′ 557″	121° 04′ 769″
Egli Well Road	316[b]	Lichen	43° 23′ 399″	119° 42′ 669″
Christmas Valley Road	322	Lichen	43° 09′ 410″	119° 57′ 406″
	324	Lichen	43° 09′ 386″	119° 57′ 424″
Blizzard Gap Valley	340[b,c]	Lichen	42° 05′ 561″	119° 41′ 462″
	344[b,c]	Dark	42° 05′ 541″	119° 41′ 398″
	346	Dark	42° 05′ 530″	119° 41′ 363″
Alvord Desert	360	Lichen	42° 30′ 744″	118° 31′ 989″
	364	Lichen	42° 30′ 687″	118° 31′ 998″
	368	Lichen	42° 30′ 634″	118° 32′ 004″
Fort Rock	386	Dark	43° 22′ 379″	121° 03′ 479″
Chihuahuan				
Jornada del Muerto LTER Dune System	418	Light	32° 36′ 27.2″	106° 48′ 18.0″
Jornada del Muerto LTER	478	Lichen	32° 30′ 46.2″	106° 44′ 32.5″
Jornada del Muerto LTER, Range 7 Rd.	484[b]	Light	32° 32′ 00.9″	106° 44′ 23.8″
Jornada del Muerto LTER, Grassland	498	Lichen	32° 32′ 02.0″	106° 43′ 43.5″
Jornada del Muerto LTER, Sandy Soil	512[b,c]	Light	32° 31′ 58.4″	106° 42′ 46.1″
	516	Light	32° 31′ 59.7″	106° 42′ 43.7″
Sevilleta LTER, Gypsum outcrops	520	Lichen	34° 12′ 41.7″	106° 45′ 34.0″
Sevilleta, LTER, 5-Points Grassland	562[b,c]	Light	34° 20′ 05.7″	106° 43′ 23.6″

[a] Light: typically light colored, devoid of lichen, smooth appearance and very cryptic; Dark: typically dark colored, without lichen, but abundant surface cyanobacteria, typically rugose or pedicelled; Lichen: typically dark colored, with significant lichen cover, typically rugose or pedicelled; Mossy: typically dark colored, with significant moss cover, typically rugose or pedicelled (Belnap and Lange, 2001).
[b] Samples used for DGGE, clone library, and qPCR (unless noted by (C)); all other samples were used for qPCR only.
[c] Samples that were not used in the qPCR analysis.

time is less than the typical doubling time for microbes in these communities. All samples were stored dry and at room temperature until the DNA was extracted. This method is recommended for preserving arid land soils such as our desert BSC samples (Campbell et al., 2009), because it prevents microbial activity in a naturally occurring manner, without the cell damage that may be associated with freezing and, particularly, thawing cycles. The Ultra Clean Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA), proven to yield similar results as those obtained with other manual methods, was used to isolate the DNA which was then stored at −80 °C until use. Community DNA was checked for quality against an EZ Load Precision Molecular Mass Ruler (Bio-Rad Laboratories, Hercules, CA, USA) by standard gel electrophoresis followed by ethidium bromide staining and imaging using the Fluor-S Multi-Imager system (Bio-Rad Laboratories). DNA was quantified using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

2.2. Determination of the archaeal and bacterial abundance by Taqman quantitative-PCR

Taqman technology for quantitative-PCR (qPCR) (Heid et al., 1996) was used to quantify the number of archaeal and bacterial 16S rRNA gene copies present in community DNA extracts from BSC samples (Table 1). For this study, either the primer pair Arch349F/Arch806R or Bac349F/Bac806R (Takai and Horikoshi, 2000) was used for specific amplification of the archaeal or bacterial 16S rRNA genes, respectively. In order to ensure specific binding, Taqman qPCR employed additional probes for both the Archaea (Arch516F) and Bacteria (Bac516F) according to the method described by Takai and Horikoshi (2000). To amplify the 16S rRNA genes, qPCRs (20 μl) were run on a 7900HT Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) in 384-well plates. Each reaction mixture consisted of 10 μl 2× iTaq Master Mix with ROX (Bio-Rad Laboratories), 800 nM of each primer, 200 nM of the respective Taqman probe (Integrated DNA Technologies Inc., Coralville, IA, USA), and the community DNA template at a concentration of 20 pg μl^{-1}. In amplifying the specific archaeal or bacterial 16S rRNA gene, all qPCRs began with an initial step of 2 min at 50 °C and 10 min at 96 °C, followed by 40 cycles of 25 s at 96 °C and 2 min at 57 °C. Fluorescence data were recorded during the qPCR run and a cycle threshold (C_t) was determined automatically with the SDS software package (Applied Biosystems). Real-time amplification plots of the products were monitored for each reaction for quality control. To generate standard curves for the transformation of C_t values into absolute units (total number of gene copies) by interpolation, dilution series were prepared from genomic DNA of appropriate target organisms (*Halobacterium salinarium* for Archaea and *Escherichia coli* for Bacteria), ranging in concentration from 0.05 to 1000 pg DNA μl^{-1}. These target organisms were chosen because their DNA was readily available in our lab and their 16S rRNA gene copy numbers were known (1 copy in *H. salinarium* and 7 copies in *E. coli*). The standard curves were run simultaneously and the log-linear correlation coefficients, R^2, between the number of 16S rRNA gene copies and C_t values were >0.98 in all standard curves. Each sample extract was run in triplicate to minimize analytical error and average values were reported for each. Failed reactions were not considered for averaging (6 for Archaea, 2 for Bacteria).

2.3. PCR-DGGE fingerprinting of archaeal communities

To generate archaeal 16S rRNA gene amplicons of sufficient size for a denaturing gradient gel electrophoresis (DGGE) analysis, we used the same archaea-specific primers applied in the 16S qPCR analyses in a 2-step nested-PCR with an internal primer based on the qPCR Taqman probe target sequence. The first reaction (50 μl) used the external primer pair Arch349F/Arch806R (Takai and Horikoshi, 2000) at 500 nM each and the second reaction (100 μl) used 30 nM of the internal primer pair Arch516F/Arch806R with a GC-clamp attached to the 5′ end of the forward primer (Muyzer et al., 1993; Takai and Horikoshi, 2000). Each 50 μl PCR contained

5 μl of 10× *Ex Taq* DNA polymerase buffer, 1.25 μl of *Ex Taq* DNA polymerase, 4 μl of a dNTP mixture (2.5 mM each; all from Takara Bio Inc., Otsu, Shiga, Japan), and 1 μl of BSA (New England BioLabs Inc., Ipswich, MA, USA). These quantities were doubled for all 100 μl reactions. All PCRs used 15–20 ng of template DNA and were performed on an iCycler Thermal Cycler (Bio-Rad Laboratories). The first external PCR used a touchdown protocol as follows: initial denaturation for 1 min at 94 °C followed by 20 touchdown cycles (1 min at 94 °C for denaturation, 1 min touchdown annealing with temperatures starting at 60 °C and decreasing 1 °C per cycle, and 3 min extensions at 72 °C). This was followed by ten cycles of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C with a final extension for 7 min at 72 °C. The DNA was purified using the QIAquick Gel Extraction Kit (Qiagen-Sample and Assay Technologies, Valencia, CA, USA) and quantified for use in the second PCR, as follows: initial denaturation (1 min at 96 °C), 26 standard cycles (1 min at 96 °C, 1 min at 50 °C, 1 min at 72 °C), and a final extension (5 min at 72 °C). A positive control, genomic DNA from *H. salinarium*, was used through the course of the nested-PCR (as described above) to verify amplification of the archaeal 16S rRNA gene.

For community fingerprinting using DGGE, a total of 12 community fingerprints (3 from each desert, see Table 1) were analyzed to obtain a representative view of the archaeal population in each desert. Each lane of the acrylamide gel was loaded with 300 ng of the internal nested-PCR product which was run on a 30–60% denaturant gradient at 60 °C for 5.5 h at 180 V in a DCODE Universal Mutation Detection System (Bio-Rad Laboratories). After electrophoresis the gels were stained with ethidium bromide, imaged, and then quantified using Quantity One 1-D Image Analysis software (Bio-Rad Laboratories). Excised bands from the gel were placed in separate vials of 50 μl 10% Tris-buffer solution for four days at 4 °C to allow for the elution of DNA. The DNA was then re-amplified by PCR. The purified PCR products were sequenced commercially and subsequently used in phylogenetic analyses.

2.4. Clone library

To supplement our DGGE analysis, separate clone libraries were constructed for each desert (Chihuahuan, Colorado Plateau, Great Basin, and Sonoran; see Table 1), each targeting the archaeal 16S rRNA genes within representative samples of pooled community DNA (extracts from 3 individual samples per desert in each pool). PCR amplification of a large portion of the entire 16S rRNA gene was accomplished with an Archaea-specific forward primer, A2FB [5′-TTGGCCTTGATCCTGCCGGA-3′ (Lopez-Garcia et al., 2002)] and a universal reverse primer, U1406R [5′-GACGGGCGGTGTGTRCA-3′ (Reysenbach and Pace, 1995)], using the protocol described by Baker and colleagues (Baker et al., 2003) for PCR amplification. Products were purified from agarose gels for ligation using the QIAquick PCR Purification Kit (Qiagen) and quantified as above. Clone libraries were assembled using a TOPO TA Cloning Kit according to the manufacturer's specifications (Invitrogen, Carlsbad, CA, USA). Cloning and transformation success was verified through PCR with 1 μl of clone-containing media as the template DNA. Cloned alleles were sequenced commercially in the forward and reverse directions with the same 16S rRNA primers used to construct the clone library. Resultant consensus sequences were aligned in the CLUSTAL W module (Thompson et al., 1997) of BioEdit (Hall, 1999) (IBIS Biosciences, Carlsbad, CA, USA) and analyzed using the MEGA 4 software package (Tamura et al., 2007). All sequences were then subjected to similarity searches using the BLASTn (Altschul et al., 1997) function of GenBank (http://www.ncbi.nlm.nih.gov), and sequences having high identity with those of our clone library were retrieved to provide phylogenetic context.

2.5. Statistics and cluster analyses

Similarity between samples, on the basis of community fingerprints, was analyzed using the unweighted pair-group method of arithmetic averages (UWPGA) applied to digitized DGGE gel images using Quantity One 1-D Image Analysis software (Bio-Rad). Measured or calculated parameters (gene copy numbers or diversity) between and within groups (Archaea and/or Bacteria for biogeographical provinces or crust types) were assessed for significant differences using the appropriate parametric statistical analyses, Student's *t*-test (Gosset, 1908) or ANOVA, after confirming homoschedasticity by the Levene's test for equal variance. The nonparametric Wilcoxon-based Kruskal–Wallis rank sum test (Wilcoxon, 1945) was used to detect significance in the differences between the ratios of Archaea/Bacteria normalized 16S rDNA gene copy numbers across the biogeographical provinces (Liermann et al., 2004), after first confirming equal variance for sample copy numbers of each domain individually. Failed reactions and samples that did not yield values for both the Archaea and Bacteria were not considered for the ratio analysis. All statistical analyses were performed using the R statistical software package (http://www.r-project.org/).

3. Results

Archaea were detected in all 12 BSC samples tested (see Table 1 for the specific samples used) in an initial PCR-DGGE profiling of community DNA from a variety of regionally disjunct sites. These archaeal assemblages were consistently of very low ecological diversity, displaying 2–5 detectable phylotypes (distinguishable bands in the gel) per sample, wherein 1–2 bands were consistently dominant (Fig. 1). This level of diversity, quantified by Richness and Shannon's Index (Fig. 1), is much lower than what has previously been reported for BSC Bacteria using the same techniques and with equivalent survey effort (Gundlapally and Garcia-Pichel, 2006). Furthermore, the Archaeal populations were remarkably stable with no significant differences in diversity (Richness or Shannon's Index) detected among samples regardless of the sample origin or BSC type (ANOVA; $P > 0.07$). Many of the dominant DGGE bands occupied equivalent positions along the denaturing gradient, suggesting that they represented similar 16S rRNA alleles common to many samples, and pointing to a high degree of conservation in community

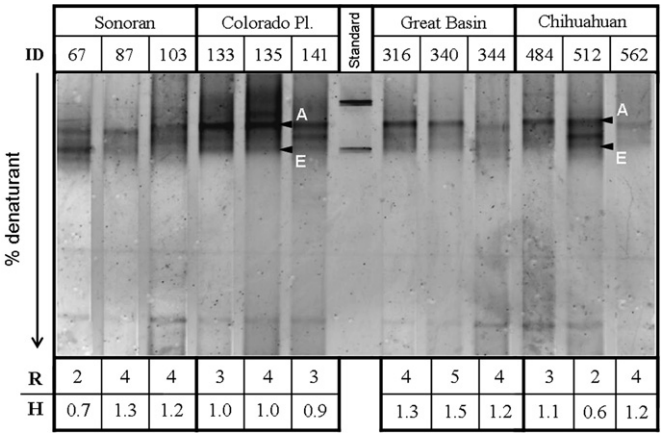

Fig. 1. Denaturing gradient gel electrophoresis fingerprint of archaeal communities in biological soil crust samples from various geographic regions. Each lane represents a sample (the number is given above each lane and can be identified in Table 1), except for the lane that contains a standard (labeled). Values for Richness (R) and Shannon–Weaver diversity indices (H) associated with each fingerprint, calculated from digitize images, are indicated below each lane. Bands later determined to represent phylotypes A and E (see Fig. 2) are indicated by labeled arrows.

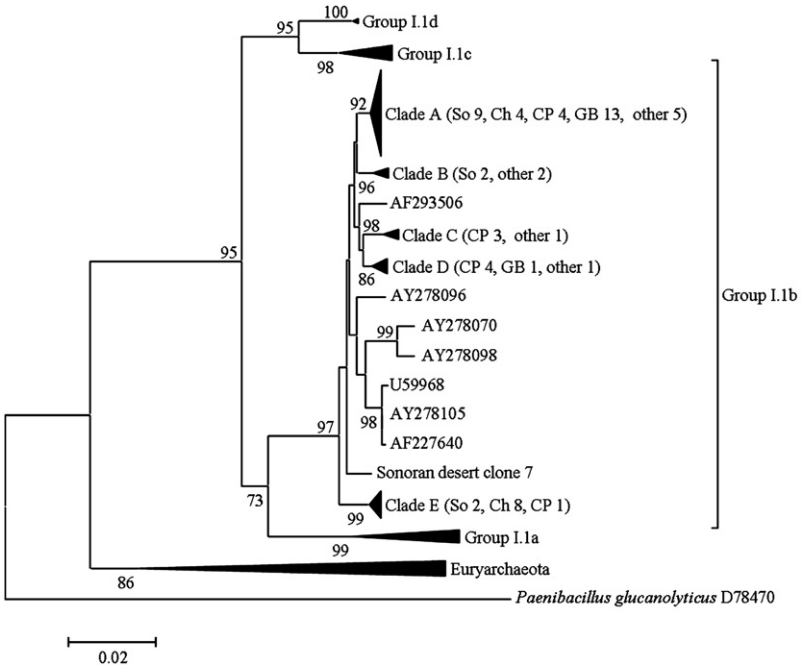

Fig. 2. Neighbor-joining phylogenetic tree of archaeal 16s rRNA gene sequences. Tree was constructed with 52 partial archaeal 16S rRNA gene sequences (~720 bp), obtained from clone libraries of genomic DNA isolated from biological soil crust samples, and 35 additional closely matched sequences from GenBank. For simplicity, some well-supported clades have been collapsed at their nodes. For relevant clades, an identifier and information on the sequences comprising it are indicated to their immediate right (So, Sonoran Desert; Ch, Chihuahuan Desert; CP, Colorado Plateau; GB, Great Basin; *other*, sequences not generated as a part of this study and originating from various environmental samples other than biological soil crusts). Bootstrap values ≥70% are indicated at the nodes.

composition within the regions studied. In fact, no significant ordination with geographical origin could be obtained using UWPGA analyses of the digitized fingerprints (not shown). We successfully excised, re-amplified, and sequenced 23 DGGE bands, which represented four distinct phylotypes (groups of sequences that differed by less than 2%). All were affiliated with the Crenarchaeota, and were most similar to other environmental alleles previously reported from molecular surveys of soils. The sequences have been submitted to GenBank under accession numbers EU434307–EU434328.

In order to improve our phylogenetic resolution and to independently confirm the low levels of archaeal diversity, we also constructed four clone libraries, one for each biogeographical province (see Table 1). For consistency, these libraries were made using DNA template pooled from the same three samples that were used in the DGGE analysis. Of the 52 clones analyzed (Colorado Plateau and Chihuahuan Desert: 12 each; Great Basin and Sonoran Deserts: 14 each), sequences differed by less than 5% among them. These represented six distinct phylotypes (Fig. 2), all of which were clearly related to the "Group I.1b" of the Crenarchaeota (DeLong, 1998). Diversity in these clone libraries saturated quite rapidly as the sequences from each desert represented only two to four phylotypes. One of these, designated as phylotype "A", was the most common and widespread; comprising 58% (30 individual sequences) of the total from all deserts. The most intense bands in the DGGE fingerprints, when sequenced, were identical to phylotype A (see also Fig. 1). Phylotype A was also indistinguishable from a variety of environmental sequences of various origins (Fig. 2), which grouped together into a well-supported clade (Clade A). Another phylotype, "E", was commonly detected (11 unique sequences) in all areas except the Great Basin, and was quite common in warmer, southern deserts. Phylotype E formed a deep-branching clade within Group I.1b and also contained known sequences from other environments. Some sequences from DGGE bands (see also Fig. 1) could clearly be assigned to this clade. Phylotype "D" was the third most common and contained 5 sequences from northern deserts that clustered together with one other known sequence from soil. The more rare phylotypes (B, C, and Sonoran Desert clone 7), while conforming three well-supported, distinct clades, were populated by only 1 or 2 sequences, precluding further interpretation. All of these sequences have been submitted to GenBank under accession numbers EU422997–EU423048.

Statistical tabulations of population density determinations, based on qPCR of the 16S rRNA gene of Archaea and Bacteria in BSCs, are found in Tables 2 and 3. In this large sample set, Archaea were detected in BSCs from all biogeographical areas and in all sites. In this

Table 2
Population density of Archaea and Bacteria in biological soil crusts according to biogeographical province of origin assessed by the copy number of 16S rRNA genes.

Province	n[a]	Archaea (10^7 copies g^{-1} soil)		Bacteria (10^7 copies g^{-1} soil)		Ratio of Archaea to Bacteria[b]	
		Range	Average (±SD)	Range	Average (±SD)	Range	Average
Sonoran	10	0.436–5.64	2.58 ± 1.72	9.01–176	64.0 ± 53.4	0.021–0.093	0.048
Chihuahuan	6	0.128–2.20	0.951 ± 0.817	4.11–28.2	14.1 ± 8.89	0.018–0.147	0.070
Colorado Plateau	12	0.155–5.17	2.02 ± 1.68	3.2–80	35.1 ± 25.5	0.038–0.102	0.056
Great Basin	11	0.311–5.19	2.73 ± 1.79	16.3–353	86.7 ± 102	0.015–0.139	0.048
All Deserts	39	0.128–5.64	2.20 ± 1.68	3.20–353	53.8 ± 65.7	0.015–0.147	0.054

[a] Number of samples used for quantification.
[b] Ratio of archaeal to bacterial 16S rRNA copies calculated for paired determinations in each sample.

Table 3
Population density of Archaea and Bacteria in different crust types assessed by the copy number of 16S rRNA genes.

Crust type[a]	n[b]	Archaea (10^7 copies g^{-1} soil)		Bacteria (10^7 copies g^{-1} soil)		Ratio of Archaea to Bacteria[c]	
		Range	Average (±SD)	Range	Average (±SD)	Range	Average
Light	12	0.155–5.64	1.86 ± 1.80	3.20–176	48.0 ± 54.6	0.018–0.093	0.049
Dark	11	0.627–5.17	2.40 ± 1.54	14.0–80.0	39.6 ± 18.3	0.033–0.102	0.058
Mossy	3	0.605–4.69	2.53 ± 2.06	16.3–187	80.7 ± 92.8	0.025–0.059	0.040
Lichen	13	0.128–5.19	2.27 ± 1.77	4.11–353	65.0 ± 93.2	0.015–0.147	0.058
All types	39	0.128–5.64	2.20 ± 1.68	3.20–353	53.8 ± 65.7	0.015–0.147	0.054

[a] See Table 1 for crust descriptions.
[b] Number of samples used for quantification.
[c] Ratio of archaeal to bacterial 16S rRNA copies calculated for paired determinations in each sample.

sense, Archaea can be deemed ubiquitous components of BSCs. The population densities of Archaea were generally around 10^7 16S rRNA gene copies per gram of soil, whereas the bacterial populations were invariably higher, in the order of 10^8–10^9 copies per gram of soil. The latter figure is consistent with independently determined bacterial populations in other sample sets of BSCs (Nagy et al., 2005; Bates and Garcia-Pichel, 2009) and with previously determined DAPI counts (Garcia-Pichel et al., 2003). There was a general trend for both the Archaea and Bacteria population densities to increase with latitude (Fig. 3); however, this trend was not strongly supported (R^2 for log-linear regression of the data <0.2).

We found no significant differences ($P > 0.09$) in population densities of Archaea or Bacteria (Tables 2 and 3) according to the biogeographical province of origin or BSC type. The only significant differences were in the densities of Archaea and Bacteria when compared to each other ($P < 0.02$). This difference was also significant when the dataset was split into geographical or compositional classes ($P < 0.05$), except for "mossy crusts", which contained the smallest sample set ($n = 3$). The ratio of Archaea to Bacteria was remarkably constant among samples, provinces, and crusts types: by this measure, Archaea contributed about 5% of the prokaryotic (Archaea + Bacteria) populations in BSCs.

4. Discussion

We have shown that members of a cluster of Crenarchaeota previously known from molecular surveys of Archaea in a variety of other environments (DeLong, 1998), including soils, represent a ubiquitous and significant component of BSC communities in a variety of biogeographical regions from North America. While the total contribution of archaeal assemblages, around 5% relative to the total prokaryotic community, was moderate in comparison to that attained in marine habitats (Sievert et al., 2000; Karner et al., 2001; Herndl et al., 2005), it is somewhat larger than that typically found in bulk soils (Buckley et al., 1998; Sandaa et al., 1999; Ochsenreiter et al., 2003), though acidic forest soils may also harbor exceptionally large archaeal components (Kemnitz et al., 2007). The fact that only a few phylotypes made up the overwhelming majority of these Crenarchaeal assemblages was unexpected, and makes each of the phylotypes quite abundant in relative terms. Phylotype A, for example, may easily represent 2–3% of all of the microbial BSC population which is equivalent to that reached in bulk soils by common bacterial genera such as *Pseudomonas* or *Burkholderia* (Janssen, 2006). Except for the cyanobacteria, which as primary producers can make up 30–50% of the crust populations, very few crust bacterial phylotypes (e.g., Proteobacteria and Actinobacteria) actually reach representations of that magnitude (Nagy et al., 2005; Gundlapally and Garcia-Pichel, 2006).

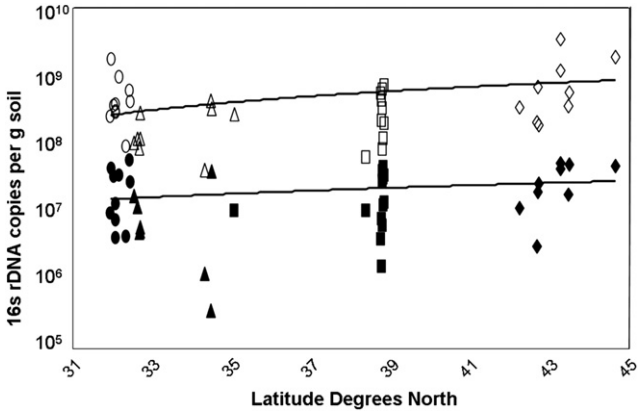

Fig. 3. Population densities of Bacteria and Archaea in samples from biological soil crust communities. Data are presented as a function of latitude gauged by the number of copies of their respective 16S rRNA genes assessed through quantitative-PCR. Bacteria are represented by empty symbols and Archaea are solid symbols. Circles are samples from the Sonoran Desert, triangles denote those from the Chihuahuan, squares correspond to the Colorado Plateau, and diamonds are from the Great Basin.

In the light of the large overall archaeal diversity found in bulk soils (Fierer et al., 2007), including those of deserts, the low diversity of archaeal BSC assemblages emerges as a differential and somewhat surprising trait. It is, therefore, possible that they have adapted to a highly specialized, and obviously successfully exploited, ecological niche. The relative importance of Archaea, their low diversity, and the composition of the assemblages were all rather constant across climatic regions as well as a variety of soil crust types. Perhaps such low diversity is a consequence of the rather extreme conditions in the soil crust surface, which may allow only a small subset of Archaea to thrive. The diversity of Archaea, indeed, seems to be negatively affected by some of these extremes, particularly desiccation, in other environments (Rothrock and Garcia Pichel, 2005).

Whatever the differential role or physiological capabilities of Archaea in BSCs may be, phylogenetic analyses based on sequences of the 16S rRNA gene are not likely to shed much light on the issue, since they could not differentiate the crust phylotypes from those retrieved in other studies of soils. At this level of phylogenetic resolution, the archaeal component of BSCs has to be regarded as an extension of general soil communities rather than a crust-specific one.

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