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ORIGINAL PAPER

Life at the hyperarid margin: novel bacterial diversity in arid soils of the Atacama Desert, Chile

Julia W. Neilson · Jay Quade · Marianyoly Ortiz · William M. Nelson · Antje Legatzki · Fei Tian · Michelle LaComb · Julio L. Betancourt · Rod A. Wing · Carol A. Soderlund · Raina M. Maier

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Abstract Nearly half the earth's surface is occupied by dryland ecosystems, regions susceptible to reduced states of biological productivity caused by climate fluctuations. Of these regions, arid zones located at the interface between vegetated semiarid regions and biologically unproductive hyperarid zones are considered most vulnerable. The objective of this study was to conduct a deep diversity analysis of bacterial communities in unvegetated arid soils of the Atacama Desert, to characterize community structure and infer the functional potential of these communities based on observed phylogenetic associations. A 454-pyrotag analysis was conducted of three unvegetated arid sites located at the hyperarid–arid margin. The analysis revealed communities with unique bacterial diversity marked by high abundances of novel *Actinobacteria* and *Chloroflexi* and low levels of *Acidobacteria* and *Proteobacteria*, phyla that are dominant in many biomes. A 16S rRNA gene library of one site revealed the presence of clones with phylogenetic associations to chemoautotrophic taxa able to obtain energy through oxidation of nitrite, carbon monoxide, iron, or sulfur. Thus, soils at the hyperarid margin were found to harbor a wealth of novel bacteria and to support potentially viable communities with phylogenetic associations to non-phototrophic primary producers and bacteria capable of biogeochemical cycling.

Keywords Arid · Atacama Desert · Bacterial diversity · Pyrosequencing · Soil

Abbreviations
MAP Mean annual precipitation
MDRS Mars Desert Research Station

Introduction

More than 47.2 % of the earth's land area (LA) is dryland, regions climatically characterized by annual deficits in available moisture (Middleton et al. 1997). Three of the dryland regions, the arid (12.1 % LA), semiarid (17.7 % LA) and dry subhumid (9.9 % LA), are collectively considered by the United Nations Convention to Combat Desertification (UNCCD) to be of particular environmental concern due to their susceptibility to desertification or ecosystem degradation. The fourth dryland region includes the naturally hyperarid deserts (7.5 % LA), areas with virtually no potential for biological productivity. Arid regions occupy the interface between the generally well-vegetated semiarid zones and the biologically unproductive

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J. W. Neilson (✉) · M. Ortiz · A. Legatzki · F. Tian · M. LaComb · R. M. Maier
Department of Soil, Water and Environmental Science, University of Arizona, Shantz Bldg Room 429, P.O. Box 210038, Tucson, AZ 85721-0038, USA
e-mail: jneilson@email.arizona.edu

J. Quade
Department of Geosciences, University of Arizona, Tucson, AZ 85721, USA

W. M. Nelson · C. A. Soderlund
Bio5 Institute, University of Arizona, Tucson, AZ 85721, USA

J. L. Betancourt
US Geological Survey, Tucson, AZ, USA

R. A. Wing
Department of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA

hyperarid deserts and are thought to be most vulnerable to global climate change (Ezcurra 2006). Despite the global significance of these arid zones, little is known about the soil bacterial diversity and potential metabolic activity of these regions or the potential contribution of these vast open areas to global biogeochemical cycling.

In this study, we use the Atacama Desert, one of the driest and oldest deserts on earth, as a model ecosystem to profile bacterial diversity in arid soils at the hyperarid margin. Like the hyperarid core, these regions are devoid of vegetation, yet unlike the hyperarid regions that experience consecutive years with no rainfall, the hyperarid margin experiences limited annual precipitation. To date, arid-land microbial ecology has focused primarily on the diversity and community dynamics of fertility islands associated with cryptobiotic desert crusts (Belnap et al. 2001; Nagy et al. 2005), shrub islands (Aanderud et al. 2008; Collins and Cavigelli 2003), or the endolithic communities of translucent stones and gypsum deposits (Dong et al. 2007; Warren-Rhodes et al. 2006; Wierzchos et al. 2006). In contrast, we focus here on the bacterial communities of unvegetated, open soils to characterize the phylogenetic diversity and evaluate the potential for functional diversity. A robust assessment of the bacterial diversity of arid lands is a critical first step toward evaluating the potential contribution of soil bacteria from these regions to ecosystem biogeochemistry.

The Atacama Desert extends along the coast of northern Chile (27°S–18°S) from the Pacific Ocean to the western slopes of the Andes. Aridity has persisted in this region for the past 90 million years (Houston and Hartley 2003), with the onset of hyperaridity in the core area of the Atacama tracked to the middle to late Miocene in association with the uplift of the Andes (Rech et al. 2010). Based on current precipitation records, the core absolute desert below 2500 m in elevation is hyperarid, while regions above 2500 m are considered arid ecosystems. The arid zone can be further separated into the unvegetated, hyperarid margin extending from 2500 to ~3000 m and a perennial vegetation belt of continuous but sparse and species-poor desert scrub and grassland (Arroyo et al. 1998; Maldonado et al. 2005) that stretches above 3000 m. In previous research, we identified a significant difference in structure between the hyperarid bacterial communities (700–2000 m) and those of the arid regions (2500–4500 m) of the Atacama based on a large-scale PCR-denaturing gradient gel electrophoresis (PCR-DGGE) community survey (Drees et al. 2006). Hyperarid community structure was found to be highly consistent over broad LAs (110 km transect distance). In contrast, community structure within the arid region showed variation between soils within the perennial vegetation belt and those near the hyperarid margin.

Our present objective was to document the bacterial diversity of unvegetated soils at the hyperarid margin and to evaluate whether the bacterial communities present have the phylogenetic diversity necessary to function as viable communities with capabilities of contributing to biogeochemical cycling. Three sites were selected along a transect through the hyperarid margin (2500–3000 m). Bacterial phylogenetic profiles were generated for each of the sites using a combination of 454-pyrosequencing and a 16S rRNA gene library. The goal of this study was (1) to use the depth of community analysis provided by high-throughput sequencing to characterize the bacterial diversity of unvegetated arid soils and (2) to infer the functional metabolic potential inherent in these arid soils from observed phylogenetic associations.

Materials and methods

Transect description

Sample sites for the current study were selected from the 2500–2800 m portion of our previously described west–east elevational transect (Fig. 1) traversing the Atacama from the barren coastal range above Antofagasta (23.65°S, 70.24°W) east to the vegetated slopes of the Volcán de Llullaillaco in the Andes (24.72°S, 68.55°W) (Drees et al. 2006). The three sites selected for the current study were

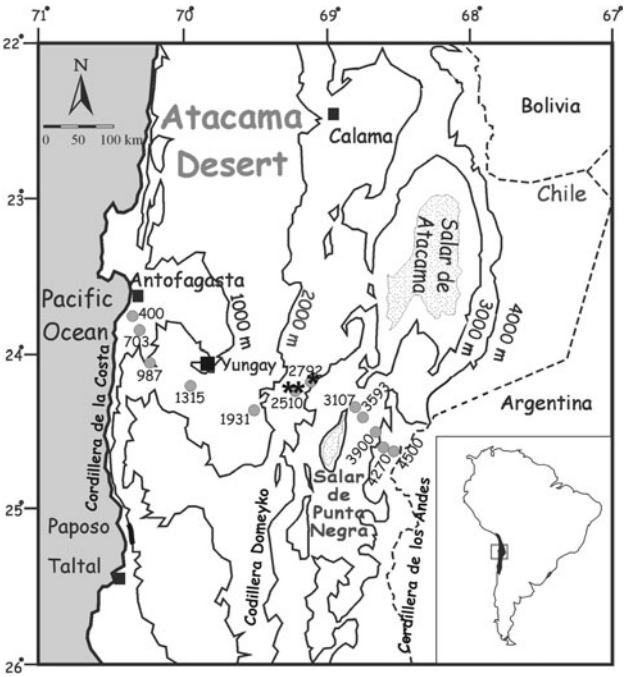

Fig. 1 Map of the Punta Negra transect sampled in October 2002. *Filled gray circles* represent sites sampled in October 2002 and *filled black squares* represent towns along the transect. *Asterisks* indicate sites sampled for this study

Table 1 Site descriptions and physical characteristics of soils sampled

Elevation (m)	Location (latitude, longitude)	pH[a]	EC[b] (mS cm^{-1})	Na (mg g^{-1})	Vegetation type (% Plant cover)	MAT[c] (°C)
2514	24.341°S, 69.251°W	7.9	0.15	0.75	None	15.3
2547	24.339°S, 69.234°W	8.1	1.77	1.33	None	15.2
2728	24.264°S, 69.191°W	8.0	2.49	1.33	Isolated dry stalks *Cistanthe salsoloides* ($\ll$1 %)	14.6

[a] Soil-deionized water ratio of 1:1, University of Arizona Water Quality Center Laboratory

[b] EC, electrical conductivity, University of Arizona Water Quality Center Laboratory

[c] Mean annual temperature. Temperature decreases linearly with elevation; $T(°C) = -1.0 \times 10^{-6}(\text{elev})^2 + 0.0016(\text{elev}) + 17.634$

located at 2514, 2547, and 2728 m between the eastern side of the central plateau and the western slopes of the Cordillera Domeyko (Fig. 1; Table 1). Precipitation in this region of the Atacama is primarily driven by the South American summer monsoon (SASM), carrying moisture from the Atlantic with final rain out on the western slopes of the Andes (Houston and Hartley 2003) creating an orographic effect where increasing elevation generally correlates with increasing precipitation above 2500 m. SASM rainfall events do not reach lower elevation desert regions below 2500 m where vascular plants have been absent for millions of years and precipitation events are extremely rare (Dunai et al. 2005; Houston and Hartley 2003; Quade et al. 2008). Recorded precipitation during a 4-year study in the central Atacama (900–1000 m elevation) was limited to a single rainfall event of 2.3 mm (McKay et al. 2003). Consistent perennial vegetation is limited to elevations between 3500 and 4800 m; however, the lower boundary of this vegetation belt fluctuates with annual precipitation patterns and extended down to 3300 m in June 2007.

Soil sampling, analysis, and DNA extraction

Samples for this study were collected in June 2007 from unvegetated arid sites representing surfaces with distinct geomorphologies (Table 1). The site elevations will be used as site names for the remainder of this paper. Soil pits were dug at each site to a depth of 40 cm. Composite samples were removed from the walls of each pit at a depth of 15–25 cm using a trowel sterilized with Lysol. Samples were sealed and stored at 4 °C until analysis. Prior to analysis, samples were sieved (2 mm) to remove gravels. Results from our previous structural analysis of soil bacterial communities sampled at an elevation of 987 m in 2002 and 2004 revealed that our sample storage strategy successfully preserved the bacterial community structure of these extremely dry desert soils (Drees et al. 2006). Chemical and physical analyses were performed as described in Tables 1 and 2 by the University of Arizona Water Quality Center Laboratory and the Arizona Laboratory for Emerging Contaminants. Total organic carbon (TOC) was quantified manometrically following sample dissolution in 3 N HCl.

Table 2 Characterization of elements important to bacterial nutrition in soil samples

Element (μg g^{-1})	Sample elevation (m)		
	2514	2547	2728
Major elements			
TC[a]	760	11500	1900
TOC[b]	148	221	214/134[c]
TN[a]	BDL	BDL	BDL
P[d]	765	768	2091
S[a]	400	1900	3000
Fe[d]	15457	14865	16566
Ca[d]	4608	30838	11568
Mg[d]	8093	10102	6562
K[d]	2247	4292	2071
Trace elements			
Mn[d]	492	406	622
Cu[d]	23.2	28.2	21.9
Zn[d]	41.2	44.6	40.3
Co[d]	8.9	10.2	11.0
Mo[d]	1.4	1.9	1.9

BDL below detection limit

[a] TN total nitrogen, TC, total carbon, and S, sulfur, determined by high-temperature combustion using an NCS analyzer (Carlo Erba model Na1500) with detection limit 0.01 %. University of Arizona Water Quality Center Laboratory

[b] TOC, total organic carbon, determined manometrically by high-temperature combustion after pretreatment with 3 N HCl (detection limit 20 μg)

[c] Replicate samples were analyzed to evaluate heterogeneity caused by occasional dry plant fragments

[d] Analyzed by inductively coupled plasma mass spectrometer following nitric acid digestion

Total genomic DNA was extracted from multiple 600 mg soil subsamples from each site using the FastDNA Spin Kit for Soils (MP Biomedicals, LLC Solon, OH) as described previously (Drees et al. 2006); extracts were combined and then quantified using the Quant-iT PicoGreen dsDNA kit (Invitrogen Corp, Carlsbad, CA, USA).

Community 16S rRNA genes were amplified separately from each community extract for 454-pyrotag libraries and a 16S rRNA gene library as described below.

454 tag-coded pyrosequencing

The 16S rDNA V6 region of DNA community extracts from the three samples was amplified using the following universal primers: a 1:1 mixture of forward primers 909/917F (5′-AAACTYAAARRAATTGACGG-3′ and 5′-GAATTGACGGGGRCCCGCA-3′) (Keijser et al. 2008) and reverse primer 1070R (5′-AGCTGACGACARCCAT) combined with 454 platform adaptors and a barcode sequence unique to each sample. The 25-µl PCR reaction mix contained 1× Invitrogen High Fidelity PCR buffer (Invitrogen Corp, Carlsbad, CA, USA), 2.5 mM $MgSO_4$, 0.5 µM each primer, 0.4 g L^{-1} unacetylated BSA (Sigma, St. Louis, MO, USA), 200 µM each deoxynucleotide triphosphate, 5 % dimethyl sulfoxide, 1.0 U Platinum High Fidelity *Taq* polymerase (Invitrogen Corp, Carlsbad, CA, USA) and 1–100 pg template DNA. The PCR conditions were 94 °C for 2 min; 30 cycles of 94 °C for 30 s, 55 °C for 40 s, and 68 °C for 40 s with a final extension at 68 °C for 5 min. Amplicons from three reactions were pooled for each sample, and purified by gel electrophoresis and band extraction using the QIAquick gel Extraction Kit (Qiagen Inc., Valencia, CA, USA). Dilutions of initial template DNA (1–100 pg $reaction^{-1}$) were amplified in separate PCR reactions for each sample to evaluate the influence of initial template concentration on the final number of sequence reads generated. Amplicon concentrations were standardized prior to pyrosequencing which was performed by the Arizona Genomics Institute (University of Arizona, Tucson, AZ, USA) using the 454/Roche GS-FLX system according to the manufacturer's guidelines. The sequences generated were deposited in the NCBI Sequence Read Archive under accession number SRP006083.

The sequences were filtered and trimmed according to the following criteria: presence of both forward and reverse primers and appropriate barcode tags; contains no Ns; minimum length of 110 bp; maximum length of 200 bp; trimmed after the 3′ end of 917F (prior to 5′-CACAA-3′) and at the 5′ end of 1070R. OTU clustering was carried out using Mothur v1.12.2 (Schloss et al. 2009) with a 0.03 cutoff. Taxonomic classifications were assigned to OTUs based on a 50 % confidence level using the RDP Classifier (Wang et al. 2007) and Silva databases (Pruesse et al. 2007). In addition, classifications with <50 % confidence were accepted for a specific OTU if both databases agreed on the taxonomic assignment. Rarefaction curves, richness estimates, diversity indices, and community comparisons were computed using the respective Mothur applications (http://www.mothur.org).

16S rRNA gene library

A 16S rRNA gene library was constructed from the site 2547 community DNA extract using primers 27F and 1492R as described previously (Mendez et al. 2008). All clones generated were Sanger sequenced and assigned to OTU groups based on a conservative sequence similarity of ≥99 % to facilitate comparison of the clone OTUs with the shorter 454-pyrotags. Contaminant sequences were identified using a negative clone library constructed from an extraction blank processed in parallel with soil samples. All 454-pyrotags or clones with 100 % similarity to negative clone library sequences were identified as contaminants and removed from the respective data sets. Phylogenetic analysis was performed as described in Fig. 5. Phylogenetic analysis was also performed on unclassified *Chloroflexi* 454-pyrotags (<50 % confidence threshold) following manual insertion into the clone library alignment. Sequences associated with *Chloroflexi* lineages (bootstrap ≥85 %) were classified as *Chloroflexi*. All clone sequences were deposited in the GenBank database with accession numbers JF706661–JF706692.

Results and discussion

Site and soil characterization

All sampled soil pits were dug into alluvial gravels derived from volcanic rock parent materials of varying ages (Table 1). Based on the characteristics of the soils and geomorphic surfaces, site 2514 is very old (>10^6 years) and stable, and probably has not received moisture except by rare rainfall events directly on the site. In contrast, sites 2547 and 2728 are located on younger (10^3 to 10^5 years), semi-active alluvial fan surfaces with historic exposure to moisture from slightly higher elevations as well as from on-site precipitation. The presence of pedogenic gypsum at varying soil depths served as an indicator of the historic aridity of the three sites. Previous work indicates that sites where gypsum occurs deeper in the soil profile have experienced wetter conditions (Dixon and McLaren 2009). The gypsum assumes a range of morphology depending on age, from thin gravel clast coatings in the younger parts of the soil profiles to massive gypcrete in the older parts. Gypcrete deposits were found close to the surface (between 10 and 25 cm) at site 2514 and were located at ≥25 cm at sites 2728 and 2547.

Organic carbon and nitrogen levels were extremely low in all soils indicating extreme oligotrophic conditions (Table 2) comparable to those found in the hyperarid regions (Drees et al. 2006). Vegetation was evaluated at all sites based on both an on-site survey (Table 1) and

microscopic examination of soil samples for the presence of root fragments. No on-site vegetation was present at sites 2514 and 2547, but microscopic examination revealed small root fragments in soils from both sites (1–5 mm in length) indicating a past vegetation history. Specific mean annual precipitation (MAP) data are not available for the individual samples sites, but available records indicate a range of 4–20 mm for all three sites (Houston and Hartley 2003; Luebert and Gajardo 2000). The presence of dry plant stalks at site 2728 was attributed to greater moisture exposure at this site from rare runoff events sourced from the surrounding mountains.

Bacterial community diversity analysis by 454-pyrosequencing

DNA yield from soil extractions was two- to fourfold higher at site 2728 than at sites 2514 and 2547 (data not shown). As a result, pyrosequencing was performed on dilutions of initial DNA template concentrations (1–100 pg reaction^{-1}) from each site to evaluate the influence of initial template concentration on the number of sequence reads generated. All final amplicon concentrations were standardized prior to pyrosequencing. No correlation was observed between template concentration and the final number of sequence reads generated per sample (data not shown); thus, replicates generating the best sequence yield were used for downstream analysis of each community. Preliminary taxonomic characterization of the sequences generated revealed the presence of chloroplast DNA belonging to the genus *Streptophyta* (96–100 % confidence) within the communities. These sequences were presumed to belong to green algal plastids and thus were removed prior to subsequent bacterial diversity analyses.

The ACE estimator was used to compare the α-diversity of the soil communities (Table 3). Estimated species diversity was higher at site 2728 than at sites 2514 and 2547. Rarefaction curves (Fig. 2) also predicted higher species richness in site 2728 than in sites 2547 and 2514. Recall that this site was characterized by higher DNA yields and the presence of dry plant stalks. Community coverage estimates based on the ACE estimator indicated that sites 2514 and 2547 were more completely sampled (89 and 78 %, respectively) than site 2728 (51 % sampled). The Shannon and Simpson indices indicated that overall species richness and evenness were similar for the three sites, despite the higher estimated α-diversity at site 2728.

Similarity among the sites was evaluated using several indices. An OTU distribution analysis (Fig. 3) revealed a significant overlap in α-diversity among the three sites. OTUs shared by all three sites ranged from 22.5 to 26.8 %, and >47% of the OTUs in each community were present in at least two of the three sites. Pairwise community comparisons based on the Sørensen abundance (Sorabund) index revealed strong similarities among all three communities, while the Bray–Curtis index suggested subtle differences. Bray–Curtis found sites 2728 and 2514 to be most similar and 2514 and 2547 least similar (Table 4). This result is surprising considering that sites 2514 and 2547 were just 1.7 km apart and site 2728 was located 9–11 km from these sites. Physical properties that differed between sites 2514 and 2547 included both the location of site 2514 on an older geomorphic surface and the presence of surface gypsum deposits at this site. Gypsum deposits at sites 2547 and 2728 were found at greater depths (≥25 cm), suggesting a higher historic moisture exposure. Site 2514 was also unique in that 23 % of the initial sequences were chloroplast DNA (Table 3), suggesting the presence of green algae at this site. Chloroplast DNA abundance at the other sites was ≤1 sequence. We hypothesize that the abundance of chloroplast DNA at site 2514 may indicate past or present colonization of the surface soil gypsum deposits by endolithic communities. Previous research in the core Atacama Desert at an elevation of 2850 m found that gypsum deposits located at the surface were colonized by mixed endolithic communities,

Table 3 Diversity analysis of 454-pyrosequencing data

Sample (m elevation)	Number of sequences[a]	Observed OTUs[b]	Chao1[c] (95 % CI)	ACE[c] (95 % CI)	Simpson (D)[c,d] (95 % CI)	Shannon[c] (95 % CI)
2514	3560 (2486)	148 (123)	136 (128; 160)	137 (129; 154)	0.14 (0.13; 0.16)	3.06 (2.99; 3.14)
2547	3386 (3249)	152 (139)	188 (162; 244)	178 (160; 210)	0.14 (0.13; 0.15)	2.93 (2.86; 2.99)
2728	2217 (2200)	148 (142)	200 (172; 254)	278 (238; 335)	0.13 (0.12; 0.14)	3.05 (2.97; 3.12)

[a] Sequences remaining after trimming and filtering (77.5 % average retention). The number in parentheses represents the sequences remaining following the removal of OTUs identified as chloroplast or contaminant DNA

[b] OTU definition = 97 % sequence similarity. The number in parentheses represents OTUs remaining following the removal of OTUs identified as chloroplast or contaminant DNA

[c] Calculated with sequences remaining after removal of contaminant and chloroplast sequences

[d] $D = \sum \frac{n(n-1)}{N(N-1)}$

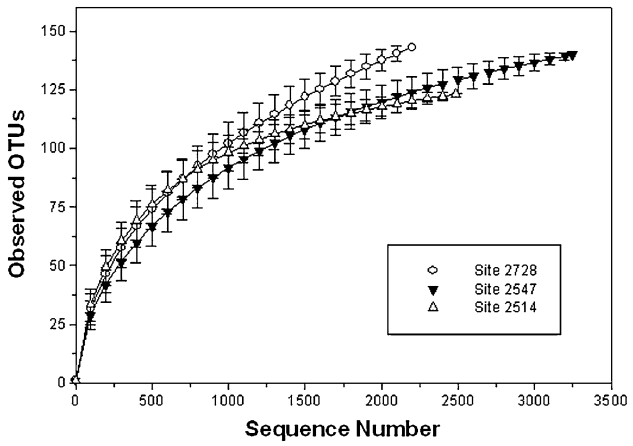

Fig. 2 Rarefaction curves generated from 16S rRNA gene 454-pyrotags using observed OTUs computed with Mothur v1.12.2 (Schloss et al. 2009) (http://www.mothur.org) with a 0.03 cutoff

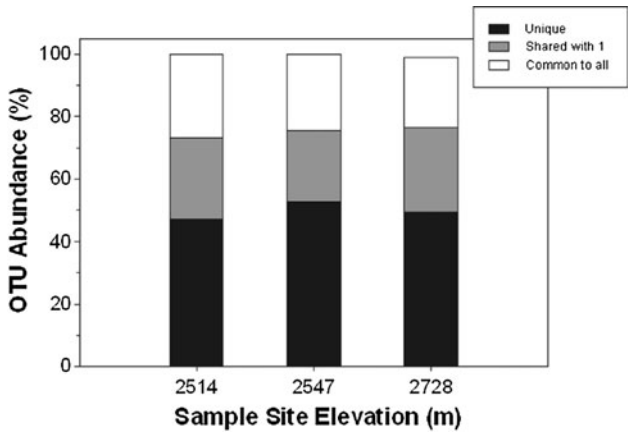

Fig. 3 Distribution of OTUs obtained from 454-pyrosequencing of bacterial soil communities from sites 2514, 2547, and 2728 in the Atacama Desert. The OTU abundances are calculated as a percent of total OTUs for each sample and are defined as follows: unique, shared with one other site, and common to all three sites. Samples are labeled according to site elevation (m)

Table 4 Comparative bacterial community structure analysis of soil communities

Sample site comparisons (m, elevation)	Analysis method	
	Bray–Curtis	Abundance-based Sørensen
2728 versus 2514	0.518	0.864
2728 versus 2547	0.222	0.867
2547 versus 2514	0.188	0.753

while gypsum that was buried at depths ≥25 cm showed no evidence of colonization (Dong et al. 2007). Research has also shown that endolithic colonization of Ca-sulfate crusts by green algae and heterotrophic bacteria can occur despite extremely low levels of MAP if the crusts are exposed to a sufficient number of days with relative humidity values >60 % (Wierzchos et al. 2011). We did not examine the gypsum at site 2514 for evidence of green algae, but the large number of chloroplast sequences present in this community suggests endolithic colonization at this site.

Taxonomic composition of arid soil pyrotag libraries

A novel taxonomic composition characterized the dominant populations of all three sites. The three communities were heavily dominated by *Actinobacteria* and *Chloroflexi* with sequence abundances of 70–77 and 7.52–14.50 %, respectively (Fig. 4). Abundances of the traditionally dominant soil phyla, *Acidobacteria* and *Proteobacteria* (Janssen 2006), were strikingly low. For example, a pyrosequencing survey of 88 soils from North and South America (Lauber et al. 2009) found *Acidobacteria*, *Actinobacteria*, and *Proteobacteria* to be the most abundant phyla followed by *Bacteroidetes* and *Firmicutes*.

At the hyperarid margin, *Acidobacteria* were completely absent from site 2547 and comprised just 0.2 and 0.09 % of the 2514 and 2728 communities, respectively. Following a similar pattern, *Acidobacteria* were also absent from 8 of 11 sites evaluated in a cold, shale desert characterized by 140 mm MAP at the Mars Desert Research Station (MDRS) located in southeast Utah (Direito et al. 2011). In contrast, *Acidobacteria* represented 17 and 22 %, respectively, of the communities isolated from the hot desert of Tataouine (South Tunisia) (Chanal et al. 2006) and an arid, unvegetated soil located at 5235 m elevation on the slopes of the Socompa volcano in the Atacama Desert (Costello et al. 2009) (MAP <200 mm).

Proteobacteria abundance ranged from 2.2 to 6.3 % in the hyperarid margin communities. Further, the most abundant *Proteobacteria* in all three communities were *Gammaproteobacteria* (Fig. 4). This is in contrast to a broad survey of surface soils that found the *Alpha-* and *Betaproteobacteria* to predominate, representing 19 and 10 % of average soil communities, respectively (Janssen 2006). A low precipitation gradient analysis of soils in Israel also found *Proteobacteria* (dominated by *Alphaproteobacteria*) to be significantly less abundant in arid versus semiarid and Mediterranean climate zones. *Proteobacteria* comprised 38 % of the Tataouine desert community, but 80 % of these were *Gammaproteobacteria* and just 10 and 0 % were *Alpha-* and *Betaproteobacteria*, respectively. *Proteobacteria* abundance was also low in the unvegetated arid Socompa volcano community (4 %), but these OTUs were *Alpha-* and *Betaproteobacteria* and not *Gammaproteobacteria*. This analysis suggests a potential correlation between the degree of aridity and the taxonomic structure of soil communities.

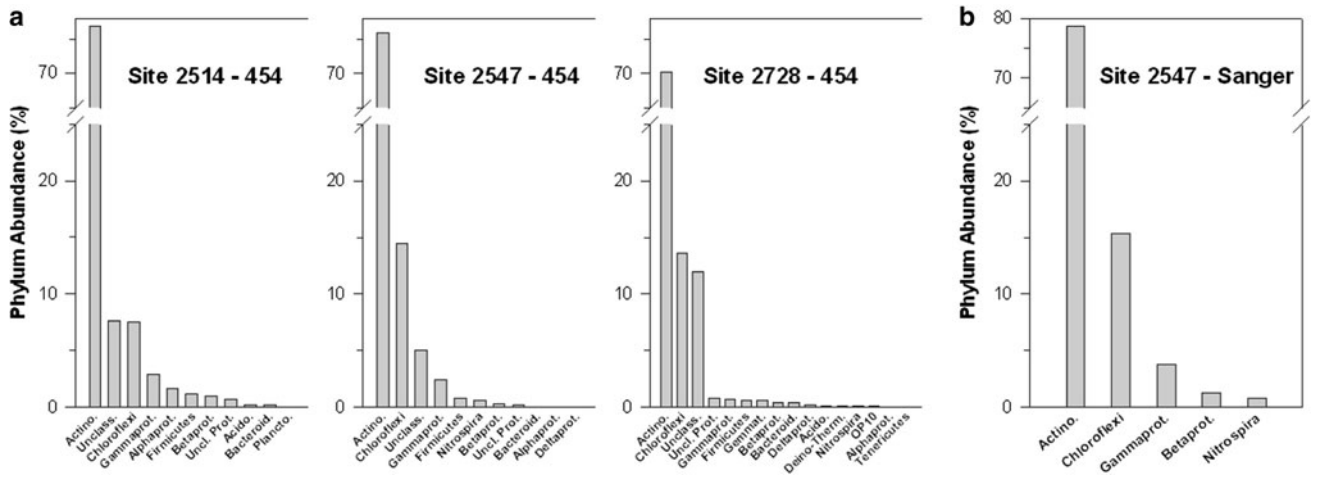

Fig. 4 Phylum abundance of bacteria present in arid soils of the Atacama calculated as a percentage of total sequence reads for each sample. Site designations represent the elevation (m) at which the samples were collected. **a** Plots labeled 454 represent data from 454-pyrotag libraries. **b** The plot labeled Site 2547–Sanger represents data from the site 2547 16S rRNA gene library. Phylum abbreviations: Acido., A*cidobacteria*; Actino, *Actinobacteria*; Alphaprot., *Alphaproteobacteria*; Bacteriod., *Bacteroidetes*; Betaprot., *Betaproteobacteria*; Deino-Therm., *Deinococcus-Thermus*; Deltaprot., *Deltaproteobacteria*; Gammaprot., *Gammaproteobacteria*; Gemmat., *Gemmatimonadetes*; Thermotog., *Thermotogae*; Verruco., *Verrucomicrobia*; Unclass., unclassified; Uncl. Prot., *Proteobacteria* that could not be classified to the sub-phylum level

Dominant arid soil phyla

The dominance of *Actinobacteria* in the hyperarid margin soil communities is typical of arid ecosystems, though the abundances observed in this study are significantly higher than those identified in most other arid soils. A pyrosequencing survey of soil bacterial diversity in seven global biomes found desert soils to have the highest abundance of *Actinobacteria* (~30 %) (Fierer et al. 2009). In the Atacama, *Actinobacteria* represented 33 % of the total community of the arid, Socompa volcano site (Costello et al. 2009). However, a clone library analysis of a hyperarid soil from the Yungay region found a community with 94 % *Actinobacteria* (Connon et al. 2007). In other deserts, *Actinobacteria* were found to be the most abundant phylum in soils from the McKelvey Valley of the McMurdo Dry Valleys, Antarctica (33 % abundance) (Pointing et al. 2010) and the second most abundant phylum in a Tataouine desert soil (26 % sequence abundance) (Chanal et al. 2006).

The most abundant *Actinobacteria* in the soils from this study were OTUs that belonged to the *Nitriliruptoraceae* family (88–93 % confidence) within the *Actinobacteridae* subclass. Pyrotags from this family accounted for ≥34 % of the sequences in each of the three communities. Further classification of these novel pyrotags will be addressed in the analysis of the 16S rRNA gene library. The next most abundant *Actinobacteria* were OTUs belonging to the *Rubrobacteridae* subclasses. The distribution of *Rubrobacteridae* was more variable among the three sites. OTUs belonging to the *Rubrobacter* genus (≥97 % confidence) comprised 21.5 % of the site 2547 community, but just 6.7 and 7.6 % of the site 2514 and 2728 communities, respectively. In contrast, the *Rubrobacteridae* of sites 2514 and 2728 were dominated by members of the *Conexibacteraceae* family with smaller representation in the *Solirubrobacteraceae* and *Patulibacteraceae* families. Site 2547 included *Solirubrobacteraceae* pyrotags, but no *Conexibacteraceae* or *Patulibacteraceae*. Recall that the Bray–Curtis pairwise comparisons of the three communities indicated a greater similarity between sites 2728 and 2514, than between either of these sites and site 2547. Finally, each of the sites included low abundances of *Acidimicrobidae*. Of interest is the fact that the majority of the *Actinobacteria* identified in the hyperarid Yungay soil belonged to the suborder *Frankineae*, a group with low representation among the sites evaluated for the current study (Connon et al. 2007).

Chloroflexi was the second most abundant phylum in all three communities with abundances of 7.5, 14.5, and 13.6 %, for sites 2514, 2547, and 2728, respectively (Fig. 4). An analysis of the five most abundant *Chloroflexi* OTUs from each of the sample sites revealed a significant amount of overlap. A multiple pairwise sequence alignment revealed that site 2547 had the highest abundance of novel OTUs with the most abundant of these (7 % abundance) located in a novel lineage that included a more limited amount of OTUs from the other two sites. The remaining high abundance *Chloroflexi* OTUs clustered primarily into two groups within the *Thermomicrobia* class (data not shown).

Unlike *Actinobacteria*, high abundances of *Chloroflexi* have not been previously associated with arid soil

communities (Yamada and Sekiguchi 2009). However, a recent culture-based study evaluating growth rates of soil bacteria found both *Chloroflexi* and the *Rubrobacteridae* subclass of *Actinobacteria* to be slow-growing bacteria requiring >12 weeks for colony formation (Davis et al. 2011). Bacteria characterized by such slow growth rates typically have a high affinity for growth-limiting resources and an increased tolerance to drought (Schimel et al. 2007). The pyrosequencing survey of global terrestrial biomes found the relative abundance of *Chloroflexi* to range from 1 to 7 % with desert biomes averaging 2.5 %. In contrast, three other studies have associated *Chloroflexi* with diverse extreme environments. First, a low precipitation gradient study in Israel found an inverse relationship between precipitation and the abundance of *Thermomicrobia* clones (Bachar et al. 2010). Second, an alpine tundra soil (Costello and Schmidt 2006) contained a *Chloroflexi* abundance of 16 %, many of which were not affiliated with established subdivisions. Finally, a study focused on a successional gradient on Hawaiian volcanic deposits found that *Chloroflexi* comprised 24.4 % of the bare soil community, while these phylotypes were not detected in soils under nearby plant canopies (Weber and King 2010). This latter study refers to the *Chloroflexi* as pioneer colonists of volcanic deposits. Interestingly, *Chloroflexi* have been characterized with a novel and complex layered cell envelope structure (Sutcliffe 2010) that includes a unique membrane construction containing long-chain 1,2-diols and a cell wall with variable and atypical peptidoglycan contents (Wu et al. 2009). These unique features combined with the high abundance of *Chloroflexi* in the Atacama arid soils may reflect specific adaptations of these populations to survival under arid conditions, but their specific functional role is yet to be elucidated.

Finally, it is interesting to note that one abundant unclassified OTU representing 6.6, 4.0, and 1.5 % of the site 2728, 2547, and 2514 communities, respectively, had 97 % sequence identity with clones from the Negev Desert in Israel (GQ425506) and uranium-mining impacted soils (EU141774). The presence of this unique, abundant, and yet to be classified organism suggests a wealth of untapped diversity in extreme, arid environments.

Comparative taxonomic composition of hyperarid margin communities

The distinctive community structure of the Atacama soils at the hyperarid margin suggests the presence of a conserved ecosystem characterizing this region, but subtle differences between the sites also revealed a degree of heterogeneity in an apparently homogeneous, unvegetated landscape. First, it is noteworthy that the total number of phyla in the site 2728 community profile was greater than at either site 2514 or 2547. Specifically, with the exception of *Planctomycetes*, all phyla identified from either site 2514 or site 2547 were present in the site 2728 community, as well as four additional phyla not found at either of the other sites. Recall that this site also had higher estimated α-diversity. Second, three patterns support the Bray–Curtis indication that sites 2514 and 2728 were most similar of the three sites. As previously noted, *Rubrobacter* spp. are less abundant at these sites than at site 2547. In addition, both *Acidobacteria* and *Bacteroidetes* populations are present in low numbers in these communities while virtually absent from site 2547. Based on the data collected for this study, we hypothesize that the subtle similarities between sites 2514 and 2728 are related to evidence for phototrophic primary production present at both of these sites, but absent from site 2547; the site 2514 community contained an abundance of chloroplast DNA (23 % of the original reads, Table 3) and site 2728 contained dry plant stalks. As previously indicated, high abundances of *Acidobacteria* and *Bacteroidetes* are generally associated with less arid soils. We acknowledge that the specific influence of diverse environmental factors on arid soil bacterial diversity cannot be identified from the limited number of sample sites characterized for this study, but the subtle differences observed among the bacterial communities at the hyperarid margin in the Atacama suggest potential correlations between enhanced primary production and the presence or absence of specific populations. Of particular interest is a possible association between the slightly higher level of α-diversity present at site 2728 and the presumed exposure to higher moisture levels from runoff.

16S rRNA gene library of site 2547

Site 2547 m was selected for construction of a nearly full-length 16S rRNA gene library to gain further insight into the identities of dominant populations and the possible functional dynamics of this arid community. A total of 236 clones representing 32 OTUs were obtained after removal of contaminant sequences and potential chimeras. The depth of diversity detected by the clone library (5 phyla) approach was less than that obtained from pyrosequencing (10 phyla), demonstrating the limitations of clone library analysis; however, the phylum distribution and dominant OTUs present within both libraries were similar (Fig. 4). Eighty-eight percent of the clone OTUs had ≥99 % sequence identity with a corresponding 454-pyrotag OTU showing excellent correspondence between the two approaches.

The most abundant *Actinobacteria* 454-pyrotags identified from the three sites were also dominant in the 2547 gene library. *Nitriliruptoraceae* clone, w1-77 (Fig. 5a), had 100 % sequence identity with the most abundant *Nitriliruptoraceae*

pyrotag. This clone was located in a well-supported clade with *Nitriliruptor alkaliphilus* (90–94 % sequence similarity), a haloalkaliphilic bacterium capable of growth on aliphatic nitriles (Sorokin et al. 2009). The closest GenBank relatives were clones with 90 % sequence similarity from unpublished studies of Indian coastal soils (HQ397411) and a Xiarinur soda lake sediment in China (GU083690). The *Nitriliruptoraceae* clones also had 89–91 % sequence similarity with a group of clones identified from the highly alkaline, saline soils of former Lake Texcoco, Mexico (Valenzuela-Encinas et al. 2009). In addition, the taxonomic survey from the MDRS found that 8–10 % of the sequences from two separate sample sites clustered in a clade with *N. alkaliphilus* (bootstrap 99 %) (Direito et al. 2011). The *Nitriliruptoraceae* clone with the closest GenBank relative was w1-22 with 95 % similarity to a clone from a simulated radioactive waste site in Idaho (GQ263668) (Field et al. 2010). This analysis of *Nitriliruptor* clones reveals that these communities at the hyperarid margin are dominated by novel bacteria with minimal similarity to previously characterized species. Interestingly, the closest relatives are found in alkaline saline soils and deserts suggesting a unique adaptation of this organism to arid ecosystems.

The second and fifth most abundant pyrotags in the 2547 community had >99 % identity with a group of *Rubrobacter* clones (41 % abundance) that were located in a highly supported clade (100 %) with the desiccation- and radiation-tolerant *R. radiotolerans* (Fig. 5a). BLAST analysis of one of these clones, w2-61, identified three highly similar (98 %) uncultured clones, two from a soil in India (HQ397161, HQ397130) and one from an undisturbed tall grass prairie soil in Oklahoma (FJ479549) (Youssef et al. 2009). Unlike the *Nitriliruptor* OTUs, clones related to *R. radiotolerans* have been previously associated with extreme ecosystems of the Atacama (Connon et al. 2007), Australia (Holmes et al. 2000), the Tataouine Desert of Tunisia (Chanal et al. 2006), and the McMurdo Dry Valleys of Antarctica (Pointing et al. 2010).

The most abundant site 2547 *Chloroflexi* pyrotag (7 % sequence abundance) had 100 % sequence identity with *Chloroflexi* clone w1-24 located in the previously described novel *Chloroflexi* lineage (Fig. 5b). This clone also represented a novel microbe with 11 % sequence divergence from the closest relative in the GenBank database (HQ397159). Four other *Chloroflexi* clones clustered in two *Thermomicrobia* clades. The clade containing clones w1-23 (100 % identity with the fifth most abundant 2547 pyrotag), w2-19, and w2-20 was also a unique lineage with 9–11 % sequence divergence from the closest GenBank relatives. Interestingly, Clone w1-23 was most closely related (91 %) to a clone from a cryptoendolithic community from the McMurdo Dry Valleys, Antarctica (de la Torre et al. 2003). The two *Chloroflexi* clones most similar to previously identified sequences were w1-21, a *Sphaerobacter* spp. (RDP Classifier, 100 %), with 95 % similarity to an uncultured clone from a saline-alkaline soil (JN037916) and a clone from a faba bean rhizosphere (EU979025) and w1-18 with 93 % similarity to a clone from a hypolithic community on a quartz stone from the Atacama Desert (FJ891038). Clone w1-18 was the only *Chloroflexi* clone not present in the 454-pyrotag library (>15 % divergent from closest pyrotag).

DGGE band sequences from our previously characterized Atacama hyperarid ecosystem (Drees et al. 2006) were inserted into the site 2547 phylogenetic tree (Fig. 5a, b). Just two bands were found to be loosely related to 2547 clones. Bands 987-3b and 987-1b had 95.7 and 95.4 % sequence identity with *Actinobacteria* w1-42 and *Chloroflexi* w1-23, respectively (Fig. 5a, b). Several 2547 clones clustered in each of these clades suggesting an overlap of some hyperarid phylotypes with OTUs from the arid-site 2547 community.

Evidence for primary production in 2547 arid community

No phototrophic primary producers were identified among the site 2547 clones; however, several clones demonstrated potential as non-phototrophic primary producers by virtue of their phylogenetic association with previously characterized chemolithoautotrophs. First, clone w3-81, most closely related to a *Nitrospira* clone (EF612393, 99 % sequence identity) from a semiarid soil in southeastern Arizona (Mendez et al. 2008), was assigned to the *Nitrospira* genus by the RDP Classifier (100 % confidence) and was located in a well-supported clade (98 % bootstrap value) containing the obligate chemolithoautotroph, *N. moscoviensi* (Fig. 5b). *N. moscoviensi* derives energy from the aerobic oxidation of nitrite to nitrate using a novel nitrite-oxidizing system (NXR) (Lucker et al. 2010; Spieck et al. 1998; Tourova et al. 2007). Research has shown that the NXR common to this genus is attached to the cytoplasmic membrane and oriented toward the periplasmic space. This configuration makes *Nitrospira* especially well adapted to substrate-limited conditions, an interesting potential adaptation for survival in oligotrophic ecosystems such as the Atacama. An analysis of nitrate accumulation in the hyperarid regions of the Atacama revealed consistent deposition rates of atmospheric inorganic nitrogen species (Ewing et al. 2007), providing evidence for a potential substrate for *Nitrospira* populations.

Second, a *Gammaproteobacteria* clone, w3-11, was located in a highly supported lineage with cultured isolates belonging to the *Ectothiorhodospira*, *Ectothiorhodosinus*, *Thioalkalivibrio*, and *Thiobacillus* genera (Fig. 5b). The

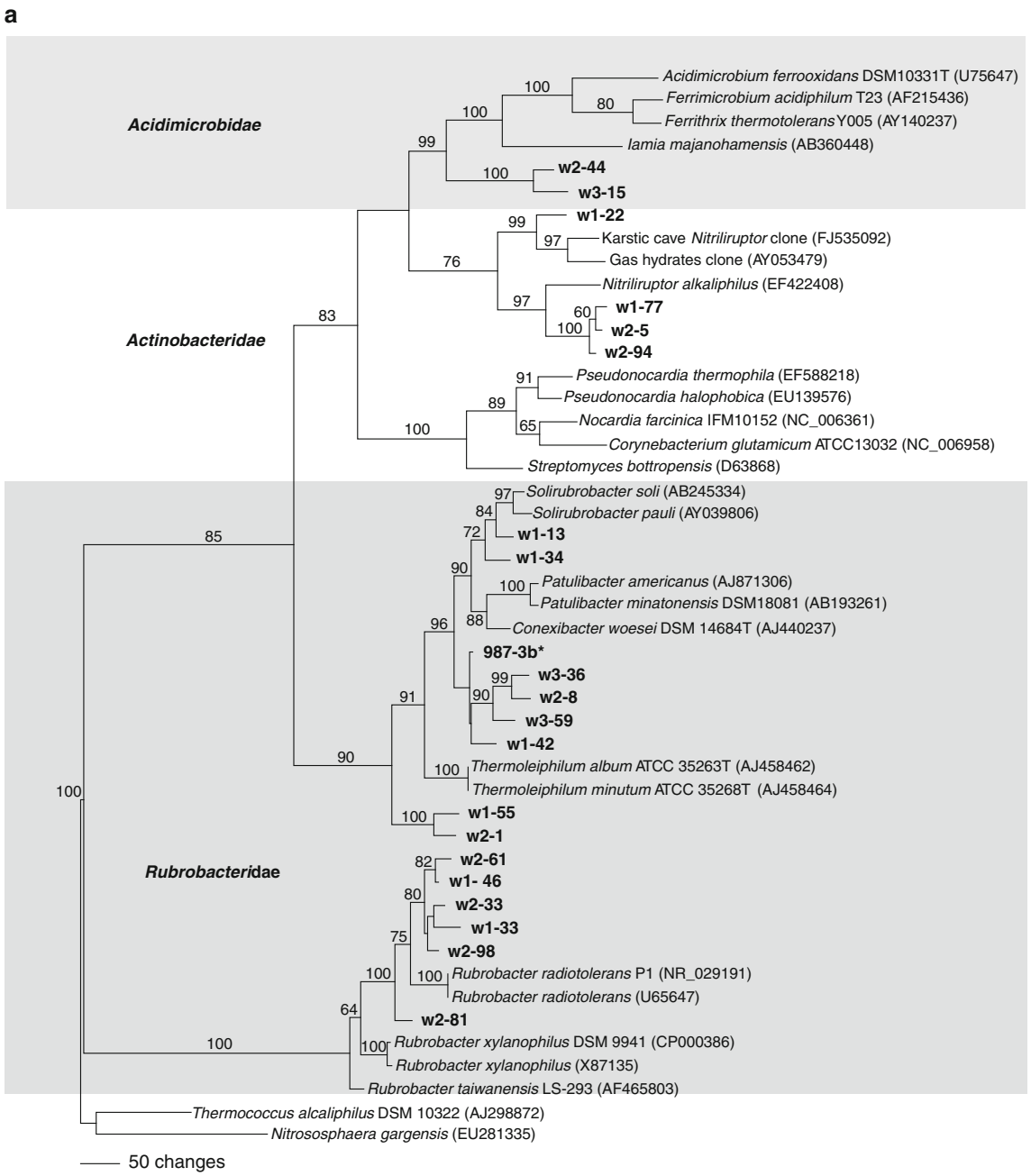

Fig. 5 Phylogenetic analysis of 16S rRNA genes from site 2547. The tree was constructed from reference bacterial sequences (followed by GenBank accession no.) and site 2547 clone library sequences (labeled with "w"). Sequences were aligned using Clustal W (Wisconsin package v.10.3; Accelrys, San Diego, CA, USA) and the alignments adjusted manually using MacClade v. 4.08 (Maddison and Maddison 2001). PCR-DGGE band sequences from the hyperarid site (Drees et al. 2006) labeled 989 and 987 (and marked with *asterisk*) were manually inserted into the base alignment. Rooted most parsimonious trees were generated by heuristic search (tree bisection reconnection branch swapping) as implemented in PAUP 4.0 Beta (Swofford 2006). Bootstrap values (1000 replicates) are given for nodes with ≥50 % support. Trees were rooted with two archaea strains. **a** *Actinobacteria*, **b** non-*Actinobacteria*

clone is classified as a member of the *Ectothiorhodospiraceae* family (91 % confidence, RDP Classifier), a family consisting primarily of obligate or facultative autotrophs (Tourova et al. 2007), and was located in a lineage with the halotolerant, iron-oxidizer *Thiobacillus prosperus* (Nicolle et al. 2009). A BLAST analysis found w3-11 to be most

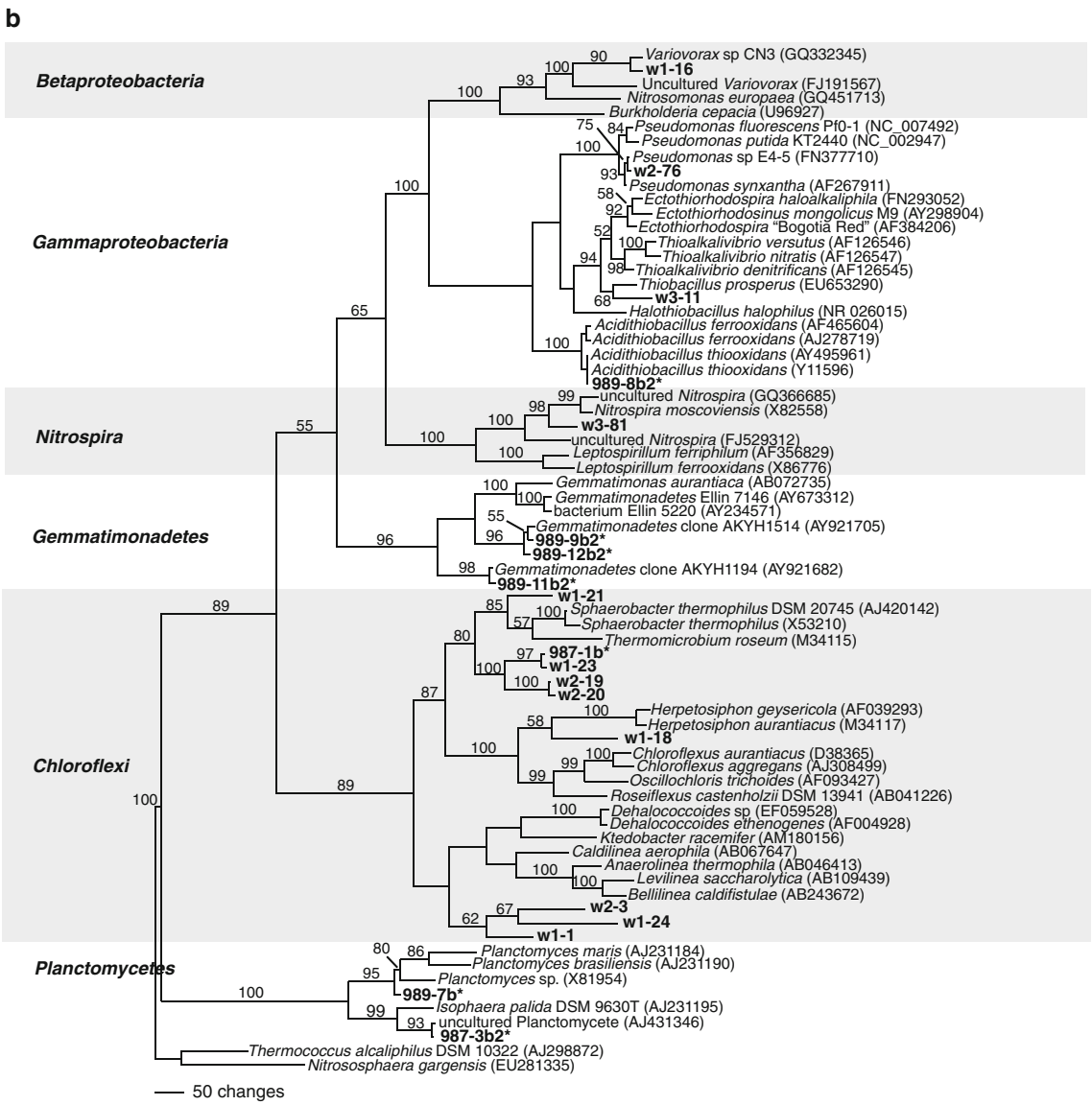

Fig. 5 continued

closely related to the fully sequenced *Thioalkalivibrio* sp. HL-EbGR7 (CP001339, 92 % sequence identity). The *Thioalkalivibrio* genus was identified in 2001 to describe a group of 25 chemolithoautotrophic sulfur oxidizers isolated from Kenyan soda lakes (Sorokin et al. 2001) and characterized by a slow growth rate at pH levels from 7.5 to 10.65, a high molar growth yield, and low oxidation rates of thiosulfate, sulfide, elemental sulfur, and polythionates. A study characterizing the microbial community inhabiting the Salar de Atacama found that a large percentage of the phylotypes identified were closely related to the *Ectothiorhodospiraceae* genera (Thiel et al. 2010). This lake is located 120 km from site 2547 (Fig. 1). Inland gypsum deposits, such as those found at the sites evaluated in this study, carry the isotopic signature of salts found in the local salars and are presumed to have been deposited throughout the central desert by eolian dispersion (Rech et al. 2003). Of interest is whether these chemoautotrophic primary producers are active in this soil community or are simply a vestige of eolian deposition along with the gypsum deposits.

Two other clone clusters were located in lineages with chemolithoautotrophic bacteria. First, clones w2-44 and w3-15 clustered (99 % bootstrap) with a diverse group of cultured autotrophic and heterotrophic iron-oxidizing bacteria belonging to the *Acidimicrobiales* order (Fig. 5a) (Johnson et al. 2009). The closest GenBank relatives were uncultured clones from rock varnish in Black Canyon, New Mexico (FJ595641, 98 % identity) and a recently deglaciated soil (GQ397083, 96 % identity), respectively. Second, a cluster of *Chloroflexi* clones (w1-21, w1-23, w2-19,

w2-20) was located in a *Thermomicrobia* clade with *Thermomicrobium roseum*. A recent analysis of the complete genome sequence of *T. roseum* produced evidence of a chemolithoautotrophic growth mode for this traditional chemoheterotroph using either CO or H_2 as an energy source (Wu et al. 2009). Unfortunately, the high divergence of the majority of the *Chloroflexi* clones from any known sequences precludes further conclusions regarding their potential for alternative growth strategies at this time.

Conclusions

Novel bacterial communities were found in soils sampled from the hyperarid margin of the Atacama Desert. The communities were dominated by unique *Actinobacteria* and *Chloroflexi* and marked by strikingly low levels of phyla typically dominant in soil communities: *Acidobacteria* and *Alpha-* and *Betaproteobacteria.* When compared with published characterizations of hyperarid Atacama soils, the communities at the hyperarid margin harbored a greater diversity and a distinct community structure suggesting greater functional potential. Hyperarid, Yungay soils (Connon et al. 2007) were found to be heavily dominated by *Actinobacteria* OTUs most closely related to *Frankia* rather than to the *Nitriliruptoraceae* and *Rubrobacteraceae* families dominant at the hyperarid margin. The soils analyzed by Drees et al. (2006) were dominated by *Gemmatimonadetes* and *Planctomycetes*, though they also included individual *Actinobacteria* and *Chloroflexi* OTUs closely related to site 2547 clones. Despite the fact that both regions are unvegetated and have extremely low organic C and N levels, these results indicate enhanced bacterial diversity at the hyperarid margin, potentially related to slightly higher MAP levels and to exposure to past vegetation history. A similar microbial transition was observed in an analysis of organic carbon turnover rates along a rainfall gradient in the Atacama from hyperarid to arid, where carbon cycling decreased significantly with decreasing moisture (Ewing et al. 2008). Minor differences in α-diversity among the three sites evaluated for this study suggest that even within the hyperarid margin, subtle variations in environmental parameters may have an impact on the specific taxonomic composition of bacterial communities.

The unique bacterial taxonomic composition observed for soils at the hyperarid margin presents intriguing evidence for the past or current presence of communities harboring an array of novel phylotypes with unknown functional potential. The site 2547 community DNA profile included a combination of radiotolerant, halotolerant, and uncharacterized rare phylotypes that may include species uniquely adapted to survival under arid conditions. In addition, clones were identified with phylogenetic associations to chemolithoautotrophic taxa that obtain energy by oxidation of nitrite, CO, iron, or sulfur suggesting genetic potential for non-phototrophic primary production and geochemical cycling in these arid Atacama ecosystems. While the molecular analyses presented in this study cannot confirm the viability or potential activity of the bacteria identified, this robust diversity analysis provides insights into the possible ecosystem dynamics of bacterial communities at the hyperarid margin. Future research is needed to characterize the metabolic activity of these novel bacterial communities in order to evaluate the functional dynamics of arid Atacama ecosystems that may model vast LAs of the terrestrial surface.

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