SHALLOW-SUBSURFACE MICROBIAL ECOLOGY AND SEDIMENT-GROUNDWATER

INTERFACE IN SULFATE-RICH PLAYA AT WHITE SANDS NATIONAL MONUMENT, NEW

MEXICO

By

STEVEN RAMIREZ

A Dissertation submitted to the Graduate School-Newark

Rutgers, The State University of New Jersey

And

The Department of Earth and Environmental Science

In partial fulfillment of the requirements

For the degree of Master of Science

Graduate Program in Environmental Geology

Written under the direction of Professor Mihaela Glamoclija

And approved by

_____

_____

_____

Newark, New Jersey

October 2017

ABSTRACT OF THE THESIS

SHALLOW-SUBSURFACE MICROBIAL ECOLOGY AND SEDIMENT-GROUNDWATER

INTERFACE IN SULFATE-RICH PLAYA AT WHITE SANDS NATIONAL MONUMENT, NEW

MEXICO

By STEVEN RAMIREZ

Thesis Director:

Mihaela Glamoclija

The hypersaline sediment and groundwater of the playa Lake Lucero at the

White Sands National Monument in New Mexico were examined for microbial

community composition, geochemical gradients, and mineralogy during the dry season

along a meter and a half depth profile of the sediment *vs.* the groundwater interface.

Lake Lucero is a highly dynamic environment, strongly characterized by the capillary

action of the groundwater, the extreme seasonality of the climate, and the

hypersalinity. Sediments are predominantly composed of gypsum with minor quartz,

mirabilite, halite, quartz, epsomite, celestine, and clays. Geochemical analysis has

revealed predominance of nitrates over ammonium in all of the analyzed samples,

indicating oxygenated conditions throughout the sediment column and in groundwater.

Conversely, the microbial communities are primarily aerobic, gram-negative, and are

largely characterized by their survival adaptations. Halophiles and oligotrophs are

extremely common throughout the samples. The very diverse communities contain

methanogens, phototrophs, heterotrophs, saprophytes, ammonia-oxidizers, sulfur-

oxidizers, sulfate-reducers, iron-reducers, and nitrifiers. Overall diversity and biomass

did not vary in a significant, consistent manner between the near surface, deeper

subsurface, and groundwater. The dynamism of this environment manifests in the

relatively consistent character of the microbial communities, where significant

taxonomic distinctions were observed but the extent of phenotypic differences is

uncertain. Therefore, sediment and groundwater substrates should not be considered

as separate ecological entities.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

Background

Hypersaline environments harbor diverse ecosystems that may range from soda lakes, saltpans, salars, hypersaline springs, playas, and ancient salt deposits [22, 50, 54, 74]. Consequently, the ecology of hypersaline environments has been extensively investigated, especially the water column of playas, and the sediments after the wet seasons when organisms flourish [11, 41, 43, 50, 53, 66, 67]. Many studies have focused on different ecological and chemical aspects of the stratification of microbial mats [53, 66, 67, 75] and only a few studies have examined the sediments and/or groundwater specifically [60]. In this study, we are focusing on a playa system; playas are intracontinental basins in which dry periods characterized by drought exceed wet periods characterized by precipitation and water inflow [10]. Due to the cyclic nature of these environments, the populations of microorganisms inhabiting them are composed of organisms that can survive drought as well as transient freshwater to saline and hypersaline conditions that alternate throughout the year [74]. From previous studies it is known that diverse microbial communities have been observed at similar environments with the phyla *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Euryarchaeota* generally being the most common [3, 11, 41, 43, 50]. Furthermore, halophilic microbes have been found to be particularly abundant [3]. The objective of this study is to investigate the composition of microbial communities living in playas

during the dry season under exclusively hypersaline playa settings along the steep

subsurface environmental gradients.

The area being investigated is the White Sands National Monument (WSNM) in

New Mexico, the site that contains the world's largest gypsum dune field. To the west of

the dunes is the Alkali Flat; a large, flat, and mostly unvegetated space that hosts about

20 playas among which is Lake Lucero (Fig. 1). Lake Lucero is the largest among the

playas, and it occupies the southern part of the basin [23, 35, 37, 54]. Since Lake Lucero

is the lowest topographic point at WSNM, evaporites accumulate here and build thick

deposits that result in a hypersaline environmental setting [23, 37]. Previous studies,

including the analysis of the nearby WSNM dune deposits, have indicated the presence

of *Cyanobacteria* as primary producers and as a diverse microbial community capable of

cycling nitrogen and sulfur compounds [28]. Only few studies have examined the

microbial ecology of Lake Lucero's sediments and/or groundwater specifically [60]. Lake

Lucero is a wet playa with the groundwater table relatively close to the surface; during

the dry season surface moisture is provided by capillary action [10, 51, 57, 72]. This

process provides much-needed water to microbial communities on the playa surface, as

well as a geochemically active environment on the surface and subsurface that

organisms can take advantage of [10]. The groundwater beneath Lake Lucero appears to

be influenced by a regional groundwater system more so than the rest of the WSNM,

which further contributes to the salinity [51]. The seasonal variations in water

availability, wind erosion, and the hypersalinity pose potential challenges for life in this

environment [2, 51, 78].

This study aimed to evaluate variations in the microbial ecology along the depth profile, geochemical gradients, changes in mineralogy, and substrate (sediment vs. groundwater). The sampling was conducted during the dry season down the 1.25 m depth profile that ended with the hard crust of coarse gypsum immersed in ground water. We were curious to see how environmental parameters such as water availability, solar radiation, and geochemistry may influence the distribution of the organisms and to inquire as to which settings are important to the organisms living in these sediments. Halophiles, oligotrophs, and sulfur-cycling microbes were expected to be ubiquitous throughout both sediment and groundwater. We hypothesized that the Lake Lucero sediments would exhibit a change in the diversity and biomass between near-surface, deeper subsurface, and groundwater environments. We were also particularly interested in seeing whether groundwater contributes to the microbial diversity at the site or if these different substrates represent separate ecological entities.

MATERIALS AND METHODS

Sampling Procedures

The sampling strategy aimed to assess the depth profile of playa deposits to capture different evaporation lithologies formed during the seasonal variations at the largest playa, Lake Lucero. The goal is to investigate different ecological niches and microbes associated with them along this depth profile. The sampling location (N 32° 41.111'; W 106° 24.093' ±3m) is an approximate topographic low within Lake Lucero (Fig. 1) where the lake has had the opportunity to persist and the microbial communities had the most opportunity to colonize and diversify within these evaporitic sediments. Manual shallow drilling could not be performed due to sediment characteristics (too hard and sticky), so a manual auger device was used to sample a 125 cm deep lithological profile. The auger device was pre-cleaned to minimize contamination [18]. Within this profile, fourteen lithologically different samples of disturbed soil were collected, which were divided by depth as consistently as possible and then placed in Falcon tubes and sterile plastic bags. The water table was reached at 125 cm depth. The coarse gypsum was too hard to auger through so our sampling ended at this level. The groundwater within the drilled hole was left to settle until the next day. A manual pump was used to collect water samples into pre-cleaned, 4L carboys, and the samples were filtered within a few hours of the collection. Filters were placed in sterile tubes and all samples were kept in a refrigerator during the field session and during the transportation back to the laboratory, where they were then stored in the freezer at

20°C. Three other groundwater samples were collected from previously installed

piezometers: one was taken from a southernmost location in Lake Lucero (N 32°

42.167'; W 106° 26.960' ±3m), and two were taken from the dune field (N 32° 49.721';

W 106° 15.972' ±3m). Dune field piezometers were installed for monitoring of shallow

and deep aquifers (see Table 1), this distinction refers not to literal depth but to the

origin of the groundwater; the shallow aquifer sample is primarily meteoric and the

deep aquifer sample is primarily from the brines.
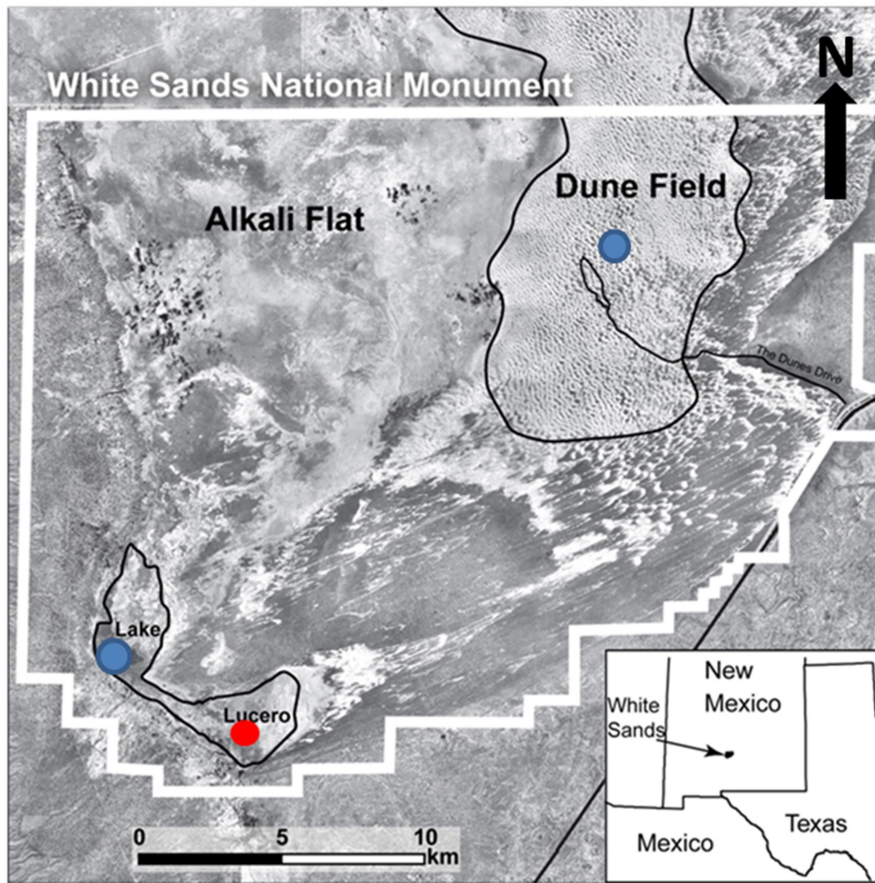
Figure 1: A map showing the WSNM area and sampling points. The red dot denotes the location where the sediment samples (1-14) and one groundwater sample (GW-1) were collected. The blue dots denote the location of groundwater samples in southern Lake Lucero (GW-2) and the dune field (GW-3 and GW-4).

Mineral Assemblages

Main mineral phases were identified by X-ray diffraction of powdered dry and dump wet samples using a Bruker D8 Advance Eco, equipped with a Cu-Kα radiation source and a LynxEye XE detector. Samples were afterwards analyzed using EVA software. Scanning Electron Microscope (SEM) with Energy Dispersive X-ray Spectroscopy (EDS) Hitachi S-4800 was used to search for the presence of microbial morphologies or biofilm and to analyze their elemental composition and minor mineral phases and precipitates. All fourteen samples were analyzed in triplicate. Once dried, the samples were coated with Iridium and analyzed. The SEM conditions were 25.0kV voltage, 20μA under standard vacuum, and working distance ranged from 9 to 13 mm.

Geochemistry

All of the collected samples were analyzed for Mg, Sr, Fe, Na, K, and Ti concentrations using inductively coupled plasma optimal emission spectroscopy (ICP-OES). Nearly 1 g (dry weight) of sample was mixed with repeated additions of nitric acid (20%) up to 10 ml (following acid digestion of soils) for 4 days with periodic sample shaking and heating [44]. Samples were filtered and the filtrates were diluted with deionized water and volumes brought up to 30 ml for ICP-OES analyses to adjust total acid to 3-5% (v/v) for ICP-OES analyses. The nitrogen nutrients from the deposits were assessed through colorimetric analyses of ammonium (NH_4^+) and nitrate + nitrite (NO_3^- + NO_2^-) concentrations. The soil samples were prepared for the analysis by mixing 1 g of sample and 10 ml of 2N potassium chloride (KCl) and leaving the samples in the solution

for 24 h at room temperature while shaking periodically. The supernatant was decanted into clean Falcon tubes.

A range of 0, 5, 10, 25, 50 and 100 µM solutions were prepared for ammonium sulfate ((NH_4^+)$_2SO_4$) and sodium nitrate ($NaNO_3$) solutions to be used as standards. Absorbance of each sample was measured in triplicate using a GENESYS 10Bio spectrophotometer; 640 nm was used for ammonium and 540 nm for nitrate. The NH_4^+ concentration of the extracts was determined by the alkaline hypochlorite/phenol nitroprusside method, after the addition of sodium citrate to prevent the precipitation of calcium and magnesium salts [64]. The NO_3^- + NO_2^- concentrations were measured using the Nitrate Test kit (LaMotte, MD) according to the manufacturer's instructions. This method does not allow for separate NO_2^- detection and therefore the results are reported as a sum of NO_3^- and NO_2^-, which will henceforth be referred to as "NO". The kit contains a cadmium compound as a reducing reagent, which converts NO following the diazotization/coupling to form a pink color.

Nucleic Acid Extraction and Polymerase Chain Reaction

DNA extractions were carried out using the MoBio PowerSoil DNA Isolation Kit. Modifications to the manufacturer's protocol were made to improve the extraction efficiency. Samples were placed in the PowerBead tubes and vortexted for 5 minutes and then spun down, for the supernatant to be transferred into new PowerBead tubes. After adding 70µL of C1 solution (used for breaking down cell membranes) the samples were vortexed for 5 minutes and centrifuged for approximately 5 seconds and then

heated in an oven at 70°C for 30 minutes. After this, the C2 solution (used for removal of DNA inhibitors) was added and the manufacturer's protocol was followed. All of the samples were extracted in triplicates to account for sample heterogeneity. Negative and positive extraction controls were used to ensure the extraction quality; negative controls did not contain any soil or groundwater and a dark, organic-rich soil from which DNA had previously been successfully extracted was used as a positive control for all samples.

Universal primers sets specific to the SSU rRNAs from all three domains of life (Eubacterial B27-F and 1429-R [13, 39], Archaeal 8A-F and 1513U-R [17, 30] and Eukaryal Euk1-F and Euk-R2 [55]) were used to determine their presence in each of the samples. PCR was performed using a Dyad Peltier Thermal Cycler with puReTaqTM Ready-To-GoTM PCR Beads (Amersham Biosciences, NJ) in a final volume of 25 µl (containing 1 µl of forward and reverse primers, 4 µl of nuclease-free water, and 20 µl of DNA template). PCR conditions were: 95°C for 2 minutes denaturation, 30-33 cycles of 95°C for 15 seconds denaturation (this parameter was modified regularly, 32 cycles worked best for most samples), annealing at lowest temperature specific to each primer set for 30 seconds, extension of 72°C for 45 seconds, and afterward a final extension of 72°C for 10 minutes. A previously analyzed sample with DNA was used as a positive PCR control, and a sample containing nuclease free water was used as a negative control. PCR products from all samples were viewed and analyzed using the Agilent 2100 Bioanalyzer and Agilent DNA 7500 LabChip kit.

DNA Sequencing and Analysis

Illumina MiSeq sequencing was performed on all samples via paired-end 16S

community sequencing using the bacteria/archaeal primers 515F/806R by Molecular

Research LP (Mr DNA) [46]. The raw sequencing data were analyzed with Mothur v1.37

[36]. Mothur parameters used included: quality filtration of sequences with (a) qaverage

cutoff 25 and (b) two base pair mismatch in sequencing primers and one base pair

mismatch in barcode, removed barcodes and sequencing primers, aligned unique

sequences to the SILVA database (release 123), removed chimeras via UCHIME,

clustered unique sequences to Operational Taxonomic Units (OTUs) using Average

Neighbor algorithm, and a standard 97% confidence value [36]. The type of sequencing

performed allows for accurate identification of bacterial and archaeal OTUs, and

eukaryotic phylotypes, down to the genus level. Some OTUs were unclassified below a

certain level. In such cases, the OTUs were characterized to the maximum level of detail

possible; e.g. an OTU classified as "phylum: *Proteobacteria*, class:

Gammaproteobacteria, order: unclassified *Gammaproteobacteria*" would be

characterized as a *Gammaproteobacteria*, with all the traits that are known to be

inherent to all species in that class (e.g. in this case, the OTU would be characterized as

being gram-negative; this is a trait inherent to all *Proteobacteria*).

Mothur was used to calculate the diversity of the samples using the Inverse

Simpson Index: a measure of diversity which takes into account the number of OTUs

present, as well as the relative abundance of each OTU [48, 62]. In this calculation, all

samples were normalized to have the same number of sequences. Samples that

contained little or no archaeal and/or eukaryotic DNA were assigned a diversity value of

"0" for those domains. PRIMER-7 was used to provide visualization of community

structures via UPGMA dendograms (Unweighted Pair Group Method with Arithmetic

Mean) and further analysis regarding changes in microbial community structure with

ANOSIM and SIMPER [9]. ANOSIM tests the null hypothesis that the average rank

similarity between objects within a group and objects from different groups is the same

by producing a p-value and a test statistic (R) between -1 and 1, where 0 indicates the

null hypothesis is true and 1 indicates a high degree of dissimilarity [56]. SIMPER

analysis facilitates the identification of OTUs that are responsible for contributing to

community structure difference between individual samples and groups of samples [56].

RESULTS

Mineralogy

The sediments analyzed for this study are predominantly composed of gypsum($CaSO_4 \bullet 2H_2O$). The surface crust additionally has thenardite (Na_2SO_4), halite (NaCl), and a minor amount of clay minerals. Along the profile relatively minor amounts of epsomite ($MgSO_4 \bullet 7H_2O$), glauberite ($Na_2Ca(SO_4)_2$), celestine ($SrSO_4$), and quartz (SiO_2) are detected too (Fig. 2). Below the surface a light brown mixture of gypsum and clay are identified, at about 60 cm deep the reddish clays, rich in iron oxides, were detected, and at about one meter deep dark gray clay occurs and it becomes thick and sticky just above the coarse gypsum strata. The bottom two samples do not contain halite. The mineralogical observations reported here are generally consistent with those of Langford *et al.* [37]. No obvious microbial morphologies or biofilms were observed using SEM on the samples, indicating that there is very low biomass in all of the analyzed samples.

Geochemistry

Ammonium and NO concentrations revealed that all of the samples had more NO than NH_4^+ (Table 2, Fig. 2), indicating the presence of aerobic conditions throughout the depth profile and the potential presence of nitrifying organisms. The change in the NO concentration is evident at the 60 cm depth (sample 6), where NO concentrations drop below 10 ppm. A slight increase of NH_4^+ was observed at 125 cm depth (sample 13,

the coarse gypsum submerged in the groundwater), however NO was still predominant.

The concentrations of the examined ions throughout the sediment column (Table 2, Fig.

2) revealed specific trends. Sodium and magnesium concentrations generally decrease

with depth, which directly reflects the variety and contribution of salts other than

gypsum to the examined lithologies. Sodium and magnesium may derive from the

dissolution of halite, thenardite, epsomite, and glauberite. This is generally consistent

with the expectation that evaporitic action would result in higher salinity at the surface

[10].
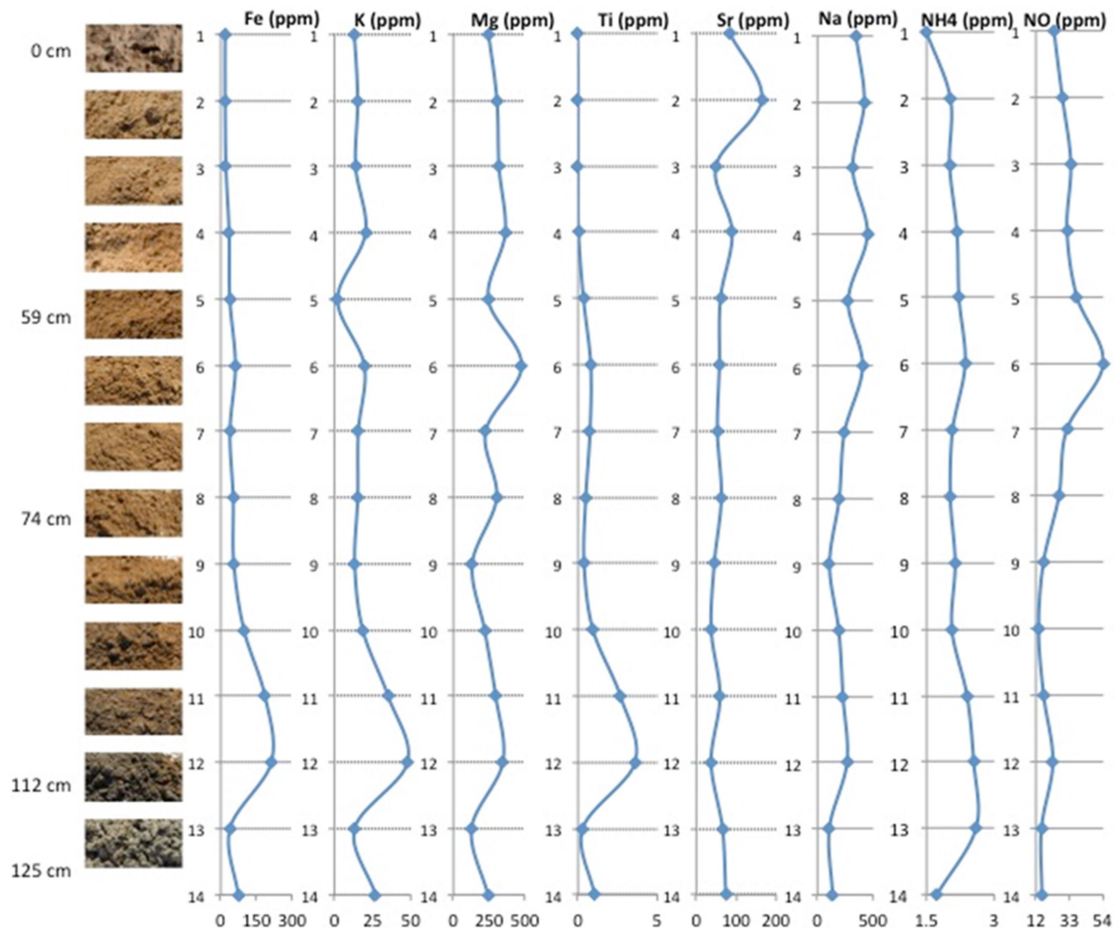
Figure 2: Depth profile illustrating the concentrations of elemental ions, ammonium, and nitrates (NO).

Iron concentrations generally increased in deeper sediments and the same is true for titanium concentrations. The increase of Fe and Ti with depth may be related to the diagenetic processes and the presence of different clays (reddish, dark gray). For example, the change in concentrations of K, Mg, Na, and NO within horizons corresponding to samples 6 and 12 are characterized by the presence of sticky clays. Sticky clays have the capacity to act as a seal for water and change the local geochemical conditions, and likely microbial ecology too. This is accomplished via specific adsorption of cations and the cation exchange capacity inherent to clay minerals due to their relatively high negative surface charge [38]. The K concentrations were relatively low and exhibited a trend similar to that of Fe, Mg, and Ti. The general changes in cation concentrations noted here are consistent with the observations made in SEM/EDS. Strontium in the samples is related to the presence of the mineral celestine ($SrSO_4$), the detected concentrations are consistent with SEM/EDS observations as celestine is observed as a minor mineral component in the samples. It is likely that strontium concentrations would be higher during the wet season due to increased dissolution [31]. Additionally, the Sr component in these samples likely derives from the local groundwater brines that increase the salinity of this playa.

Taxonomy

Overall 7627 bacterial OTUs, 541 archaeal OTUs, and 34 eukaryotic phylotypes were identified in the fourteen sediment samples and four groundwater samples. Based on the number of OTUs and raw sequences, Bacteria was the most dominant and

diverse domain, especially within the sediment column (Fig. 3 and 4). The most prevalent bacterial phyla were *Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes,* and *Gemmatimonadetes* (Fig. 3). The most dominant phylum was the highly diverse *Proteobacteria*, which accounted for 45% of all bacterial OTUs, especially the *Gammaproteobacteria* (55% of *Proteobacteria* OTUs) and *Alphaproteobacteria* (27%) [40].
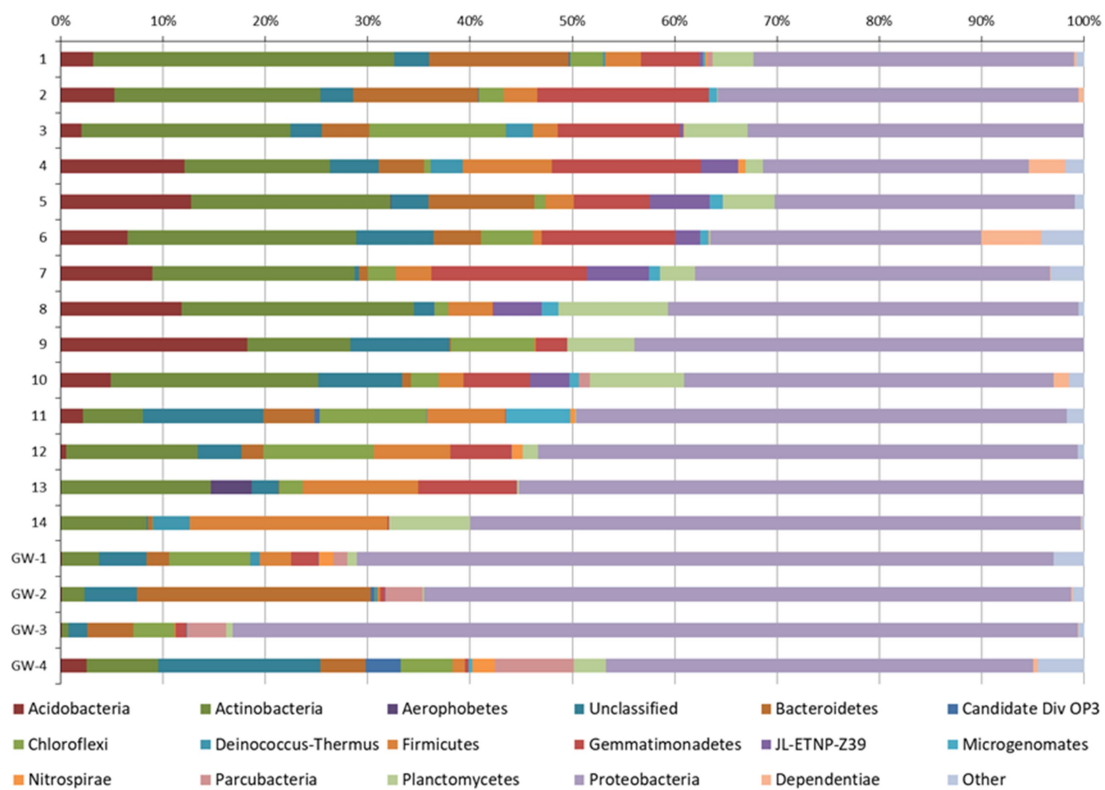
Figure 3: Phylum-level classification of bacterial data, 1-14 samples are from the sediment column and GW samples are groundwater.

Figure 4: Diversity of samples measured with the Inverse Simpson Index. Overall, bacteria were substantially more diverse than archaea and eukarya. Sample GW-4 (shallow aquifer – dunes) had the highest bacterial and archaeal diversity of all the samples, while sample 5 was the least diverse. In the groundwater, diversity was higher for both archaea and eukarya but lower for bacteria (except GW-4).

Archaea had a marginal presence in most samples and were substantially less diverse than the bacteria (Fig. 5). The most prevalent archaeal phyla are *Euryarchaeota* and *Thaumarchaeota* (Fig. 5). *Euryarchaeota* alone constitutes ~72% of the archaeal OTUs; there are several halophilic genera within this phylum [40] so its substantial dominance in the archaeal community composition was expected.

Figure 5: Phylum-level classification of archaeal data, 1-14 samples are from the sediment column and GW samples are groundwater. Samples that contained little or no archaeal DNA are excluded.

The distribution of eukaryotes is relatively poor as they were only observed in three sediment samples and all the groundwater samples. The most prevalent eukaryotic divisions observed were *Viridiplantae* and *Fungi* (Fig. 6). The low quantity of eukaryotic sequences and their absence from many samples makes it unfeasible to examine trends in the community composition in much detail. The eukaryotic communities were very low in diversity, much lower than the other two domains (Fig. 5).
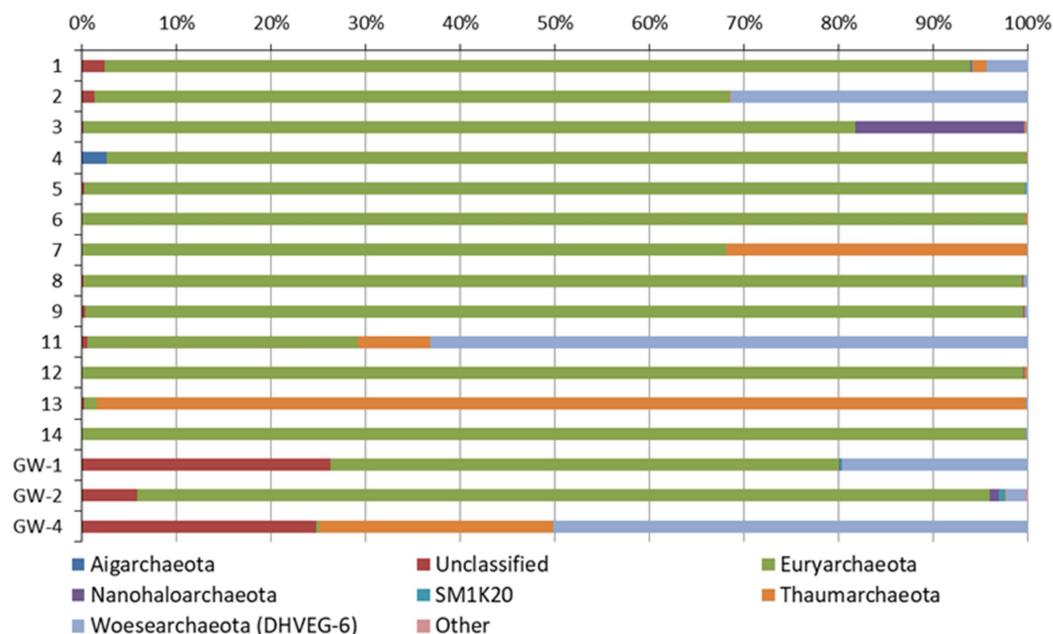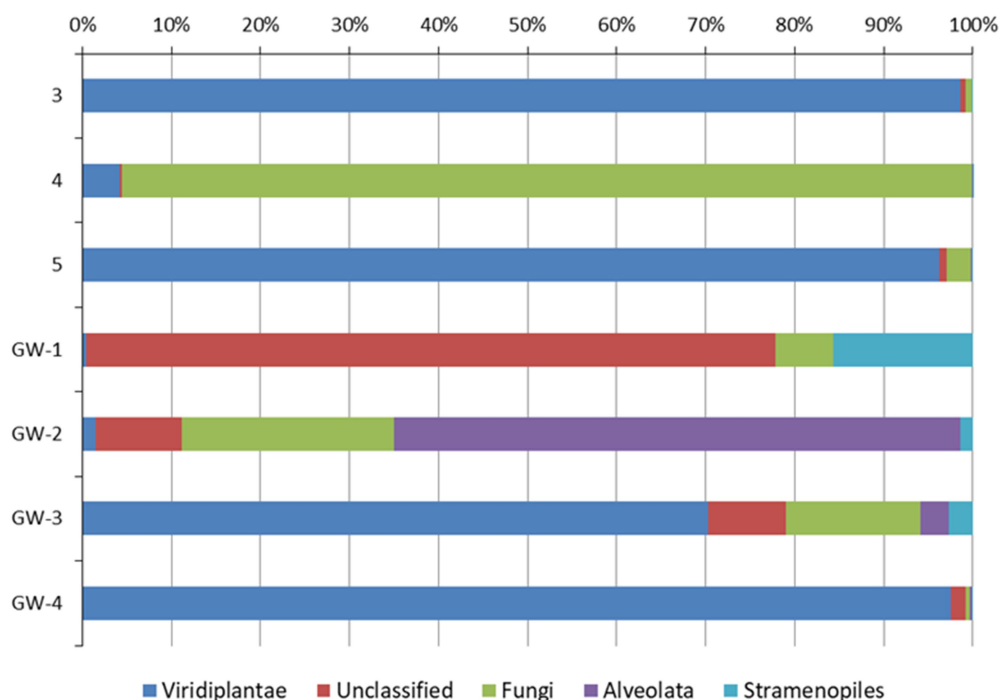
Figure 6: Phylum-level classification of eukaryotic data, 1-14 samples are from the sediment column and GW samples are groundwater. Samples that contained little or no eukaryotic DNA are excluded.

The UPGMA dendogram was utilized to segregate the samples based on their microbial community structure, as determined by OTU abundance. For bacteria the UPGMA clustering was well-aligned with the differences in local habitat, although the deepest sediment sample (sample 14) was somewhat isolated from the others indicating that its community structure is different from the rest of the sediment column (Fig. 7). The SIMPER analysis showed that when compared to the other sediment samples, sample 14 had a higher abundance of OTUs that are classified as the genera *Staphylococcus* and *Pseudomonas*. Sediment and groundwater were found to be significantly different (the lowest dissimilarity value between any two groups in SIMPER was ~94%). UPGMA revealed that the playa groundwater samples (GW-1 and GW-2) and

dune groundwater samples (GW-3 and GW-4) were different from each other. The

ANOSIM analysis confirms the UPGMA observation by producing a sample statistic (R)

value of 0.99 with a p-value <0.001, which indicates that all the sample groups are

extremely different from each other in terms of bacterial community structure. The

SIMPER analysis revealed that the within-group similarities of samples are very low with

the highest one being 21.36% for sediments, 8.99% for the dune groundwater group

(GW-3 and 4), and 6.16% for the playa groundwater group (GW-1 and 2). The sediment

group was differentiated from the other groups largely by OTUs classified as

Acidimicrobiales OM1 clade, *Pseudomonas*, uncultured Sva0071

(*Gammaproteobacteria*), *Delftia* (*Betaproteobacteria*), unclassified

Gammaproteobacteria, and unclassified *Actinobacteria*. The dune groundwater was

differentiated largely by OTUs classified as *Pseudomonas*, *Sphingobium*

(*Alphaproteobacteria*), unclassified *Rhodobacteraceae* (*Alphaproteobacteria*),

unclassified JG30-KF-CM66 (*Chloroflexi*), *Seohaeicola* (*Alpharoteobacteria*), and

Methylotenera (*Betaproteobacteria*). The playa groundwater was differentiated by OTUs

classified as *Halomonas* (*Gammaproteobacteria*), *Marinobacter*

(*Gammaproteobacteria*), *Thiomicrospira* (*Gammaproteobacteria*), *Sediminimonas*

(*Alphaproteobacteria*), uncultured E6AC02 (*Bacteroidetes*), unclassified

Gammaproteobacteria.

Figure 7: UPGMA dendograms of bacterial (top) and archaeal (bottom) data based on OTU abundance. Blue is for sediment, red is for playa groundwater, and green is for dune groundwater. For bacteria, sediment samples 1-13 clustered together closely while 14 (the deepest sediment sample) was further apart, indicating that bacterial communities at this depth differed somewhat from the communities higher in the column. Dune and playa groundwater samples clustered apart from the sediment samples and from each other. For archaea, samples 10 (sediment) and GW-3 (dune GW) are excluded due to having a low amount of archaeal DNA present. Most sediment samples clustered together with the exceptions of 7 and 13, which clustered further apart, and sample 12 which was individually isolated. The two playa groundwater samples were fairly similar in structure and the one dune groundwater sample was isolated from all other samples.

Although the three major ecological groups remained separate from each other based on the archaeal dataset, the communities were more divided than the bacterial communities. (Fig. 7) The ANOSIM produced a sample statistic of 0.62 with a p-value < 0.01, implying that the sample groups are moderately different in terms of archaeal community composition. However, this is skewed due to the significant separation within the sediment group; group-to-group comparisons in SIMPER showed that archaeal communities differed greatly between the different habitats just as the bacterial communities do (the lowest dissimilarity value between any two groups is ~97%). Within-group similarities were low: 22.61% for sediments and 11.42% for the playa groundwater. The sediment group was differentiated from the others mainly by OTUs classified as unclassified *Thermoplasmatales* (*Euryarchaeota*)*, Marine Group I* (*Thaumarchaeota*), and *Halapricum* (*Euryarchaeota*). The deep dune field aquifer sample was extremely differentiated due to OTUs classified as *Marine Group I* (*Thaumarchaeota*)*,* unclassified *Woesarchaeota* (several OTUs)*,* and an unclassified archaean. The playa groundwater was differentiated by OTUs representing unclassified *ST-12K10A* (*Methanomicrobia*), genus *Candidatus Halonobonum* (*Euryarchaeota*), and an unclassified archaean.

DISCUSSION

Desert microbial communities strategically inhabit near-surface environments where they have the availability of sunlight and evaporation-based chemical disequilibria, additionally they are protected from the desiccation and UV radiation by a thin layer of sediment [40]. The newly collected data show that diversity increases in the sediment column towards the deeper parts (e.g. sample 9, about 90 cm deep), even though the apparent cause of disequilibria is lacking (e.g. evaporation). The taxonomic data show that aerobic organisms are likely the dominant constituent of the microbial communities, while anaerobes and microaerophiles have a relatively minor presence throughout the sediment column and groundwater samples. Based on the predominance of NO over NH_4^+ and the taxonomic data analyzed it appears that the environment is oxygenated throughout the depth column. The organisms observed here are generally consistent with those observed in other playas and hypersaline environments [3, 11, 42, 43, 50, 52].

Halophiles

The hypersalinity strongly affects the microbial ecology and halophiles have a substantial presence in the examined Lake Lucero population. The archaeal OTUs identified are of the class *Halobacteria*, the order *Methanomicobia* (halophilic methanogens), and the phylum *Nanohaloarchaeota*; which is consistent with findings in other hypersaline environments where these groups are ubiquitous [14, 19, 25, 40, 53,

67]. The extreme acidophilic order *Thermoplasmatales* is identified with a significant presence in the sediments and is represented by uncultured groups. Some of the uncultured groups have been observed in a saline environment, which could explain their existence in Lake Lucero [40, 61].

Halophiles are present in all the dominant bacterial phyla: *Proteobacteria, Actinobacteria, Firmicutes, Chloroflexi,* and *Bacteroidetes* [40]. The purple sulfur bacteria order *Chromatiales* (*Proteobacteria*) contains some of the most extreme bacterial halophiles and has been observed in similar hypersaline environments [67]. A highly abundant (>10,000 sequences) halophile is the nitrite and nitrate-reducing genus *Sediminimonas* in the groundwater of southern Lake Lucero; this is one of the most abundant anaerobes observed in the data [79]. Halophiles observed in moderate abundance (5,000-10,000 sequences) include the genera *Salinibacter*, *Staphylococcus*, *Streptococcus*, and *Nitriliruptor* [2, 40, 42, 43]. *Nitriliruptor* are alkaliphilic [70]. Low abundance (1,000-5,000 sequences) halophiles include the genera *Rothia*, *Kocuria*, and *Truepera* [1, 8, 73].

The halophiles observed are diverse, and their distribution is relatively consistent which implies that there is no significant gradient of salinity in the sediment column. However, the most extreme halophiles (*Halobacteria*) have much lower abundance in the samples immersed in groundwater (samples 13 and 14). This observation, along with the lower concentrations of Na in these samples, implies that the salinity is likely lower at this depth horizon.

The halophiles of Lake Lucero include the dominant phyla that have been observed in saline environments before and halophiles identified here are phylogenetically and metabolically diverse and predominantly aerobic, with some anaerobes present (e.g. methanogens and denitrifiers) [3, 5, 11, 42, 50]. The taxonomic data shows that the halophiles observed here contain groups capable of methanogenesis, phototrophism (e.g.purple sulfur bacteria), heterotrophism, ammonia-oxidation, sulfur-oxidation, and possibly denitrification/DRNA (dissimilative reduction of nitrate to ammonium) Conversely, the hypersalinity inhibits the metabolic activity of purple non-sulfur bacteria (which are absent from the data) [66].

Nitrogen Cycle

Portions of the nitrogen cycle at Lake Lucero were assessed through the concentrations of NH_4^+ and NO, and the OTU abundance data. A moderate amount of green phototrophic bacteria capable of nitrogen fixation have been observed in samples 11-13 [76]. A small amount of nitrogen-fixing purple phototrophic bacteria are observed, mostly at the surface (sample 1) but also in samples 8, 11, 12, groundwater (GW-3), and the deep aquifer sampled at the dune field [76]. No other known nitrogen-fixing microbes are explicitly identified. Denitrifying microbes are not explicitly observed, although the diverse genera *Pseudomonas* and *Paracoccus* both contain species capable of denitrification [12, 40]. A very small amount of anaerobic ammonium oxidizing (anammox) bacteria of the order *Brocardiales* are observed, exclusively in the deep aquifer of the dune groundwater [33]. Rhizobial genera are observed in large

amounts (mostly *Bradyrhizobium*) and are well distributed throughout the sediment column but with comparatively low abundance in groundwater [40]. The absence of observed plants here indicates that rhizobia likely live freely in the soil, in which state they cannot fix nitrogen [40].

The distribution of green and purple bacteria in the sediment profile implies that nitrogen fixation likely occurs at the surface. This input of nitrogen into the system may be augmented by atmospheric deposition [21]. The distribution of ammonia-oxidizers generally mirrors that of the nitrogen fixers, as would be expected in a nitrogen cycling ecosystem. The small amount of observed nitrifying microbes are well-distributed throughout the sediment column, whereas denitrifying microbes are not directly identified. These observations, as well as the difference in NH_4^+ and NO concentrations, indicate that the environment along the depth profile is aerobic and that nitrification processes are prevalent in this system. The nitrogen cycle of the sampled dune groundwater seems to be similarly driven mainly by nitrification, although the brine-based groundwater is distinguished slightly by its anammox bacteria. Both NO and NH_4^+ concentrations are generally low, as is typical in arid environments [40].

The slight increase in NH_4^+ concentrations at the bottom of the sediment profile implies that denitrification and/or DRNA (dissimilative reduction of nitrate to ammonium) may be more important here than in the rest of the sediment column. It also correlates with the moderate abundance of *Petrimonas* in sample 11 (contains the species *P. sulfuriphila* which reduces elemental sulfur and nitrate and is a mesophilic anaerobe) and with the darker coloration of sediments at this depth, which implies that

there may be decaying organic matter present at this depth [29]. The decaying organic matter may have been increased in sediments 12 and 13 due to the presence of the sticky gray clay that may have acted as a seal and trapped the organics transported by groundwater. *Coryneform* bacteria are aerobic saprophytes that are present in low abundance at this depth; by degrading organic matter they release ammonium into the soil [40]. However, even in this part of the sediment column NO concentrations are still higher than NH_4 concentration. Redistribution of the NH_4 released by saprophytes through the capillary action of groundwater may account for the relatively consistent concentrations of this compound throughout the sediment column.

Sulfur Cycle

The purple sulfur bacteria use H_2S as an electron donor (or elemental sulfur if H_2S is limited) and often rely on sulfate-reducing and/or sulfur-reducing microbes to produce H_2S [40]. This may explain their presence in samples 11 and 12 as it coincides with the presence of the aforementioned sulfur-reducing *Petrimonas* in sample 11 [29]. Despite the abundance of gypsum only a small amount of sulfate-reducers and sulfur-reducers are explicitly observed, although the highly abundant genus *Pseudomonas* contains species that are capable of sulfur-reduction (such as *P. mendocina*) [40]. The low abundance of sulfate-reducing microbes is due to the fact that this is primarily an aerobic setting, and there seems to be a shortage of organic matter to use as electron donors [40]. This can be inferred from the very infrequent observations of carbon in SEM/EDS and the absence of plants on the surface. Possible carbon sources would

include cellulose from fungi (observed in sample 4), chitin from the exoskeletons of

arthtropods, and the phototrophic microbes observed in the sediment [40].

Nonetheless, the particularly low abundance of sulfate-reducers is surprising since the

relatively minor presence of anaerobic microbes signifies that there must be localized

anaerobic conditions throughout the sediment column, and sulfate would likely be an

important nutrient source for these anaerobes. This is especially surprising in the

deepest section of the sediment column, as the apparently greater amount of organic

matter there could, theoretically, provide electron donors for sulfate reduction [40].

This seems to be one of the main differences between the sediments of Lake Lucero and

hypersaline settings with microbial mats, as those environments generally have a higher

abundance of sulfate-reducing bacteria because certain strains are able to coexist within

cyanobacterial biofilm in aerobic settings [40, 53, 67].

Thiomicrospira is the only sulfur-oxidizing genus observed but it is highly

abundant and mostly distributed in sample GW-1, with a minor presence at the surface

[40, 68, 69]. It is possible that sulfate reducers are amongst the many unclassified OTUs

present in the data or present within the genus *Pseudomonas* [40]. If this is the case,

then sulfate reducers at the bottom of the sediment column may be producing enough

H_2S to nourish the large amount of sulfur-oxidizing *Thiomicrospira* at the sediment-

groundwater interface. The relative lack of sulfur-oxidizing microbes in the sediment

column implies that the sediments generally have less reduced sulfur compounds than

the groundwater, as would be expected in a primarily aerobic setting with a large

amount of sulfate minerals. The presence of *Chromatiales* and *Thiomicrospira* at the

surface suggests that there is a source of reduced sulfur there. The minor presence of

purple sulfur bacteria in the dune groundwater implies the same at that location.

Nonetheless, it is clear that the sulfur cycle in Lake Lucero sediments is dominated by

oxidative processes; which is consistent with the assessment of the nitrogen cycle and

of the microbial populations. The verity of the original hypothesis that microbes

involved in sulfur cycling would be abundant across all analyzed samples is uncertain,

the taxonomic data implies that this is improbable but it is possible that some microbes

may be using alternative metabolic pathways.

Photosynthetic Microbes

A moderate amount of green non-sulfur bacteria (anoxygenic phototrophs found

in a wide range of environments) and purple sulfur bacteria were explicitly observed but

the phylum Chlorobi, which consists of green sulfur bacteria, is present in very low

abundance [40]. Cyanobacteria are also observed in very low abundance. It is possible

that the mixing effect of the groundwater capillary action limits the growth of

phototrophs since it prevents the segregation of microbial communities which would be

beneficial for them (e.g. as seen in microbial mats). This mixing would also have the

potential to redistribute phototrophs to deeper levels of the sediment, which limits the

consistency of their exposure to UV rays. Phototrophs inhabiting the deeper section of

the column could be living in a state of dormancy; this is a survival mechanism often

observed in extreme environments [34].

Sediment-Groundwater Exchange

The mixing effect generated by the capillary action of groundwater, in conjunction with the climatic seasonal variability, makes Lake Lucero a very dynamic environment. This dynamism plays a significant role in structuring the microbial communities. Although the compositions of microbial communities identified within the sediments and groundwater differ significantly (see Fig. 7), it seems that the capillary action of the groundwater causes limited redistribution of microbes throughout the column. Therefore, the microbial communities are not strictly segregated by depth and some of the microbes appear to be displaced. An obvious example of this would be the presence of purple phototrophic bacteria deep in the sediment column, where they would have limited access to sunlight; they can survive under these conditions but cannot grow optimally [6]. The stable stratification seen in microbial mats in wetter settings cannot be maintained under these dry and occasionally moistened conditions, and thus the community composition in Lake Lucero seems to be markedly different from that seen in environments with a salt crust and microbial build ups. This is consistent with the findings of Canfora *et al.* that show that the presence or absence of such a crust is a significant differentiating factor amongst the microbial communities of saline environments [5].

The effects of this capillary action on the microbial communities are apparent despite having sampled the sediment during the dry season when the capillary movements are minimal. Since capillary action is conditioned by the rate of evaporation at the surface, it is probable that the microbial communities would show more evidence

of groundwater-driven redistribution during the wet season when evaporation is higher [10]. This could be further exacerbated by the increased precipitation and subsequent flooding. However, it is possible that some microbes respond to the changes by entering a state of dormancy when nutrient availability is low and then emerging from this state when local conditions are more favorable for survival [35]. In such circumstances, seasonality might be reflected less in changes to the composition of the microbial communities but more in a shift between active and inactive community members.

Variation of Microbial Communities Along the Sediment Profile

Although the capillary action of groundwater limits microbial segregation, some trends can still be observed. The deepest part of the sediment column is characterized by sticky clays with trapped decaying organic matter which is inferred by the darker coloration of the sediment, the slightly higher concentrations of NH_4^+, lower concentrations of NO, and the presence of saprophytes. However, the fact that NO concentrations are still higher than NH_4^+ and that the saprophytes are low in abundance indicates that even at this depth, the playa sediments are still primarily an aerobic environment. Although not explicitly identified, sulfate-reducers may be present here in localized anaerobic niches and producing H_2S that is then oxidized by the *Thiomicrospira* present in the groundwater.

Proteobacteria were present in all samples and became more abundant with increasing depth of the sediment column. They were more prevalent in groundwater, with the exception of the deep dune groundwater; in the shallow dune groundwater

this phylum composed more than 80% of the bacterial OTUs. This may be due to the gram-negative nature of the *Proteobacteria*; their cell walls would make them less susceptible to osmostic lysis caused by sudden influxes of water during the wet season [40]. The *Gammaproteobacteria* genus *Pseudomonas* has a large presence throughout all samples, especially in the sediment [40]. SIMPER analysis showed that OTUs identified as *Pseudomonas* were significant differentiating factors for sample 14 and for the dune groundwater samples. However, the significant metabolic diversity of this particular genus makes it difficult to draw conclusions from this observation without species-level identification of the OTUs and/or a more in-depth analysis (e.g. RNA sequencing). Conversely, *Acidobacteria* and *Gemmatimonadetes* were abundant in most sediment samples, but largely absent from groundwater samples.

Bacteroidetes display significantly greater abundance in the upper half of the sediment column, likely due to the increased availability of cellulose and chitin closer to the surface. *Bacteroidetes* are typically saccharolytic (specializing in the degradation of complex polysaccharides such as cellulose and chitin) [40], and polysachharides could be available at WSNM from the plants and arthropods that have been seen on and near the surface, as well as the fungi observed here [40, 71]. The *Actinobacteria* are similarly less abundant in deeper sediment samples, and even less abundant in groundwater samples. The most significant of these are the family *Acidimicrobiales*, in particular the OM1 clade; the single largest OTU in the dataset belongs to this class (~102k sequences). They are more abundant in samples 1-13 of the sediment column than in sample 14 or the groundwater, as was shown via SIMPER analysis. This uncultured group of

Actinobacteria has been observed often in freshwater and marine environments and is thought to be oligotrophic and planktonic; it may be possible that they flourish during the wet season and are dormant during the dry season [27, 45, 49]. Conversely, the *Firmicutes* are generally more abundant in deeper sediment samples and have a marginal presence in the groundwater samples.

Another example of localized differences in community composition would be the bacterial phylum *Chloroflexi*, which is mostly present in samples 11-13 and in the groundwater beneath the sediment column. Their distribution in the sediment correlates with the increase in the concentrations of several cations in samples 11-13 (Fe, K, Mg, Ti), the change in color (dark brown to black/dark gray), and direct exposure to groundwater capillary action. This could potentially be due to a higher availability of nutrients at this depth. Aside from the aforementioned green non-sulfur bacteria, the most abundant classes of the *Chloroflexi* are uncultured groups (JG30-KF-CM66 and S085) and unclassified OTUs. The *Chloroflexi* are, generally, more abundant in sediment than groundwater. They are particularly abundant in samples 11 and 12, where the abundance is driven mainly by the unknown OTUs and JG30-KF-CM66; this coincides with the occurrence of sticky clay and the concentration increases of several ions mentioned previously. The *Chloroflexi* abundance in sample 3 is driven by both uncultured groups. Less abundant classes include the *Thermomicrobia* (thermophilic green non-sulfur bacteria [40]) and *Thermoflexia* (thermophilic, microaerophilic, facultative anaerobic bacteria [16]). The large proportion of *Chloroflexi* with unknown

physiological roles generates significant uncertainty about what exactly is driving their observed distribution.

The analysis of groundwater samples shows that the microbial communities in the groundwater at WSNM differ greatly based on their local habitat (playa or dune field). Furthermore, groundwater samples differed greatly even within the same category (e.g. the two dune groundwater samples had low similarity) indicating that conditions are highly localized. For the dune groundwater samples the clearest distinguishing factor is the origin of the water. The microbial communities of the dune groundwater samples are largely differentiated from each other by *Pseudomonas*, *Sphingobium*, and the class *Rhodobacteraceae* which are significantly more abundant in the shallow aquifer. There are no OTUs that are significantly more abundant in the deep aquifer; however, some OTUs that are slightly more abundant there are *Seohaeicola*, *Xanthomonas*, and *Tepidimonas*. The only OTU that has a significant presence in both groups is denoted as *Pseudomonas*. The lack of species-level identification leaves some uncertainty, as there is no way of knowing which *Pseudomonas* species is more abundant in the shallow aquifer and which is abundant in both samples. This is further complicated by the high diversity of the family *Rhodobacteraceae*, and the fact that the genus *Seohaeicola* is part of the *Rhodobacteraceae* [7, 25]. *Sphingobium* and *Tepidimonas* are genera of obligate aerobes, so their high abundances indicate that both the shallow and deep aquifers must be primarily aerobic environments [7, 47]. *Seohaeicola* contain aerobic and anaerobic species, as well as moderate halophiles [79, 80]. The presence of *Xanthomonas*, however, is surprising as these are plant pathogens

[59]. All of the genera noted here are gram-negative. Archaeal data is not available for the shallow aquifer, but for the deep aquifer a much higher abundance of *Thaumarchaeota* and *Woesarcheota* is seen here than in the playa groundwater or the sediment; this implies a higher amount of ammonia oxidation at this location.

The playa groundwater samples are also largely differentiated from each other. The groundwater below the sediment column (GW-1) has a much higher abundance of the genera *Halomonas*, *Marinobacter*, and *Thiomicrospira* while the groundwater in southern Lake Lucero (GW-2) is more abundant in *Sediminimonas*, *Halobacteria*, *Methanomicrobia*, and the uncultured E6ACO2 (*Bacteroidetes*). The only OTU abundant at both locations denotes an unclassified *Gammaproteobacteria*. As with the dune groundwater, gram-negative and aerobic microbes are prominent. Halophiles are abundant at both locations but the most extreme halophiles (*Halobacteria*) have a larger presence in GW-2, implying that this location may be more saline [32, 40, 81]. The abundances of *Thiomicrospira* and *Sediminimonas* implies that both locations are primarily oxidizing environments with readily available reduced compounds, although at GW-1 sulfur cycling seems to be more prominent and at GW-2 nitrogen cycling seems more prominent [40, 77].

A moderate amount of methanogenic archaea are observed in the uncultured order STK1210A in the class *Methanomicrobia* [40]. The vast majority of these are in GW-2; methanogens have previously been observed in the groundwater of Lake Lucero [60]. Previous work has shown that hydrogenotrophic methanogens (such as the *Methanomicrobia*) tend to be inhibited in the presence of sulfate-reducing bacteria, as

these microbes consume acetate and H_2 needed by the methanogens [25, 63]. This implies that GW-2 may contain a much lower amount of sulfate-reducers than the other samples, which is consistent with the previous interpretation that sulfur cycling is relatively less prevalent at this location.

The sediment samples are more consistent in their microbial communities but still mostly distinct amongst each other, especially sample 14 which is a relative outlier from the rest of the sediment column. The most significant difference is the negligible presence of *Acidimicrobiales* OM1 in sample 14 as opposed to its high abundance in the rest of the sediment. This reinforces the previous notion that these planktonic bacteria flourish during the wet season and lay dormant during the dry season, when they are redistributed throughout the sediment column via capillary action [27, 45, 49]. The fact that they are most abundant at the surface lends further credence to this notion, as this would be the ideal location for them if they are indeed surviving in this manner. Sample 14 is also distinct due to its much higher abundance of *Acinetobacter*, a diverse bacterial genus of which most free-living soil species are saprophytes [15]. This would conform with the previously noted observation of saprophytes at this depth. Only *Pseudomonas* is identified as being consistently abundant throughout the sediment column. The archaeal communities were less consistent than the bacteria, sample 12 was isolated and samples 7 and 13 were paired while the rest of the samples grouped together in a dendogram (Fig 6). Samples 7, 12, and 13 all had a negligible abundance of *Thermoplasmatales*, while the other samples had them in high abundance. The samples 7 and 13 also had a uniquely higher abundance of *Thaumarchaeota* (ammonia

oxidizers), while 12 was unique for its high abundance of the halophilic genus

Halapricum [4, 65]. Although the clustering of the *Halapricum* population in sample 12 is

curious, it implies that the communities in this sample are not too different from 7 and

13 since halophiles are ubiquitous in all samples and *Halapricum* is not significantly

different from other *Halobacteria* genera (furthermore, 7 has a high abundance of

unclassified *Halobacteria*) [65]. Therefore, it seems that the biggest difference between

the archaeal communities of samples 7, 12, and 13 and the rest of the sediment is the

abundance of *Thermoplasmatales*. Considering that these are also most likely also

halophiles, it seems that differences within the archaeal communities in the sediment

samples are not significantly based on their phenotypic characteristics but more on

specific taxonomic distinctions.

Other Microbial Constituents

The genus *Ralstonia* is observed in high abundance, mostly at the sediment

surface (samples 1 and 2) and the groundwater-sediment interface (samples 14 and

GW-1), this may be due to the oligotrophic species *R. pickettii,* which is commonly found

in water and soil [58]. The *Gemmatimonadetes* phylum is oligotrophic and highly

abundant [20]. They are commonly observed in arid soils and are well adapted to living

in low moisture conditions, but they are not well adapted to resisting wet-dry cycles;

hence, they are observed with lower abundance at the surface and very low abundance

in the groundwater [20]. The previously mentioned *Acidimicrobiales* OM1 are also

oligotrophic [45]. The low amount of available nutrients in Lake Lucero makes this a

natural habitat for oligotrophs so it is not surprising that they are abundant and well-distributed in this environment [40]. These conditions would also lend themselves well to the endospore-forming bacteria, however only a small amount of these are explicitly observed such as *Bacillus* and *Peanibacillus* (also capable of nitrogen fixation) [24, 40].

The abundance of explicitly identified iron-reducing microbes is extremely low (phylum *Deferribacteres* and the genus *Shewanella*), as would be expected based on the fact that this is primarily an aerobic environment and iron metabolizing microbes are typically anaerobic [40]. This is consistent with the identified low concentrations of iron in the sediment and the relatively infrequent observation of iron using SEM/EDS. However, the observed iron-reducers are mostly in samples 13 and 14, which does not conform with the characterization of iron in this system (concentration peak in samples 11-12 followed by decrease in 13-14). Their absence in groundwater is consistent with the low concentrations of iron measured there previously (<1-2 mg/L) [51, 60], although it contrasts with the observation of iron-reducers in the groundwater by Schulze-Makuch [60]. It is possible that these microbes are present amongst the unclassified OTUs in the groundwater; this would also explain their presence in the deep sediment samples.

CONCLUSION

The results reported here show that the sediment and groundwater of Lake Lucero is a highly dynamic environment, strongly characterized by the capillary action of the groundwater, the extreme seasonality of the climate, and the hypersalinity. This extreme environment harbors microbial communities that are primarily aerobic, gram-negative, and are largely characterized by their survival adaptations. Halophiles and oligotrophs are extremely common throughout all samples, as anticipated. Furthermore, it is suspected that some community members may be using dormancy as a survival mechanism. These communities are very diverse and contain methanogens, phototrophs, heterotrophs, saprophytes, ammonia-oxidizers, sulfur-oxidizers, sulfate-reducers, iron-reducers, nitrifiers, and denitrifiers. Contrary to the original hypothesis, diversity and biomass did not vary in a significant, consistent manner between the near-surface, deeper subsurface, and groundwater. The dynamism of this environment manifests in the relatively consistent character of the microbial communities, where significant distinctions are more taxonomic than phenotypic; hence, the aforementioned substrates should not be considered separate ecological entities. An exception to this would be the minor shift observed in the deepest part of the column as the communities are affected by the presence of sticky clays, higher concentrations of various cations, and potentially decaying organic material. Saprophytes and *Chloroflexi* are more abundant here. The salinity appears be lower as well, as evidenced by the

decrease in the Na concentration and a lower abundance of extreme halophiles relative

to the rest of the sediment profile.

Appendix 1 – Tables & Figures

**Table 1:** Groundwater field measurements.

Sample Location	Lake Lucero – Central (GW-1)	Lake Lucero – South (GW-2)	Dune Field – Deep Aquifer (GW-3)	Dune Field – Shallow Aquifer (GW-4)
Temperature (C°)	17.7	15.0	17.5 – 18.5	16.8 – 18.2
pH	7.09	7.84	7.02	7.40
Conductivity (µS)	59.0	139.9	37	11.98

Table 2: Cation concentrations for the soil samples from White Sand Monument. The higher numbered samples are at lower depths (i.e. 1 is from the surface, 14 is from the groundwater). "BDL" stands for Below Detection Limit. The NO measurements include both NO_3^- and NO_2^-.

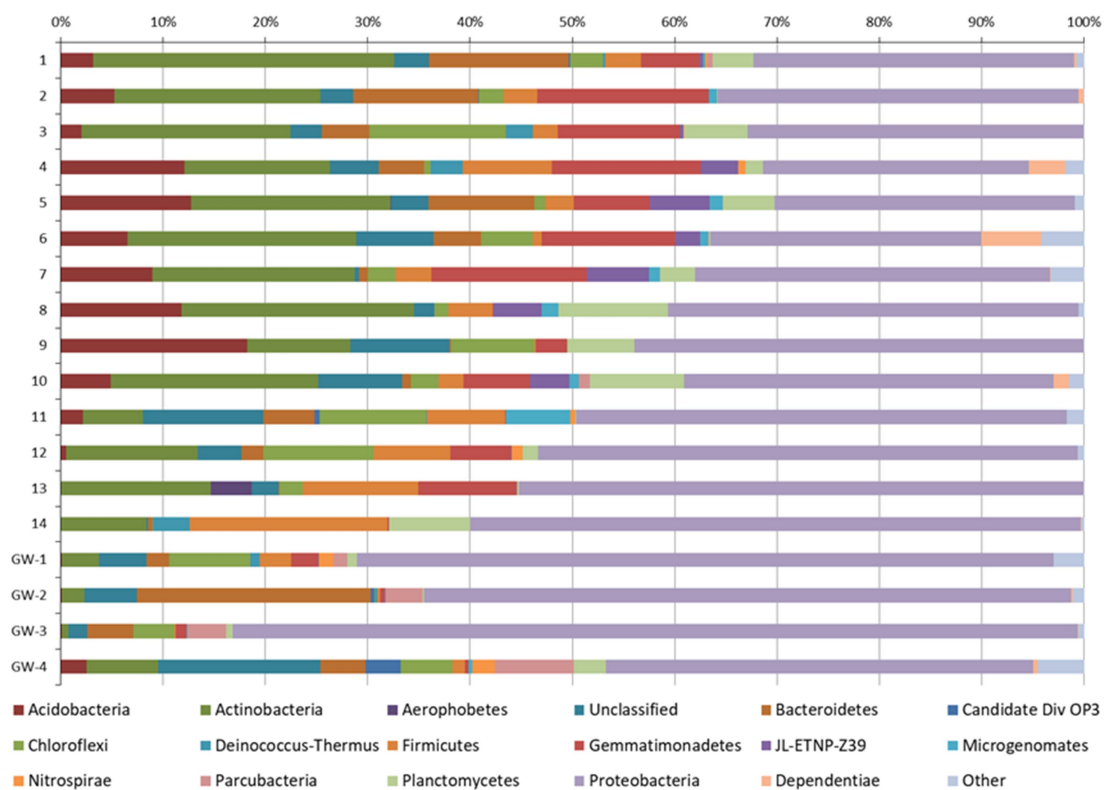| Sample ID	Fe (ppm)	K (ppm)	Mg (ppm)	Ti (ppm)	Sr (ppm)	Na (ppm)	NH_4^+ (ppm)	NO (ppm)
1	17.58	13.26	247.18	BDL	83.44	345.77	1.50	23.06
2	18.46	15.19	303.94	BDL	164.13	420.53	2.03	28.48
3	21.83	13.85	318.02	BDL	49.58	317.13	2.01	33.77
4	35.04	20.27	360.93	0.03	89.86	448.41	2.17	31.33
5	37.79	2.08	243.52	0.34	60.52	277.20	2.22	37.51
6	61.36	19.50	475.99	0.80	57.84	405.60	2.35	53.90
7	38.41	15.59	225.29	0.76	52.78	242.68	2.08	31.29
8	52.00	15.21	300.81	0.49	62.84	195.50	2.02	26.34
9	54.77	13.24	131.71	0.38	45.04	108.81	2.13	16.65
10	97.09	18.52	217.84	0.94	37.31	191.64	2.06	13.67
11	185.42	35.13	295.29	2.67	59.30	229.74	2.39	16.81
12	211.80	47.73	344.75	3.60	37.12	264.97	2.54	22.61
13	38.60	13.24	126.90	0.30	65.71	106.59	2.59	15.46
14	72.96	26.87	240.96	1.04	74.03	136.56	1.72	15.76
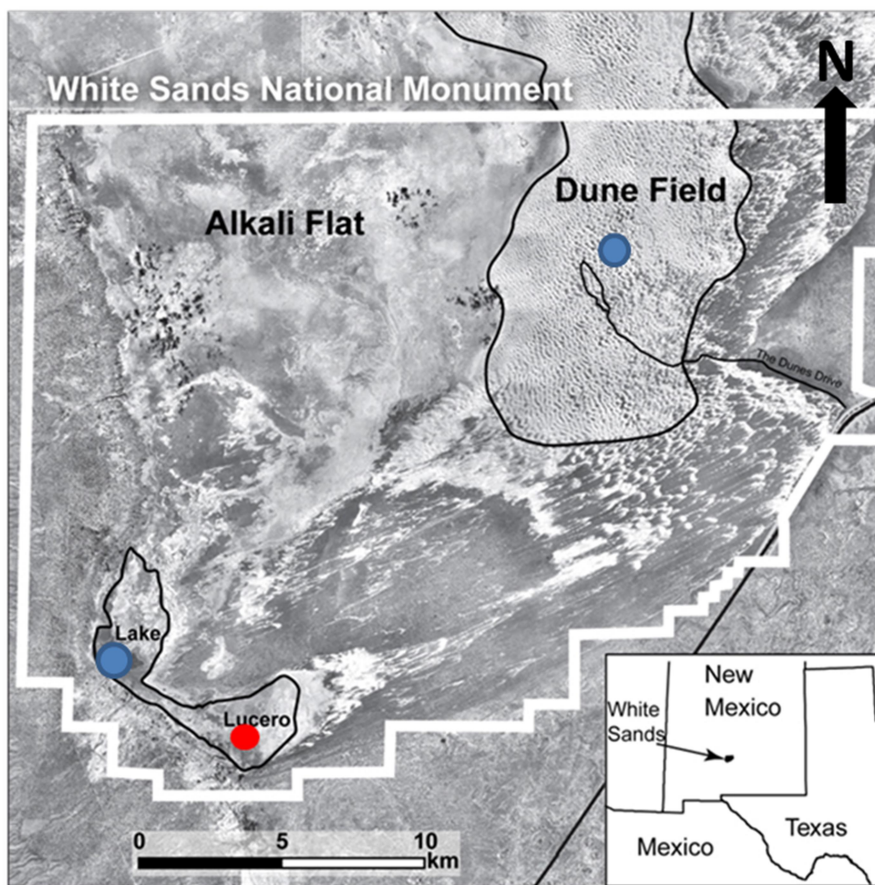
Figure 1: A map showing the WSNM area and sampling points. The red dot denotes the location where the sediment samples (1-14) and one groundwater sample (GW-1) were collected. The blue dots denote the location of groundwater samples in southern Lake Lucero (GW-2) and the dune field (GW-3 and GW-4).
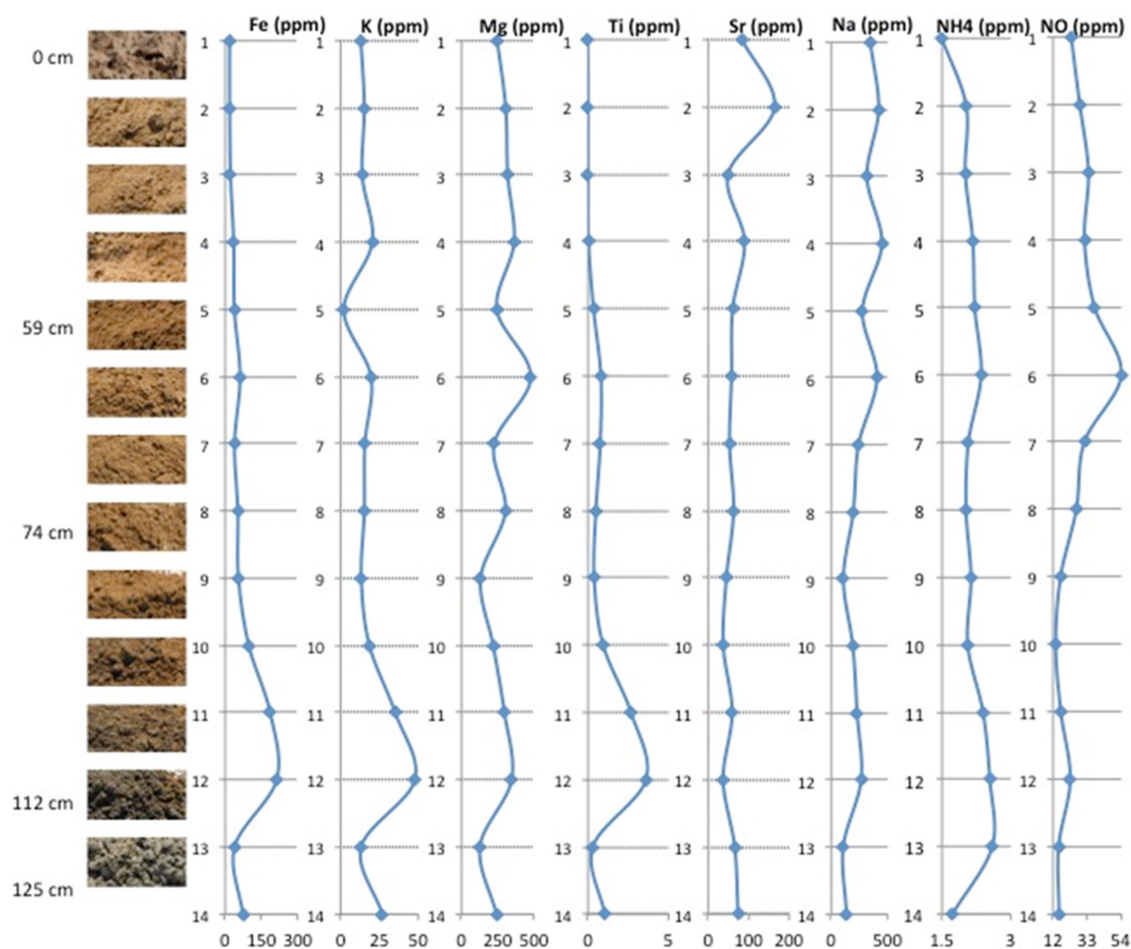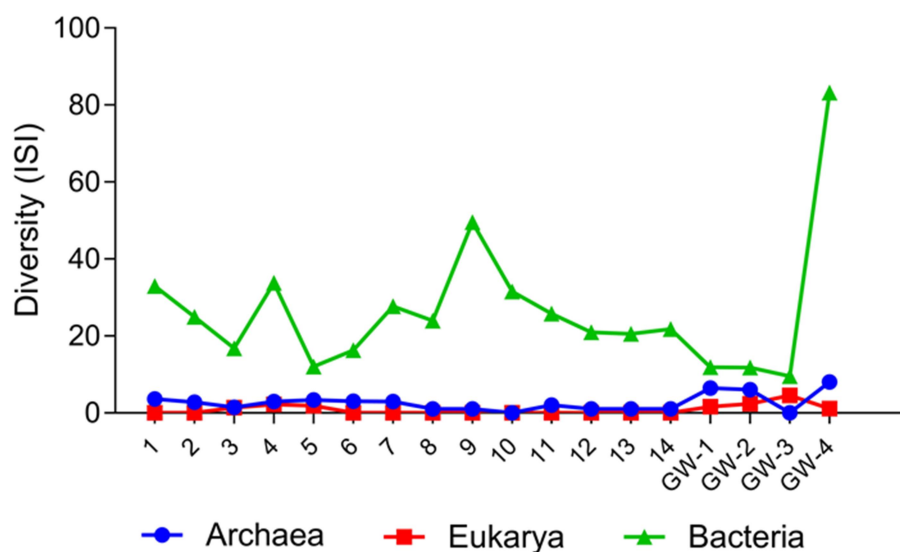
Figure 2: Depth profile illustrating the concentrations of elemental ions, ammonium, and nitrates (NO).
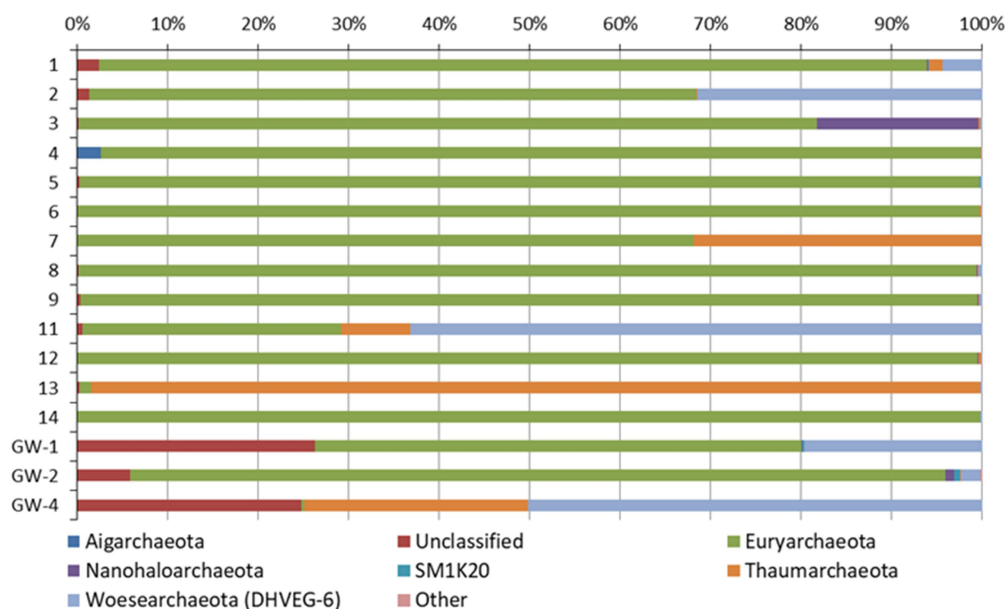
Figure 3: Phylum-level classification of bacterial data, 1-14 samples are from the sediment column and GW samples are groundwater.

Figure 4: Diversity of samples measured with the Inverse Simpson Index. Overall, bacteria were substantially more diverse than archaea and eukarya. Sample GW-4 (shallow aquifer – dunes) had the highest bacterial and archaeal diversity of all the samples, while sample 5 was the least diverse. In the groundwater, diversity was higher for both archaea and eukarya but lower for bacteria (except GW-4).

Figure 5: Phylum-level classification of archaeal data, 1-14 samples are from the sediment column and GW samples are groundwater. Samples that contained little or no archaeal DNA are excluded.
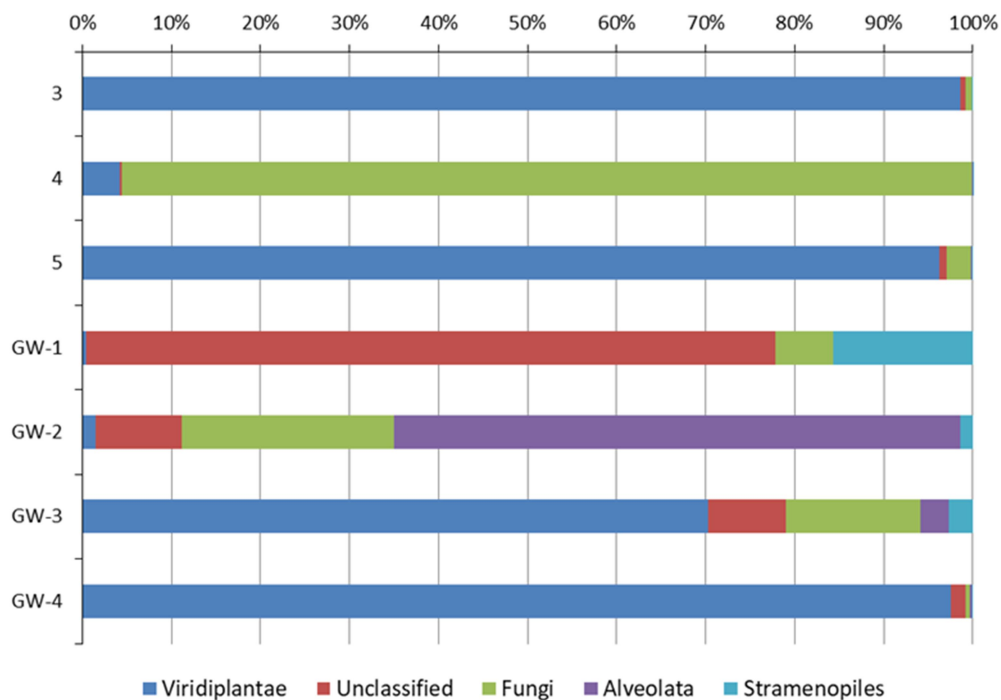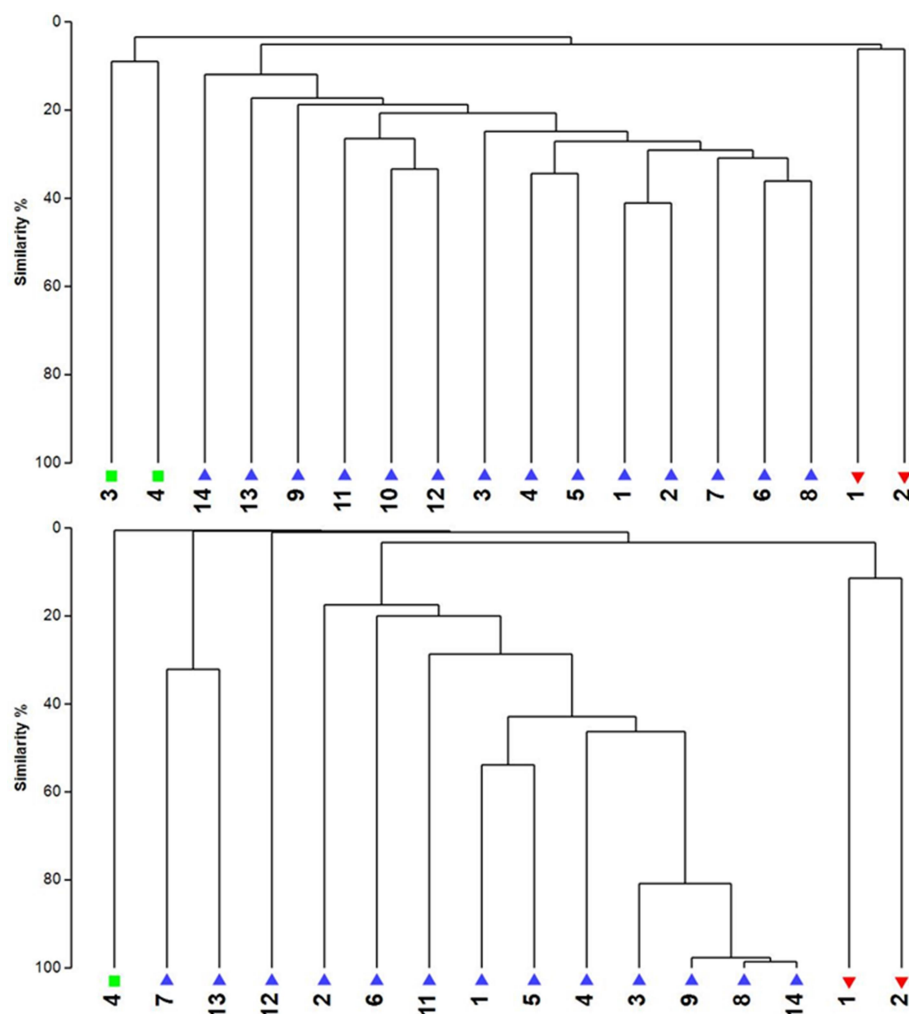
Figure 6: Phylum-level classification of eukaryotic data, 1-14 samples are from the sediment column and GW samples are groundwater. Samples that contained little or no eukaryotic DNA are excluded.

Figure 7: UPGMA dendograms of bacterial (top) and archaeal (bottom) data based on OTU abundance. Blue is for sediment, red is for playa groundwater, and green is for dune groundwater. For bacteria, sediment samples 1-13 clustered together closely while 14 (the deepest sediment sample) was further apart, indicating that bacterial communities at this depth differed somewhat from the communities higher in the column. Dune and playa groundwater samples clustered apart from the sediment samples and from each other. For archaea, samples 10 (sediment) and GW-3 (dune GW) are excluded due to having a low amount of archaeal DNA present. Most sediment samples clustered together with the exceptions of 7 and 13, which clustered further apart, and sample 12 which was individually isolated. The two playa groundwater samples were fairly similar in structure and the one dune groundwater sample was isolated from all other samples.

References

1.   Albuquerque L, Simoes C, Nobre MF, Pino NM, Battista JR, Silva MT, Rainey FA, da Costa MS (2005) Truepera radiovictrix gen. nov., sp. nov., a new radiation resistant species and the proposal of Trueperaceae fam. nov. FEMS Microbiology Letters 247, 161–169.

2.   Anton J, Pena A, Santos F, Martinez-Garcia M, Schmitt-Kopplin P, Rossello-Mora R (2008) Distribution, abundance and diversity of the extremely halophilic bacterium Salinibacter ruber. Saline Systems 4:15. doi:10.1186/1746-1448-4-15.

3.   Babavalian H, Amoozegar MA, Pourbabaee AA, Moghaddam MM, Shakeri F (2013) Isolation and identification of moderately halophilic bacteria producing hydrolytic enzymes from the largest hypersaline playa in Iran. Microbiology, 82, No.4.

4.   Campbell MA, Chain PSG, Dang H, El Sheikh AF, Norton JM, Ward NL, Ward BB, Klotz MG (2011) Nitrosococcuswatsonii sp. nov., a new species of marine obligate ammonia-oxidizing bacteria that is not omnipresent in the world's oceans: calls to validate the names 'Nitrosococcus halophilus'and 'Nitrosomonas mobilis. FEMS Microbiol Ecol 76: 39–48.

5.   Canfora L, Bacci G, Pinzari F, Lo Papa G, Dazzi C, et al. (2014) Salinity and Bacterial Diversity: To What Extent Does the Concentration of Salt Affect the Bacterial Community in a Saline Soil? PLoS ONE 9(9): e106662. doi:10.1371/journal.pone.0106662

6.   Casamayor EO, Garcia-Cantizano J, Pedros-Alios C (2008) Carbon dioxide fixation in the dark by photosynthetic bacteria in sulfide-rich stratified lakes with oxic–anoxic interfaces. Limnol. Oceanogr., 53(4), 1193–1203.

7.   Chen H, Jogler M, Rohde M, Klenk H, Busse H, Tindall BJ, Sproer C, Overmann J (2013) Sphingobium limneticum sp. nov. and Sphingobium boeckii sp. nov., two freshwater planktonic members of the family Sphingomonadaceae , and reclassification of Sphingomonas suberifaciens as Sphingobium suberifaciens comb. nov. Int. J. Syst. Evol. Microbiol. 63: 735-743, doi: 10.1099/ijs.0.040105-0.

8.   Chou Y, Chou J, Lin K, Lin M, Wei Y, Arun AB, Young C, Chen W (2008) Rothia terrae sp. nov. isolated from soil in Taiwan. Int. J. Syst. Evol. Microbiol. 58: 84-88, doi: 10.1099/ijs.0.65172-0.

9.   Clarke, KR, Gorley, RN, 2015. PRIMER v7: User Manual/Tutorial. PRIMER-E, Plymouth, 296pp.

10.   Cooke RU, Warren A, Goudie SA (1993) *Desert Geomorphology*, UCL Press Limited, London, pp. 202-215.

11.   Costa KC, Hallmark J, Navarro JB, Hedund BP (2008) Geomicrobiological changes in two ephemeral desert playa lakes in the Western United States. Geomicrobiol. J., 25: 250-259.

12.   Crack JC, Hutchings MI, Thomson AJ, Le Brun NE (2016) Biochemical properties of Paracoccus denitrificans FnrP: reactions with molecular oxygen and nitric oxide. J Biol Inorg Chem 21:71–82. doi: 10.1007/s00775-015-1326-7.

13.   DeLong EF (1992) Archaea in coastal marine environments. PNAS USA, 89:5685–5689.

14.   Di Meglio L, Santos F, Gomariz M, Almansa C, Lopez C, Anton J, Nercessian D (2016) Seasonal dynamics of extremely halophilic microbial communities in three Argentinian salterns. FEMS Microbiol Ecol, 92 (12): fiw184.

15.   Doughari HM, Ndakidemi PA, Human IS, Benade S (2011) The Ecology, Biology and Pathogenesis of Acinetobacter spp.: An Overview. Microbes Environ. 26, No. 2, 101–112.

18.   Dodsworth JA, Gevorkian J, Despujos F, Cole JK, Murugapiran SK, Ming H, Li W, Zhang G, Dohnalkova A, Hedlund BP (2014) Thermoflexus hugenholtzii gen. nov., sp. nov., a thermophilic, microaerophilic, filamentous bacterium representing a novel class in the Chloroflexi, Thermoflexia classis nov., and description of Thermoflexaceae fam. nov. and Thermoflexales ord. nov. Int. J. Syst. Evol. Microbiol., 64: 2119–2127.

17.   Eder W, Ludwig W, Huber R (1999) Novel 16S rRna gene sequences retrieved from highly saline brine sediments of Kebrit Deep, Red Sea. Arch Microbiology 172:213–218.

18.   Eigenbrode J, Benning LG, Maule J, Wainwright N, Steele A, Amundsen HE (2009) A field-based cleaning protocol for sampling devices used in life-detection studies. Astrobiology 9(5):455-65.

19.   Falb M, Muller K, Konigsmaier L, Oberwinkler T, Horn P, von Gronau S, Gonzalez O, Pfeiffer F, Bornberg-Bauer E, Oesterhelt D (2008) Metabolism of halophilic archaea. Extremophiles 12:177–196. doi:10.1007/s00792-008-0138-x.

20.   Fawaz MN (2013) "Revealing the Ecological Role of Gemmatimonadetes Through Cultivation and Molecular Analysis of Agricultural Soils." Master's Thesis, University of Tennessee.

21.   Fenn ME, Haeuber R, Tonnesen GS, Baron JS, Grossman-Clarke S, Hope D, Jaffe DA, Copeland S, Geiser L, Rueth HM, Sickman JO (2003) Nitrogen emissions, deposition, and monitoring in the western United States. Bio-Science 53:391–403.

22.   Friedman, G.M., Krumbein, W.E. (1985). *Hypersaline Ecosystems: The Gavish Sabkha*. Heidelberg, Germany: Springer-Verlag Berlin Heidelberg.

23.   Fryberger, 2001. Geological Overview of White Sands National Monument. https://www.nature.nps.gov/geology/parks/whsa/geows/; accessed February 17, 2017.

24.   Gao M, Yang H, Zhao J, Liu J, Sun Y, Wang Y, Sun J (2013) Paenibacillus brassicae sp. nov., isolated from cabbage rhizosphere in Beijing, China. Antonie Leeuwenhoek 103:647–653.

25.   Garcia-Maldonado JQ, Bebout BM, Everroad RC, Lopez-Cortes A (2015) Evidence of Novel Phylogenetic Lineages of Methanogenic Archaea from Hypersaline Microbial Mats. Microb Ecol 69:106–117. doi:10.1007/s00248-014-0473-7.

26.   Garrity GM, Bell JA, Lilburn T, Class I. (2005) Alphaproteobacteria class. nov. In: Brenner DJ, Krieg NR, Stanley JT, Garrity GM (eds), Bergey's Manual of Sytematic Bacteriology, 2nd edn. Vol. 2 The Proteobacteria), part C The Alpha -, Beta -, Delta -, and Epsilonproteobacteria Springer: New York, USA, p 1.

27.   Ghai R, Mizuno CM, Picazo A, Camacho A, Rodriguez-Valera F (2013) Metagenomics uncovers a new group of low GC and ultra-small marine Actinobacteria. Nature Scientific Reports 3: 2471. doi: 10.1038/srep02471.

28.   Glamoclija M, Fogel ML, Steele A, Kish A (2012) Microbial Nitrogen and Sulfur Cycles at the Gypsum Dunes of White Sands National Monument, New Mexico. Geomicrobiol. J., 29:733–751.

29.   Grabowski A, Tindall BJ, Bardin V, Blanchet D, Jeanthon C (2005) Petrimonas sulfuriphila gen. nov., sp. nov., a mesophilic fermentative bacterium isolated from a biodegraded oil reservoir. Int. J. Syst. Evol. Microbiol. 55: 1113–1121.

30.   Huber H, Hohn MJ, Rachel R, Fuchs T, Wimmer VC, Stetter KO (2002) A new phylum of Archaea represented by a nanosized hypothermophilic symbiont. Nature 417:63–67.

31.   Ichikuni M, Musha S (1978) Partition of strontium between gypsum and solution. Chem Geol, 21: 359—363.

32.   Jeong SH, Lee JH, Jung JY, Lee SH, Park MS, Jeon CO (2013) Halomonas cibimaris sp. nov., isolated from jeotgal, a traditional Korean fermented seafood. Antonie Leeuwenhoek, 103, 3, pp 503–512.

33.   Jetten MSM, Van Niftrik L, Strous M, Kartal B, Keltjens JT, Op de Camp HJM (2009) Biochemistry and molecular biology of anammox bacteria. Crit. Rev. Biochem. Mol. Biol. 44(2–3): 65–84.

34.   Jones SE, Lennon JT (2010) Dormancy contributes to the maintenance of microbial diversity. PNAS. 107, No. 13, 5881-5886.

35.   Kocurek G, Carr M, Ewing R, Havholm KG, Nagar YC, Singhvi AK (2007) White Sands Dune Field, New Mexico: Age, dune dynamics and recent accumulations. Sediment Geol, 197, Issues 3–4, 313–331.

36.   Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol. 79(17):5112-20.

37.   Langford RP (2003) The Holocene history of the White Sands dune field and influences on eolian deflation and playa lakes. Quat Int 104, 31-39.

38.   Langmuir D (1997) *Aqueous Environmental Geochemistry*. Upper Saddle River, New Jersey, USA.

39.   Madden AS, Smith AC, Balkwill DL, Fagan LA, Phelps TJ (2007) Microbial uranium immobilization independent of nitrate reduction. Environ Microbiol 9:2321–2330.

40.   Madigan, M.T., Martinko, J.M., Bender, K.S., Buckley, D.H., Stahl, D.A. (2015). *Brock Biology of Microorganisms*, 14th edition. Glenview, Illinois, USA.

41.   Makhdoumi-Kakhki A, Amoozegar MA, Kazemi B, Pasic L, Ventosa A (2011) Prokaryotic Diversity in Aran-Bigdol Salt Lake, the largest hypersaline playa in Iran. Microbes Environ. 27, No. 1, 87-93.

42.   Makhdoumi-Kakhki A, Amoozegar MA, Ventosa A (2012) Salinibacter iranicus sp. nov. and Salinibacter luteus sp. nov., isolated from a salt lake, and emended descriptions of the genus Salinibacter and of Salinibacter ruber. Int. J. Syst. Evol. Microbiol. 62, 1521–1527. doi:10.1099/ijs.0.031971-0

43.   Mesbah, M.N., Abou-El-Ela, S.H., Wiegel, J. (2007). Novel and Unexpected Prokaryotic Diversity in Water and Sediments of the Alkaline, Hypersaline Lakes of the Wadi An Natrun, Egypt. Microb Ecol 54, 598-617. doi: 10.1007/s00248-006-9193-y.

44.   Method 3050B: Acid Digestion of Sediments, Sludges, and Soils, Revision 2. EPA (1996).

45.   Mizuno CM, Rodriguez-Valera F, Ghai R (2015) Genomes of Planktonic Acidimicrobiales: Widening Horizons for Marine Actinobacteria by Metagenomics. mBio 6 no. 1 e02083-14. doi: 10.1128/mBio.02083-14.

46.   Molecular Research LP (MR DNA) http://www.mrdnalab.com/16s-ribosomal-sequencing.html; accessed March 21, 2017.

47.   Moreira C, Rainey FA, Nobre MF, da Silva MT, da Costa MS (2000) Tepidimonas ignava gen. nov., sp. nov., a new chemolithoheterotrophic and slightly thermophilic member of the β-Proteobacteria. Int. J. Syst. Evol. Microbiol., 50, 735–742.

48.   Morris EK, Caruso T, Buscot F, Fischer M, Hancock C, Maier TS, Meiners T, Muller C, Obermaier E, Prati D, Socher AS, Sonnemann I, Waschke N, Wubet T, Wurst S, Rillig MC (2014) Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories. Ecol Evol; 4(18): 3514–3524.

49.   Morris RM, Frazar CD, Carlson CA (2012) Basin-scale patterns in the abundance of SAR11 subclades, marine Actinobacteria (OM1), members of the Roseobacter clade and OCS116 in the South Atlantic. Environ Microbiol 14(5), 1133–1144.

50.   Navarro JB, Moser PD, Flores A, Ross C, Rosen MR, Dong H, Zhang G, Hedlund BP (2009) Bacterial succession within an ephemeral hypereutrophic Mojave Desert playa lake. Microb Ecol 57: 307-320.

51.   Newton BT, Allen B (2014) Hydrological Investigation at White Sands National Monument. New Mexico Bureau of Geology and Mineral Resources, open-file report 559.

52.   Oren A (2008) Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Saline Syst 4:2 doi:10.1186/1746-1448-4-2.

53.   Oren A, Sorenson KB, Canfield DE, Teske AP, Ionescu D, Lipski A, Altendorf K (2009) Microbial communities and processes within a hypersaline gypsum crust in a saltern evaporation pond (Eilat, Israel). Hydrobiologia 66; 15-26.

54.   O'Sullivan P.E., Reynolds C.S. (2005). The Lakes Handbook, Volume 1: Limnology and Limnetic Ecology. Blackwell Oxford, UK.

55.   Potvin M, Lovejoy C (2008) PCR-based estimates of artificial and environmental 18S rRNA gene libraries. J Eukaryot Microbiol 56:174–181.

56.   Rees NG, Baldwin DS, Watson GO, Perryman S, Nielsen DL (2004) Ordination and significance testing of microbial community composition derived from terminal

restriction fragment length polymorphisms: application of multivariate statistics. Antonie Leeuwenhoek 86, 339-347.

57.   Reynolds RL, Yount JC, Reheis M, Goldstein H, Chavez P, Fulton R, Whitney J, Fuller C, Forester RM (2007) Dust Emission from Wet and Dry Playas in the Mojave Desert, USA. ESPL 32, 1811–1827.

58.   Ryan MP, Pembroke JT, Adley CC (2007) Ralstonia pickettii in environmental biotechnology: potential and applications. J Appl Microbiol 103: 754–764.

59.   Ryan RP, Vorholter F, Potnis N, Jones JB, Van Sluys M, Bogdanove AJ, Dow JM (2011) Pathogenomics of Xanthomonas: understanding bacterium–plant interactions. Nature Rev Microbiol 9, 344-355, doi:10.1038/nrmicro2558.

60.   Schulze-Makuch D (2002) Evidence of the discharge of hydrothermal water into Lake Lucero, White Sands National Monument, Southern New Mexico. New Mexico Geological Society 53rd Annual Fall Field Conference Guidebook, pp. 325-329.

61.   Siam R, Mustafa GA, Sharaf H, Moustafa A, Ramadan AR, Antunes A, Bajic VB, Stingl U, Marsis NGR, Coolen MJL, Sogin M, Ferreira AJS, El Dorry H (2012) Unique Prokaryotic Consortia in Geochemically Distinct Sediments from Red Sea Atlantis II and Discovery Deep Brine Pools. PLOS ONE, 7, Issue 8; e42872.

62.   Simpson EH (1949) Measurement of diversity. Nature 163:688.

63.   Smith JM, Green SJ, Kelley CA, Prefurt-Bebout L, Bebout BM (2008) Shifts in methanogen community structure and function associated with long-term manipulation of sulfate and salinity in a hypersaline microbial mat. Environ Microbiol, 10, Issue 2; 386–394.

64.   Solorzano L (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol Oceanogr, 14, Issue 5: 799–801.

65.   Song HS et al. (2014) Halapricum salinum gen. nov., sp. nov., an extremely halophilic archaeon isolated from non-purified solar salt. Antonie Leeuwenhoek, 105:979–986. doi:10.1007/s10482-014-0156-x.

66.   Sorenson KB, Canfield DE, Oren A (2004) Salinity Responses of Benthic Microbial Communities in a Solar Saltern (Eilat, Israel). Appl Environ Microbiol, Mar. 2004, p. 1608–1616.

67.   Sorenson KB, Canfield DE, Teske AP, Oren A (2005) Community Composition of a Hypersaline Endoevaporitic Microbial Mat. Appl Environ Microbiol, 71, No. 11, 7352-7365.

68.   Sorokin DY, Tourova TP, Lysenko AM, Muyzer G (2006) Diversity of culturable halophilic sulfur-oxidizing bacteria in hypersaline habitats. Microbiology 152: 3013-3023, doi: 10.1099/mic.0.29106-0.

69.   Sorokin DY, Tourova TP, Kolganova TV, Spiridonova EM, Berg IA, Muyzer G (2006) Thiomicrospira halophila sp. nov., a moderately halophilic, obligately chemolithoautotrophic, sulfur-oxidizing bacterium from hypersaline lakes. Int. J. Syst. Evol. Microbiol. 56: 2375-2380, doi: 10.1099/ijs.0.64445-0.

70.   Sorokin DY, Van Pelt S, Tourova TP, Evtushenko LI (2009) Nitriliruptor alkaliphilus gen. nov., sp. nov., a deep lineage haloalkaliphilic actinobacterium from soda lakes capable of growth on aliphatic nitriles, and proposal of Nitriliruptoraceae fam. nov. and Nitriliruptorales ord. nov. Int. J. Syst. Evol. Microbiol., 59, 248–253. doi: 10.1007/s10482-012-9849-1.

71.   Stroud CP (1950) A Survey of the Insects of White Sands National Monument, Tularosa Basin, New Mexico.Am Midl Nat, 44, No. 3, pp. 659-677.

72.   Szynkiewicz A, Moore CH, Glamoclija M, Bustos D, Pratt LM (2010) Origin of coarsely crystalline gypsum domes in a saline playa environment at the White Sands National Monument, New Mexico. J Geophys Res, 115, F02021.

73.   Tang S, Wang Y, Lou K, Mao P, Xu L, Jiang C, Kim C, Li W (2009) Kocuria halotolerans sp. nov., an actinobacterium isolated from a saline soil in China. Int. J. Syst. Evol. Microbiol. 59, 1316–1320.

74.   Ventosa, A., Mellado, E., Sanchez-Porro, C., Marquez, M.C. (2008). "Halophilic and Halotolerant Micro-Organisms from Soils" in *Microbiology of Extreme Soils*, ed. P. Dion (Heidelberg, Germany: Springer-Verlag Berlin Heidelberg). 87-115.

75.   Vogel BM, Des Marais DJ, Turk KA, Parenteau MN, Jahnke LL, Kubo MDY (2009) The Role of Biofilms in the Sedimentology of Actively Forming Gypsum Deposits at Guerrero Negro, Mexico. Astrobiology; Volume 9, Number 9. doi: 10.1089=ast.2008.0325.

76.   Wahlund TM, Madigan MT (1993) Nitrogen Fixation by the Thermophilic Green Sulfur Bacterium Chlorobium tepidum. J Bacteriol, 175, No. 2; p. 474-478.

77.   Wang Y, Wang Z, Liu J, Chen Y, Zhang X, Wen M, Xu L, Peng Q, Cui X (2009) Sediminimonas qiaohouensis gen. nov., sp. nov., a member of the Roseobacter clade in the order Rhodobacterales. Int. J. Syst. Evol. Microbiol., 59: 1561-1567, doi: 10.1099/ijs.0.006965-0.

78.   Western Regional Climate Center – White Sands National Monument
http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?nm9686; accessed February 15, 2017.

79.   Xie B, Lv X, Cai M, Tang Y, Wang Y, Cui H, Liu X, Tan Y, Wu X (2014) Seohaeicola
nanhaiensis sp. nov., A Moderately Halophilic Bacterium Isolated from the Benthic
Sediment of South China Sea. Curr Microbiol, 69, Issue 6, pp 802–808.

80.   Yoon J, Kang S, Lee S, Oh K, Oh T (2009) Seohaeicola saemankumensis gen. nov.,
sp. nov., isolated from a tidal flat. Int. J. Syst. Evol. Microbiol., 59: 2675-2679, doi:
10.1099/ijs.0.011312-0.

81.   Zhong Z, Liu Y, Liu H, Wang F, Zhou Y, Liu Z (2015) Marinobacter halophilus sp.
nov., a halophilic bacterium isolated from a salt lake. Int. J. Syst. Evol. Microbiol.,: 2838-
2845, doi: 10.1099/ijs.0.000338.