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The prokaryotic diversity of biological soil crusts in the Sonoran Desert (Organ Pipe Cactus National Monument, AZ)

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Abstract

We studied prokaryotic community structure and composition in biological soil crusts (BSCs) from the Sonoran Desert, and their variability over space and time, using statistically analyzed, PCR-based molecular surveys of environmental 16S rRNA genes. Four sites, tens of km apart, were sampled, 3 times over a 1 year period, collecting 10 duplicate samples every 50 m in each site. Denaturing gradient gel electrophoresis (DGGE) revealed communities much less diverse than those of typical soil assemblages, displaying dominance of some bacterial types. No differences in crust microbial diversity or composition were detected between crusts under plant canopies and those in plant interspaces, indicating a likely crust independence from higher plant resources. However, statistically significant variability with space and time could be detected, and samples within a site were more similar than samples between sites. Both temporal and spatial variability in community composition involved non-dominant members of the community. Extensive sequencing and phylogenetic analysis revealed a large array of bacterial types, many novel. The most common included members of Cyanobacteria, Proteobacteria, Actinobacteria and Acidobacteria. Bacteriodetes, Chloroflexi and Gemmatimonadetes were not seen in high numbers, but were present in all sites, and Deinococci were also detected. Archaea were present, but as minor components. Sonoran BSC communities were distinct in rough compositional character from those in bulk arid soils or agricultural soils, and contained reoccurring, uncultured microbes.

Keywords: Biological soil crusts; Denaturing gradient gel electrophoresis; Real-time PCR; Non-metric multidimensional scaling; Rarefaction analysis; Cyanobacteria

1. Introduction

Biological soil crusts (BSCs) are complex, spatially organized macroscopic associations of microorganisms, lichens and sometimes mosses, located on topsoils in many geographic and climatic soil environments; they are restricted, however, to areas where the environment limits the growth of higher plants to the extent that litter accumulation does not prevent the soil surface from being illuminated [1]. They form in plant interspaces and under plant canopies. BSCs stabilize the soil against erosion [2,3] and are key in nutrient import [4,5]. Bacteria are important components in BSCs, with cyanobacteria typically being the founders and dominant primary producers, largely responsible for both carbon and nitrogen inputs.

The composition of primary producers (cyanobacteria and microalgae) in BSCs has been studied with traditional cultivation-dependent and microscopy methods in a variety of localities [2,6–9]. Recently, polyphasic and molecular studies of crust cyanobacteria have also been presented [10,11]. A few studies have described bacteria directly associated with BSCs [11–13], but no single, exhaustive study of their bacterial components has yet

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been conducted [1]. Thus, little is known about the diversity of non-phototrophic bacterial groups directly associated with BSCs, or about their geographical and climatic variability in arid regions. Nothing is known about the abundance and contributions of Archaea, even though they are known components of other soil systems [14,15].

The fertility island hypothesis, one of the oldest and most widely accepted theories in desert ecology, states that microbial community structure in the soil and the biogeochemical transformations associated with it are dependent on the proximity to higher plants, usually sparse in arid environments [16]. This fact has found support in studies by Herman et al. [17] in Chihuahuan desert soils and Aguilera et al. [18] in Chilean arid soils. Community composition in arid grassland soils varies with depth in the soil profile (0–30 cm; [13]), and variations have been detected in geographically distinct arid soils [19]. In the case of BSCs, shifts in community composition occur at the small vertical scale (stratification), particularly within the top 1 cm [12], and soil chemical composition can affect the composition of cyanobacteria [10]. However, geographic, climatic or other factors, including proximity to plants have not been addressed. In fact, it is plausible that the island hypothesis does not apply to BSCs, since they are independent of plant-bound resources for at least carbon and nitrogen inputs, and can be considered themselves as ''mantles of fertility''.

We analyzed the prokaryotic community structure of typical BSCs from the Sonoran Desert in a diversity of soil settings, over space and time, by sampling select transects in Organ Pipe Cactus National Monument located in the Sonoran Desert of Arizona. This region is one of the hottest and most arid desert regions in the United States, with mean annual temperature ranges of 15–24 °C and receiving only 75–255 mm of average annual rainfall [20]. Prokaryotic communities were analyzed from environmental nucleic acid extracts using denaturing gradient gel electrophoresis (DGGE) separation of PCR amplified 16S rDNA gene fragments. Nonmetric Multidimensional Scaling (NMDS) was used to address sample variability in time and space, as well as the influence of higher plant proximity. Direct sequencing from DGGE bands, phylogenetic analyses and statistical analyses were used to address the species composition, proportion and estimated population in BSCs. Real-time PCR analysis was used as an additional detection and estimation of Archaea.

2. Materials and methods

2.1. Site selection, description and sampling

Four sites were chosen based on accessibility, variability in soil type and visual observation of BSC development. These sites will be referred to as Bates Well Road (BWR), Camino de Dos Repúblicas (CDR), Puerto Blanco Drive (PBD), and Quitobaquito (QBQ). BWR was sampled at three time points: November 2002 (Time 1), April 2003 (Time 2), and October 2003 (Time 3). The other three sites were sampled at times 2 and 3 only.

Site BWR (32°12.044′ N latitude and 112°4.123′ W longitude) was flat to gently sloping and contained ''hyperthermic arid 6'' soil, according to the classification of Jay et al. [21]. Vegetation was sparse with some creosote bush (*Larrea tridentata*) and Prickly pear (*Opuntia* spp.) present. BSCs were inconspicuous and mostly flat in plant interspaces, with some pedicelled (displaying somewhat convoluted surface microtopography) crusts under small plant canopies. They were light in color, with *Peltula* spp. (containing cyanobacteria as symbionts) as the only lichen present. During time point 2, but not at other sampling times, a significant cover of short grasses was also present.

The CDR site (31°53.322′ N latitude and 112°54.136′ W longitude) had ''hyperthermic arid type 4'' soils [21], and contained extensive areas of desert pavement (gravel sized rocks mixed with sand as topsoil feature), which locally excluded BSCs. Most crusts here were in close proximity to plants (*Larrea tridentata* and *Opuntia* spp.), with hardly any annual grasses present. BSCs were visually similar to those at BWR. No lichens were apparent in soil samples.

The PBD site (31°56.359′ N; 112°59.602′ W) was gently sloping, on ''hyperthermic arid type 4'' soils [21]. There was little visual difference between the two time periods sampled. BSCs were well developed both under plants and in interspaces, pedicelled and contained dark areas, due to an abundance of sunscreen-bearing cyanobacteria and cyanolichens. The lichens *Placidium lacinulatum*, *Peltula* spp., and *Collema coccophorum* were identified here. *Larrea tridentata* and *Opuntia* spp. were present, with hardly any annual grasses.

The QBQ site (31°56.534′ N; 113°01.192′ W) contained soils classified as ''hyperthermic arid type 4'' [21]. The uppermost surface was very fine-grained and whitish in color, due to the presence of evaporitic salt deposits originating from waters in nearby Quitobaquito springs. BSCs here were highly pedicelled, with a large presence of black and orange colored lichens and mosses (*Peltula* spp., *Peltula peltulata*, *Placidium* spp., and *Collema coccophorum* were identified). Cholla (*Opuntia* spp.) cacti were common, in addition to prickly pear and creosote, and there were no noticeable differences in vegetation cover between sampling times.

Sampling for each site and time was done with 5 cm diameter Petri dishes; the deeper end of the dish was pushed onto the BSC, previously wetted with filter-purified water, to capture the topsoil. The sample was then displaced using a trowel and secured with a dish cover.

This allowed for intact collection of the crust, approximately 1–2 cm of topsoil. Samples were dehydrated before storage to prevent microbial activity after collection, sealed with tape and placed in individual plastic zip-lock bags, to prevent cross-sample contamination. For each site and time a total of 20 sample plates were taken along a linear transect covering 500 m, consisting of 10, 50 m-distant sampling points. Each point was sampled in duplicate. All samples were coded with GPS, transect position and qualitative proximity to plants (''under plant'' or ''interspace''). Bulk soil samples (1–10 cm deep) for soil chemical analysis were also taken at each site. Samples were stored dry until analysis.

2.2. BSC community DNA extraction and PCR amplification

The DNA in approximately 1 g of vertically sliced soil from each 1–2 cm deep sample was extracted using a MoBio soil DNA kit (MoBio Laboratories Inc., Solano Beach, CA, USA). Extracted DNA was quantified on a 1% agarose gel with TAE buffer base, by comparison with Bio-Rad EZ Load precision molecular mass ruler (Bio-Rad Laboratories, Hercules, CA, USA). Agarose gels were stained with ethidium bromide, then visualized and quantified with a Bio-Rad Fluor-S MultiImager system and Quantity One 4.2.1 (Bio-Rad Laboratories, Hercules, CA, USA). All DNA extracts were stored at −80 °C.

PCR amplification specific for ca. 590 bp-long 16S rRNA gene fragments from the heterogenous bacterial community extract was done using a (GC-clamped) forward primer BAC GM5F(GC) and a reverse primer BAC 907R, universal for the domain Bacteria [22]. For Archaea, forward primer ARCH 931F and reverse (GC clamped) primer UNIV 1392R (GC) were used for amplification of a ca. 460 bp-long 16S rRNA gene fragments [23]. Between 5 and 10 ng of environmental DNA template were used for PCR. For primers GM5F(GC) and 907R, the thermal cycling program from Wade and Garcia Pichel [24] was used. For primer pair 931F/1392(GC)R, 26 cycles of the following were run; 95 °C for 45 s (denaturation), 56 °C for 45 s (annealing) and 72 °C for 45 s (extension), and lastly 1 cycle of 72 °C for 7 min (final extension). All reactions were done in a Bio-Rad iCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) and began with denaturation at 95 °C (hot start) for 5 min (bacteria) and 2 min (archaea) with 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 reaction contained the following: 10 μL of 10× Takara Ex Taq DNA polymerase, 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, Inc., Westbury, NY, USA), and 5–10 ng of template DNA. Quantification of products was done as described for DNA extracts.

2.3. Denaturing gradient gel electrophoresis fingerprinting procedures and analysis

For each sample, 350 ng of PCR product was used in DGGE analysis. The DGGE protocol followed that of Muyzer [25]. Bacterial DGGE gels were constructed with a 30–50% denaturant gradient (80% denaturant consisting of 48 ml of formamide and 50.4 g of urea). Archaeal DGGE gels were constructed with a gradient of 30–80%. All DGGE gels were run at 60 °C, at 200 V, for 4 h in a Bio-Rad DCODE universal mutation detection system (Bio-Rad Laboratories, Hercules, CA, USA). The gels were stained with ethidium bromide and de-stained with nanopure water. Imaging and gel documentation was the same as in agarose gel procedures, and relative intensities of bands were quantified with Quantity One image analyses software. To insure the standard quantification among different gels the same detection sensitivity was used. For PCR amplification of specific alleles, bands were excised from gels with a sterile scalpel and placed in 10% Tris-buffer solution for elution of DNA. Multiple bands in the same gradient line were excised for sequence comparison. The solution (1 μL) was then used for PCR with non-GC clamped primers for bacteria (BAC GM5F and BAC 907R) or Archaea (ARCH 931F and UNIV 1392R), using the thermocycling program above.

DGGE standards were prepared using 16S rRNA fragments three bacterial isolates that had been cultivated from the Colorado Plateau. The standards had been chosen to separate consistently at a given percentage of denaturant and their combination made a useful ladder. These standards were used to align DGGE fingerprint images. Initially, a set of 10 sample duplicates were run to confirm identical sample fingerprints and eliminate any technical variability. Separate DGGE gels were run loaded with samples from each of the 4 sites to allow for direct intra-site comparison. Comparative gels were run with selected samples from each site to allow for inter-site or time-course comparisons.

2.4. Sequencing and phylogenetic analysis

PCR products were purified using the Qiagen PCR Purification kit (Qiagen Sciences, MD, USA) and 100 ng of purified product was used for sequencing. All sequencing was performed commercially with an ABI prism 377 DNA sequencer (Applied Biosystems,

Foster City, CA, USA). Both forward and reverse sequences were obtained and a consensus sequence was determined using the program Sequence Navigator (Applied Biosystems, Foster City, CA, USA). No chimeras were found when sequences were checked with Bellerophon [27]. Clustal W was used to align sequences in ARB [26], where phylogenetic reconstructions were performed by inserting band sequences into base trees previously constructed with virtually complete 16S rRNA sequences, obtained from GenBank. Trees were constructed using a maximum likelihood algorithm and band sequences were inserted by a parsimony algorithm.

2.5. Real-time PCR analysis

We used real-time PCR to estimate the absolute number of copies of Bacterial 16S rDNA present in the DNA extracts. The primer set used was BAC GM5F/BAC 907R. Genomic DNA from *Escherichia coli* was used as a template standard. Real-time analysis was performed on an ABI Prism 700HT sequence detection system (Applied Biosystems, Foster City, CA, USA) using "SYBR green" as a fluorescent dye for detection of dsDNA product. The cycle threshold for each sample was determined, from sample fluorescence, using SDS detection and recording software (Applied Biosystems, Foster City, CA, USA). The quantity of template in the environmental sample was interpolated in a linear regression obtained from standards. Quantity was transformed from mass to 16S rDNA gene copies, knowing that *Escherichia coli* has 7 rRNA operons [28].

2.6. Soil analyses

Analysis of soil physical and chemical properties was performed on approximately 200 g of soil from each of the 4 sites. Measurements of percent organic matter, pH, electrical conductivity, and particle composition (sand, clay, and silt), total reactive nitrogen, phosphorous, and nitrate, plant-available potassium, and the sodium absorption ratio of calcium, potassium, magnesium, and sodium were obtained. All analysis was performed commercially at the Brigham Young University Soil and Plant Analysis Lab, using previously published procedures [29–35].

2.7. Data analyses and statistics

For community fingerprint comparison of different samples we quantified both 16S rRNA gene allele richness (number of detectable bands) in each lane and Shannon–Weaver Diversity Index [36] using automatic detection and quantification of bands by image analyses. For rarefaction analyses we calculated cumulative richness in samples from the 4 sampling sites using the approach of Nübel et al. [37].

Statistical significance in diversity and richness estimates among samples was determined using a student's *t*-test [38]. For comparison of fingerprints sets, UPGMA (Unweighted pair-wise group arithmetic averages) values were calculated using Quantity One software. NMDS was also used to analyze the similarity among fingerprints of interest. For inter-site, time-course comparisons, and in order to discount procedural variability, a selected representative subset of three samples from each site (or time point), chosen after all sample fingerprints were viewed, was run in a single DGGE gel. Construction of DGGE banding pattern analyses was done according to the methods of van Hannen et al. [39]. Binary matrices from fingerprints (1: band presence and 0: band absence) were imported into SYSTAT software, where a distance matrix was calculated and used for NMDS. This analysis constructs a two-dimensional plot depicting the relationships among a number of observations, presented in a Euclidean plane where measurements that are very similar plot close to each other.

2.8. Nucleotide sequence accession numbers

The sequences found from DGGE analysis included in this study have been deposited with GenBank as Accession Nos. AY647884 through AY647917, AY823515 through AY823518, AY648699, AY61551, and AY833647.

3. Results

3.1. Soil chemistry

All sites were similar in terms of particle size distribution and overall chemical characteristics (data not shown), even though QBQ was somewhat more saline (Sodium-SAR: 201.70 ppm and conductivity at 1.24 $\Delta s/M$). There were, however, marked differences in nutrient availability; nitrogen to phosphorus molar ratios varied from almost 40 (PDB) to around 16 (QBQ) [40].

3.2. Archaea

One DGGE fingerprint using Archaea-specific primers run with samples from BWR (not shown) showed distinct bands throughout the 30–80% denaturant gradient. However, sequences obtained from 20 different bands excised from this gel yielded only one Archaeal match, most similar to the Crenarcheota (Table 1), but only with low similarity (85%). All other sequences matched members of the domain Bacteria, indicating a high degree of non-specific amplification,

Table 1
Unique sequences obtained in this study with accession number and nearest BLAST match in Genbank, percent similarity to it, and phylum assignment

Accession Number	Band ID	Closest relative	Similarity (%)	Phylum
AY647896	35a1	α-Proteobacterium	93	Proteobacteria
AY661551	475	*Chelatococcus asaccharovorans*	97	
AY648699	19a1	*Massilia timonae*	98	
AY647884	19a2	*Massilia timonae*	96	
AY647885	711	*Massilia timonae*	95	
AY647887	431	β-Proteobacterium	89	
AY647886	491	δ-Proteobacterium	91	
AY647888	31a6	Uncultured actinobacterium	98	Actinobacteria
AY647889	652	Uncultured actinobacterium	95	
AY647890	991	Rubrobacteriadae	88	
AY647891	29b4	Chloroflexaceae	91	Chloroflexi
AY647892	651	Dehalococcoides	97	
AY647893	1154	*Hymenobacter* sp.	85	Bacteriodetes
AY647894	615	*Hymenobacter* sp.	85	
AY647895	1011	*Flavobacterium* sp.	95	
AY647897	29F	*Taxeobacter* sp.	91	
AY647909	1132	Alga (AF497903)	90	Cyanobacteria
AY647905	1152	*Chroococidiopsis* sp.	89	
AY647901	1193	Clone (AY099254)	91	
AY647899	691	*Cylindrospermum* sp.	92	
AY647900	1192	*Cylindrospermum* sp.	92	
AY647902	451	*Cylindrospermum* sp.	92	
AY647898	1191	Clone (AF428508)	91	
AY647903	1153	Clone (AF42850)	91	
AY647907	1172	Clone (AF428532)	93	
AY647904	452	Cyanobacterium	99	
AY647906	1131	*Scytonema hyalinum*	89	
AY647908	872	*Synechococcus* sp.	90	
AY647910	19a3	*Microcoleus vaginatus*	98	
AY647911	631	*Microcoleus vaginatus*	93	
AY647912	21a2	*Microcoleus steenstruii*	93	
AY647913	453	*Microcoleus steenstrupii*	95	
AY647914	19a7	*Microcoleus steenstrupii*	95	
AY647915	612	*Microcoleus steenstrupii*	95	
AY647916	614	*Microcoleus steenstrupii*	79	
AY647917	412	*Occilatoria* sp.	94	
AY823515	35a4	Gemmatimonadetes	93	Gemmatimonadetes
AY823516	693	*Deinococcus* sp.	86	Deinococcus-Thermus
AY823517	492	*Holophaga* sp.	82	Acidobacterium
AY823518	35a2	Bacterium (AB094797)	85	*incertae sedis*
AY833647	233a	Uncultured crenarchaeote	94	Crenarcheaota

Additional sequences, differing by less than 2% from those listed here, are not included, but were used in the analyses.

probably due to a lack of an appropriate amount of template.

3.3. Bacterial quantification

We quantified, by real-time PCR, the abundance of amplifiable 16S rDNA copies of Bacteria. In site BWR, CDR, PBD and QBQ the 16S gene copy totals were 1.62×10^8, 1.58×10^8, 1.39×10^8, and 9.03×10^8, respectively. Bacteria copy numbers estimated with this method were congruent with bacterial abundance measured in BSCs by DAPI staining elsewhere [12].

3.4. Bacterial DGGE fingerprints

We ran DGGE profiles specific for Bacteria for each transect at time periods 1 and 2. DGGE fingerprints for each transect at each time period are summarized by measurements of average richness, average Shannon–Weaver Diversity Index and UPGMA similarity (Table 2). There were no statistically significant differences in estimates of average Shannon–Weaver Diversity among different sites. However, differences were significant for estimates of average richness among sites and between time points at sites PBD and CDR. UPGMA similarity

Table 2
Summary of diversity and richness for each site and time obtained from quantified DGGE fingerprints

Site	Time period	Samples for analysis	Richness (R) (# of Bands)	Shannon–Weaver diversity indices (H')	UPGMA[a]	UPGMA[b]
BWR	1	9	19 ± 3	2.73 ± 0.42	0.40	N/A
	2	10	22 ± 4*	3.02 ± 0.28	0.43	N/A
	3	9	22 ± 4*	2.66 ± 0.31	0.30	0.42
CDR	2	10	25 ± 4*,**	2.38 ± 0.39	0.43	N/A
	3	3	14 ± 1*,**	2.31 ± 0.27	0.38	0.50
QBQ	2	10	12 ± 3*	2.04 ± 0.23	0.49	0.60
	3	3	14 ± 3*	2.60 ± 0.74	0.38	0.57
PBD	2	10	19 ± 6**	2.03 ± 0.49	0.25	0.40
	3	3	14 ± 6*,**	2.32 ± 0.32	0.38	0.42

[a] Minimum similarity between all samples in a transect.
[b] Minimum similarity between transect samples, with 1 sample outlier eliminated.
* Indicates significance of 0.05 or less from the total mean of all measurements.
** Indicates significance of 0.05 or less between time point means.

values for intra-site sample comparisons were below 0.49 in all cases, and increased significantly if the most deviant sample in each set (outlier) was omitted from the analysis. For comparison, the UPGMA similarity among samples in all 4 sites (run separately at time point 2) was only 0.39.

NMDS analyses specifically aimed at probing differences in community structure in samples from under plant canopies vs. those situated in plant interspaces (done on samples from all sites at time period 2), could not detect any, even though they showed that samples in interspaces tended to be more variable than samples under plants (Fig. 1(a)). An NMDS comparison of a subset of three samples from all sites (at time points 2 and 3) also indicated that variability within sites was smaller than overall variability (Fig. 1(b)), as was suggested by UPGMA results (see above). Temporal variations were also detected by NMDS with fingerprints obtained in samples from one site at one time period being more similar than overall temporal similarity (Fig. 2) in the same site. The plot also indicates a step-wise shift in community composition with time.

3.5. General molecular survey of community members

Sequencing efforts of bacterial DGGE bands were extensive and involved BWR at times 1 and 2, as well as CDR, PBD and QBQ at time 2. We could successfully retrieve, sequence and identify bands whose contribution to total PCR-amplified DNA, as detected by our imaging software, averaged 31% per sample. In total, we obtained and analyzed 74 sequences. Extrapolation of cumulative richness plots (rarefaction) indicated that in the 4 sites at time period 2 there were some 62 unique sequence types to be potentially detected by our methods when using a large number of samples (Fig. 3); 40 unique sequence types were actually obtained (Table 1). This is much less than the 74 bands sequenced, since many of the sequences were retrieved repeatedly, and independently, from different fingerprints. When rarefaction analyses were carried out on a single transect (data not shown), estimates around 30 maximally detectable phylotypes were obtained, where 10 had been successfully sequenced.

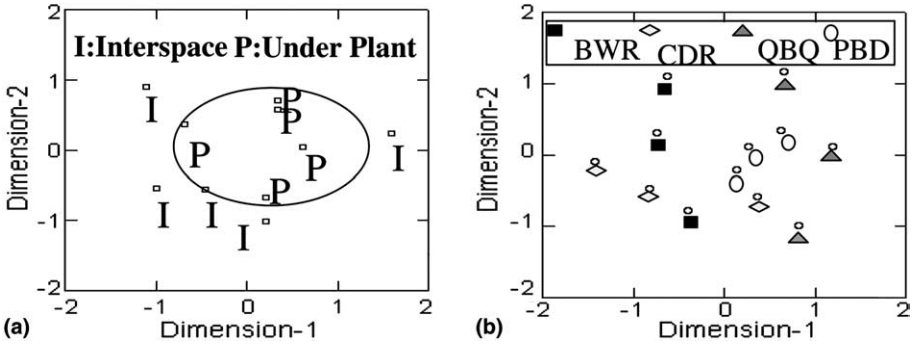

Fig. 1. Non-metric Multi-Dimensional Scaling (NMDS) plot comparison of fingerprints. (a) Samples from plant interspace crusts (I) and under plant canopy crusts (P) in site CDR at time period 2; divergence in community structure within the subset P (circled) is smaller that that of subset I. (b) Samples from all sites (legend in insert); overall divergence is much larger that that obtained within each site.

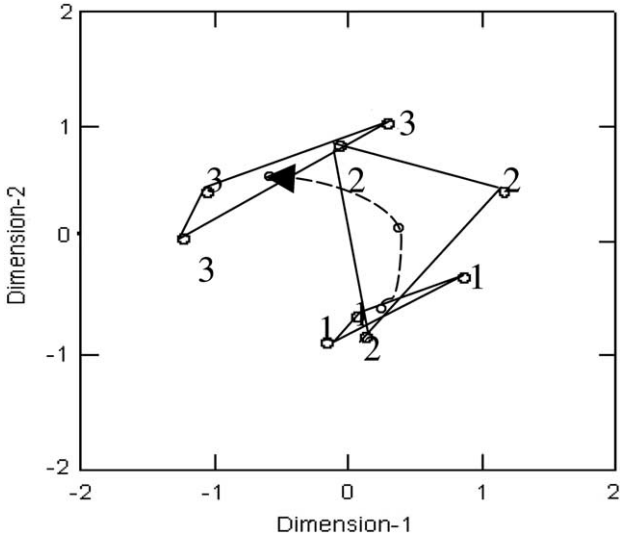

Fig. 2. NMDS analyses to assess temporal variability in community structure in site BWR. Points are labeled according to the time-point of sampling (1 = November 2002, 2 = April 2003, 3 = October 2003). Overall divergence in fingerprints is much larger than the divergence obtained at any one time-point, indicating the role of temporally varying parameters in determining community composition. A vector of dynamic change was been constructed by joining the center of mass of the respective triangles formed by samples in each time point to describe the general trends in community composition with time within the two-dimensional space.

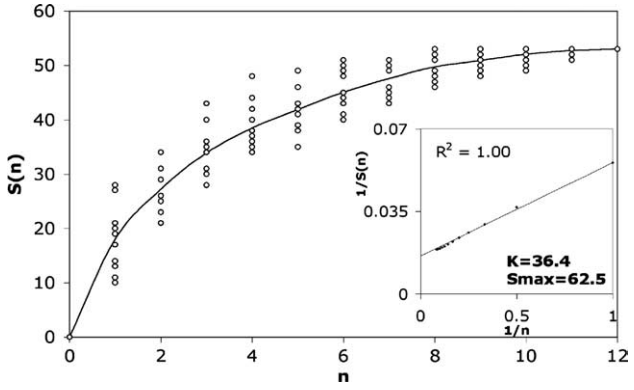

Fig. 3. Rarefaction analyses of bacterial diversity estimates for the entire set of sites. The cumulative number of unique denaturing gradient gel electrophoresis (DGGE) bands $S(n)$, is plotted as a function of the number of samples analyzed, n. All possible sequence combinations are included with equal weight to eliminate the possibility of variation in curve shape due to the order of accumulation. Double reciprocal plot fit is in insert, with Pearson correlation coefficients, R^2, estimates of asymptotic total regional richness, S_{max}, and half saturation constants, K, are shown.

Overall, representatives of 9 bacterial phyla, and several novel, deep-rooted lines of descent were found. A simple tally of sequences assigned to known phyla is in Fig. 4. Cyanobacteria were the most abundant, followed by the Proteobacteria, Actinobacteria, Bacteriodetes, Chloroflexi as common, and the Acidobacteria, Deinococcus and Gemmatimonadetes present in detectable quantities. One sequence had so little similarity to known phyla that it was treated as a separate, ''unknown'' group. This simple tally does not really measure the abundance of each phylotype in the original DNA extracts, since some sequences represented common and strong DGGE bands while others were obtained from weak and/or sparsely occurring bands. To account for this we calculated the relative contribution of each phylotype to the total 16S rDNA amplified by PCR, by using frequency of occurrence in the various transects, and the relative intensity of the corresponding bands in a sample. When this was done, the importance of Actinobacteria and Acidobacteria to the overall community increased greatly.

Phylogenetic neighbor-joining trees for the Cyanobacteria, Proteobacteria, Actinobacteria, Bacteriodetes, and Chloroflexi, were constructed with public, full 16S rRNA gene sequences; band sequences were inserted therein using parsimony. Trees are shown for the Proteobacteria and Cyanobacteria (Fig. 5(a) and (b)), the groups from which the most sequences were obtained. In the Cyanobacteria (Fig. 5(a)), a numerous and diverse clade was evident within the heterocystous (Nostocales), with a closest match to the genus *Scytonema*. Another large, diverse clade closely matched cultured sequences from soil crust isolates assigned to the morphogenus *Microcoleus steenstrupii*. These sequences represented the most common and abundant single microbial group in the crusts. Two sequences grouped closely with strains of the morphospecies *M. vaginatus*. Individual sequences grouped closely with the heterocystous groups (*Scytonema* and *Anabaenopsis*), and a unicellular cyanobacterium (*Synechococcus*).

In the β-Proteobacteria (Fig. 5(b)), all sequences were close to cultured members of the Oxalobacteraceae (one *Massilia* spp. clone, two phenanthrene-degrading bacteria and a *Massilia timonae* strain). Together they make up one of the most clearly defined and common non-phototrophic groups in the crusts. Two other proteobacterial phylotypes, grouped basal to the main proteobacterial subdivisions, and a third was allied closely with a strain *Bosea thiooxidans*. One sequence grouped closely with an uncultured ''candidate division OP8'' environmental clone.

For Actinobacteria, phylotypes did not group with known species, but together in a cluster basal to the *Rubrobacter* subdivision. Three sequences were obtained for the Bacteriodetes. One grouped closely with a *Flavobacterium frigoris* strain and the other two matched closest to a soil crust strain obtained from this same environment (Nagy and Garcia-Pichel, unpublished research). Two phylotypes fit in the Chloroflexi, grouped basally to the branch of phototrophs (*Chloroflexus* and *Roseiflexus* clades), and more distant to the halogenated-compound respirers (*Dehalococcoides* clade).

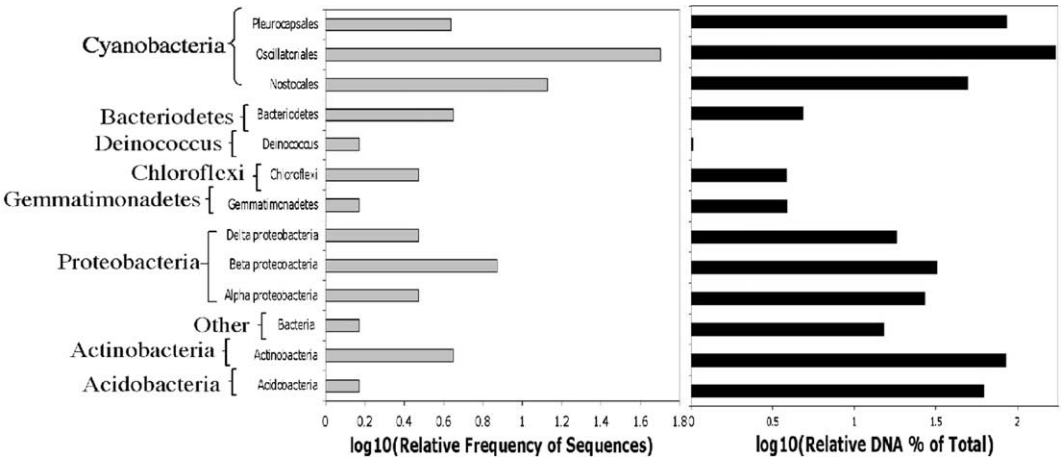

Fig. 4. Relative contribution of major bacterial divisions to the communities in biological soil crusts from Organ Pipe National Monument on the basis of a tally of BLAST placement in sequences recovered from all fingerprints. The right graph is a simple frequency distribution of unique 16S rDNA sequences. In the left graph, each sequence has been weighted by its relative abundance in the total PCR amplification as calculated by image analyses of band intensity and frequency of occurrence.

Alternative neighbor joining trees with bootstrap analyses using Paup 4.0b10, and based on the 500–600 bp fragment common to database sequences and DGGE sequences, yielded almost identical results to trees made with ARB (data not shown). The only exception was the placement of sequence ''Band 651'', which clustered within, rather than outside, the *Dehalococcoides* branch in the Chloroflexi (with a bootstrap value of 80%).

4. Discussion

4.1. Diversity, spatial and temporal variations in BSC community structure

Community diversity in Sonoran BSCs as judged, for example, by simple transect-averaged richness estimates, which varied between 14 and 24 detectable DGGE bands per sample, must be considered relatively low compared with the typical patterns obtained using similar techniques in mesic, non-stressed soil communities. There, the number of detectable bands can be in the hundreds, with no clear dominance patterns present [41]. BSC richness values are in the range of those obtained (using DGGE fingerprints with Bacteria-specific primers) in other cyanobacteria-dominated biofilms, such as microbial mats [42] or in plant-associated soil communities [43]. This low level of diversity may be due to the extreme nature of the crust microhabitat, which is exposed to high levels of solar radiation and very high temperatures, as well as strong and recurrent short-lived wetting-desiccation events [44]. It could also be due to the functional dependence of the community on rather simple carbon source excretion products and decaying biomass of cyanobacteria. It is probably this comparatively low level of richness that enabled the depth of coverage attained in our survey, as estimated by rarefaction analyses (Fig. 3), which may have required a significantly larger effort in typical soil environments [45]. Statistically significant differences in richness could be detected among sites, however, and also between sampling times in the same site. This was not mirrored by significant changes in Shannon–Weaver Diversity indices, which remained stable over space and time. Judging from qualitative observations of the DGGE fingerprints, this was due to the fact that dominant bands, which play a large role in determining the value of Shannon–Weaver Diversity wherever clear dominant members exist, were widespread and did not vary much. It was minor bacterial components that fluctuated the most.

NMDS analysis, which uses un-weighted absence/presence for typifying a particular fingerprint and therefore equates minor and major components, helped visualize such changes. That variability in community structure among samples in a single site was smaller than the overall variability between sites (Fig. 2(b)), speaks for a role of particular microclimatic or edaphic characteristics in influencing, if surely not determining, community composition. Interestingly, BSC communities under plant canopies or in plant interspaces could not be distinguished on the basis of community fingerprint similarity (by UPGMA) nor by overall richness or diversity, indicating that at this site the influence of plants is not significant. This contributes to the theory that BSCs are ''mantles'' of fertility, independent of ''fertility islands''. However, under-plant samples showed less internal variability than interspace samples. Plants may indeed dampen environmental factors that cause variation in community composition causing community structure to remain more homogeneous. We

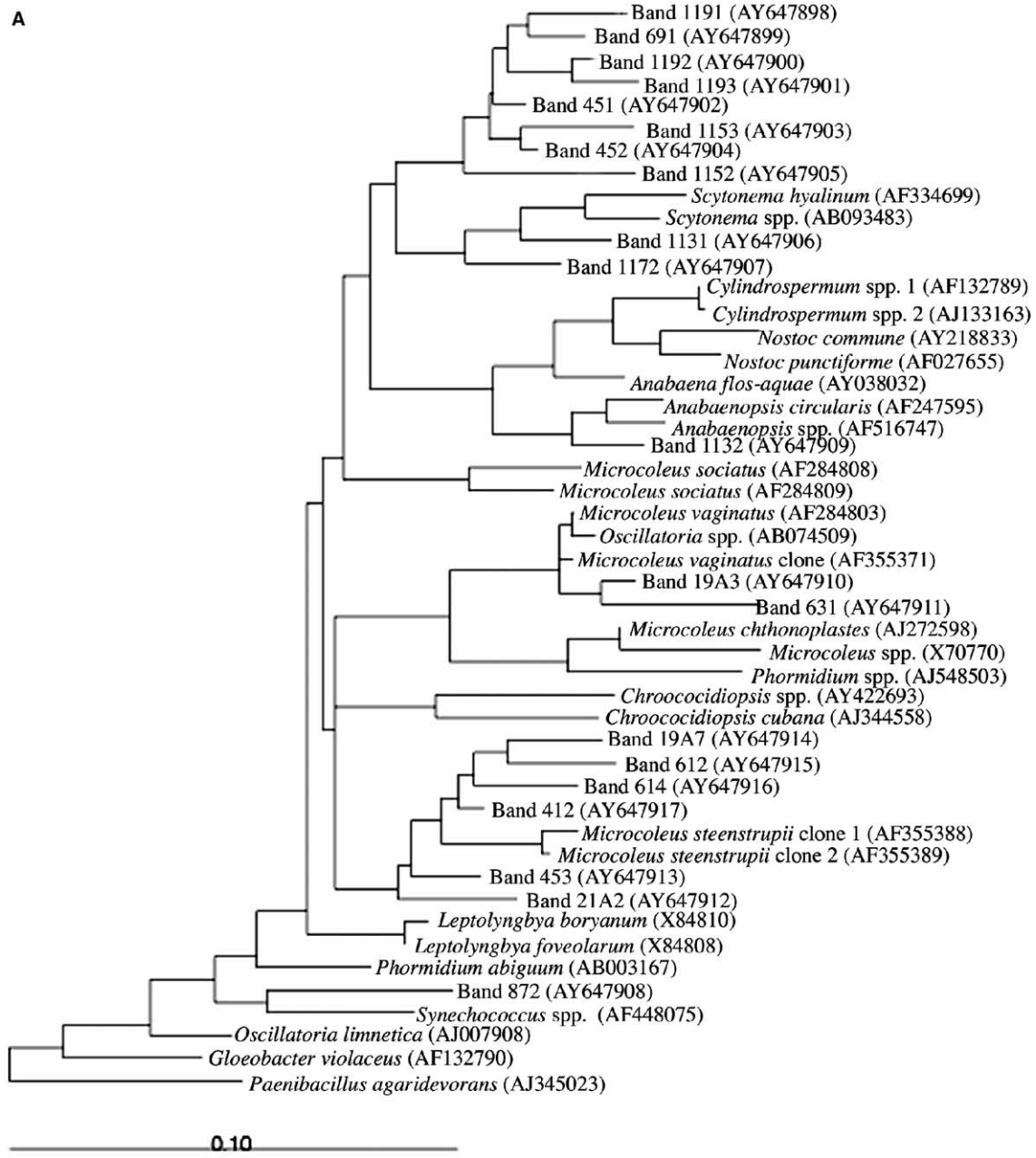

Fig. 5. Reconstruction of phylogenetic relationships of the two major groups of bacteria detected by sequencing. Separate maximum likelihood trees (with one outgroup sequence) were constructed using ARB, including virtually complete 16S rRNA gene sequences available publicly and selected according to initial BLAST similarity to our novel (partial) sequences. Partial sequences were then inserted into the corresponding tree using maximum parsimony without changing the overall tree topology. A: Cyanobacteria, B: Proteobacteria.

could also detect temporal shifts in community structure in a single site that appeared to happen in a successional mode, with samples separated by longer periods being more dissimilar. These changes (again, involving the least abundant phylotypes) could be due to a steady increase in precipitation events over the course of the year sampled, after a very prolonged drought period that had taken place prior to the first sampling date (see Larson, [46]). But temporal sampling was neither dense nor sustained enough to correlate the shifts with any particular seasonal or pulsed events.

4.2. Community composition

We are cognizant of the possible biases and problems associated with the use of PCR-based molecular surveys such as differential extraction and amplification, and the presence of multiple 16S rRNA operons in some taxa [47]. Also, fingerprinting techniques such as DGGE have limited resolution [48]. Nevertheless the picture drawn by this survey was internally consistent, in that common bands (and sequences) were repeatedly obtained in independent analyses and variations in

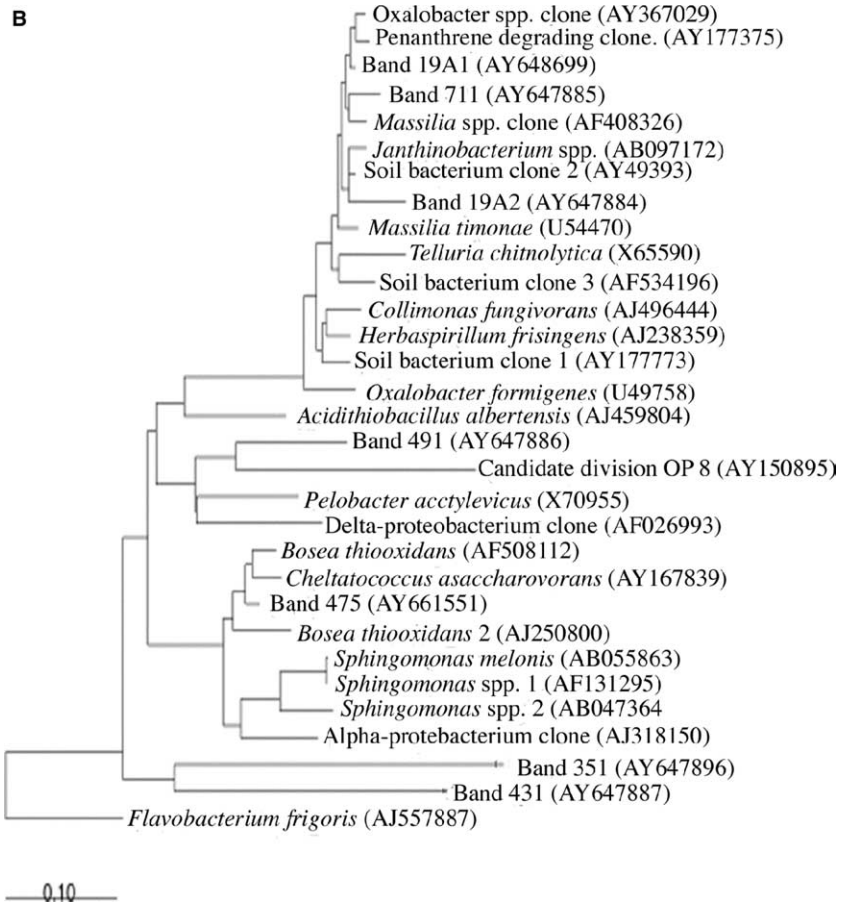

Fig. 5 (*continued*)

community composition among samples in a transect involved usually the least abundant members. While we regard frequencies and tallies as indicative rather than absolute, the rough traits of community composition seem robust and clearly differentiate the surveys of BSC microbial assemblages here from those obtained with a variety of methods in other soil communities (Table 3). Major and minor bacterial components of Sonoran Desert BSCs are most similar to BSCs from the Colorado Plateau, and less similar to communities in bulk arid soils and even less to those of bulk mesic agricultural soils, respectively.

In contrast to Archaea, the Bacteria were major components of Sonoran BSCs, with a diverse array of phylotypes present. Cyanobacteria were clearly the most abundant members of BSCs in this environment (other

Table 3
Ranking comparison of bacteria detected and identified from various molecular based surveys, in different soil environments

Sonoran BSCs (percent of DNA amplified and identified)[a]	Colorado Plataeu BSCs (percent of DNA amplified and identified)[b]	Arid soils (percent of clones)[c]	Agricultural soils (percent rRNA present in soil)[d]
Cyanobacteria (54.8)	Cyanobacteria (38.4)	Acidobacteria-like (51.1)	Proteobacteria (27)
Actinobacteria (15.1)	Proteobacteria (16.3)	Proteobacteria (15.5)	Actinobacteria (11.1)
Proteobacteria (13.8)	Actinobacteria (11.8)	Flexibacteria and relatives (13.3)	Planctomycetes (7.2)
Acidobacteria (11.1)	Bacteriodetes (10.6)	Actinobacteria (6.7)	Acidobacteria (3.5)
Bacteriodetes (0.9)	Firmicutes/Bacilli (5.2)	Planctomycetes (4.5)	Verrucomicrobia (1.9)
Chloroflexi (0.7)	Thermomicrobiales (2.9)	Unknown (8.9)	Bacteriodetes (0.4)
Gemmatimonadetes (0.7)	Acidobacteria (2.5)		
Deinococcus/Thermus (0.2)	Unaffiliated alleles (12.6)		
Unknown (2.7%)			

[a] This work.
[b] Gundlapally and Garcia-Pichel, unpublished.
[c] Kuske et al. [52].
[d] Buckley and Schmidt [51].

oxygenic phototrophs such as the green algal *Geminella*, however, may be dominant in acidic crusts; [49]). This was expected from previous work, both microscopic and molecular in nature. The clade of sequences around that of *M. steenstrupii*, a small, filamentous cyanobacterium previously reported from BSCs [47,50] was the most abundant and diverse. The abundance and dominance of sequences in the *M. steenstrupii* clade was surprising, since the dominant cyanobacterium in BSC from the Colorado Plateau and other locations is clearly *M. vaginatus* [3,10,12]. Obviously BSCs from different biogeographical provinces may select for different filamentous cyanobacteria as a dominant prokaryotic member. These two dominant species may have very different adaptive abilities, perhaps related to freezing (uncommon in the Sonoran, but common in the high Plateau) or to maximal temperatures (higher in the Sonoran). It is noteworthy that the morphogenus *Microcoleus* is not well supported by phylogetic studies [10], and actually the *M. vaginatus* and *M. steentrupii* clades are not closely related, in spite of sharing a generic epithet.

Another important cyanobacterial clade of environmental sequences was among the heterocystous cyanobacteria, as could also be expected from microscopy surveys, but many of those phylotypes formed a phylogenetic group hard to ally with well-known morphogenera. These organisms are likely responsible for the large rates of nitrogen fixation measured in most BSCs and probably ecologically key to mature BSCs.

Among non-phototrophs, the Proteobacteria were the most important group. They are also quite common in BSCs from the Colorado Plateau [12], and in bulk arid soils [51]. In these BSCs, β-Proteobacteria were the most prominent subdivision, primarily matching members of the family Oxalobacteraceae, whereas α-Proteobacteria are the most important in agricultural soils [50]. The high relative abundance of Oxalobacteria seems to be a trait particular to the BSC communities and it may be interesting to speculate if it has to do with their ability to degrade oxalate, a compound accumulated or excreted by many desert plants, lichens and fungi. The Actinobacteria were the next major component of Sonoran BSC communities. They are also important in agricultural soils and have been detected in BSCs from the Colorado Plateau [12], and bulk arid soils [52]. One sequence matched well the genus *Rubrobacter*, a deeply branching subdivision of the Actinobacteria previously described from Australian arid soils [53]. The rest formed its own deep rooting branch in the tree, basal to the *Rubrobacter* subdivision and represent clearly novel, uncultivated members of the community. The Acidobacteria are a little known, but important component of desert soils at large [13,52] and relatively minor components of agricultural soils [50] and Colorado Plateau BSCs [12]. They were abundant in our crusts, if perhaps less prominent than in bulk arid soils. Also detected in small quantities were members allied to *Hymenobacter*, *Taxeobacter* and *Flavobacterium* in the Bacteriodetes. Members of the Bacteriodetes have been detected previously in BSCs [12] from the Colorado Plateau, and in low abundance in agricultural soils [50], but not in bulk arid soils. Interestingly, representatives from the Chloroflexi were also obtained, which were well distributed if not very abundant; there are no previous reports of Chloroflexi members from arid soils, either from the phototrophic branch or the heterotrophic branch. Phototrophic Chloroflexi are common in marine microbial mats and hot springs [54,55] in many occasions associated with cyanobacteria. Preliminary pigment analyses by HPLC (not shown) failed to detect bacteriochlorophylls in support of a possible role for anoygenic phototrophs in BSC communities. The *Dehalococcoides* are a clade of organohalide respirers, typical of contaminated sites [56]; this offers little for speculation, since BSCs are pristine natural communities not typically subject to contamination. Obviously the functional significance of these Chloroflexi relatives remains an open question. Members of the Gemmatimonadetes, a phylum recently cultured from common soil [57], were also detected as another minor component in BSCs. Among the Deinococci, common in arid soils, a single sequence was also obtained. But a sizable proportion of the total diversity detected was represented by *sequentiae incertae sedis*, which correspond in one case to possible novel divisions, in another to a relative of the proposed candidate division OP8 from hot springs, and in the last to some quite deeply branching Proteobacteria, which may constitute by themselves a novel subdivision.

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