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MICROBIOLOGY OF AQUATIC SYSTEMS

Bacterial Succession within an Ephemeral Hypereutrophic Mojave Desert Playa Lake

Jason B. Navarro · Duane P. Moser · Andrea Flores · Christian Ross · Michael R. Rosen · Hailiang Dong · Gengxin Zhang · Brian P. Hedlund

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Abstract Ephemerally wet playas are conspicuous features of arid landscapes worldwide; however, they have not been well studied as habitats for microorganisms. We tracked the geochemistry and microbial community in Silver Lake playa, California, over one flooding/desiccation cycle following the unusually wet winter of 2004–2005. Over the course of the study, total dissolved solids increased by ~10-fold and pH increased by nearly one unit. As the lake contracted and temperatures increased over the summer, a moderately dense planktonic population of ~1×10^6 cells ml^{-1} of culturable heterotrophs was replaced by a dense population of more than 1×10^9 cells ml^{-1}, which appears to be the highest concentration of culturable planktonic heterotrophs reported in any natural aquatic ecosystem. This correlated with a dramatic depletion of nitrate as well as changes in the microbial community, as assessed by small subunit ribosomal RNA gene sequencing of bacterial isolates and uncultivated clones. Isolates from the early-phase flooded playa were primarily Actinobacteria, Firmicutes, and Bacteroidetes, yet clone libraries were dominated by Betaproteobacteria and yet uncultivated Actinobacteria. Isolates from the late-flooded phase ecosystem were predominantly Proteobacteria, particularly alkalitolerant isolates of *Rhodobaca*, *Porphyrobacter*, *Hydrogenophaga*, *Alishwenella*, and relatives of *Thauera*; however, clone libraries were composed almost entirely of *Synechococcus* (Cyanobacteria). A sample taken after the playa surface was completely desiccated contained diverse culturable Actinobacteria typically isolated from soils. In total, 205 isolates and 166 clones represented 82 and 44 species-level groups, respectively, including a wide diversity of Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Gemmatimonadetes, Acidobacteria, and Cyanobacteria.

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J. B. Navarro · D. P. Moser · A. Flores · C. Ross · B. P. Hedlund (✉)
School of Life Sciences, University of Nevada Las Vegas,
Las Vegas, NV 89154-4004, USA
e-mail: brian.hedlund@unlv.edu

D. P. Moser
Division of Earth and Ecosystem Sciences,
Desert Research Institute,
Las Vegas, NV 89119, USA

M. R. Rosen
Nevada Water Science Center, United States Geological Survey,
Carson City, NV 89701, USA

H. Dong · G. Zhang
Department of Geology, Miami University,
Oxford, OH 45056, USA

Introduction

A playa is an intracontinental basin in which the water balance (precipitation and inflow minus outflow, evaporation, and evapotranspiration) is negative for the majority of months and for the annual total [53]. Playas fit into three broad categories based on their relationship to groundwater altitude and flow [53]. Hydrologically closed basins with negative water balance form discharge playas, which accumulate solutes and are often perennially wet. Recharge playas, in contrast, lie on porous strata above the water table and provide conduits to subsurface aquifers during precipitation or flooding. Because recharge playas provide water directly to the subsurface, evaporite formation is minimal and lakes created by flooding events are ephemeral. An intermediate category, termed a through-flow playa, acts as a local catchment but has one or more outflow; thus,

through-flow playas are intermediate with respect to water retention and evaporite accumulation.

Although there are approximately 50,000 playas in the world [42] representing a wide diversity of chemistries and flooding/desiccation regimes, most playa microbiology studies have focused on perennially wet discharge playas or similar hypersaline environments, such as solar salterns of marine origin and continental salt and soda lakes [2, 5, 9, 13, 17, 18, 22, 25, 28, 34, 38, 45, 46, 58]. Many of these ecosystems host sediment-associated mat communities that are characterized by high rates of microbial activity, vertically differentiated microbial communities, and steep geochemical gradients [34, 40, 50]. Many of these mat communities consist primarily of Cyanobacteria, Chloroflexi, Proteobacteria, Bacteroidetes, Firmicutes, and Spirochaetes [14, 28, 38, 43, 44]; however, a more thorough cultivation independent investigation of mats in a 9% salinity pond in Guerrero Negro, Mexico, uncovered an extremely high diversity of microorganisms, including bacteria from 752 species- and 42 phylum-level groups [34].

In most hypersaline mat systems, photosynthetically derived biomass is mineralized primarily by fermentation and sulfate reduction [22, 40, 50]. In turn, sulfide is oxidized both phototrophically and chemolithotrophically in the overlying layers of mat and water [58]. However, at extreme salinities, major steps in C, N, and S cycles are stunted due to the severe bioenergetic burden of maintaining $Na^+/K^+/H^+$ homeostasis and/or synthesis of molar concentrations of compatible solutes [33, 46]. For example, dissimilatory sulfate reduction to sulfide is inhibited at high salinity [33, 46]. Instead, microbial communities in salt saturated systems take advantage of rarer but more thermodynamically favorable redox-active couples that accumulate to unusually high concentrations in closed basins, such as arsenate/arsenite and selenate/selenite [3, 21, 33, 45].

Planktonic microbial inhabitants of hypersaline habitats have also been studied, revealing dense and productive communities of primarily Alphaproteobacteria, Gammaproteobacteria, Bacteroidetes, Firmicutes, Cyanobacteria, and Actinobacteria [2, 9, 13, 27, 28, 38]. The ratios of these phyla depend on the particular habitat. At high salinities, planktonic phylum- to genus-level diversity decreases with increasing salinity, leaving a population dominated by specific halophilic clades of Euryarchaeota (*Haloquadratum*), Bacteroidetes (*Salinibacter*), and the alga *Dunaliella* in salt-saturated systems [2, 9, 13, 18, 49]. In contrast, planktonic communities of dilute soda lakes are extremely diverse and productive [17]. Microorganisms in these habitats are not challenged with bioenergetic burdens of hypersaline systems, yet biologically relevant solutes are concentrated from the entire watershed by meteoric runoff, thereby lifting bottom-up controls that limit microbial growth in typical aquatic environments [20].

In contrast to the discharge systems discussed above, we are not aware of systematic microbiological studies of recharge or through-flow playas, yet these are much more common than discharge playas and soda lakes. Microorganisms in recharge and through-flow systems are not faced with the extreme osmolarity challenges characteristic of discharge systems, except perhaps in hypersaline microenvironments that may form in localized areas of low permeability. Instead, the transient and unpredictable nature of wetting cycles is likely to be a dominant force in shaping microbial communities in ephemerally flooded playas. During periods of desiccation, microbial activities are likely to be severely limited by low water activity. Furthermore, only microorganisms that survive desiccation and avoid removal by wind-driven erosion or those that are wind- or water-deposited will be available to take advantage of wetting events. Despite these challenges, we predicted that episodically flooded playas would be excellent habitats for microorganisms because biologically relevant solutes are concentrated by meteoric runoff, yet top-down controls that might otherwise limit microbial activity are restricted to the subset of eukaryotic grazers and viruses that survive desiccation and ablation or those that are allochthonously deposited and able to grow to relevant densities during a flooding event. Microbial communities in ephemerally flooded playas might be expected to be similar to those inhabiting other shallow, mineral-rich waters or nearby soils that are periodically influenced by meteoric water input, including communities inhabiting ephemerally flooded salt deposits in the Great Salt Plains of Oklahoma (USA) [10], shallow alkaline pools in the Neusiedler See-Seewinkel National Park (Austria) [17], and soils adjacent to Salt Spring, British Columbia (Canada) [65]. These systems are characterized by extreme spacio-temporal variations in salinity, UV irradiance, and temperature, yet these disturbances have been proposed to promote microbial community diversity [65], wide microbial growth ranges with respect to temperature and salinity [10], and extreme temporal variations in productivity [17].

In this study, we took advantage of the unusually wet winter of 2004–2005, which resulted in the flooding of many of the playas throughout the American Southwest, to examine the geochemistry and microbial community of Silver Lake, CA, a large through-flow playa in the Mojave Desert. To begin to understand this playa as a microbial habitat, we focused a cultivation-intensive study on the heterotrophic microbial population with the goal of determining whether a specific microbial community developed in the flooded playa and, if so, what the composition of that community was and how it changed as physicogeochemical conditions of the playa changed. This cultivation effort was

supplemented with a cultivation-independent census of representative phases of the community succession, as revealed by the cultivation results.

Materials and Methods

Site Description and Sampling

Samples were obtained from Silver Lake playa, located approximately 10 km north of Baker, CA. Samples SL1 and SL2 were taken from GPS location 35°20.156′ N, 116°05.621′ W (datum: WGS84). Although the playa is nearly flat, it is tilted so that the north end is at a slightly lower elevation [67]; therefore, the sampling site for SL1 and SL2 was dry during sampling times SL3 and SL4. As a result, samples SL3, SL4, and SL5 were taken at GPS location 35°22.616′ N, 116°07.936’ W (Fig. 1). Samples were analyzed on-site for temperature, conductivity, dissolved oxygen, and pH using a YSI 556 Multiprobe System (YSI Inc., Yellowsprings, OH). Water was taken from roughly mid-depth in the water column (Table 1) using sterile polypropylene tubes, which were transported on ice to the laboratory for all cultivation experiments. Because membrane filters plugged almost immediately, samples for major ions were transported on ice to the lab, centrifuged to remove suspended solids, filtered through a 0.2-μm hydrophilic polyether-sulfone filter for anions or a 0.2-μm nylon filter for cations (Pall Scientific), and quantified by ion chromatography (anions: Dionex IonPac AS11 Analytical and IonPac AG11 Guard columns; cations: Dionex IonPac CS12A Analytical and IonPac SG11 Guard columns; conductivity detection). Alkalinity was determined by titration to pH 4.5 (LaMotte, Chestertown, MD). Total organic carbon (TOC) and total inorganic carbon (TIC) of unfiltered frozen samples were determined using a Shimadzu TOC5000 carbon analyzer.

Mineralogy

Sediment slurries were dried onto a glass slide for X-ray diffraction analysis. Samples were X-rayed with a Siemens D500 X-ray diffraction system using Cu-κα radiation, a monochromator, and were scanned in 0.02 two-theta steps from 2 to 40°, with a count time of 2 sec per step. X-ray diffractograms were interpreted by a combination of the best match software and manual peak alignment. In addition, chemical composition obtained from scanning electron microscopy energy dispersive spectroscopy (SEM-EDS) helped narrow the field of possible candidates. Because quartz was present in all the samples, it was used as an internal standard to check for instrumental bias. No bias or drift was observed.

Dried sediment samples were sprinkled onto SEM stubs and Au coated for SEM observation using a Zeiss Supra35 VP-FEG scanning electron microscope. The SEM was operated at an accelerating voltage of 10 to 15 kV. A short working distance (6–10 mm) and low beam current (30–40 mA) were used to achieve the best image resolution. A longer working distance (8.0 mm) and higher beam current (50–70 mA) were used for qualitative energy dispersive spectroscopy (EDS) analysis.

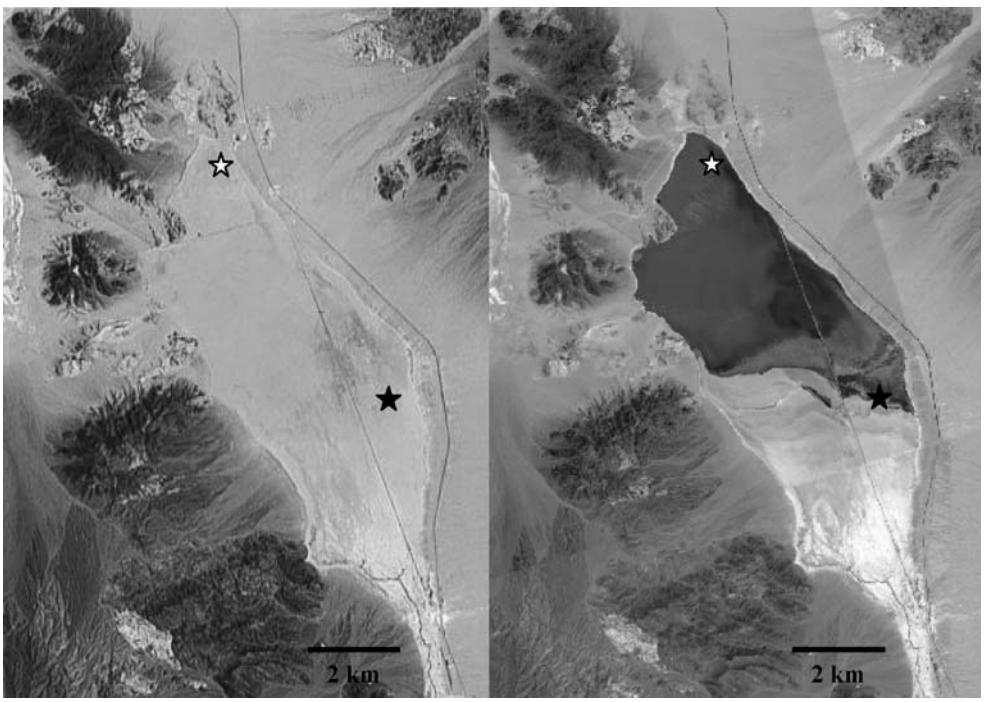

Figure 1 Satellite view of Silver Lake playa during a dry period (*left*) and a wet period (*right*), with sampling locations designated by *stars* (white=1, 2; black=3, 4, 5). The wet playa shown is intermediate in flooding compared with time points SL2 and SL3. *Scale*, 2 km. Images modified from Google Maps™

Table 1 Sampling conditions and chemistry[a]

Date	SL1 3/13/2005	SL2 4/30/2005	SL3 6/21/2005	SL4 6/25/2005
Water Temperature (°C)	20.8	24.6	36.7	26.2
Conductivity (mS/cm)	1.47	2.42	15.13	14.36
Water Depth (cm)[b]	60	33	3.5	15
Dissolved Oxygen (μM)	284	286	255	258
pH	8.39	8.5	9.03	9.18
TOC (ppm)	28.5	n.d	142	176
TIC (ppm)	63.1	n.d.	251	249
Alkalinity (ppm $CaCO_3$)	204	296	1000	1056
Turbidity (NTU)	1018	848	9	1076
Suspended minerals	I,S,Q,Ch	I,S,Q,Ch	I,S,Q,Ch,C	I,S,Q,Ch,C
Cations				
Sodium (mM)	13.7	22.0	152	148
Calcium (μM)	358	530	740	608
Potassium (μM)	53.5	86.9	382	371
Magnesium (μM)	43.6	69.1	315	321
Anions				
Chloride (mM)	6.46	10.9	83.4	80.9
Sulfate (mM)	1.73	3.00	23.5	22.7
Nitrate (μM)	216	354	0.370	<0.0348
Phosphate (μM)	12.6	22.9	11.1	13.6
Bromide (μM)	0.750	1.36	9.40	9.40
N:P mol. Ratio[c]	17.1	15.5	0.033	$<2.6\times10^{-4}$

n.d. not determined, *I* illite, *S* smectite, *Q* quartz, *Ch* chlorite, *C* calcite

[a] Samples were taken approximately every 1.5 months with the intention that samples represent major phases of playa lake evaporation. Sample SL4 was taken a few days after sample SL3 to resolve questions of whether wind-driven turbidity was a major controller of microbial concentration and composition. A fifth sample, SL5, was taken on 9/5/05 when the playa surface was completely desiccated. The water content of this sample was assumed to be in equilibrium with the atmosphere, which was approximately 10% relative humidity. Neither lithium, nitrite, nor ammonium was detected. Relevant detection limits: Li^+ 4.32 μM, NH_4^+ 23.3 μM, NO_3^- 34.8 nM, NO_2^- 86.9 nM

[b] Water depth was determined at the site of sampling

[c] N/P mol. ratios >16 suggest the ecosystem is P limited. N/P <16 suggests the ecosystem is N-limited [60]

Cultivation and Media

A dilute nutrient medium designed to simulate environmental conditions (generically called SL medium after Silver Lake) was prepared with varying pH (8, 9, and 11) and salt concentrations (1%, 10%, and 25%). It consisted of 0.2 g l^{-1} yeast extract, 0.2 g l^{-1} peptone, and 0.2 g l^{-1} dextrose, final concentrations. No trace elements were added to the medium. A concentrated stock salt solution was made using 300 g l^{-1} NaCl, 2.0 g l^{-1} $MgSO_4$, 0.036 g l^{-1} $FeCl_3$, and 0.1 g l^{-1} $CaCl_2$ [47, 63], and diluted to the desired salt concentration of each medium. Nine hundred milliliters of yeast extract, peptone, dextrose, and the appropriate volume of salt solution diluted with water and 100 ml of a 10× $NaHCO_3$ stock (50 g l^{-1}) were autoclaved separately and brought to the appropriate pH with filter-sterilized KOH or HCl after mixing. For most probable number (MPN) analysis, water samples were inoculated into six different media: 1% salt, pH 8; 1% salt, pH 9; 1% salt, pH 11; 10% salt, pH 8; 25% salt, pH 8; and 25% salt, pH 11. MPNs were done in triplicate, scored by 4′,6-diamidino-2-phenylindole staining and epifluorescent microscopy, and statistically evaluated according to de Man [12]. Solid media were prepared as described above, with the addition of 2% agar, with the exception of 25% salt, pH 11, which failed to solidify. MPN tubes and plates were kept at room temperature in the dark in plastic bags for at least 4 months and scored periodically for all cultivation experiments. All MPN tubes, except the 25% salt tubes, were conclusive within 2 weeks. The pH of all media was checked at the time of inoculation and after 2 weeks of incubation to monitor possible changes in pH. No significant changes in pH were observed. The pH of solid media may have changed over the course of longer incubations and allowed new colonies to emerge; therefore, we urge some caution in interpreting medium pH associated with individual isolates.

Molecular Analysis of Isolates

Approximately 50 colonies, representing a number of the dominant colony morphotypes as well as a survey of distinct colony morphologies, were picked from the most

dilute plates from each sampling time and streaked for isolation. Fewer isolates from the 10% and 25% salt plates were selected since culturable halotolerant organisms were substantially less abundant than those cultivated in lower salinity media at all time points. Isolate DNA was extracted using a colony lysis buffer [30] and small subunit ribosomal RNA (SSU rRNA) genes were polymerase chain reaction (PCR) amplified using primers specific for bacteria: 9bF [16] and 1512uR [15], or archaea: 8aF [16] and 1512uR [15]. The 25 μL PCR reaction mixture contained 1 μL DNA extract, 1× Taq reaction buffer, 6 nM of each primer, 800 μM each deoxyribonucleotide triphosphate, and 0.65 U of GoTaq DNA polymerase (Promega). Cycling conditions were: denaturation at 96°C for 4 min followed by 35 cycles of denaturation (30 sec at 94°C), primer annealing (30 sec at 55°C), and elongation (1.5 min at 72°C), with a final elongation step (10 min at 72°C). PCR products were sequenced at the Nevada Genomics Center from the forward primer in a 96-well format (Applied Biosystems (ABI) Prism 3730 DNA Analyzer). The mean sequence length with Phred score >20 was 676 bp. Sequences were aligned using Ribosomal Database Project version 8.1 Sequence Align program [37] and the alignment was manually corrected in ARB [36]. Phylogenetic trees were constructed using ARB (Neighbor-joining, Kimura 2-parameter correction [36]). Some sequences were long enough to be assigned at the phylum level and were considered for Fig. 3, but not Figs. 4, 5, 6 and 7.

Environmental Clone Library Construction and Analysis

Water was filtered through 0.2-μm Supor polysulfone filters (Pall), which were frozen on dry ice in the field. Filters were thawed and split into two vials for extraction using two different environmental DNA isolation kits: MoBio PowerMax Soil Kit (Carlsbad, CA) and the Qbiogene Fast DNA SPIN Kit for Soil (Irvine, CA). DNA yield was similar. Also, SSU rRNA gene T-RFLP analysis using *RsaI*, *MspI*, and *Taq1*, separately, showed that the different PCR reactions with different template DNA yielded very similar SSU rRNA gene products (data not shown). Therefore, biases inherent in extracting DNA from microbial communities and amplifying SSU rRNA genes from a mixed template pool [32, 64] were at least consistent. DNA from the Qbiogene Fast DNA SPIN Kit was used for both clone libraries.

Small subunit rRNA gene fragments were amplified as described above using primers 9bF and 1512uR, ligated into a T/A cloning vector, and transformed into *E. coli* (Invitrogen). Colonies were picked into 96 well plates and grown overnight. Plasmids were extracted using the QIAwell 96 Ultra Plasmid kit (Qiagen, Valencia, CA) and sequenced at the Nevada Genomics Center using the forward PCR primer. Initially, reads from each library were classified at the phylum level by using SEQUENCE_MATCH [37], and the data sets were purged of probable chimeras by using Bellerophon [26] and manually checking possible chimeras in a ClustalX alignment. Sequences were then incorporated into the appropriate ARB alignments of isolate and reference sequences and analyzed phylogenetically as described above. In addition, clone libraries were compared by using the default settings in LIBSHUFF [56].

The Genbank accession numbers for SSU rRNA gene sequences generated in this study are DQ516983–DQ517182, for isolates, and EF555669–EF555725, for environmental clones.

Results and Discussion

Silver Lake is one of two linked playas that occupy the bed of Pleistocene Lake Mojave (Fig. 1). Currently, Silver Lake is predominantly dry, with several short-lasting flooding events in a typical year. Major rain events that flood the playa for more than a month are comparatively rare, occurring on the order of once per decade [67]. During the wetting cycle of our study, the playa held an estimated maximum of 5 to 15 million liters of water in March of 2005 and had dried completely by August of 2005.

Silver Lake was sampled four times during its flooded phase to examine the solute chemistry, suspended particle mineralogy, and water column microbial community (SL1–SL4, Table 1). In addition, the dry playa bed was sampled once to develop an understanding of which organisms survived desiccation (SL5). The water column was generally well mixed with high turbidity and high daytime dissolved oxygen concentrations (Table 1), reflecting small sediment particle size and susceptibility to wind-driven mixing (<1 m depth, high surface area, and sparse vegetation). The abundant suspended solids were characterized by X-ray diffraction (XRD) and scanning electron microscopy energy dispersive spectroscopy (SEM-EDS) as illite and smectite clays, quartz, and chlorite. In addition, calcite was abundant at sampling times SL3 and SL4, which was predicted because thermodynamic saturation models showed SL3 and SL4 to be an order of magnitude more saturated with respect to calcite as compared to SL1 and SL2 (data not shown, [48]). SL3 was different from all other sampling times because it was an extremely hot (air temperature 47°C) and calm day, leading to reduced turbidity and high water temperature (Table 1).

Geochemical Evidence of Nitrate and Phosphate Utilization in Silver Lake

The salinity increased by nearly 10-fold over the course of the study (Table 1), reaching about 1/3 that of sea water.

Most ions concentrated in a conservative manner showing a general inverse proportionality to the volume of the lake, consistent with evaporative water loss. However, nitrate and phosphate were non-conservative. Soluble nitrate was high in SL1 and SL2, but extremely low in SL3 and below detection in SL4 (Table 1). The depletion of nitrate was not due to mineral precipitation since common nitrate minerals, such as nitratine ($NaNO_3$, sol. 815 g L^{-1}) and niter (KNO_3, sol. 360 L^{-1}), are of equal or greater solubility than common evaporite minerals such as gypsum ($CaSO_4 \cdot 2H_2O$, sol. 2.4 L^{-1}) and halite (NaCl, sol. 360 L^{-1}) [11], and all of these minerals were far from their solubility thresholds. Thus, we infer that the N depletion was due to biological activity, either denitrification, nitrate assimilation, or both. Although oxygen-limiting conditions were never observed in the Silver Lake water column, sampling was confined to daylight hours. The contribution of the extremely high numbers of aerobic heterotrophs (>1×10^9 cells/ml; Fig. 2), coupled with the night time respiration of phototrophs would have imparted a high oxygen demand and may have outpaced diffusion-driven oxygen supply on calm nights. Alternatively, since nitrate was the only form of soluble N measured in the playa, the large increase of culturable heterotrophs and Cyanobacteria between sampling times SL2 and SL3 (Fig. 2) may have necessitated assimilatory use of the nitrate pool. In either case, the depletion of soluble inorganic N may have triggered nitrogen fixation in the playa during the summer bloom.

Similarly, the decrease in soluble phosphate from sampling times SL2 to SL3 at concentrations well below saturation for common phosphate salts such as brushite ($CaHPO_4 \cdot 2H_2O$) or monetite ($CaHPO_4$), and the absence of these minerals in XRD and SEM-EDS suggests biological assimilation. However, since phosphate was never completely depleted, and the N/P ratio decreased to well below the Redfield ratio of 16:1 [60]; P was not limiting.

Enumerations of Cultivable Microorganisms Reveal a Dense Heterotroph Population Well Suited for the Chemistry of the Playa

Silver Lake medium was prepared with a variety of pH (8, 9, and 11) and salt concentrations (1%, 10%, and 25%) to monitor the microbial community response to anticipated changes associated with evaporation. Most probable number (MPN) enumerations in the optimum SL media formulations (1% NaCl; pH 8, 9) showed that a moderately dense population of culturable heterotrophs (~1×10^6 cells per milliliter) in SL1 and SL2 was succeeded by an extremely dense population of more than 1×10^9 mL^{-1} in SL3 and SL4 (Fig. 2). Unfortunately, the actual cell densities in SL3 and SL4 are unknown because our MPN dilution series was not carried out beyond that value and direct counts were obscured by the high density of suspended minerals. The great abundance of heterotrophs in SL3 and SL4 was not attributable to the wind-driven suspension of sediment clays, and subsequently sediment-associated benthic microorganisms, because SL3 and SL4, only 4 d apart, had dramatically different turbidities but almost identical heterotroph densities (Table 1; Fig. 2) and similar microbial communities (Figs. 3, 4, 5, 6, and 7). To our knowledge, this is the highest concentration of culturable planktonic heterotrophs ever described in a natural aquatic ecosystem, exceeding summer heterotroph concentrations in shallow alkaline ponds in Neusiedler See-Seewinkel, which also hosted extremely high rates of planktonic bacterial secondary production [17].

Although media were prepared to cover a range of pH and salinity predicted to accompany evaporation, the high salt (10% and 25%) and high pH media (pH 11) represented conditions that were ultimately never observed in the bulk water column. However, hypersaline conditions may have existed in small areas of low permeability as the flood

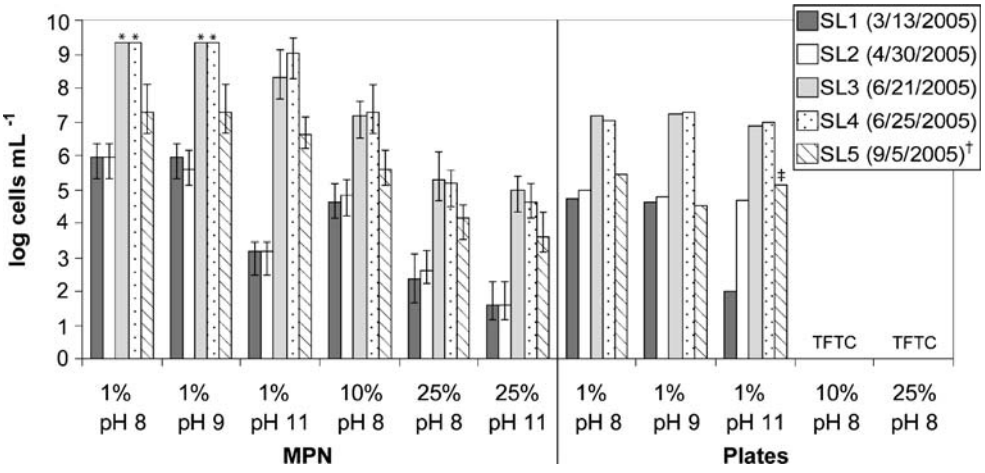

Figure 2 Culturable heterotrophs in liquid MPN tubes (mean±95% confidence interval, n=3) and solid media (average, n=2) at different sampling times (SL1–SL5). Values at the *base of the bars* indicate the salt content as weight percent and pH. *Asterisks* All MPN tubes were positive, so cell numbers were >1×10^9 cells per milliliter in samples from SL3 and SL4 in 1% salt pH 8 and pH 9 media. SL5 data are normalized to gram dry sediment. *Double cross* Data represent a single plate. *TFTC* too few to count

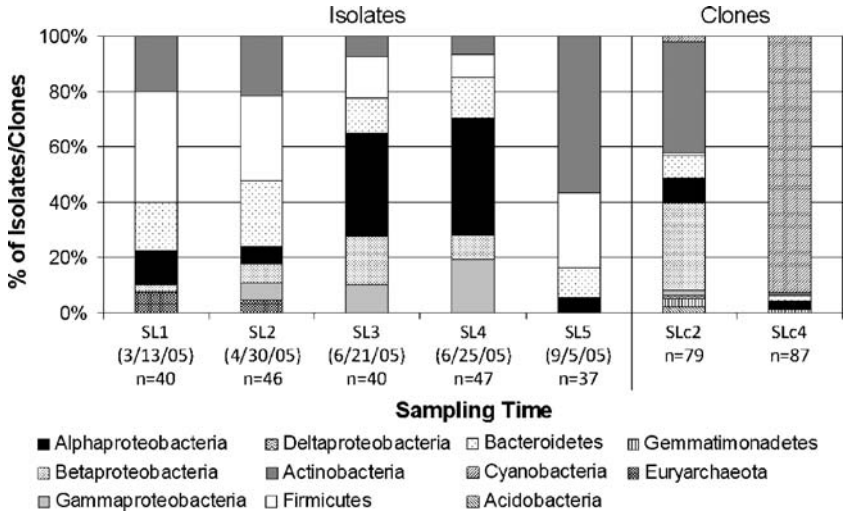

Figure 3 Temporal trends of cultivated microbes from solid media and sequences retrieved from clone libraries. Sequences were binned into phyla and classes (for Proteobacteria) by phylogenetic analysis using ARB [36]

subsided later in the season, providing microniches for extremophiles. Plate and MPN enumerations showed that the heterotrophic community was best suited for growth at the low salinity and moderately alkaline conditions of the playa lake because heterotroph counts on media with 10% or 25% salinity were greater than one and greater than three orders of magnitude lower, respectively, than 1% salinity media at all time points (Fig. 2). Similarly, MPN and plate counts in media with pH 11 were greater than three orders of magnitude lower than in pH 8 or 9 media for SL1 and SL2; however, the microbial community shifted to become more alkalitolerant later in the season, as evidenced by similar MPN and plate counts in media with pH 8, 9, and 11 from SL3 and SL4 (Fig. 2).

Most probable number enumerations were generally one to two orders of magnitude higher than plate counts (Fig. 2). This trend was exaggerated in high salt media, where MPN tubes yielded up to 1×10^7 cells per milliliter, yet plate counts were below the statistical detection limit (30 cells per milliliter).

Early-Flooded Phase Isolates (SL1 and SL2)*)*

More than half of the isolates from the first two sampling times, SL1 and SL2, belonged to the Gram-positive phyla Firmicutes and Actinobacteria (Fig. 3). All isolates in the Firmicutes belonged to the class Bacillaceae. Bacillaceae were isolated on all five solid media (Fig. 4), and were highly diverse. Those isolated on 1% salinity media were closely related to isolates from other environments, mainly soils (mean SSU rRNA gene percent identity to known organisms ~98%). In contrast, those from 10% or 25% salinity media were either related to isolates from soda lakes or salt flats, such as *Marinibacillus* [5, 70], *Halobacillus* [10, 35, 68, 69], *Oceanibacillus* [10], *Marinococcus* [10], and *Filobacillus* [10, 54], or were only distantly related to cultivated organisms (SSU rRNA gene percent identity <95% to known organisms).

Almost all early-phase Actinobacteria isolates belonged to a monophyletic clade in the Microbacteriaceae designated PG1 (Fig. 5). This group was closely related to groups 1 and 2 Wadden Sea Actinobacteria, which were dominant culturable Gram-positive bacteria in the Wadden Sea planktonic community [62]. In contrast, the dominant Actinobacteria from rRNA clone libraries belonged to three of the four freshwater uncultivated Actinobacteria clades described by Warnecke *et al*. [66]: acI (A and B), acII (B), and acIV (A and B) (Fig. 5). Actinobacteria thus appear to have been an important component of the early-flooded phase, and similar organisms are significant components of the picoplankton in freshwater lakes [23, 51, 55], estuaries [66], soda lakes [27], and oceans [8, 51]. The ecological roles of the uncultivated clades remain unknown.

Gram-negative isolates from the early-flooded phase belonged to the Bacteroidetes and the Proteobacteria (Fig. 3). One large group of Bacteroidetes isolates, designated PG3, comprised two clusters related to the estuarine heterotroph *Belliella baltica* [6], one that was isolated from the early-flooded phase and the other that was isolated from the late-flooded phase (Fig. 6). Other early phase Bacteroidetes isolates were related to *Algoriphagus*, *Pontibacter*, or *Flavobacterium*, or were only distantly related to known organisms (SSU rRNA gene percent identity <93% to known organisms). The cultured Proteobacteria from SL1 were related to *Loktanella*, in the Alphaproteobacteria, or *Thauera* in the Betaproteobacteria, yet SL2 isolates were more diverse (Fig. 7). In contrast, Betaproteobacteria were the dominant Gram-negative bacteria in the environmental clone library from SL2, particularly a group of clones related to the facultative methylotroph *Methylibium*. Isolates of *Methylibium* metabolize petroleum hydrocarbons, fatty acids, and C1 compounds [41], so they

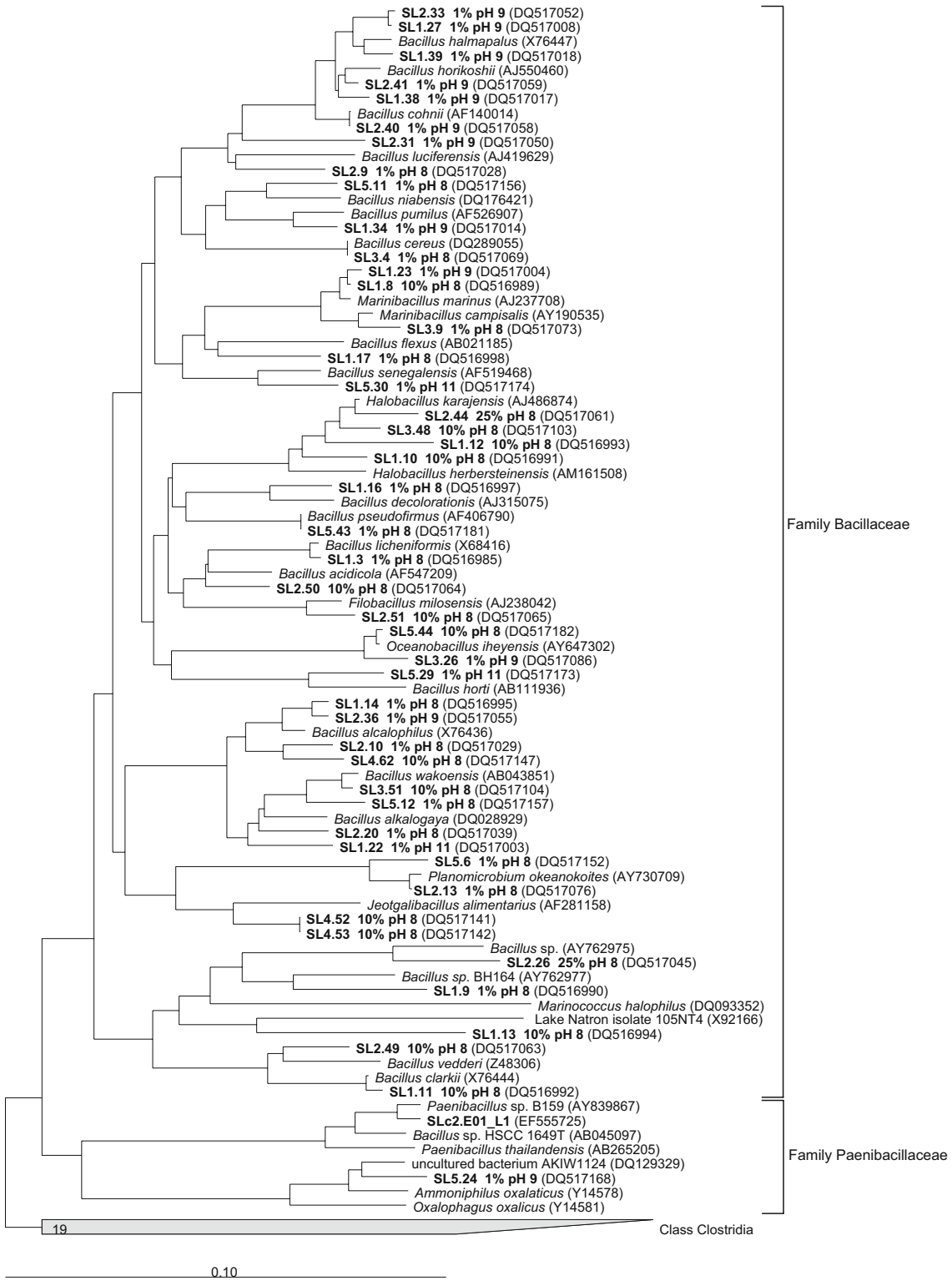

Figure 4 Neighbor-joining tree of SL Firmicutes (*bold*) and other bacteria, including the closest cultivated relative from sequence databases. The tree was produced using *Escherichia coli* nucleotide positions 49–512 [7] in ARB [36]. SL isolates were named according to sampling time (SL1–5). The pH and salinity of the isolation medium is indicated after the strain name. SL clones were named similarly but with a lowercase c after the SL designation. Multiple identical or nearly identical sequences include the number of isolates or clones in the group in parentheses. Accession numbers are indicated in parentheses. Wedges are colored according to whether SL sequences were from isolates (*white*), environmental clones (*black*), or both (*grey*). Genbank numbers for wedged sequences or similar sequences represented by a single sequence are in Table S1. The scale bar indicates 10% sequence difference

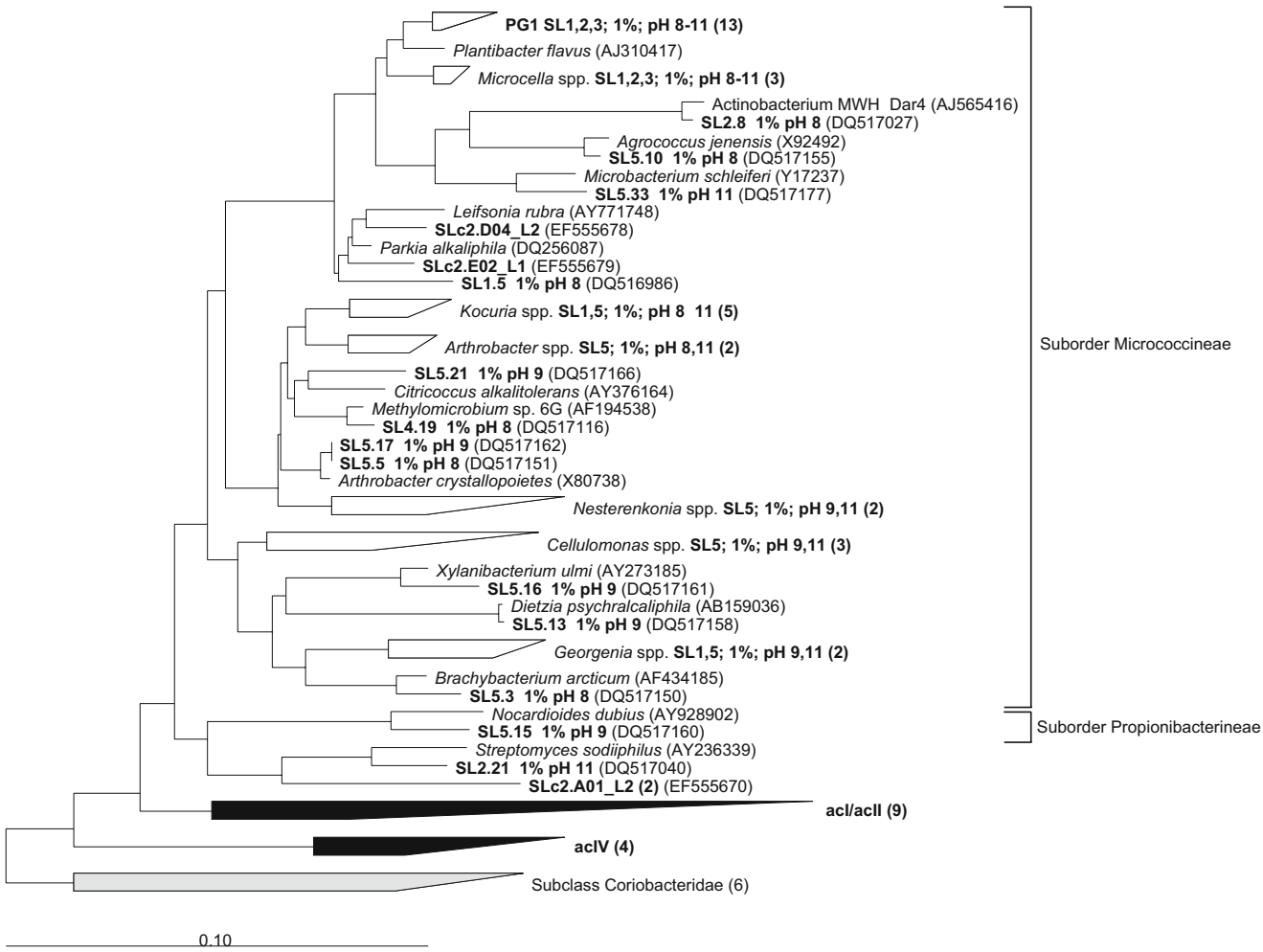

Figure 5 Neighbor-joining tree of SL Actinobacteria (*bold*) and other bacteria, including the closest cultivated relative from sequence databases (*black*). Produced using *E. coli* nucleotide positions 49–512 [7]. For additional information, see the legend for Fig. 4

may not have grown on our isolation media, which relied on utilization of glucose and/or components of peptone or yeast extract.

Late-Flooded Phase Isolates (SL3 and SL4)

Pigmented colonies dominated low salt plates from late-flooded phase, particularly 0.1- to 0.2-cm reddish-brown, raised, circular, shiny colonies with entire edges. Since the low-salt media gave rise to MPN and plate counts at least two orders of magnitude higher than 10% and 25% salt media (Fig. 2), these reddish-brown pigmented colonies were the most abundant organisms culturable with the media and culture conditions used. SSU rRNA gene sequencing of >25 of these pigmented colonies revealed three phylotypes of Alphaproteobacteria: *Rhodobaca*, *Porphyrobacter*, and *Rhodobacter* (Fig. 7). *Rhodobaca* and *Porphyrobacter* were also recovered in clone libraries from SL4, consistent with their relative abundance among heterotrophs from the late playa ecosystem. *Rhodobaca bogoriensis*, the closest relatives of the Silver Lake *Rhodobaca* isolates, is a bacteriochlorophyll-a containing a purple non-sulfur bacterium isolated from soda lakes in the African Rift Valley that is capable of photoheterotrophy on a wide variety of organic compounds [39]. *Rhodobaca* is also closely related to *Roseinatronobacter thiooxidans* and *Rosienatronobacter monicus*, both obligately aerobic heterotrophs from soda lakes [4, 57], a large cluster of isolates from the Wadi an Natrun hypersaline lakes in Egypt [38] and a large cluster of clones from the oxic and anoxic zones of Mono Lake, CA [27]. Thus, this clade may be specifically adapted for continental alkaline waters. *Porphyrobacter* has also been isolated from the Wadi an Natrun [38]; however, the high numbers of isolates from the highest dilution isolation plates from Silver Lake is the first suggestion that this organism might be abundant in some alkaline environments.

Most other colonies on low-salt high-dilution plates from SL3 and SL4 were pink or white/translucent. Pink colonies represented PG3, related to *Belliella*, *Roseococcus*, or a relatively diverse clade only distantly related to known

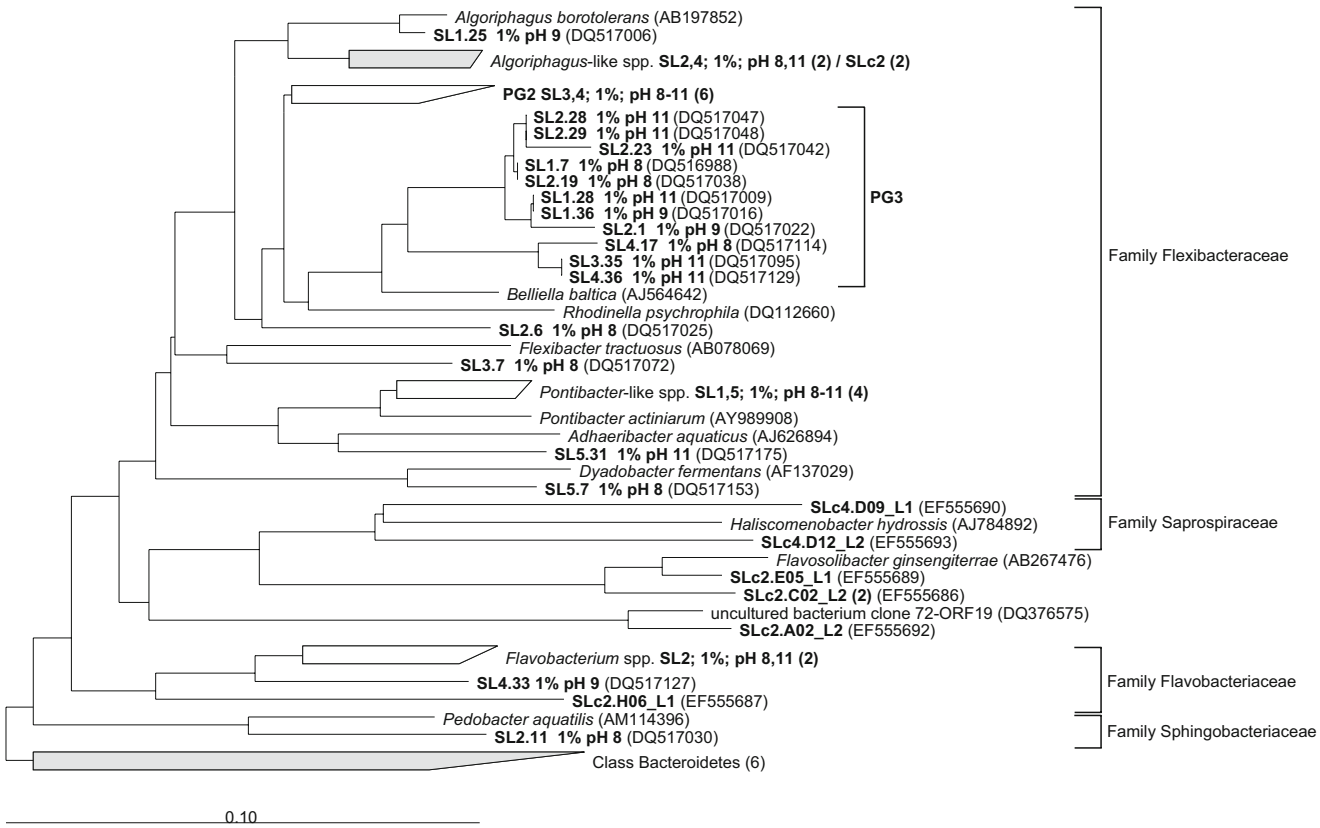

Figure 6 Neighbor-joining tree of SL Bacteroidetes (*bold*) and other bacteria, including the closest cultivated relative from sequence databases (*black*). Produced using *E. coli* nucleotide positions 49–512 [7]. For additional information, see the legend for Fig. 4

organisms, designated PG2 (SSU rRNA gene percent identity <93% to known organisms). Most white or translucent colonies were related to *Thauera* (~93% SSU rRNA gene percent identity), designated PG4, *Alishewanella*, or *Hydrogenophaga*. PG4 was isolated only on pH 9 and pH 11 media, suggesting they may be obligate alkaliphiles, whereas described species of *Thauera* and *Azoarcus* are not known to grow above pH 8.8 [24, 52]. *Hydrogenophaga* was also isolated from SL2 and recovered in the environmental clone library from SL2.

Although all high-salt isolates from the early-flooded playa belonged to the Firmicutes or the halophilic Euryarchaeota (Fig. 3; Fig. 4; data not shown), halotolerant isolates from the late phase playa ecosystem were related to the genera *Marinobacter* and *Idiomarina*. Though these organisms are typically considered marine, prototypical marine genera have been isolated previously from saline soils [10].

Desiccated Phase Isolates (SL5)

Colony morphotypes on low-salt plates from SL5 were highly diverse, yet the majority of isolates were Gram-positive bacteria (>80%; Fig. 3), perhaps suggesting that desiccation selected for Gram-positive bacteria. SL5 Actinobacteria spanned eight families, with the majority grouping within the Micrococcaceae (Fig. 5). Because SL5 Actinobacteria have close relatives that are known soil inhabitants and they were isolated only from the dry playa bed, it is possible that they were terrestrial bacteria that persisted in the wet playa but only became numerically important upon desiccation. Alternatively, since sediment was not sampled while the playa was flooded, these Actinobacteria might be normal inhabitants of the sediment during both dry and flooded conditions. Firmicutes from SL5 were also highly diverse (Fig. 4).

Comparison of Cultivation-dependent and Cultivation-independent Studies

The limitations of microbial cultivation and isolation as an approach to survey microbial communities are well documented [1, 59]. Although our study made use of media that were based on the chemistry of habitats similar to Silver Lake and used dilute carbon and energy sources that are commonly used by chemoheterotrophs, the cultivation approach certainly excluded important ecotypes, such as obligate photo- and chemoautotrophs and obligate anaerobes. Therefore, to gain a more comprehensive understanding of the microbial communities present during flooded phases, cultivation studies were

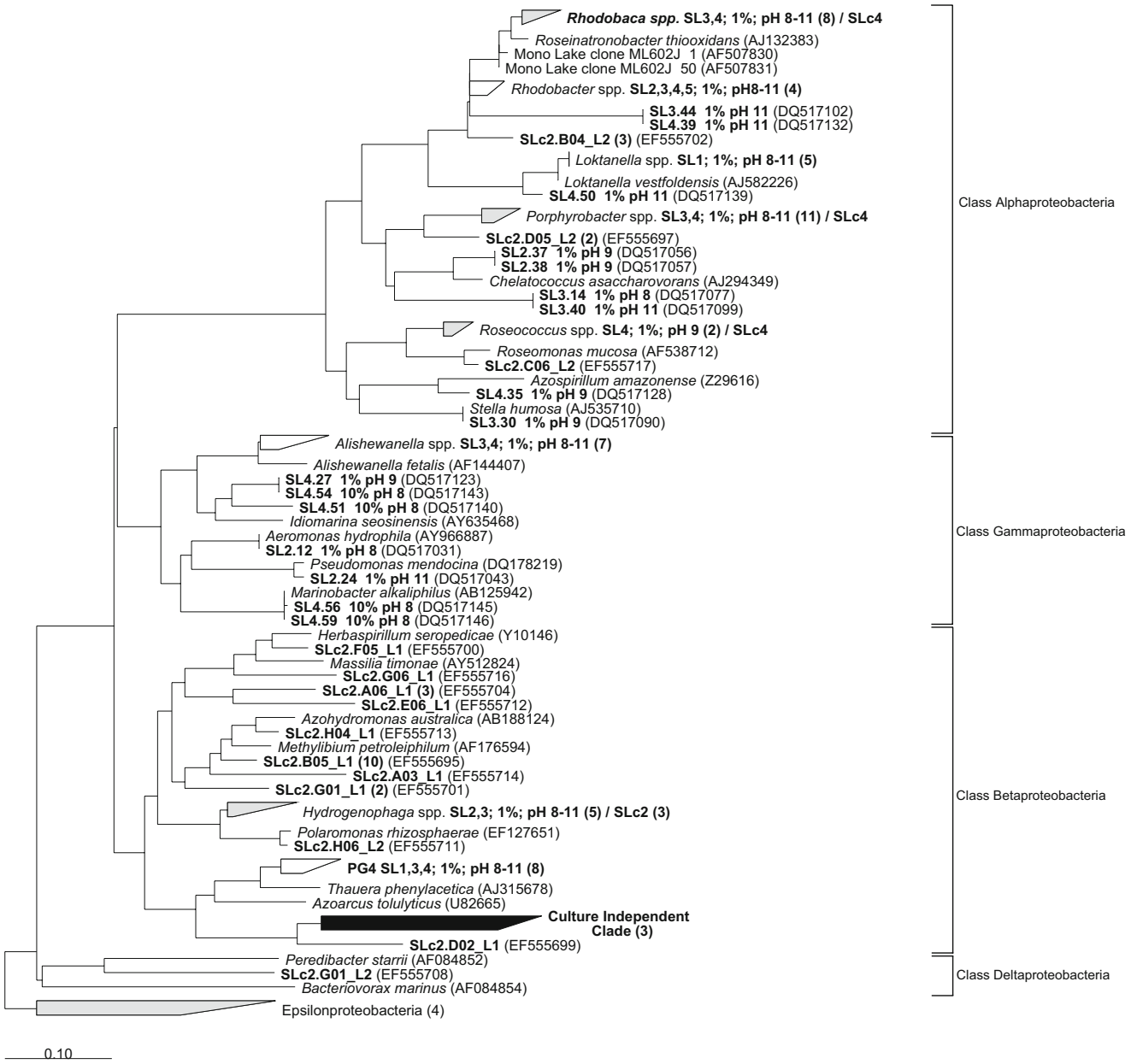

Figure 7 Neighbor-joining tree of SL Proteobacteria (*bold*) and other bacteria, including the closest cultivated relative from sequence databases (*black*). Produced using *E. coli* nucleotide positions 49–512 [7]. For additional information, see the legend for Fig. 4

complemented with cultivation-independent censuses of bacteria in SL2 and SL4. Although PCR-based censuses also suffer from biases such as differential DNA extraction, primer selectivity, and variable SSU rRNA gene copy number [64], the combination of cultivation-dependent and cultivation-independent approaches offers some relief from the biases inherent in each approach.

Statistical analysis of the clone library data confirmed that the early- and late-phase flooded playa hosted very different microbial communities (ΔC_{xy}=11.731 (P=0.001) and ΔC_{yx}= 13.744 (P=0.001)[56]). In addition, corresponding isolate libraries and environmental clone libraries were very different (Fig. 3). Betaproteobacteria and Actinobacteria were underrepresented among isolates from SL2, compared with the environmental clone library, whereas Firmicutes and Bacteroidetes were overrepresented (Fig. 3). In addition, Gemmatimonadetes, Acidobacteria, and Deltaproteobacteria were detected only in the cultivation-independent study from SL2, but their low abundance in the library suggested they were not abundant in the playa aquatic community.

The clone library from SL4 was dominated by Cyanobacteria, primarily *Synechococcus* (81 clones; Fig. 3, Fig. S1), which were not targeted by our cultivation strategy. Some strains of *Synechococcus* fix nitrogen in the dark under

anaerobic conditions [19, 31, 61]. If the late-phase water column did become anaerobic on some nights, as may be suggested by the depletion of nitrate, unicellular diazotrophic cyanobacteria may have possessed the temporal versatility to dominate over non-diazotrophic phototrophs under conditions of low nitrogen and cyclic anoxia as they do in many photosynthetic mats [61]. Thus, primary production and nitrogen fixation by *Synechococcus* may have supported the bloom of heterotrophs in the late-stage community. In contrast, the collection of heterotrophic isolates from SL4 was dominated by Alphaproteobacteria related to *Rhodobaca* and *Porphyrobacter*. These two genera were also recovered in the environmental clone library, suggesting that they may have been abundant in the late-flooded playa ecosystem, although clearly the cyanobacterial dominance in the library obscured much of the heterotrophic diversity.

Conclusions

Our study of the microbial community and geochemistry in Silver Lake playa revealed a dense, diverse, and dynamic microbial population. Microorganisms responded to, and in the case of N and P, controlled geochemical changes within the desiccating playa. However, the detailed interactions between the aqueous phase chemistry, the solid-phase mineralogy, and the various microbial inhabitants of the playa ecosystem remain poorly understood and should be fruitful ground for future investigation.

The extremely high density of culturable heterotrophs in the late-flooded playa ecosystem was unexpected since total microscopic counts in natural waters, marine or fresh, are almost always 2×10^5 to 5×10^6 cells per milliliter, regardless of nutrient content [20], and the concentration of culturable heterotrophs is typically two to three orders of magnitude lower [59]. Although the extremely high density of microorganisms in the playa can be generally understood to result from high nutrient concentrations that accumulated in the playa watershed over the years between wetting events (e.g., by dry fall), the factors that allowed heterotrophs to bloom to $>1\times10^9$ cells per milliliter remain unclear. To our knowledge, this is the highest cell density to date reported for any aquatic habitat. The highest density previously reported was from shallow alkaline pools in Austria (direct "heterotrophic" count of 0.5×10^9 cells per milliliter and direct Cyanobacteria count of 1.1×10^9 cells per milliliter). That study also reported the highest secondary production rates, 738 μg C l^{-1} h^{-1}, and specific growth rates, 1.65 h^{-1}, in any natural aquatic system [17]. Similar to Silver Lake, the microbial population in the Austrian soda pools peaked in summer and the authors attributed high microbial concentrations to turbidity and temperature for Cyanobacteria and heterotrophs, respectively, rather than nutrient concentration due to evaporation [17]. If temperature really is important in controlling heterotrophic cell density in this type of habitat then the high water temperature of the late playa ecosystem, resulting from the high surface area/volume ratio of the playa and the hot Mojave Desert summer, may help explain why heterotroph concentrations in Silver Lake exceeded reports from other alkaline aquatic systems.

The abundance of bacteria in alkaline waters indicates that the microbial loop that operates in marine and freshwater pelagic systems is altered to allow higher cell concentrations, as has been suggested by others [27]. Because the factors controlling cell density in these systems are unknown, it might be informative to measure and account for bacterivory and bacteriophage activity in ephemeral alkaline habitats to try to illuminate the nature of the alteration. Of note, although Mono Lake, CA maintains a high concentration of bacteria, it also hosts an extremely dense population of viruses [29].

The changes in microbial density and composition documented in this study may suggest that a fresh round of microbial community succession occurs through each flooding cycle whereby Gram-positive bacteria are the main survivors from the previous wet cycle and thus the dominant colonial seed stock for freshly inundated playas. Later, as the community matures, Proteobacteria successionally replace the Gram-positive bacteria among cultivable heterotrophs; however, it is uncertain from our study which environmental parameters controlled the succession (e.g., cyanobacterial bloom, increased temperature, N limitation). It is also unknown whether recharge playas, which are much more common than discharge or through-flow playas, host similar microbial communities that undergo successional changes within an accelerated timescale or whether the shorter duration of their flooding cycles and lower evaporite accumulation selects for entirely different microorganisms and microbial processes.

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