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Geomicrobiological Changes in Two Ephemeral Desert Playa Lakes in the Western United States

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The geochemistry and microbiology of two ephemeral playa lakes in the Western United States, Surprise Valley Alkali Lake (SVAL) and Eldorado Playa (EP), were examined over one wetting cycle, revealing dramatic temporal changes in suspended mineralogy, aqueous chemistry, and bacterial populations. In SVAL the predominant suspended mineral changed from smectite to vermiculite and clinoptilolite, which led to a depletion of soluble Mg^{2+} . Nitrate became depleted in both playas as a result of biological nitrogen demand imparted by unusually dense microbial communities reaching $\sim 1 \times 10^8$ cultivable heterotrophs per ml of water. One hundred eighty eight bacterial isolates were obtained, representing sixty phylotypes and four phyla: Actinobacteria, Bacteroidetes, Proteobacteria, and Firmicutes. Phylogenetic analyses suggested that the microbial communities reflected different phases of succession, with SVAL changing from a diverse community with abundant *Yonghaparkia* to a less diverse late summer community with abundant Bacteroidetes and Proteobacteria such as *Loktanella*, *Rhodobaca*, *Saccharospirillum*, *Flexibacter*, and phylogenetically novel members of the Flexibacteriaceae. In EP, a diverse assemblage of bacteria often associated with soils was replaced very quickly by a much less even community dominated by *Yonghaparkia*, *Sandarakinorhabdus*, and relatives of *Belliella baltica*. Strikingly, the early summer microbial community from

SVAL was not significantly different from the EP community that developed within one week of flooding, even though these playas are almost 1000 km apart, whereas sympatric communities in different phases of succession were different. To our knowledge, this is one of the first geomicrobiological studies of a recharge playa, the dominant playa type worldwide.

Keywords Playa, Great Basin, Mojave, alkaline, Nevada

INTRODUCTION

Desert playas are defined as hydrologic basins having a negative water balance (i.e., the net water influx through precipitation and runoff is less than the net water efflux through subsurface drainage and evaporation; Rosen 1994). Playa surfaces are among the flattest landforms known, with slopes of typically less than 0.2 meters per kilometer, and sediments composed primarily of clays infused with salts, the composition of which depends upon the host lithology (Rosen 1994). Playas can range from permanently wet and highly saline for closed-basin discharge playas, to rarely wet with few evaporites for hydrologically open recharge playas (Rosen 1994). A playa with restricted drainage, termed a through-flow playa, is intermediate with regard to water and solute retention. The Great Basin and Mojave Deserts of the western United States contain hundreds of mostly sodium carbonate/chloride recharge playas, the remnants of endorheic Pleistocene lakes (Neal 1975; Rosen 1994).

In the Mojave, most playas are flooded a few days per year or less due to low annual precipitation (~ 11.4 cm/yr), rapid drainage, and high rates of evaporation, imparted by high annual temperatures ($\sim 20.1^\circ\text{C}$) and low annual relative humidity ($\sim 30\%$; climatic data from (NOAA 2007)). Thus, in addition to extremes in temperature, the microbial inhabitants of Mojave recharge playas must endure long periods of metabolic inactivity during which they are limited by low water activity and subject to ablation, punctuated by episodic and ephemeral periods of

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intense activity during flooding. Playas of the Great Basin, by contrast, receive more water due to higher precipitation (~ 19.0 cm/yr) and evaporate more gradually due to cooler temperatures ($\sim 10.7^\circ\text{C}$) and higher annual relative humidity ($\sim 50\%$; climatic data from NOAA 2007), leading to higher frequency and longer duration of playa flooding.

Despite a growing body of literature on the microbiology and geochemistry of discharge playas (Blum et al. 2001; Oremland et al. 2005; Kulp et al. 2006), which are relatively uncommon, the biogeochemistry of flooded recharge and flow-through playas has not received much attention. In the current study, we investigated the geomicrobiology of two flooded playas, Surprise Valley Alkali Lake (SVAL), a large through flow playa in northeastern California, and Eldorado Playa (EP), a small recharge playa in southern Nevada. The study was designed to address several questions broadly aimed at determining whether flooded desert playas develop specific microbial communities and, if so, how they change through time, along with physical and geochemical changes that accompany evaporative desiccation.

MATERIALS AND METHODS

Site Descriptions and Sampling

SVAL is a narrow ~ 70 km chain of lakes in the northwestern Great Basin near Cedarville, CA that delineates the deepest portions of Pleistocene Surprise Lake (Reheis 1999). It encompasses four major seasonal lakes, Lower Alkali Lake, Upper Alkali Lake, Middle Alkali Lake, and Cowhead Dry Lake. The lakes are recharged every spring by relatively reliable snowmelt from the Warner Mountains to the west, supplemented by irrigation runoff. Although hydrologic data on the lakes is insufficient to classify them definitively, Middle Alkali Lake (Figure 1), where this study took place, can be considered a through-flow playa or a recharge playa since it is only intermittently wet. SVAL was flooded throughout the summer of 2006 and was sampled in early June and late August. A third sampling trip revealed that the playa was dry by October. SVAL samples were obtained from the water column of Middle Alkali Lake, approximately 4 kilometers east of Cedarville, CA, from the south side of the road from a causeway that bisects the lake at GPS location N $41^\circ 32.141'$, W $120^\circ 06.454'$ (datum: WGS84).

EP is a small recharge playa located in the eastern Mojave Desert (Figure 1) about 15 km south of Boulder City, NV. EP is not flooded every year and floods that do occur are brief due to rapid evaporation and subsurface recharge. EP was monitored throughout 2006 and 2007, during which it flooded twice. During one of these events in October of 2006, EP was sampled within 24 hours of flooding and also a week later, immediately before the water disappeared into the subsurface. EP samples were taken from the water column from roughly the lowest point in EP from GPS location N $35^\circ 52.211'$, W $114^\circ 55.975'$ for EP1 and N $35^\circ 51.254'$, W $114^\circ 56.701'$ for EP2 (datum: WGS84) (Figure 1; Table 1).

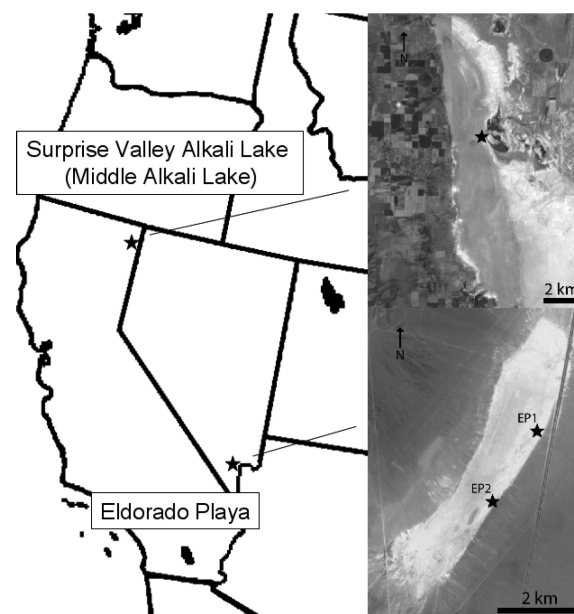


FIG. 1. Satellite view of SVAL Middle Alkali Lake during a hydrated phase (left) and EP during a dry phase (right). Scale bars, 2 km. Images modified from Google Maps. Stars approximate sampling locations.

Temperature, conductivity, and pH were measured in situ by using a Smart Water Analysis Laboratory (LaMotte, Chesterton, MD). Water samples for cultivation were taken from the water column using sterile 50 cc polypropylene tubes, which were transported on ice to the laboratory. Samples for major ions were transported on ice to the lab, centrifuged (10 min at $5000 \times g$) to remove suspended solids, filtered through a $0.2 \mu\text{m}$ hydrophilic polyether-sulfone filter for anions or a $0.2 \mu\text{m}$ nylon filter for cations (Pall Life Sciences, Bath, England), 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 of filtered samples to pH 4.5 with 1.6 N sulfuric acid (LaMotte, Chesterton, MD).

Mineralogy

The X-ray diffraction (XRD) analyses were made on clay fractions separated by centrifugation and sedimentation following rinsing with distilled water to achieve dispersion. Oriented pastes of K- and Mg-saturated clays ($< 2 \mu\text{m}$) were prepared by smearing the clays onto glass slides (Theisen 1962). The K-saturated sample slides were examined by XRD at 25°C and after heating at 350°C and 550°C for two hours. The Mg-saturated samples were also analyzed at 25°C and after being placed in a desiccator containing a pool of ethylene glycol and heated at 65°C for 2 hours. The desiccator vent was closed upon removal from the oven and the slides stored in the desiccator at least 12 hours prior to XRD analysis. All samples were examined by XRD ($\text{CuK}\alpha$ radiation) using a PANalytical X'PERT

TABLE 1
Sampling conditions, chemistry^a, and cell density

	SVAL1	SVAL2	EP1	EP2
Date	6/2/2006	8/30/2006	10/15/2006	10/22/2006
Water Temperature (°C)	17.8	25.1	21.4	23.2
Conductivity (mS/cm)	2.72	18.85	0.350	1.485
pH	9.05	9.47	9.11	8.89
Alkalinity (ppm CaCO ₃)	300	580	96	410
Suspended minerals ^b	S,I,K	V,I,K,C,P	S,I,K,Ch	S,I,K,Ch
Dissolved oxygen (% sat.)	ND ^c	ND	100.1	148.5
Cations				
Sodium (mM)	27.73	223.31	3.21	14.67
Calcium (μM)	107.51	180.84	39.79	171.36
Potassium (μM)	27.88	159.85	32.61	86.95
Magnesium (μM)	24.69	<0.0123	<0.0123	<0.0123
Anions				
Chloride (mM)	11.05	127.06	0.97	4.75
Sulfate (mM)	1.43	16.17	0.16	0.77
Nitrate (μM)	30.63	<0.0348	49.10	<0.0348
Phosphate (μM)	44.12	113.83	5.48	2.60
Bromide (μM)	17.65	237.98	<0.005	<0.005
N:P (mol. ratio) ^d	0.68	<0.00030	8.9	<0.013
Isolate diversity (OTUs) ^e	23	13	20	15
Cell density				
Plate counts (± S.D.; n = 3)	1.3 × 10 ⁶ (± 1.0 × 10 ⁶)	7.1 × 10 ⁶ (± 0.55 × 10 ⁶)	1.5 × 10 ⁵ (± 1.3 × 10 ⁴)	1.8 × 10 ⁵ (± 2.5 × 10 ⁴)
MPN (± 95% CI; n=3)	7.0 × 10 ⁷ (2.0-28 × 10 ⁷)	1.1 × 10 ⁸ ± (0.3-4.8 × 10 ⁸)	5.0 × 10 ⁶ (0.2-2.4 × 10 ⁶)	1.1 × 10 ⁸ ± (0.3-4.8 × 10 ⁸)

^aNeither lithium, nitrite, nor ammonium were detected. Detection limits: Li⁺, 4.32 μM; NH₄⁺, 23.3 μM; NO₂⁻, 86.9 nM.

^bS, smectite; I, illite; K, kaolinite; V, vermiculite; C, clinoptilolite; P, plagioclase feldspar; Ch, chlorite. Dominant mineral is bolded.

^cND, not determined.

^dN:P mol. ratios > 16 suggest the ecosystem is P limited. N:P < 16 suggests the ecosystem is N limited (Sternner and Elser 2002).

^eOTUs were defined at the 3% level by using DOTUR (Schloss and Handelsman 2005).

Pro diffractometer, equipped with an X'Celerator detector. The minerals present were primarily identified based on their basal or d(001) spacings.

The d(001) spacing is the distance between successive identical layers along the c-axis, including the interlayer region, whose thickness changes in response to adsorbed interlayer cations and the amount of water present. The various Mg- and K-saturation, heat, and glycolation treatments were used to distinguish and identify the different clay minerals present. For example, vermiculite was distinguished from smectite based on ethylene glycol treatment of Mg-saturated clays. Both smectite and vermiculite exhibit d(001) peaks at about 1.4 nm when Mg-saturated; smectite further expands to 1.6 to 1.8 nm upon treatment with ethylene glycol. Vermiculite also collapses to 1.0 nm with K-saturation whereas K-saturated smectite typically has a d(001) peak at 1.24 to 1.28 nm. Heating to 350°C is necessary to fully collapse K-saturated smectite to 1.0 nm.

Cultivation and Media

Since microscopic or flow cytometric direct counts could not be reliably obtained due to very high burdens of suspended mineral particles, a cultivation-based approach was utilized to obtain estimates (likely an underestimate) of heterotrophic cell density. Samples were serially 10-fold diluted in triplicate into liquid SL medium (pH 9, 1% NaCl) and incubated for most probable number (MPN) analysis or spread onto the same medium solidified with 2% agar. SL medium contains 0.2 g/ml each of yeast extract, peptone, and D-glucose, and major salts representative of desert playas: 0.1 g/l NaCl, 0.67 g/l MgSO₄, 0.012 mg/l FeCl₃ and 0.033 mg/l CaCl₂. Next, 900 ml of the organic/salt solution 100 ml of a 10X NaHCO₃ stock (50 g l⁻¹) were autoclaved separately and brought to the appropriate pH with filter-sterilized KOH or HCl after mixing.

Cultivations were incubated aerobically at room temperature in the dark and scored periodically for at least 4 months. MPNs

were scored according to de Man (1975), and all were conclusive within 2 weeks. Fifty colonies, spanning the diversity of obvious colony morphotypes, were picked from the most dilute positive plates from each sampling time and streaked for isolation and molecular identification.

Molecular Analysis of Isolates

Isolate DNA was released using a colony lysis buffer (Johnson et al. 2001) and 16S rRNA genes were directly amplified by PCR using primers specific for bacteria: 9bF (Eder et al. 1999) and 1406uR or 1512uR (Eder et al. 1999). PCR products were sequenced at the Nevada Genomics Center from the forward primer in a 96-well format (Applied Biosystems Prism 3730 DNA Analyzer). The mean sequence length with Phred score >20 was 668 base pairs. Sequences were aligned, manually corrected, and analyzed phylogenetically (Neighbor-joining, Kimura 2-parameter correction) in ARB (Ludwig et al. 2004). Operational taxonomic units (OTUs) were defined by using the nearest neighbor algorithm in DOTUR (Schloss and Handelsman 2005). Phylogenies of microbial isolate libraries were compared using Unifrac (Lozupone et al. 2006).

The GenBank accession numbers for SSU rRNA gene sequences generated in this study are EF522845-EF522944, for SVAL sequences, and EU279862-EU279955, for EP sequences.

RESULTS

Playa Mineralogy and Geochemistry

SVAL was sampled in June (SVAL1) and August (SVAL2) of 2006. EP was sampled within 24 hours of initial flooding on October 15, 2006 (EP1) and shortly before the last surface water had disappeared on October 22, 2006 (EP2, Table 1). At the times of sampling, both playas had shallow water columns (<2 m for SVAL; <1 m for EP) that were extremely turbid due to wind-driven mixing and electrostatic mineral suspension. Although the electrolyte concentrations were relatively high (Table 1), it is possible for stable suspensions of clay to form in these solutions. The critical coagulation concentration (CCC) is the lowest electrolyte concentration at which a colloidal suspension begins to undergo rapid coagulation, and is dependent on both the nature of the minerals in suspension and on the composition of the electrolyte solution. Smectites and vermiculite are both easily dispersed, particularly in the presence of Na^+ , which is the dominant cation present in these suspensions. Additionally, it has been shown that the concentration of a monovalent electrolyte suspension must be 64 times greater than that of a divalent electrolyte suspension to cause flocculation (Essington, 2004).

Suspended clays in SVAL1, EP1, and EP2 were identified by X-ray diffraction (XRD) as predominantly smectite with minor amounts of illite and kaolinite (Table 1). In addition, small amounts of chlorite were observed in samples from EP. In contrast, XRD analysis of suspended and sediment minerals from SVAL2 revealed that vermiculite was the dominant min-

eral present in the clay fraction. Smectite was not identified in the clay fraction of these samples. The zeolite clinoptilolite and potassium feldspar were also observed in SVAL2. This change in the dominant mineralogy was accompanied by a dramatic decrease in the concentration of soluble magnesium, possibly due to the sorption of Mg^{2+} by vermiculite and/or clinoptilolite, each of which have very high cation exchange capacity (CEC, 130 to 220 cmol per kg; Boettinger and Ming 2002; Malla 2002).

If vermiculite and clinoptilolite were precipitating from solution, then Mg^{2+} may also have been incorporated into the structures of both minerals. Estimating the structural formulas of the vermiculite and clinoptilolite based on dissolution and total elemental analyses is possible; however, such analyses are generally performed on monomineralic samples. Determining accurate structural formulas of minerals in samples with several different minerals is very difficult because of the uncertainties in assigning amounts of elements to each mineral.

Both playas displayed marked depletions in soluble nitrate (Table 1). In contrast, most other ions increased in concentration, suggesting solute concentration through evaporative water loss. Nitrate depletion was not due to nitrate mineral precipitation because the common nitrate minerals nitratine (NaNO_3 , sol. 815 g/l) and niter (KNO_3 , sol. 360 g/l; Crowley and Hook 1996) were far from saturation concentration. Therefore, nitrate depletion was mostly likely biological, probably via denitrification, nitrate assimilation, or both. In EP the concentration of soluble phosphate also decreased at concentrations well below saturation for common phosphate salts such as brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) or monetite (CaHPO_4) and the absence of these minerals in XRD suggests biological assimilation. In SVAL the high concentration of soluble phosphate, up to 0.11 mM, may have been a result of agricultural runoff since agricultural fields, primarily alfalfa, lie between SVAL and its recharge source, the Warner Mountains.

Bacterial Community Composition and Succession

Heterotrophs were enumerated by MPN analysis, revealing dense communities of $\sim 7.0 \times 10^7$ and $\sim 1.1 \times 10^8$ cultivable cells/ml in SVAL1 and SVAL2, respectively. In contrast, in EP, a population of $\sim 5.0 \times 10^6$ bloomed to $\sim 1.1 \times 10^8$ cultivable cells/ml within 1 week. 16S rRNA gene sequencing and phylogenetic analysis revealed that the 188 isolates obtained by serial dilution and plating belonged to 60 OTUs at a cutoff of 97% identity. The dominant isolates in both playas belonged to the same three phyla: Proteobacteria, Bacteroidetes, and Actinobacteria. In addition, a few strains of Firmicutes were obtained from EP (Figures 2 and 3).

At SVAL, despite similar cell density at the two sampling times, the composition of cultivable heterotrophs changed dramatically, with the most notable changes being a decrease in overall diversity from 23 to 13 bacterial OTUs and the concomitant replacement of Actinobacteria by Proteobacteria (Figure 2). SVAL1 isolates included one dominant Actinobacteria

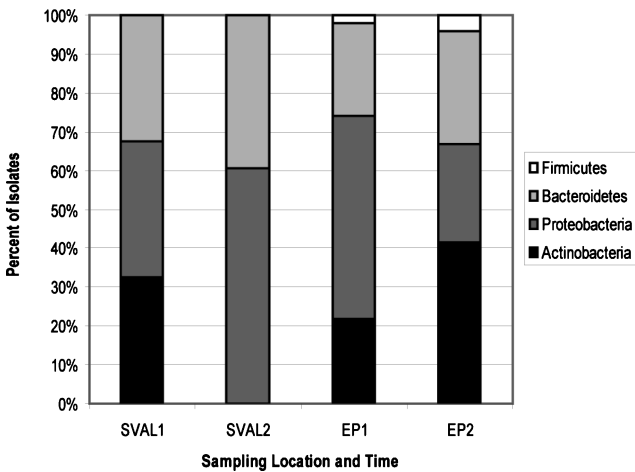


FIG. 2. Phylum-level summary of isolates from SVAL and EP, based on partial 16S rRNA gene sequencing. Sequences were binned by DOTUR (Schloss and Handelsman 2005) and subsequent phylogenetic analysis in ARB.

phylotype related to *Yonghaparkia alkaliphila* (Figure 3) and diverse Proteobacteria (Figure 4) and Bacteroidetes (Figure 5). SVAL2 was enriched in Proteobacteria, particularly two Alphaproteobacteria OTUs, *Loktanella* and *Rhodobaca*, and six

Gammaproteobacteria OTUs, particularly a group related to *Saccharospirillum* (Figure 4). Although Bacteroidetes, as a whole, were similarly represented in SVAL1 and SVAL2, the diversity decreased from ten OTUs to four. Two were unique to SVAL2, including relatives of *Flexibacter tractuosus*, which dominated among cultivable Bacteroidetes (Figure 5). The other two were isolated from both time points and likely represent new genera in the Flexibacteriaceae.

A dynamic microbial community succession was also observed in EP, both in terms of cell density and composition, which may be considered surprising considering the short flooding duration. However, in contrast to SVAL, the succession occurred within the three dominant phyla, rather than between them (Figure 2). The diversity of isolates decreased from 20 OTUs in EP1 to 15 OTUs in EP2, corresponding to the replacement of a diverse assemblage of bacteria typically isolated from soils, such as *Paracoccus*, *Xanthomonas*, *Pseudomonas*, and a wide variety of Actinobacteria, by three OTUs that accounted for more than two-thirds of all EP2 isolates: *Yonghaparkia* in the Actinobacteria (Figure 3), a group related to *Sandarakinorhabdus limnophila* in the Alphaproteobacteria (Figure 4), and relatives of *Belliella baltica* in the Bacteroidetes (Figure 5).

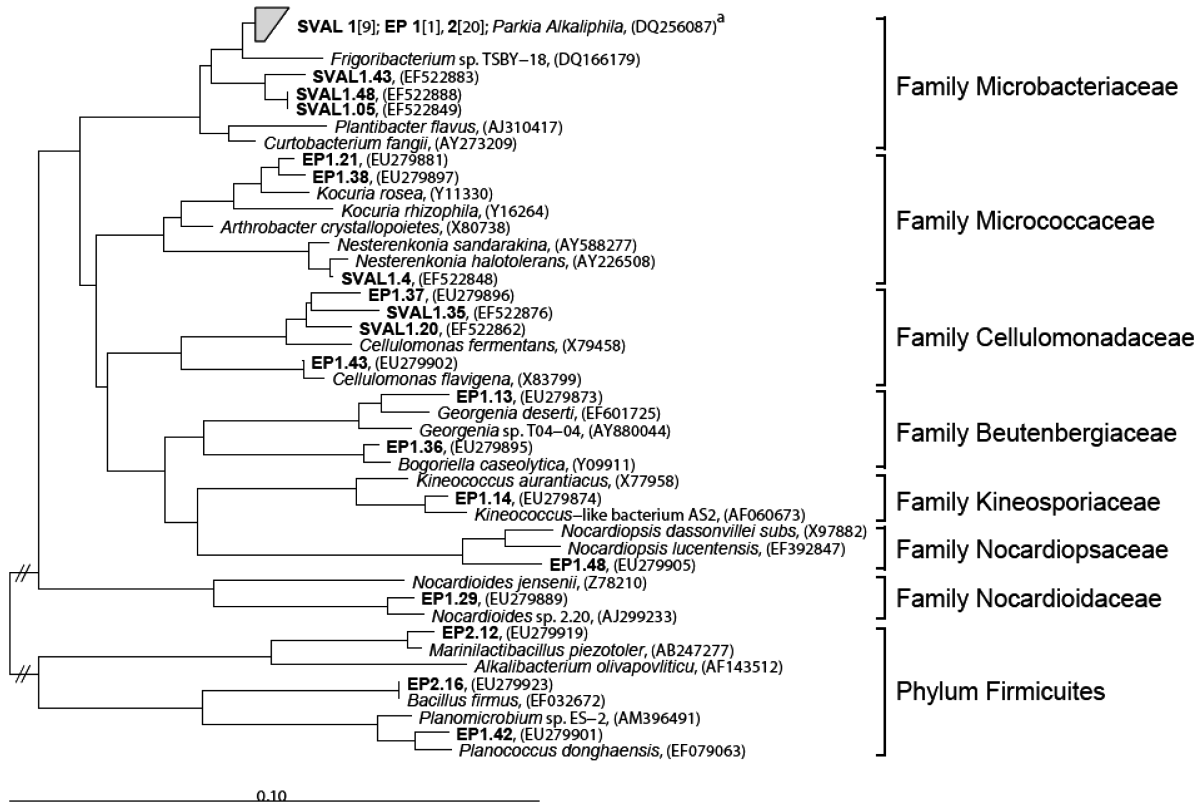


FIG. 3. Neighbor-joining tree of SVAL and EP Actinobacteria (bold) and other bacteria including the closest cultivated relative from sequence databases. Produced using *E. coli* nucleotide positions 98-621 (Brosius et al. 1978) in ARB (Ludwig et al. 2004). The scale bar indicates 10% sequence difference. Sampling time points and sequence names are in bold. Numbers in brackets describe the number of sequences with the wedge. a-b. Sequence names and GenBank numbers for wedged sequences are provided in Table S2.

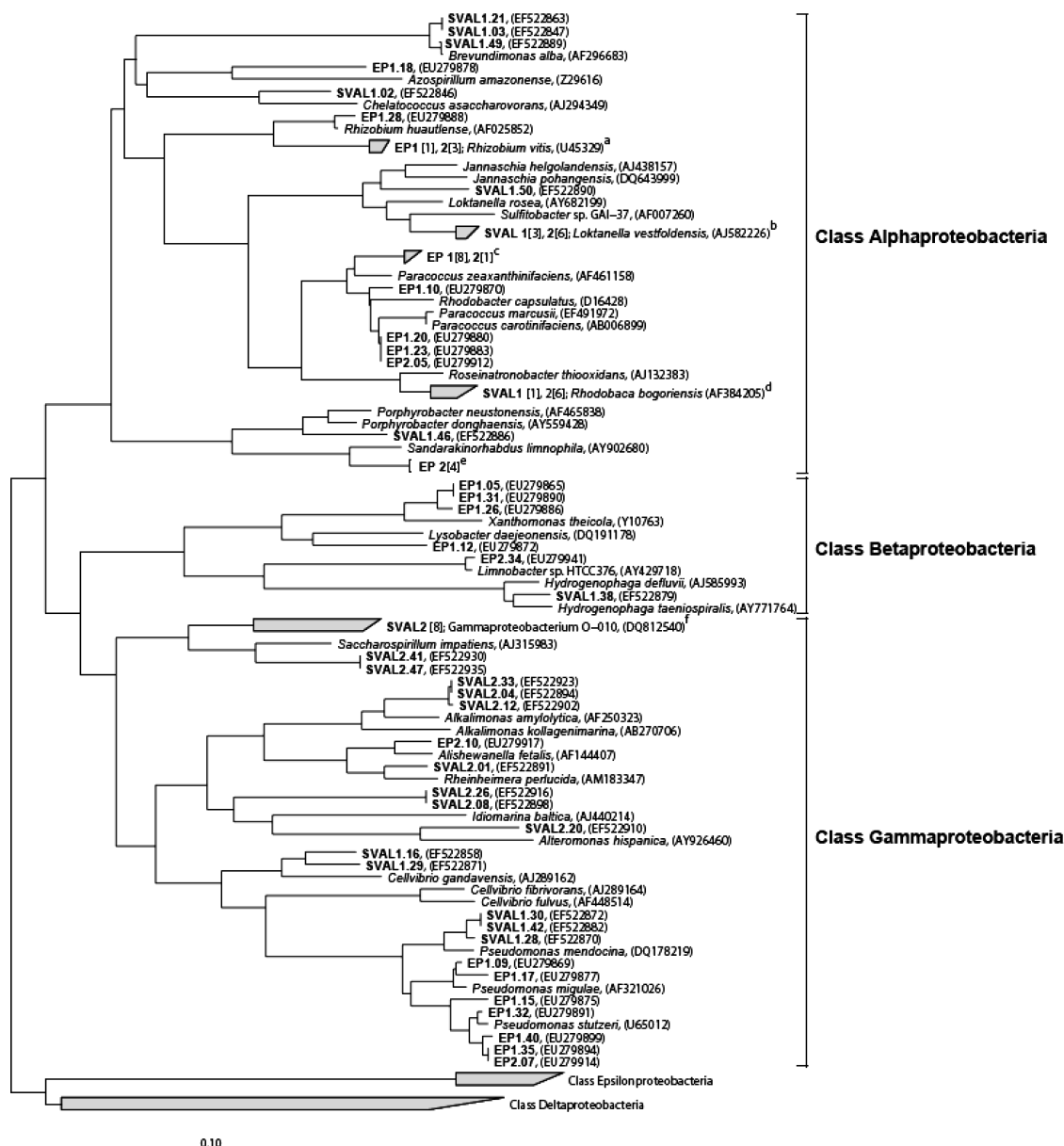


FIG. 4. Neighbor-joining tree of SVAL and EP Proteobacteria (bold) and other bacteria including the closest cultivated relative from sequence databases. The tree was produced using *E. coli* nucleotide positions 101–638 (Brosius et al. 1978) in ARB (Ludwig et al. 2004). The scale bar indicates 10% sequence difference. Sampling time points and sequence names are in bold. Numbers in brackets describe the number of sequences with the wedge. a–g. Sequence names and GenBank numbers for wedged sequences are provided in Table S2.

Within and Between Playa Isolate Comparisons

To better compare the microorganisms isolated from SVAL and EP, all four isolate libraries were compared by using Unifrac (Lozupone et al. 2006). A Unifrac Significance analysis (including Bonferroni Correction; 1000 permutations) indicated that the isolate libraries from SVAL1 and EP2 were not significantly different ($P = 0.0660$), whereas all other pair-wise comparisons were significantly different ($P < 0.006$). Furthermore, a

Cluster Environment Analysis (non-normalized; 1000 permutations) grouped SVAL1 and EP2 to the exclusion of EP1, leaving SVAL2 as the most distant, with all nodes receiving $>99.9\%$ jackknife support (data not shown). However, the similarity between SVAL1 and EP2 was primarily due to the dominance of *Yonghaparkia* in both playas because all libraries were significantly different when *Yonghaparkia* were removed from the Unifrac significance analysis ($P < 0.006$).

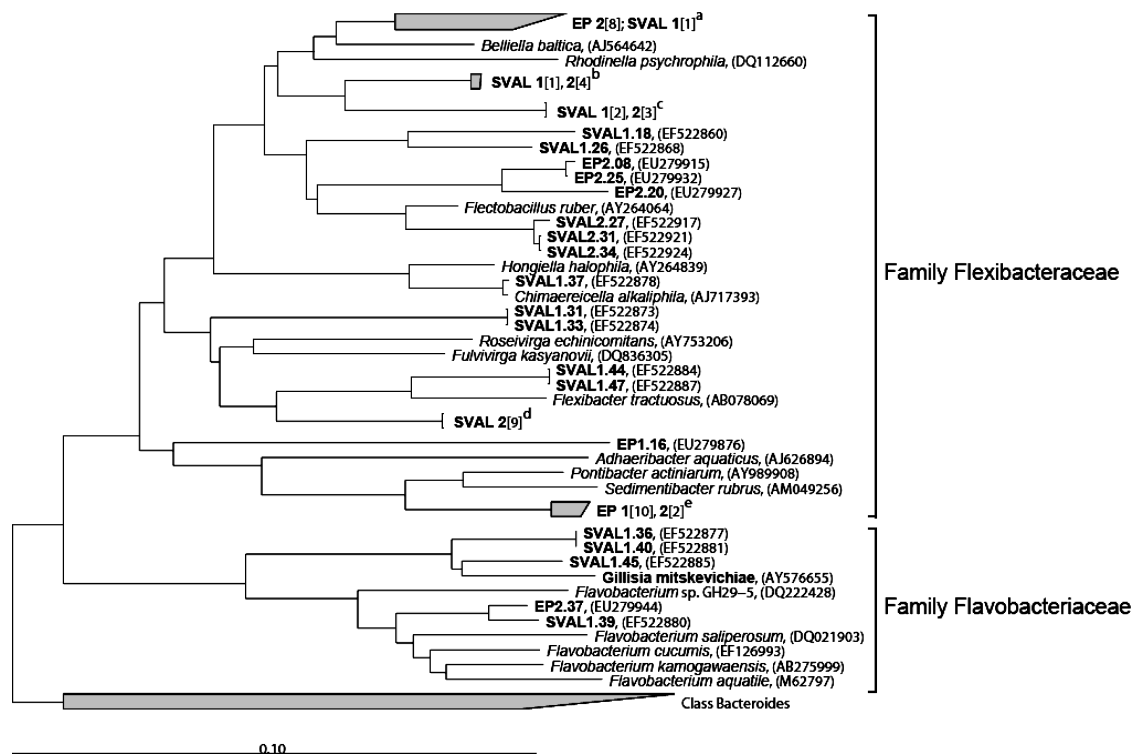


FIG. 5. Neighbor-joining tree of SVAL and EP Bacteroidetes (bold) and other bacteria including the closest cultivated relative from sequence databases. Produced using *E. coli* nucleotide positions 101-624 (Brosius et al. 1978) in ARB (Ludwig et al. 2004). The scale bar indicates 10% sequence difference. Sampling time points and sequence names are in bold. a–f. Sequence names and GenBank numbers for wedged sequences are provided in Table S2.

DISCUSSION

High Cell Densities in Flooded Desert Playas

This study revealed cultivable water-borne heterotrophic microbial populations of $\sim 1 \times 10^8$ cells/ml, which is remarkable because total microscopic counts in typical aquatic ecosystems range from 2×10^5 to 5×10^6 cells/ml, regardless of nutrient content (Fenchel et al. 1998) and the concentration of culturable cells is generally two to three orders of magnitude lower (Staley and Konopka 1985). Nevertheless, the high concentration of heterotrophs was consistent with other studies suggesting that continental alkaline aquatic habitats host extremely dense and productive microbial communities (Jones et al. 1998; Kirschner et al. 2002; Eiler et al. 2003; Humayoun et al. 2003).

MPN enumerations were one to two orders of magnitude higher than plate counts (Table 1). Other authors have noted similar discrepancies in seawater and freshwater (Button et al. 1993; Bussmann et al. 2001), and this phenomenon may reflect an intrinsic inability of some bacteria to form colonies on agar plates (Janssen et al. 2002; Rappé et al. 2002; Zengler et al. 2002).

Microbial Community Successional Patterns in Inundated Playas

This study revealed three significantly different collections of microbial isolates among four samples. Strikingly, similar isolates were obtained from different playas in early stages of suc-

cession rather than from within a single playa at different sampling times. The similarity in microbial composition of SVAL1 and EP2 shows that geographically distant playas may develop similar microbial communities. These similar communities may be an expression of gross similarities in playa geochemistry, for example salinity, that would depend on the stage of the playa in evaporative desiccation more than geographic location per se. Thus, we suggest that through-flow and recharge playas in the Mojave and Great Basin may progress through similar, but not identical, stages of biogeochemical evolution following inundation. EP1 represents the earliest of these stages.

EP1 was sampled within 24 hours of the rain event that flooded the playa; therefore, it is not surprising that EP1 contained fresh water and a relatively dilute population of bacteria typical of soils. EP1 likely hosted a mixture of soil-derived microorganisms flushed in from the watershed and organisms from the dry playa bed that had survived desiccation and ablation since the last flooding or moistening event. Although it is likely that SVAL had similar seed populations, that playa was not sampled at the time of initial flooding.

EP2, sampled only a week after EP1, represented a successional change from the EP1 seed population with a higher concentration of cultivable heterotrophs and a dramatically different microbial community. The ~ 20 -fold increase in the concentration of cultivable heterotrophs was more than what would be expected due to evaporative cell concentration alone because Na^+

only concentrated by a factor of 4.6; however, since we lack direct cell counts due to interference from suspended minerals, the actual change in cell density is unknown. EP2 was similar to SVAL1 in that abundant organisms included members of the Microbacteriaceae related to *Yonghaparkia alkaliphila* and Bacteroidetes related to *Belliella baltica*.

During this stage, the playa water had begun to subside from maximum flood levels and the conductivity had started to increase (1.5–2.7 mS/cm), suggesting evaporative solute concentration, which provided microbial inhabitants of the flooded playas with excess inorganic C, N, and P (Table 1) and measurable amounts of microbiologically relevant trace elements such as B, Co, Cu, Fe, Mn, Mo, Ni, W, V, and Zn (Table S1). This excess of nutrients removed bottom-up controls that can limit microbial growth in typical aquatic habitats (Fenchel et al. 1998) and may be an important factor in the development during this stage of dense water-borne heterotroph populations of $\sim 1 \times 10^6$ to $\sim 1 \times 10^8$ cultivable cells/ml.

However, eventually these flooded playas experienced nitrate depletion, as was documented in EP2 and SVAL2. Unfortunately, in the absence of continuous oxygen monitoring or nitrogen budgeting, including dissolved organic nitrogen (DON), we cannot resolve whether the nitrate depletion was due to biological nitrate assimilation or denitrification or both. Calculations of the N demand required to account for the cell growth based on stoichiometry of pelagic bacteria (Vrede et al. 2002) suggest that the nitrate depletion could be attributed to assimilation in EP because the calculated N demand and observed nitrate depletion are of the same order of magnitude (data not shown). On the other hand, nitrate depletion in SVAL was not accompanied by a bloom of heterotrophs; however, neither “uncultivable” heterotrophs nor phototrophs could be included in these calculations so they should be treated with caution.

Regardless of the mechanism of nitrate depletion, it is clear that the microbial community in EP2 and SVAL2 became N limited because the N:P ratio was much lower (Table 1) than the Redfield ratio of 16:1 (Sterner and Elser 2002). This geochemical constraint would have favored N-fixing organisms. Consistent with this, a high concentration of the N-fixing, heterocyst-forming cyanobacterium *Anabena* was observed in samples from SVAL2 (data not shown). Thus, although the microbial communities in EP2 and SVAL1 were similar, it is likely that EP2 was poised to transition to a third major successional phase but became desiccated before this could occur. This third succession phase is represented by SVAL2.

SVAL2 was taken months after initial playa flooding. The playa water was shallow and receded and the conductivity was high (18.85 mS/cm), indicating further evaporation. The late phase microbial community in SVAL was less diverse but extremely dense, $\sim 1.1 \times 10^8$ cultivable heterotrophs/ml. The dominant heterotrophs were Gram negative bacteria, particularly Alphaproteobacteria, successional replacing Actinobacteria. Important microbial groups appeared to be *Loktanella*, *Flexibacter*, *Saccharospirillum*, relatives of *Rhodobaca*, and novel

organisms in the Flexibacteriaceae. The late flooded playa system also hosted the predominantly marine orders Alteromonadales and Oceanospirillales. Members of the Oceanospirillales and Alteromonadales, especially *Halomonas*, have also been isolated from continental salt flats (Caton et al. 2004), soda lakes (Jones et al. 1998; Borsodi et al. 2005), and solar salterns (Bellnoch et al. 2002).

Possible Effects of Clays on the Biogeochemistry of Flooded Playas

It is interesting to speculate on the influence of suspended clays of desert playas on the density, composition, and activity of microbial inhabitants of playas. Both clays and cells are predominantly negatively charged in nature (Solokov et al. 2001) so it is possible that clays could electrostatically buoy non-motile microorganisms to prevent settling, thereby inflating water-borne cell numbers by preventing cell sedimentation. It is also possible that cells attach directly to suspended clays via outer membrane or cell wall-associated proteins, fimbriae, flagella, exopolysaccharides (Walker et al. 2004 and references therein; Lunsdorf et al. 2000). This buoying effect could be particularly important to position phototrophs in the water column and interactions with clays may protect cells from grazing (Lunsdorf et al. 2000).

In addition, suspended clays in inundated playas might alter the microbial loop to favor high cell concentrations by titrating virus particles (Schiffenbauer and Stotzky 1982; Lipson and Stotzky 1983) or localize high concentrations of nutrients at the cell surface (Stotzky 1986). Finally, if the water column were to experience even transient or patchy anoxia (e.g., as might occur at night during the maximum bloom), in the absence of dissolved oxygen and nitrate, microorganisms might respire ferric iron in clays as an alternative terminal electron acceptor (Kostka et al. 1999, 2002).

Clays with high CEC such as vermiculite and clinoptilolite, as were observed in SVAL2, would certainly impact the aqueous chemistry of playas and, by extension, the microbial community. In this study, the dramatic decrease in soluble Mg^{2+} in SVAL2 was attributed to sorption by, and/or precipitation of Mg^{2+} -containing vermiculite and clinoptilolite; the low soluble Mg^{2+} concentration could limit cell growth and/or select for species with high affinity Mg^{2+} transport systems. However, other cations were almost certainly impacted as well, including trace metals that could be toxic at high levels. The chelation by high CEC clays of trace metals, which are concentrated in desert playas (Table S1), could protect cells from potentially toxic metals while simultaneously leaving them available to organisms with high affinity transport systems (Babich and Stotzky 1979; Stotzky 1986).

CONCLUSIONS

Despite the current study and the study of Silver Lake playa (Navarro et al. in review), microbial community diversity, dynamics, and activities in ephemerally flooded desert recharge

and through-flow playas remain poorly understood. It is clear that inundated desert playas host very dense microbial communities. These communities are relieved of bottom-up controls by nutrient concentration that is characteristic of endorheic systems. Relief from top-down controls of grazing and bacteriophage lysis may be mediated by suspended clays, either through the microbial interactions with clays or by clay sorption to virus particles. It is also clear that flooded desert playas proceed through several stages of microbial community succession. It seems likely that nitrate depletion plays a major role in this succession, though the mechanism of nitrate depletion remains obscure. The remarkable cell densities and dynamic mineralogical, chemical, and microbial transformations occurring in these ecosystems make them fascinating subjects for geomicrobiological studies.

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