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DIFFERENCES IN EUBACTERIAL AND ARCHAEAL SOIL COMMUNITIES
ALONG A PRECIPITATION TRANSECT IN THE MOJAVE DESERT

A Thesis

Presented to

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Of the Requirements for the Degree

Master of Science

by

Elaine Pressly Bryant

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The Designated Thesis Committee Approves the Thesis Titled

DIFFERENCES IN EUBACTERIAL AND ARCHAEAL SOIL COMMUNITIES
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by

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APPROVED FOR THE DEPARTMENT OF BIOLOGICAL SCIENCES

SAN JOSÉ STATE UNIVERSITY

May 2011

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ABSTRACT

DIFFERENCES IN EUBACTERIAL AND ARCHAEAL SOIL COMMUNITIES
ALONG A PRECIPITATION TRANSECT IN THE MOJAVE DESERT

by
Elaine Pressly Bryant

Deserts occupy one third of the total land mass of Earth, yet little is known about microbial soil communities under conditions of low precipitation and humidity.   The Mojave Desert of the Southwestern United States provides an opportunity to observe microbial communities along a precipitation transect. The diversity of these communities can elucidate which specific groups of Eubacteria and Archaea are best adapted to limited liquid water.

Two methods were utilized to determine patterns and trends of the microbial soil communities under varying precipitation levels. Viable plate counts allowed comparison of culturable microorganisms over a four-year period. Molecular techniques distinguished unculturable Eubacteria from Archaea. The results indicated three trends. First, the number of culturable microbes decreased from 10^7 to 10^6 CFUs/g dry soil as the annual precipitation decreased. Also, the diversity of the eubacterial communities increased as the annual precipitation decreased, indicated by Shannon-Wiener indices increasing from 3.51 to 3.76. Finally, the diversity increases for archaeal communities were small, producing Shannon-Wiener indices from 1.21 to 1.36. Possible indicator bacteria for desertification processes were suggested by the increasing numbers and diversity of cyanobacteria at the drier sample sites.

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PREFACE

The following thesis is presented as two journal articles. Chapter 1 is a literature review of research on microbial soil communities in deserts around the world. The analyzed communities include the Eubacteria, the Archaea, fungi, and bacteriophage populations associated with arid, semi-arid, coastal, and cold deserts. The desert regions of interest include locations on all continents.

Chapter 2 presents research on bacterial and archaeal communities along a precipitation transect through the Mojave Desert in southern California. Microbial soil communities were investigated using culture and molecular techniques to examine changes in diversity as a function of annual precipitation. Also examined was the selection of an indicator microbe that could be used to detect the advancement of desertification in semi-arid environments. Sequence analysis of the 16S rRNA gene indicated that the diversity of the eubacterial communities increased as precipitation decreased, though the archaeal communities indicated no change in diversity along the transect. Cyanobacterial species found only in the driest sample site were suggested as possible indicator organisms for increasing desertification in the Mojave Desert.

CHAPTER 1: LITERATURE REVIEW

Introduction

Microorganisms have been studied in terms of their pathogenic characteristics or for their use in industries such as the production of food, agriculture, bioremediation, and biotechnology. However, the function and structure of microbial communities in the natural world have proven to be highly complex. As detection methods have improved, microbes have been discovered in environmental and anthropomorphic niches thought to be too harsh for any form of living organism. The study of these organisms offers potential rewards in terms of enzymes for industrial and medical use as well as providing an idea of life on other planets [46, 42].

The extreme arid biomes on Earth present microorganisms and higher life forms with unique challenges. All life on Earth requires liquid water, and therefore the discovery of microorganisms existing in soil of the Atacama Desert that received a single precipitation event of 2.3 mm of rainfall in a period of four years was quite surprising [42]. The complex desert soil communities of bacteria, fungi, and protozoa have proven difficult to assess and have yet to be characterized in terms of the identities of the microbes, their function in the ecosystem, and their means of coping with the extremes of arid environments.

Understanding the relationship between microbial communities and precipitation may also prove useful in predicting effects of global warming.

Resulting data might indicate possible conditions such as the presence or absence of certain bacteria or archaea or changes in the microbial community that might reveal the advancement of desertification. Identifying such indicator organisms may allow conservationists and communities time to develop appropriate responses to the decreasing moisture conditions [8].

This review will focus on the state of research on microorganisms existing in arid biomes on Earth in terms of desert locations, specific microorganisms targeted, techniques used for analysis, and conclusions derived from the resulting data. The intention is to use this information to determine gaps in the overall picture of microbial diversity in arid environments and to identify directions for future research.

Diversity of Microorganisms

Definitions of microbial diversity often focus on species richness - the number of species in an environment and species evenness - indicating the number of individuals per species. However, this interpretation does not take into account the community structure and function of the microbial population. Hunter-Cevera [64] presented a more comprehensive definition of microbial diversity: "Microbial diversity includes the genetic composition of microorganisms, their environment or habitat where they are found and their ecological or functional role within the ecosystem." Thus, an examination of the

microorganisms existing in a defined community should encompass several aspects of microbial communities:

- the genetic differences based upon examination of nucleic acid sequences for conserved genes
- possible symbiotic relationships between microorganisms in terms of contributions to:
  - nutrient access and microbial stratification (three-dimensional location of communities)
  - species progression related to changing physical characteristics of the environment

Kirk [35] outlined the problems of assessing microbial diversity and identified four areas of concern: microbial distribution in soil, inability to culture most soil microorganisms, the chemical and physical challenges of extracting DNA or RNA, and the overall difficulty of classifying microbes.

The distribution of microorganisms in soil is not uniform, but often seen as existing in "several nested levels of organization" or horizontal and vertical stratification related to the presence or absence of required nutrients. This produces an uneven distribution or clumps of microhabitats throughout an area of interest. Also, the spatial and temporal distribution of microbial communities has proven difficult to evaluate. Many early studies based their conclusions on fungal growth by examining the fruiting bodies above ground, which do not correlate to the below-ground fungal expansion. Thus, the actual sampling pattern and the

number of samples taken in an area are important considerations for determining microbial diversity [35].

The second problem defined by Kirk, et al. [35] focused on assessing microbial diversity by using culture techniques. Culturing microbes has become a necessity for the identification and understanding of pathogenic microbes. The temperatures, nutritional sources, and other growth conditions are easily replicated based upon knowledge of the host organism – animal or plant. The application of such culturing techniques is not appropriate for environmental microbial communities. Research over the last twenty years indicates less than 1% of microscopically observable soil bacteria can actually be cultured in the laboratory [57, 35]. Thus, microbial diversity based upon culturing the microbes from soil samples has been grossly under-estimated and skewed. The growth conditions for environmental microbes appear to be quite complicated. Kaeberlien, et al. [34] demonstrated this situation by using a diffusion chamber to simulate an intertidal marine sediment. Marine sediment provided the source of bacteria, and organisms from that source were transferred to warm agar made with seawater, and separated from the sediment by polycarbonate membranes. This allowed exchange of environmental constituents but restricted movement of the microbes. The resulting colonies represented at most 40% of the initial inoculum. Purification of isolates was attempted, resulting in the isolation of two bacteria. Further growth of these isolates was maintained on the marine sediment, but the only growth on culture media was observed when other

organisms from the marine sediment were also present. This interdependency between multiple microorganisms was termed "coculture." The researchers posit that the isolates required something from the other organisms, possibly chemical signaling, before becoming metabolically active [57, 34].

Kirk's [35] third problem in assessing soil microbes deals with the extraction of conserved genes that can be used for identification of the various microbes.   With the advent of molecular techniques, such as the polymerase chain reaction (PCR), cloning plasmids, transformation, and various forms of gel electrophoresis, a broader picture is forming of the true nature of microbial diversity in soil. However, these processes also have limitations based upon selection of the conserved gene for comparison and the physical act of extracting DNA and/or RNA.

Most of the molecular techniques used for identifying microorganisms focus on the gene for the small ribosomal subunit [23, 20, 6, 25, 59]. Eukaryotic or prokaryotic universal or specific primers are used for the amplification of a 1,500 base pair 16S rRNA gene with the assumption that the resulting copied segment can be cloned and separated to identify individual microorganisms at the genus and possibly the species level. However, there are inherent problems with trying to identify microorganisms this way due to different affinities between primers and templates, differing amounts of G+C content in the DNA (resulting in different strand separation times) and preferential amplification – DNA strands with higher quantities of guanine and cytosine take longer to copy than those with

low quantities of these nucleotides. Finally, microorganisms found in low percentages in a community may not be amplified to the same degree as the more plentiful microorganisms resulting in little or no presence in a gel and no further analysis.

Kirk, et al. further elaborates on problem of conserved genes for microbial identification by examining the current procedure for separating microbes from soil particles. Most DNA extraction kits combine a specified amount of soil with a tube of buffered solution, containing small beads. The tubes are then vortexed, causing the beads to collide with soil particles, detaching microbes attached to those particles. This often results in damaging the cell and the nucleic acids. Lysing the cells to extract their nucleic acids is done mechanically, chemically or by combination of both methods. The efficiency of lysis protocols depends upon several factors:

- the volume of cells per sample: a low volume indicates the need to minimize loss, whereas a high volume may require dilution to obtain a balance between the concentration of chemical agent (typically a detergent) and the number of cells to lyse
- the type of cell to lyse: different microbes have different cell walls and membrane structure, which may require buffer solutions of different ionic strengths
- the presence of humic acids in the samples effect future PCR processing thus must be removed from the sample

- the nucleic acid purification techniques often result in a decrease in the final concentration of nucleic acids. For samples with low cell volume, this limits the number of uses for the nucleic acids.

These factors all affect the quantity and quality of resulting DNA or RNA template [35].

The final area of concern for determining microbial diversity as outlined by Kirk, et al. focuses on the confusion regarding the taxonomy of microorganisms and the definition of the term "species" as it applies to prokaryotes. In 1753, Linnaeus presented the binomial system of genus and species as the most exacting means of identifying a multicellular organism. Since then, numerous efforts have been proposed for the determination of this level of specificity. Staley points out that there are three basic taxonomic classification systems currently in use: the biological species concept (BSC), which basis speciation on reproductive isolation; the morphospecies concept (MSC), which uses external characteristics such as size, shape, color, etc to speciate organisms, and the phylogenetic species concept (PSC), which relates species by their evolutionary relatedness based on gene sequence comparisons. Because Eubacteria and Archaea do not demonstrate meiotic sexual reproduction, the BSC is not applicable to this group of microorganisms.

In *The Prokaryotes: Symbiotic Associations, Biotechnology, Applied Microbiology*, Stackebrandt discusses the attempts to use the MSC for bacterial speciation. Cohn first applied the binomial system to bacteria in the late 1800s

using observable morphological differences to distinguish between six genera. His attempt resulted in the acknowledgment that morphological features of bacteria did not directly relate to physiological attributes, and thus were not sufficient for differentiation beyond the genus level [21].

As technology provided more levels of testing to separate one bacterium from another, more observable and biochemical characteristics were included as necessary for the distinction of a species. Buchanan combined all these characteristics into one system that was then used for the production of *Bergey's Manual of Determinative Bacteriology*. Problems with this empirical system arose when bacteriologists realized that many of the notable distinguishing characteristics were not mutually exclusive. An example of this is the ability to produce clumping factor in the genus *Staphylococcus*: *S. aureus* produces this factor, but *S. lugdunensis* also produces it in a delayed manner. No other staphylococci related to human or animal diseases produce clumping factor. Therefore, the use of clumping factor alone does not clearly delineate between these two species of *Staphylococcus*.

Further development of techniques to distinguish pathogenic bacteria led to the establishment of the taxonomic level of strain, which assumed great importance when treating diseases caused by variants of bacteria exhibiting characteristics possibly acquired by horizontal gene transfer, such as antibiotic resistance. The inclusion of the taxonomic level of strain allowed for the grouping of bacteria at the genus and species level, while allowing some

variation in one or two attributes [53]. Thus, the distinction of genus and species

using the MSC has expanded to incorporate not only morphological

characteristics, but observable and measurable attributes such as the Gram

reaction, oxygen requirement, motility, nutritional requirements, biochemical test

reactions, and other such demonstrable traits that are consistent for a group of

Eubacteria or Archaea.

In the 1960s scientists recognized that evolutionary changes were

detectable by examining cellular DNA and RNA, leading to the PSC [21].

Comparison of the nucleotide bases for a conserved gene, such as the 16S

ribosomal subunit, identified sites of mutations that can be assessed in terms of

ancestral relationships. Interpretation of the results is based upon how many

different bases in the gene are detected with the assumption that two very similar

genes indicate the two organisms are closely related. The process of DNA-DNA

hybridization (DDH) heats double-stranded DNA (dsDNA) until the two strands

separate (melting temperature of strands = T_m). The temperature is then cooled

to within 5°C of T_m and the single strands are allowed to reattach. The stability of

the resulting dsDNA is indicative of the level of similarity in the original DNA

molecules [53]. Using this technique, two dsDNA strands demonstrating 70% or

greater similarity at the temperature within 5°C of T_m indicate they are from the

same species. Stackebrandt further related DNA reassociation with 16S rRNA

gene analysis, indicating bacteria that meet the same species criteria using DNA

reassociation demonstrate ≥ 97% similarity between 16S rRNA genes [60].

In 1970 Colwell suggested a new method of species assignment that "assembles and assimilates" information regarding the genetic, molecular, morphological, biochemical and ecological aspects of an isolate before assigning it to a species. Colwell proposed the term polyphasic taxonomy and used this to relate 86 strains of the genus *Vibrio*. Specifically, thirty strains of *V. cholerae*, broken down as classic *V. cholerae*, *El Tor* vibrios, and non-cholerae vibrios, thirty-five strains of *V. parahaemolyticus*, and 21 strains representing control genera and *Vibrio* species from marine environments were selected for comparison. Standard laboratory tests, such as growth on specific media, motility, carbohydrate fermentation, colonial characteristics, antibiotic susceptibility, nutritional requirements, and electron microscopy, were scored and tallied. The conclusion offered by scoring the phenotypic data and comparing it to the DNA-DNA reassociation data indicated that the current assignment of strains *cholerae, El Tor* and all but 2 of the *non-cholerae* should be classified as the same species. Strains of *V. parahaemolyticus* demonstrated a separate cluster, and the control bacteria were separate from all Vibrios [16].

Mola, et al. also used a polyphasic approach in 1997 to assign six halophilic Gram-positive cocci isolated from salt ponds in Huelva, Spain to the genus and species *Nesterenkonia halobia.* The species had been previously created based upon a single strain isolated from an unknown salty deposit outside Noda, Japan. Mola, et al. utilized standard biochemical tests such as fermentation of carbohydrates, hydrolysis tests, citrate utilization, and antibiotic

susceptibility, as well as the determination of guanine-plus-cytosine content, 16S

rRNA similarity, and DDH to conclude that the six isolates from a saltern in

Huelva, Spain should be assigned to *N. halobia* [41].

In February 2002 a group of European microbiologists formed an ad hoc

committee funded by the Federation of Microbial Societies (FEMS) to evaluate

the definition of bacterial species with regards to the advancement of molecular

techniques. The committee determined that the rigid application of DDH is still

the gold standard for the taxonomic assignment of species. The current

definition of a species: "a category that circumscribes a (preferably) genomically

coherent group of individual isolates/strains sharing a high degree of similarity in

(many) independent features, comparatively tested under highly standardized

conditions," was upheld. However, the committee further encouraged

researchers to develop new techniques to support and/or supplant DDH [61].

A recent attempt to further elucidate the taxonomic level of species has

been the inclusion of ecological characteristics to the overall polyphasic

approach to classification. Konstantinidis and Tiedje identified conserved

protein-coding genes in seventy related, fully sequenced genomes from the NCBI

database, the Sanger Center, the Institute for Genomic Research and the

Advanced Center for Genome Technology, University of Oklahoma. These

highly conserved protein-coding genes are directly related to phenotype, thus

give a more concise comparison of the ecological conditions of the bacterial

environment than simply using the 16S rRNA gene. BLAST was used to perform

pairwise comparisons of all the protein-coding genes and the average nucleotide identity (ANI) of the conserved genes between strains was computed. The ANI scores were used as a measure of evolutionary distance between strains. DNA-DNA reassociation and 16S rRNA gene sequence were also performed on the related genes. The researchers concluded that the ANI scores clearly support 16S rRNA sequence identity matches and DNA-DNA reassociation results, and an ANI score of 93-94% represents the species gold standard of 70% DNA-DNA reassociation level. Interestingly, a direct comparison of conserved genes between strains grouped as the same species (94% ANI) yielded gene code differences of 5-35%. By comparison, the ANI value for gene similarities of humans and chimpanzees is 98.7%, indicating that the level of acceptable gene coding differences between highly similar bacteria is too low and, thus, too homogeneous to associate with phenotype and environmental niche [37].

Koeppel et al. implemented an algorithm to identify ecotypes of bacteria existing in arid desert environments. Cohan first defined the term ecotype as a more specific term than species, thus indicative of strains. An ecotype reflects the related characteristics of a group of organisms due to environmental pressures related to a specific environment. This environment is subjected to natural selection such that a mutant resulting from environmental stressors will out-compete all other strains of that ecotype, resulting in the extinction of the original ecotype. Strains of other ecotypes will not be affected [15]. Koeppel, et al. investigated the application of the ecotype simulation model (ES) to examine

the members of two clades of the genus *Bacillus*. Sequence differences for three genes were used to establish diversity within and between two clades of *Bacillus* isolated from Evolution Canyons in Israel. For the *B. simplex* clade, *gapA, rpoB,* and *uvrA* were compared. For the second clade, the only different gene examined was *gyrA* instead of *uvrA*. Complete linkage clustering was employed to group the samples from each clade such that the evolutionary pattern of divergence was produced. Interpretation of the resulting pattern indicated periods of constant ecotype formation as well as inflection points indicative of increased diversity. Application of the ES model resulted in 9 ecotypes for the *B. simplex* clade and 13 ecotypes for the *B. subtilis-B. licheniformis* clade. The authors mention that the current species demarcation for 16S rRNA is >3% divergence, but that Stackebrandt had more recently suggested that level be changed to >1% to reflect the species differences for eukaryotic organisms [62]. Using this suggested degree of similarity, the 16S rRNA gene comparison for the *B. simplex* clade showed 100% identity between all isolates. Within the second clade of *Bacillus*, the isolates separated into two species groups based upon 16S rRNA similarity, except for 6 ecotypes. Four of these phylogenetically related to B. *licheniformis*, and two were more closely related to *B. spizizenii*. Thus, the model indicated that the even using the 1%16S rRNA gene similarity does not recognize measurable ecotype diversity, and suggest that the utilization of such ecologically oriented models should be incorporated into further attempts to speciate bacteria [36].

These four concerns – microbial distribution in soil, inability to culture most soil microorganisms, the chemical and physical challenges of extracting DNA or RNA, and the overall difficulty of classifying microbes - indicate the complexity associated with assessing soil microbial diversity. A 2008 report from the American Society for Microbiology, entitled "The Uncharted Microbial World: Microbes and their activities in the Environment," acknowledge these problems and further indicate the need for new techniques used to perform community-level studies detailing the physiological contributions from various microbes. In addition, the current limitations associated with the detection of microbial interactions should be eliminated [30].

Harwood, et al. continue the examination of the current state of microbial research to include the lack of knowledge of how microorganisms function in the global biogeochemical cycles, including nitrogen fixation, recycling of greenhouse gases, and recycling basic nutrients from dead material. Baseline data on microbial community members, structure and functions have yet to be determined, thus the effects of disturbance of such communities are unknown, whether the environment be internal to animals (gums/teeth), waste management systems, or soil communities [30].

Diversity Measurements

The current techniques used for microbial community investigations incorporate culturing, fluorescent labeling, microscopic observation, and

molecular techniques focusing on DNA, protein and phospholipid fatty acid analysis (PFLA), as well as the use of ribosomal sequence tags. The results from these techniques provide insight into microbial diversity in one of two ways. DNA/RNA analysis may identify microorganisms to the genus and sometimes species level. Fluorescent labeling, culture plates, microscopic observation, PFLA, and ribosomal sequence tags provide relative assessments of microbial diversity.

Many culturing and molecular techniques depend on the application of mathematical models to relate the resulting data to species diversity. Diversity indices mathematically measure the species diversity in a community by assessing the species richness – the number of species present, and the species evenness – the number of individuals of each species. The most common diversity index is the Shannon or Shannon-Weiner Index. Based on information theory, this index reflects the degree of randomness, or order, in a population. The index is determined by the expression:

$$H = -\sum_{i=1}^{s}(p_i)(\ln p_i)$$

where H = information content of the sample or index of species diversity, s = number of species, and p_i = proportion of total sample belonging to the i th species.

The value of H reflects the diversity of a community based upon the maximum value of H_{max}, where $H_{max} = \ln S$ when the number of individuals is the same for all species [38].

Physical Characteristics of Deserts

Defining or classifying types of deserts typically reflects the type of research or concern of interest to scientists. Agricultural researchers emphasize the differing precipitation and temperatures, and the resulting vegetation. Geographers and meteorologists use physical systems such as wind patterns, proximity to open water or mountain ranges, and seasonal variations to characterize arid environments. Geologists examine the soil and mineral content, possibly related to mining and new oil reserves. Regardless of the discipline, one characteristic of deserts holds true – they are the driest regions on the planet [40].

Aridity indices have been utilized to delineate deserts from surrounding areas offering more hospitable conditions for life. The Thornthwaite Moisture Index is a calculation based upon the water balance of an area:

$$I_m = \frac{s - 0.6d}{e} \times 100$$

I_m is the moisture index, s = sum of monthly surpluses of precipitation greater than the estimated evaporation, d = sum of monthly precipitation deficits, and e=estimated annual potential evaporation. The e value includes adjustments for

seasonal changes, monthly temperatures and a soil-moisture factor. Meigs

expanded the aridity index and further defined designations of arid lands in terms

of agricultural potentialities [43]. Thus the extremely arid, or hyperarid regions

have recorded 12 months of more of no precipitation. Arid areas receive rainfall

inadequate for the propagation of crops. Semi-arid zones are predominately

grass or steppes. Thornthwaite's designation of "subhumid" relates to the

Mediterranean or chaparral climate, with very dry summers and wet winters.

Meigs' designations have been widely used as the standard for classification of

arid environments [65]. Table 1 identifies the resulting designations for arid

regions.

Table 1. Designations used for arid regions, the related Thornthwaite Moisture
Index, the approximate annual precipitation and the percent of terrestrial land
associated with this designation.

Designation	I_m	Annual Precipitation	Global Land Area
Extremely arid	< -56	< 2.5 cm	7.50%
Arid	-40 to -56	2.5 to 20 cm	12.10%
Semi-arid	-20 to -40	20 to 50 cm	17.70%
Dry subhumid	0 to -20	50 to 75 cm	9.90%

Based upon these global circulation patterns and the existing terrain of the

continents, the United States Geological Society acknowledges 5 physical and

climatological situations that result in the formation of deserts: proximity of

mountain ranges, predominant wind direction, predominant pressure system, proximity to large bodies of water (oceans, lakes), and ocean currents [65].

Several deserts exist due to the rain shadow effect caused by mountain ranges that uplift the air, resulting in precipitation on one face of the range, and dry air flowing down the back side of the range. The Mojave, the Chihuahuan, the Taklimakan, and the Turpan Depression are examples of rain shadow deserts. Also, included under this designation might be deserts resulting from seasonal wind alterations, such as the Thar Desert. Monsoons bring heavy rain to eastern India, but much of the precipitation is lost as the storm crosses the continent. By the time the air mass reaches the India-Pakistan border, very little precipitation remains [45].

The deserts of Australia exhibit the characteristics associated with midlatitude deserts. There are few mountain ranges to produce rain shadow deserts, and evidence of seasonal lakes and rivers are abundant. The average annual precipitation ranges from 200mm in the Simpson Desert to 400mm with the extremely dry central area averaging 150mm. Temperature averages range from 25 - 42ºC in the Great Sandy Desert to 4 - 36ºC in the Simpson Desert. Surface composition includes sand dunes, pebbles, and red soils. Vegetation includes acacia shrublands, grasslands, and succulents. Wildlife diversity in the Simpson Desert includes 34 mammals, 231 birds, 22 amphibians, 13 fish and 125 reptiles [40, 45].

Trade wind deserts are north and south of the equator, and form due to the influx of dry winds from the Horse Latitudes. These deserts are primarily extreme arid or arid environments and include the Sahara and the Arabian deserts. Characteristics of trade wind deserts vary considerably due to size and geographic location. The Sahara Desert is larger in area than the continental United States, and exhibits ecoregions from extremely arid to Mediterranean. Rainfall can occur at any time of year and the annual precipitation for the core of the Sahara is less than 25mm while the eastern portion receives less than 5mm. Temperature ranges are extreme with highs over 50ºc and lows below freezing.

Coastal deserts are located on the western side of continents. Cold water currents moderate the climate, causing fog that blocks solar radiation resulting in cooler temperatures, and very little precipitation. The Atacama Desert, located along the coast of Peru and Chile, is west of the Andes Mountains. The Peru Current moves south to north parallel to the South American coast, resulting in heavy fog that moderates the temperature and introduces minimal levels of precipitation to the area. Average temperatures range from 14.5 ºC in winter months to 21ºC in summer. Movement of the fog further inland is dependent upon air temperature, humidity and wind speed, thus the average precipitation amounts decrease from north to south, with 0.8mm in Arica, to 1.20mm in Antofagasta [44]. Research performed over a 4 year period in the central portion of the Atacama recorded a single precipitation event occurring at midnight that measured 2.3mm. Averaged over 4 years the annual precipitation amount would

be 0.58mm/year. The differing infiltration of the fog establishes a gradient of flora and fauna decreasing from the southern desert to the northern areas [42]. The surface of the Atacama consists of sand and pebbles. Approximately 550 plant species manage to exist in this xeric land, with numbers increasing with proximity to the fog zone. The only animals found in the dry areas are arachnids, insects, lizards, mice, fox and some birds. The fog zones show an increase in the number of observed birds.

Polar deserts exist due to the extremely cold, dry air, bringing very little moisture to the area. The Antarctic plateau averages 51mm of precipitation per year, whereas coastal areas may experience up to 510mm rainfall. In many cases, the precipitation is very light so little is absorbed into the ground, even during the summer season. Mean Antarctic temperatures range from -55ºC at Vostok Station, to -49ºC at the South Pole. Daily temperature ranges from -50ºC to 20ºC have been measured during the austral summer. The continent has the lowest average humidity of any location on earth – less than 10%, with humidity often dropping to as low as 1%. Should melting of ice occur due to seasonal temperature increases, the ice may sublimate, resulting in no liquid water production. These polar areas may have snow drifts, frozen ground or gravel and boulders. Often the freeze-thaw cycle seen in the Antarctic create hexagonal patterned textures in the ground that have recently been observed on Mars [40, 45].

Soil Microbial Diversity

The study of soil microorganisms has been very important in terms of agriculture. Plants and microbes have interesting interactions, each providing nutrients to the other, and often dictating what grows where. According to Atlas, there are 10^6-10^9 bacteria per gram of soil [3]. Soil provides numerous microhabitats that offer protection, water and minerals to a variety of microbes. Aerobes make use of extensive surface areas on organic litter, as well as irregular rocks and soil particles. Facultative and strict anaerobes find appropriate anoxic conditions at depth and particularly in flooded soils. The diversity of niches results in a wide variety of bacteria associated with soil. *Acinetobacter, Arthrobacter, Bacillus, Caulobacter, Clostridium, Micrococcus Pseudomonas, Streptococcus* and *Xanthomonas* are just a few of the genera identified from soil samples.

Studies of soil microbial diversity in extreme environments were initially performed using various clinical laboratory media for culturing microbes extracted from soil samples. A 1962 study of Ross Island in the McMurdo Sound of Antarctica plated dilutions of samples on nutrient agar, Sabouraud Dextrose Agar, Thornton's standardized agar, and ferric ammonium-citrate-nitrate medium. Algae as well as Eubacteria and Archaea were isolated, including *Chromatium minutissimum, Bacillus stearothermophilus, Bacillus licheniformis, Micrococcus cryophilis, Flavobacterium peregrinum*, *Pseudomonas fluorescens*, and molds from the genera *Penicillium* and *Aspergillus.* Culture media for soil microbes has

progressed from general media to lower levels of nutrients more suggestive of

the original chemical makeup of the soil. Previously uncultured bacteria have

been identified by employing low-nutrient culture media, thus introducing new

genera to the families *Acidobacteria, Actinobacteria, Verrucomicrobia,* and

Planctomycetes. [11]

Microbial diversity of soil communities has also been determined using

molecular techniques based upon the evolutionary alterations to the 16S rRNA

gene. Fierer, et al. used 16S rRNA genes and metagenomic techniques to

create clone libraries for bacteria, Archaea, fungi and viruses taken from three

different soils: the Mojave Desert in southern California, the Kansas prairie and

the rainforest of Peru. Using parametric models to estimate the diversity of the

different microbial communities, they estimated that the highest number of

operational taxonomic units (OTUs) for the Archaea was found in the desert, the

highest diversity for fungi was in the prairie soils, and viruses demonstrated

highest diversity in rainforest soils. The bacterial diversity remained relatively

constant regardless of the sample. The archaeal diversity in the arid

environment was mentioned as unusual. The authors proposed that this may

result from the decreasing competition from bacterial communities that are under

greater stress in such extreme conditions. The high diversity of fungi in prairie

lands was supported by a previous study of the Konza Prairie. No other concise

analysis of viral diversity in soils has been undertaken. Comparisons of the viral

sequences to GenBank led to the observation that the viral communities differ

due to bacteria in distinct habitats [23].

Microbial Diversity in Arid Deserts

Arid deserts are found on all continents except Europe. Their

environments are characterized by sand dunes, clear blue skies, little to no

vegetation, reptilian creatures and no visible water. The Sahara Desert in

northern Africa, the Judean Desert in Israel and the Arabian Desert of Saudi

Arabia are typical examples of arid deserts. Investigations of microbial diversity

in these extremely hot and dry regions vary with technique and microbial groups

investigated. The molecular techniques of nested PCR, 16S rDNA clone libraries

and colony hybridization were used on soil from Sturt National Park in New South

Wales, Australia [32] to identify species of *Rubrobacter*. Culture techniques and

microscopic examination were employed to examine protozoa in central

Australia, bacteriophage forms in the Sahara, and fungi found in Egypt and

around the Dead Sea of Israel and Jordan [55, 29, 58, 54].

The Australian studies focused on specific groups of microorganisms – the

subclass *Rubrobacter* in the high G+C phylum *Actinobacteria*, and the variety of

protozoa in the soil and in cyanobacterial crust. *Rubrobacter* clones were

isolated from eight samples and analyzed using 16S rRNA gene amplification.

Resulting clone libraries indicated a high abundance of *Rubrobacter* in these

samples, which was verified by group-specific probe hybridization. The summary

of this research expressed as a phylogenetic tree indicated four deep-branching, highly diverse monophyletic groups: *Actinobacteria, Acidimicrobidae, Coriobacteridae* and *Rubrobacteridae*. The *Rubrobacteria* further split into three groups, which were not evenly represented in existing clone databases, and the predominant *Rubrobacter* species identified in the Sturtz National Park were not the predominant species identified in previous studies of desert soil bacteria [32].

The protozoa of Australian deserts were analyzed from 27 samples collected from bare soil between plants, under termite mounds, under plant litter, from plant hummocks and from cyanobacterial crust. Numbers of each type of protozoan were determined using stained slides, plaque counts, and microtiter plates. The results indicate 145 species of protozoa exist in arid soils. Forty-two flagellates were counted, with 9 species being identified. Amoebae were the most abundant, with the *Acanthamoeba* genus present at 24 of the 26 sample sites. The ciliates were primarily under plants and termite mounds, and 57 species were found with 52 identified to the species level. The most prominent ciliate was the genus *Holosticha*. Protozoa tests were also more abundant in litter or under plants, though only 10% of the tests were inhabited. The predominant testacea was the genus *Euglypha*. The sample from the cyanobacterial crust was shown to house an amoeba, *Thecamoeba*, and a ciliate, *Nassula picta*, which were consuming the bacteria filaments, as well as two heliozoans, and several other ciliates [55].

Steiman, et al. presented a survey of fungal organisms in arid soils around the Dead Sea in Israel and Jordan. Fifty-six samples were taken, incorporating the upper 10 centimeters of soil, and were isolated via culturing plates. Further analysis of the soil included determination of the level of soluble salts and recording the pH of the soil in each sample. With some exceptions, the salt content was found to decrease with distance from the Dead Sea and the data indicated that the number of isolates was inversely proportional to the salt content of the soil. The first journal article concentrated on identifying organisms of the taxonomic classes Ascomycetes, Basidiomycetes, and Zygomycetes. Twenty-three genera and 90 species were isolated from the 56 soil samples, with the most plentiful fungi being of the order Eurotiales – a sac fungi, with the genera *Aspergillus, Eurotium* and *Penicillium* found most often. Most of the more common fungi had been previously noted in related research on the arid soils of the area and are, in fact worldwide in distribution. New species were identified for genera *Aspergillus* and *Microascus* [63].

The second paper from the same research group examined the fungi of the class Deuteromycetes that were not of the genera *Aspergillus* or *Penicillium*. From the 56 soil samples analyzed, 51 genera and 106 species were identified. The most common genera were from the class Dematiaceous hyphomycetes and were *Alternaria, Botrytic, Cladosporium,* and *Ulocladium*. The genus *Fusarium* from different taxa was also very common [29].

Prigent, et al. analyzed 13 surface sand samples of the Sahara in Tunisia

and Morocco for the presence of bacteriophages. The technique used to identify

possible phage particles involved two methods of phage extraction resulting in

phage suspensions– direct chemical extraction with centrifugation and extraction

with incubation. The phage suspensions were placed on microscopy grids and

stained with 1% (w/v) phospho-tungstenic acid, then examined by electron

microscope. Bacteriophages were extracted from the soil through a process of

sonication, gravity sedimentation, centrifugation and filtration. Bacteriophage

nucleic acids were then isolated with buffer-saturated phenol, ether extraction

and ethanol precipitation. Electrophoresis in 1% agarose gels were treated with

ethidium bromide for visualization. The 13 sites produced from 4 to 44 viral

particles observable with a transmission electron microscope. The morphologies

of the viral particles corresponded to the Myoviridae, the Siphoviridae and the

Podoviridae families [54].

Bacterial and Archaeal microorganisms were identified from sand dunes in

south Tunisia. Colonies were cultured on 0.1xTSA medium, the DNA was

extracted, and partial sequencing of the 16S rRNA gene was performed.

Bacterial diversity was determined from 117 sequences, and indicated a

predominance of *Proteobacteria* – primarily in the γ-*Proteobacteria* sub-phylum,

Actinobacteria and *Acidobacteria*. The 43 Archaeal clones were all members of

a non-thermophilic group of *Crenarchaeota*. Radiation tolerance was also

detected by exposing 1g of sand to a 15kGy cobalt-60 source, which was then

cultured and analyzed. Nine morphotypes were identified, four associated with the genus *Bacillus*, two were associated with phylum *Deinococcus-Thermus*, and the remaining three were related to two genera in α-*Proteobacteria* [14].

As the previous descriptions of research suggest, the arid deserts have been the focus of many in-depth studies concerning a variety of microorganisms. While the primary focus is simply to expand the knowledge of microbial communities in such extreme environments, the relationship between these microorganisms and humans, animals and crops is also of tremendous interest and concern. The identification of fungi associated with such dry environments contributes to a problem observed in the dry southern areas of California. Though not as extreme as the Dead Sea, winds often introduce fungal spores into the air and result in an increase in fungal respiratory diseases. Thus, the identification of possible pathogens may insure proper preventive measures for the local animal population.

The presence of bacteriophages in the Sahara indicates the presence of a variety of bacterial host, which may not be discernable using typical culture techniques or may be found in such low numbers that molecular techniques do not adequately represent them. Furthermore, the studies concerning Bacteria and Archaea continue to expand the range of harsh environments populated by members of these domains. These arid environments present such incredible swings in diurnal temperatures, moisture, and humidity, and exhibit very high levels of ultra-violet radiation, as well as extremely low levels of nutrients, making

these deserts inhospitable for most of the species on Earth. Yet, even here we find populations of microorganisms managing to survive.

Microbial Diversity in Semi-arid Deserts

Semi-arid deserts are common in North and Central America, China and South America. These deserts typically contain many shrubs and small nocturnal mammals, and have more precipitation than the arid deserts due to seasonal monsoon rains. Molecular techniques were used to examine cyanobacterial crusts in Utah, and to determine the bacterial composition of soil samples from Northern Arizona [28, 39, 20]. Also in Utah, protozoa were isolated and identified using culture techniques [7]. Eastern Washington's desert environment provided soil samples from an elevation transect that were chemically analyzed to determine the microbial biomass, nitrification potential, mineralizable carbon and nitrogen [58].

Garcia-Pichel, et al. examined four cyanobacterial crusts from the Colorado Plateau in Utah to identify the components of the crusts and the soil substrate. The polyphasic approach involved microscopic examination of the *Cyanobacteria* to group into morphologically distinct clusters, and the molecular analysis of the 16S rRNA genes using Denaturing Gradient Gel Electrophoresis (DGGE). The soil was categorized as silt, gypsum, shale, or sandy soils. Six cyanobacterial morphotypes were isolated and identified microscopically as belonging to the genera*, Shcizothix and Scytonema* – found in all soil types*,

Phormidium – not found in shale*, Nostoc* – found only in gypsum and silt,

Chlorogloeopsis – found only in silt*,* and *Microcoleus,* specifically the species

vaginatus – not found in gypsum. Single cells and filaments were teased apart

and cultured separately. DNA was also extracted from dry desert crust, and

used as template for PCR.   The resulting 450bp fragments were then processed

using DGGE. Phylogenetic analysis using sequences from the DGGE bands

resulted in six distinct cyanobacterial clusters. Five clusters proved to be novel,

contradicting the microscopic analysis. Analysis of the data demonstrates that:

1. morphological identification clearly underestimates the diversity of

cyanobacterial crusts; 2. the physiochemical conditions of soil greatly influence

the cyanobacterial species; 3. *Microcoleus vaginatus* is the dominant species in

these crusts, and 4. the presence of the filamentous species grouped as

Xeronema are ubiquitous and are related to the morphological genera

Schizothrix and *Phormidium* [28].

　　　Protozoa found in Utah's Great Basin were identified by culturing soil

samples collected in two locations: north of the Great Salt Lake where the

average precipitation is 300 mm and close to Bonanza, Utah – annual

precipitation of 200mm. The samples were tested for water content and pH, then

2 g samples of each were plated and cultured for protozoa. Thirty-four species of

protozoa were found and all but four are considered cosmopolitan, or commonly

found species. The most common protozoa of the cool deserts were ciliates,

followed by the flagellates. Further analyses of the data indicate that protozoa

inhabit pores located between or inside the mineral particles where water is trapped by capillary processes. Thus, the distribution of protozoa is dependent upon the physical characteristics of the soil [7].

Conditions similar to the Great Basin Desert extend through eastern Oregon and Washington. An elevation transect in southeastern Washington State provided samples to investigate climate change and microbial activities. Twenty-five sample sites between elevations of 228 m to 844m were selected at 25m elevation increments. This transect represented a mean annual maximum temperature decrease from 30 to 24°C. The samples were analyzed for pH, electrical conductivity, soluble carbon, inorganic nitrogen, total carbon and nitrogen and biomass. Other sample measurements were made for dehydrogenase activity, nitrification potential and total mineralizable carbon and nitrogen. The biomass was calculated by using substrate-induced respiration (SIR), which relates the ratio of CO_2 to biomass carbon. Flasks of prepared soil were weighed, and then glucose was added to each sample. The flasks were incubated for 2 hours and the evolution of CO_2 was then measured by gas chromatography. The biomass results indicated no difference in biomass with elevation, but a notable difference in biomass between grassy soil and crustal soil consisting of seedless plants such as mosses, algae, ferns and fungi [58].

Two articles were based upon a study done by the Environmental Molecular Biology Group of Los Alamos National Laboratory that compared two sample sites on the cinder cone of Sunset Crater to two sites in the Coconino

National Forest approximately 30 km away. Each site was sampled under plants

and between plants. The two sites exhibited different soil and tree

concentrations; however the inorganic nitrogen concentrations were similar.

Other organic materials were in much lower concentration on Sunset Crater than

at the forest site. DNA was extracted and used as template for PCR of the 16S

rRNA gene. The initial research resulted in the sequencing of 16 clones from

each soil sample (total of 60 clones), which were partially sequenced and

analyzed. Four clones were dropped from the analysis after being identified as

chimeric artifacts or contained extraordinarily long insertion sequences and

unknown origin.   Out of the remaining 56 clones, 20 proved to be associated

with previously known or identified bacterial groups, primarily with

Proteobacteria, gram-positive organisms and flexibacteria. The remaining 64%

of the sequences represented novel bacterial groups and actually fell into 5

clades. Four of the clades appear closely related to the *Acidobacteria*; however

the fifth group appears to be more closely related to the common ancestor [39].

The second study performed with the same samples involved the

comparison of molecular techniques to culturing. The soil samples were used to

inoculate culture plates, which were incubated and purified by streaking.

Restriction fragment length polymorphism identified genera, resulting in 801

clones and 179 isolates. Sequencing of these resulted in the identification of 526

RFLP patterns. Analysis of the isolate and the clone RFLP patterns showed

differences in phylogenetic diversity. Data from the cultured organisms indicate

most of the bacteria were from the phylum 'Gram Positive', whereas the clone libraries based upon the RFLP analysis indicated the phylum Acidobacteria were the dominant bacteria [20].

Several studies have been performed to identify Archaea in semi-arid deserts around the world. The Great Salt Plains National Wildlife Refuge encompasses a highly saline river bottom area. Extreme conditions of the area include daytime temperatures of over 50°C, desiccating winds, low humidity, intense UV radiation, and an alkaline pH. Caton et al. cultured microbes from soil samples then extracted DNA from the isolates. Nineteen phylotypes related to the archaeal halophiles, were identified, specifically *Haloarcula, Haloferax, Halorubrum, Haloterrigena* and *Natrinema*. The archaeal diversity demonstrated by the isolates was shown to be representative of the environment by rarefaction curves, indicating a rather low level of diversity in this sampling area [13].

A different alkaline lake bed was the sample site for investigating Archaea capable of reducing nitrate. Lake Texcoco near Mexico City is a central desert area very similar to the Chihuahuan Desert to the north. Rather than use the 16S rRNA gene for sequencing, primers for the archaeal narB and *nirA* genes were used for amplification and then used to create clone libraries. The resulting sequences from *narB* and *narA* indicated close association with the *Halobacteriaceae* [5].

Microbial Diversity in Coastal Deserts

The coastal deserts are restricted to the western coast of South America and Africa. The Atacama Desert in Chile is situated between the Pacific Ocean and the Andes Mountains and extends from 20º - 30º South latitude – the horse latitudes. The altitude of the desert ranges from 800 to 1200 m above sea level. The ocean currents, a coastal mountain range, and a strong high-pressure system are responsible for the extreme aridity of the area [42]. Due to the extremely low humidity and lack of liquid water for many years at a time, this area has become regarded as a terrestrial analog to Mars. Research performed there may result in new experiments or techniques that can be incorporated into a robotic or possibly a manned mission to the red planet.

D.R. Bagaley examined microbial life in the Yungay region of the Atacama by making culture-dependent dilution plates of 33 samples. Five media were used to culture heterotrophic microbes: plate count agar, marine agar, nutrient agar, 0.1 strength plate count agar and 0.01 strength plate count agar. Broth cultures of each media were also used. Counts were made of the colony forming units (CFUs) per gram of soil for each plate resulting in a range of CFUs from 0 to 7.4 x 10^5 CFU/g soil on 0.1 strength plate count agar. Isolates were then use as template for amplification of the 16S rRNA gene to sequence and identify the bacteria. Most of the bacteria were identified as from the phylum *Actinobacteria* and specifically from the genus *Blastococcus*. The remaining bacteria fall under the phyla *Proteobacteria* and *Firmicutes* [6].

Another study examined the possible relationship between bacteria and fungi, particularly the vesicular arbuscular mycorrhizae (VAM), in the Atacama Desert. VAM are fungi, which exist, in a symbiotic relationship with plant roots. The researchers examined the difference in soil constituents and microbial biomass from two sampling areas: "fertility islands," represented by native shrubs, and between plants after periods of drought and periods of above average rainfall. During the fall or dry season, soil samples were taken under randomly selected shrubs. *Adesmia bedwellii* is a thorny shrub providing 2.6-5.7% coverage of the study site. Several other samples were taken a distance of 5 m from the shrub canopy. Soil samples were again taken from the same locations at the end of the rainy season, resulting in 48 soil samples total. Chemical analysis was performed for pH, organic matter concentration, electrical conductivity, available phosphorus and soil moisture. Dilution plates were used to culture bacteria and fungi, using plate count agar for bacteria and potato dextrose agar for fungi. The results of the soil characteristic analysis indicated that only the soil moisture content and pH differed in the dry and rainy seasons. The soil under the canopy showed a significantly higher amount of organics and nitrogen, and the values doubled during the wet season. Microbial population under the canopy was observed to increase by two orders of magnitude compared to the intraspace samples during the dry season. However, the difference between the canopy and between-shrub areas was lower during the rainy season. VAM spores were also counted and plant root infection by these

spoors was determined. Analysis indicated that spoor infections were low during periods of increasing organic matter and nitrogen, indicating that the mycorrhizal infection allows the desert shrub to overcome the environmental stress of drought conditions [4].

Microbial Diversity in Cold Deserts

The cold deserts are located where the availability of liquid water is restricted by extremely cold air/winds, year round snow, ice pack and low surface temperatures. These conditions inhibit plant growth and place severe restrictions on microbial growth. The higher peaks of many mountain ranges and portions of Antarctica, Greenland, and islands of Russia and Canada experience these types of extreme conditions [45]. Given a rather high relative humidity range of 66-78%, average annual precipitation ranges from 10.33 to 24.45 mm. Average temperatures range from -6.71°C in summer to -15.32°C in winter [44].

Microbial activity in the frozen soils of the Arctic has been assessed by measurements of the carbon dioxide produced by bacteria as the final product in respiration. Three sites from Arctic areas were selected: Barrow, Alaska, West Siberia and Kopparas Mire in Sweden. These sites differ in length of time the soil is frozen and average air temperatures. The presence of CO_2 and thus, respiration was measured for temperatures down to -39°C, indicating that microbes were active at all three sites even at that extreme temperature [49].

The relative abundance and diversity of bacteria were assessed by serial analysis of ribosomal sequence tags (SARST) on soil samples from three arctic tundra sites and three boreal forest sites, encompassing a latitude range from 47° to 82°N. SARST generated a total of 12,850 ribosomal sequence tags (RSTs) and produced the largest collection of 16S rRNA gene sequences from environmental samples to date. RSTs are genus or species specific thus were used to provide phylogenetic information on the microbial community. The results indicated higher diversity in the undisturbed Arctic tundra sites than in the forest soil, which counters the assumption that diversity decreases as sampling approaches the polar region. The resulting RSTs showed affiliation with the alpha, beta, gamma and delta *Proteobacteria, Acidobacteria, Firmicutes, Aquificae, Nitrospira, Planctomycetes, Verrucomicrobia* and *Cyanobacteria* though eleven others were unclassified at the genus level [47].

Biolog sole-carbon-source utilization microplates were used to assess microbial diversity and community structure for three arctic samples sites. Replicate soil samples were taken from three sites: one located in proximity to human settlement, another taken at the foot of bird rock perches, and the third site was from unenriched tundra. The Biolog microplates offered 95 different carbon sources for utilization by a community of microorganisms. Inoculated plates were incubated at 30°C, 10°C and 4°C for 29 days. The resulting pattern of carbon source utilization was then analyzed statistically using multiple methods. Based upon color development within the 96 wells, indicating growth

due the utilization of the specific carbon source, calculations of the Shannon

index, Shannon evenness and the Jaccard coefficients were made [19].

Interestingly, the results indicated that color development was dependent upon

incubation temperature. The colder temperatures yielded the greatest

differences in indices, richness and substrate utilization. Between the three

sample sites, the Shannon diversity indices were not significantly different at

incubation of 30ºC, but at the lower temperatures the diversity indices of the

tundra dropped lower than for the other two sites that were influenced by human

and animal presence and thus had a higher content of organic material.

Substrate richness, or the number of available substrates providing growth

requirements, was less for the tundra than the other two soils [19].

Microbial Diversity in Antarctica

Antarctica has been the site of active microbial investigation since the

early 1960s. Researchers have examined bacteria, archaea, protozoa and fungi

found in coastal areas as well as the dry valleys, to ascertain how these

microbes manage to survive the cold, dry and windy conditions.

Boyd, et al. in 1962 and Cameron, et al. in the late '60s performed early

studies of Antarctic microbes. Boyd's study was based on Ross Island in the

McMurdo Sound and consisted of three soil sites – a rookery, an area supporting

lichen growth, and a site with no obvious supply of organic matter. Cameron's

study was based in Wheeler Valley – one of the dry valleys of Antarctica. The

sample sites for Wheeler Valley followed a transect of the valley and consisted of 6 samples. Both studies based their results primarily on culturing soil microbes on a variety of media. Cameron's data was categorized in terms of the type of microorganisms and the biochemical characteristics of the bacteria, such as oxygen usage, lactose fermentation and nitrate/sulfate reducers [11, 12].

Boyd's data was analyzed to examine Ross Island bacteria by genus and species. *Azotobacter chroococcum, Bacillus, Micrococcus cryophilis, Streptomyces, Flavobacterium, Achromobacter* and *Pseudomonas* spp were identified, though many were associated with anthropomorphic sources. Few filamentous fungi were present, though *Penicillium* and *Aspergillus* were identified at one site [11].

Other early studies of Antarctica indicated that the community of Antarctic soil microbes belong to a limited number of cosmopolitan taxa, are primarily aerobic, and related to the genera *Arthrobacter, Brevibacterium, Cellulomonas, Corynebacterium* as well as the gram-negative Eubacteria and Firmicutean bacteria. *Cyanobacteria* were also identified, but thought to be restricted to only coastal, moist environments [26].

Pointing, et al. examined three niches occupied by bacteria in the Dry Valleys. Endoliths are bacteria that colonize tiny spaces or pores inside rocks. Chasmoliths inhabit cracks or fissures in rocks. These two groups of bacteria are associated with the higher altitude valleys of the region. Hypolithic bacteria live under translucent rock, where sunlight is able to pass through the rock, allowing

photosynthesis for carbohydrates, and where moisture is trapped and does not sublimate. These niches were examined for the presence of bacteria, archaea, and algae by amplification of extracted DNA using primers specific for the bacterial and archaeal 16S rRNA gene and the eukaryotic 18S rRNA gene. No archaeal genes were amplified after numerous PCR adjustments, indicating that archaea were not part of the microbial communities in the Dry Valleys. Evaluation of resulting clone libraries indicated the *Chroococcidiopsis* and *Nostocales* were the predominant *Cyanobacteria* found as endoliths and chasmoliths, and the hypoliths were *Oscillatoriales*. *Chloroflexi* was also found in the soil samples and as an endolith. Fourteen other phyla were identified in the soils of the Dry Valleys, though only *Acidobacteria* and *Actinobacteria* were ubiquitous. Eukaryotic analysis indicated the presence of algae – specifically *Chlorphyta*, and two fungi – *Ascomycota* and *Basidiomycota*. The algae were associated with the soil and all lithic niches, however the fungi were only associated with endoliths and chasmoliths [52].

The 5S rRNA sequence was used to investigate endolithic microorganisms from the Linnaeus Terrace of the Upper Wright Valley in the McMurdo Dry Valley region of Antarctica. This study reflected an interest in cryptoendolithic microbes inhabiting the "porous sandstone rocks" of the area. These organisms take the form of lichens involving algae or Cyanobacteria and fungi, accompanied by free-living microbes. In many cases, these communities are visible as green zones between crystals or rock layers. The 5S rRNA

fragments retrieved from three soil samples were sequenced and analyzed by cluster analysis involving principal component analysis and the construction of a phylogenetic tree. The resulting 5S rRNA sequence was matched to a marine species *Vibrio* and verified by the construction of an evolutionary tree [17].

A recent study by Smith, et al. compared results from previous culturing methods of microbial identification to identification of bacteria using molecular techniques based upon the 16S rRNA gene. Three sites within the Dry Valleys of the Ross Dependency of Eastern Antarctica were sampled. The sites ranged from sea level under the carcass of a crabeater seal, to a mid-slope site in Miers Valley to a high altitude site in Penance Pass. The samples were processed for protein, lipid, DNA and water content. PCR and DGGE were used to make clone libraries from the sequencing of plasmids. The resulting 181 clones indicated that there were unique patterns for 57% of the carcass site, 51% of the mid-slope site, and 43% for the high altitude site. The dominant phyla at the three sites were uncultured environmental clones, *Actinobacteria*, and *Cyanobacteria*, respectively. The latter two phyla agree with the previously identified culture-dependent studies. However, the previously isolated organisms from *Achromobacter, Bacillus, Corynebacterium, Micrococcus, Planococcus,* or *Pseudomonas* were not evident in this analysis. Conversely, Acidobacteria, Verrucomicrobia, and *Bacteriodetes* are not found in historical or recent culture-based libraries yet are major constituents of the soil community based upon 16S rRNA gene analysis [59].

In summary, desert soil communities have been examined for different microbiological populations for many years. No comprehensive studies focusing on similar treatment of samples have been performed for a single microbial group in multiple desert environments. An analysis of the previously mentioned research (Table 2) is difficult to assess in terms of a concise description of microbial soil communities existing under conditions of limited liquid water.

Table 2. Summary of microbial research in different types of deserts

Eubacteria: Phylum	Arid	Semi-arid	Coastal	Cold	Antarctica	References
Acidobacteria	x	x		x	x	14,17,20,38,46
Actinobacteria	x		x		x	6,11,14,26, 31, 58
Aquificae				x		46
Bacteriodetes		x			x	11,20,38
Chloroflexi					x	52
Cyanobacteria		x		x	x	26,27,46,52,58
Deinococcus-Thermus	x					14
Firmicutes	x		x	x		6, 11, 46
Gram Positive		x				20, 38
Nitrospirae				x		46
Planctomycetes				x		46
Proteobacteria	x	x	x	x	x	6,11,14,17,20,38,46
Verrucomicrobia				x	x	46, 58

Table 2. (Continued)

Archaea: Phylum	Genus	References
Crenarchaeota	NA	14
Euryarchaeota	Haloarcula	5, 13
	Haloferax	
	Halorubrum	
	Haloterrigena	
	Natrinema	

Protozoa: Order	Genus	References
Acanthamoebidae	Acanthamoeba	54
Thecamoebidae	Thecamoeba	
Holostichidae	Holosticha	
Euglyphidae	Euglypha	

Fungi: all from Ascomycota	Genus	References
Ploesporaceae	Alternaria	11,52
	Ulocladium	
Trichocomaceae	Aspergillus	
	Penicillium	
Davidiellaceae	Cladosporium	
Microascaceae	Microascus	
mitosporic Hypocreales	Fusarium	

Viruses: Order	Family	References
Caudovirales	Myoviridae	53
	Siphoviridae	
	Podoviridae	

Global Warming and Soil Health

International organizations as well as individual countries are investigating the possible impact of global warming on arid and semi-arid regions of the world. In a 2007 report by the Intergovernmental Panel on Climate Change (IPCC) an international group of scientists examined evidence for altered climactic conditions due to man-made emissions of CO_2 into the atmosphere compared to natural atmospheric cycles, and concluded that anthropogenic forces are the major factor driving climate change. Based upon current research and predictions from various climate modeling programs, the IPCC forecasts the following: 1- increasing temperatures, particularly in currently arid or semi-arid environments, 2 - a shift in precipitation patterns, and 3 - increasing frequency of extreme weather, climate and ocean level events over the next decade [33, 50, 51].

Desertification, or the degradation of formerly productive land, is of major concern to governments living on the borders of the arid and semi-arid regions of Earth [65]. Human encroachment on fragile borderlands has enhanced desertification, however a growing concern focuses on climate changes resulting in the increase of desert borders, and a loss of pasture and crop lands. Degradation of sub-Saharan areas has been linked to global weather patterns, specifically the North Atlantic Oscillation (NAO) and the El Nino Southern Oscillation (ENSO). Observed vegetation dynamics of the Sahel were found to correlate to very closely to fluctuations in the NAO [48].

IPCC models also indicate desertification of semi-arid regions of Africa and an increase in periods of drought for the western United States and Canada, inland areas of Australia, portions of the Iberian Peninsula, large inland areas of Asia and possibly even the inhabited Arctic regions [10, 31, 1, 22, 18, 2]. Gao, et.al used three aridity measurements to analyze simulations of the Mediterranean countries to estimate alterations in precipitation and temperature due to global warming. Their results indicate a high probability that by the end of this century, the southern Mediterranean area, the peninsula areas, and southeastern Europe will experience an increase in arid lands, due primarily to global warming and decreased precipitation [27].

A 2007 study of climate models for southwestern North America further supports the IPCC findings. Out of nineteen climate models only one model indicated a wetter climate. Of 49 individual projections, only three gave rise to a wetter climate. The remaining projections indicated a warming and drying trend, measured by estimated precipitation – estimated evaporation (P-E). Models indicated movement of the Hadley Cell and Ferrell Cell poleward, expanding the tropical low pressure areas and dry zones [56].

To monitor the threat of global warming and possible resulting desertification of semi-arid regions, efforts are being made to identify indicator species that might be used to measure the expansion of arid conditions. Foissner suggests the use of certain protozoa as indicators of their environment. Because they are ubiquitous and single-celled organisms, they survive where few higher

organisms do not, and since they are Eukaryotes, their genome more closely resembles higher organisms than the genomes of bacteria or archaea. This latter relationship offers a closer approximation for the response of animals and plants to changing conditions. For detecting oxygen depletion or enhancement, ciliates have shown to be very sensitive to O_2 levels due to their need for microaerophilic or anaerobic conditions for growth and reproduction. Certain flagellates were shown to increase in concentration as CO_2 levels increased, whereas naked amoebae decreased in concentration [24].

Bending, et al. examined possible indicators for managed farmland in Warwickshire, UK. The organic matter content (SOM) has been utilized as a bioindicators for the health of agricultural soils, and incorporates a variety of specific measurements: organic nitrogen, light-fraction organic matter (LFOM), and carbon from organic and inorganic substances. Also utilized were Biolog Gram negative sole-carbon-source microplates. These characteristics were measured and compared from two different soil treatments at three different times: August 1996, April 1997 and December 1997, thus allowing an opportunity to gauge changes in the microbial communities over time. One segment of the farmland was sown with rye grass, red clover, and ley. This area was mown and mulched on a monthly basis. The second segment of farmland was planted with *Vicia sativa* for winter months, then plowed under and replanted with spring barley and white clover. The results of the SOM measurements were to yield negligible differences between the two treatments of the soil, with subtle

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differences attributed to the season as opposed to the management treatments. However, the patterns of carbon usage presented by the Biolog GN plates reflected a notable difference in the treatments. Metabolic diversity was greater in the ley segment than in the crop rotation (second) soil treatment, particularly after the disruption of the microbial community due to plowing prior to planting the barley crop. Also, the carboxylic acid utilization was observed for the microbial community of treatment 1 over the fall and winter months, but subsided by the following winter. This may be due to a shift in the microbial community where, over time, different bacteria out-competed the carboxylic acid users. Thus, Biolog GN may prove to be helpful as an indicator of soil health [9].

Summary

The literature regarding microbial diversity in arid terrestrial environments is patchy in its coverage of both the geographically and characteristically similar deserts as well as its overall examination of microorganisms. A lateral comprehensive look at the microbial populations of deserts that have similar temperature ranges, annual precipitation, circulation patterns, and other physical characteristics would allow the determination of local microbial fauna vs. cosmopolitan visitors. A horizontal approach that would examine the microbial populations in deserts of varying degrees of precipitation, radiation, and temperature extremes would also allow the determination of species better equipped to survive the extremes of the desert environment.

Most studies focus on one type of microorganism for identification and diversity measurements, and ignore possible interdependency or community aspect of microbial populations. Though probably requiring more time and expenses, a thorough investigation of the fungi, protozoa, archaea and bacteria in a single area would prove valuable and establish a basis for further community determinations in similar environments.

The various techniques for the determination of microbial diversity are constantly undergoing improvements. However, culturing bacteria for diversity measurements is no longer appropriate as the sole approach. When characterization of bacteria is required, or when attempting to examine protozoa, culturing is very useful, but molecular techniques and fluorescence microscopy offer a more quantitative approach to evaluating the broader picture of microbial communities.

Future research in microbial diversity of soil communities has many avenues to pursue, based upon the techniques and microorganisms of interest. However, more complete microorganismal studies and comparisons between desert areas offer basic general information that could be incredibly helpful for assessing an overall picture of microbial soil diversity. In turn, this would allow astrobiology researchers to gain a clearer idea of what to possibly expect on dry, cold planets such as Mars.

Specific Goals of This Study

The goal of this research is to examine the bacterial and archaeal communities across an annual precipitation gradient to observe the following characteristics:

1. changes in diversity and numbers of Eubacteria and Archaea as a function of available water

2. potential indicator organisms that may reflect trends with regards to desertification

3. novel Eubacteria or Archaea identified by sequence analysis and phylogenetic relatedness

By incorporating two procedures: viable count plates and clone libraries, a more comprehensive understanding of eubacterial and archaeal desert soil communities may result, compared to the limited picture currently provided by a single technique

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CHAPTER 2

Differences in Eubacterial and Archaeal Soil Communities along a Precipitation Transect in the Mojave Desert

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Short version of the title: Diversity of Archaea and Eubacteria along a Mojave Desert precipitation transect

Introduction

The extreme arid biomes on Earth present microorganisms and higher life

forms with unique challenges. All life on Earth requires liquid water, and

therefore the discovery of microorganisms existing in soil of the Atacama Desert

that receives a single precipitation event of 2.3mm of rainfall in a period of four

years was quite surprising [16]. The complex desert soil communities of

Eubacteria and Archaea have proven difficult to assess, and have yet to be

characterized in terms of the identities of the microbes, their function in the

ecosystem, and their means of coping with the extremes of arid environments.

Understanding the relationship between microbial communities and

precipitation may also prove useful in predicting effects of global warming.

Resulting data might indicate possible conditions such as the presence or

absence of certain bacteria or archaea, or changes in the microbial community

that might reveal the advancement of desertification conditions. Identifying such

indicator organisms or communities may allow conservationists and communities'

time to develop appropriate responses to the decreasing moisture conditions [3].

The focus of this research is to detail changes in microbial communities

along a precipitation transect through the Mojave Desert. The observable

changes in vegetation, soil chemistry, and microbial populations provide discrete

snapshots of macro and micro communities along the precipitation gradient. As

climate change increases desertification, the communities in the higher annual

rainfall sites should begin to resemble the communities further down the

precipitation gradient, and offer insight into the changing microbial soil communities. The precipitation transect allows the identification of Eubacteria and Archaea capable of surviving longtime alteration of precipitation patterns.

The unculturability of many environmental bacteria also leads to difficulty in using traditional laboratory methods for the examination of bacterial communities. Whereas the use of special media allows for the growth and characterization of commensal bacteria, too little is known regarding specific requirements and conditions for the propagation and close study of environmental Eubacteria and Archaea. This results in the application of methods which examine the byproducts of life – chemical signatures which may be discerned from testing, or by examination of community DNA extracted from environmental samples.

With these concerns in mind a polyphasic approach was used to discern changes in microbial communities along a precipitation transect through the Mojave. Associated with decreasing precipitation and high summer temperatures should come decreased vegetation, decreased organic compounds in the soil, and changes in pH and salt content of the soil. The hypothesis based upon these conditions is that the microbial communities will decrease in number and diversity. The populations of the taxonomic classifications of Eubacteria and Archaea will also be affected due to an organism's ability to function in increasing adverse conditions and community competition. Viable counts and 16S rRNA gene sequences extracted from the microbial soil community will show changes

in eubacterial and archaeal populations, as well as lead to identification of an organism which might serve as an indicator of increasing desertification.

Methods and Materials

Precipitation Transect. The data for this research project was collected as part of the NASA Spaceward Bound Mojave 2007, 2008 and 2009 programs. Spaceward Bound is a project sponsored by NASA to bring secondary science teachers and science education majors into the field with research scientists. Participants learn a variety of techniques used in research labs and then effect changes in their approach to teaching science in the middle school or junior high school. The teachers, which were interested in environmental microbiology, assisted with establishing the sample area, collecting samples, and preparing the samples for culture and molecular analysis.

The selection of sampling sites spanning a precipitation transect through southern California was based upon a rainfall map produced by the California Department of Forestry and Fire Protection, Fire and Resources Assessment Program (FRAP) [5]. Taken from annual average precipitation measurements from 1900 to 1960, the map indicated a progression of annual precipitation from 9 inches (23 cm) in eastern Kern County to 3.5 inches (9cm) around Baker, California. This 260 km transect follows state route 58 from Bakersfield to Barstow, and interstate 15 from Barstow to Baker, providing access to sampling sites along the precipitation gradient. Figure 1 shows the resulting transect.
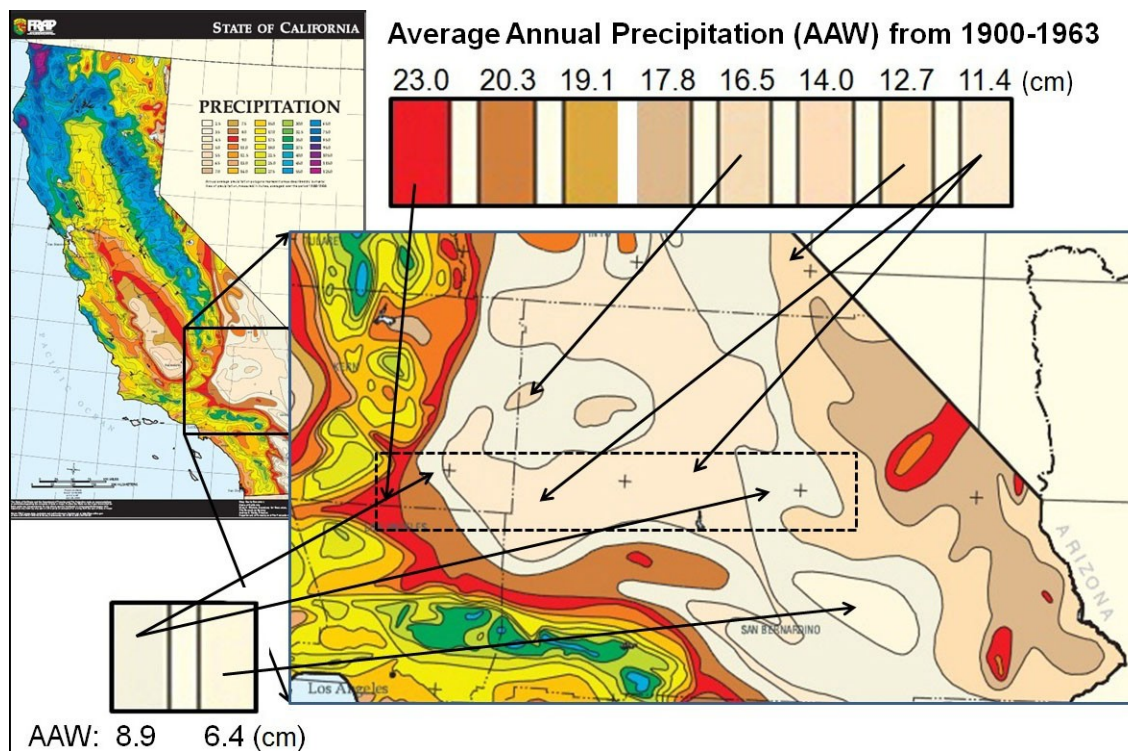
Figure 1. FRAP average precipitation map of California from 1900-1960 with the precipitation transect identified.

The properties between Tehachapi Pass and Zzyzx are primarily governed by the Bureau of Land Management (BLM) and thus required no special permits for sampling. Two of the sites selected in the driest zone of the transect pass through the Mojave National Preserve (MNP). Permits for sample collection were obtained through the NASA Spaceward Bound Program.

The highest annual precipitation site was selected in the Tehachapi Pass, above the town of Mojave, California. Four more sample sites were identified traveling east toward Baker. The immediate areas east and west of Barstow, where were avoided because civilization would have a greater impact upon microbial communities. The final two sampling sites within the MNP were

established driving along Kelbaker Road. The identification method for the

sample sites was based on a previous study. Figure 2 shows the sample sites in

relation to major roads and towns in southeastern California. Table 3 indicates

the sample designation, name, and location information, as well as the amount of

precipitation, for each site.

Figure 2. Sample site locations representing a precipitation transect

Table 3. Geographical data for sample sites

Site #	Site Name	Latitude (°)	Longitude (°)	Altitude (m)	Precip.* (cm)
15	Sand Canyon	N35.1230	-118.3240	1212	28
16	California City	N35.0218	-117.9160	736	15
17	Kramer Junction	N35.0431	-117.5599	782	13
18	Fields Road	N35.0260	-116.5400	630	12
19	Rasor Road	N35.1400	-116.1950	466	11
20	Black Tank	N35.1860	-115.8450	771	9
21	Henry Springs Rd	N35.2530	-115.9790	403	9

*based on FRAP precipitation map

Sample Collection. At each sample location, a 10x10 m square was established to minimize the influence of carbon and nitrogen from ground cover or plants. Sterile scoops were used to collect soil down to 8 cm depth. Samples were placed into sterile collection bags (18 oz, Nasco Whirl-pak® Fort Atkinson, WI) and temporarily stored in a cooler at air temperature for a maximum of 6 h until arrival at the onsite lab, where the samples were stored at 4°C. Samples were processed within 24 h of collection. During the 2007 expedition, 5 samples were collected corresponding to the locations identified with an "*" and a capital letter as is shown in Figure 3a. Sampling for the 2008 and 2009 expeditions was altered to collect three samples per site, as is shown in Figure 3b.
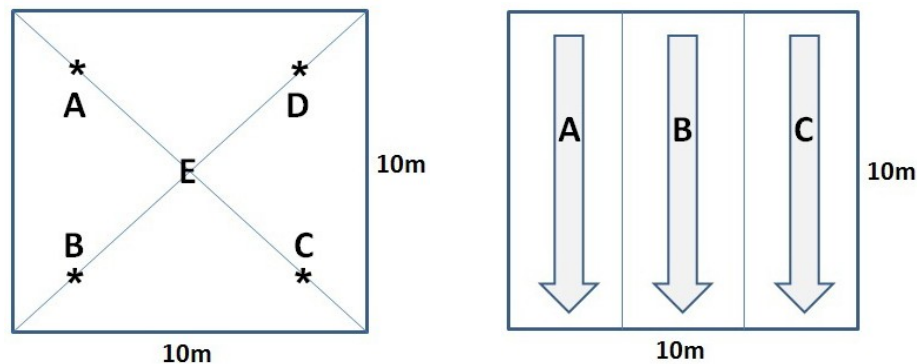
Figure 3a and b. Location of soil samples taken from each sample site for the (a) 2007 and (b) 2008 – 2010 Spaceward Bound expeditions

In 2009, approximately 400 g of soil was collected in addition to regular samples and shipped within 24 h for chemical analysis by Basic Laboratory, Inc. (Reading, California).

Soil Sample Analysis.   Inoculation of viable count plates as well as DNA extraction was performed at the Desert Studies Center (DCS) at Zzyzx. Further DNA extraction and molecular procedures were carried out upon arrival at SJSU.

For the 2007 expedition, soil from samples A and B, as well as samples C and D were combined in equal 50 g amounts resulting in three samples per site: A, B, and E. These three samples were then used as inoculum for viable plate counts. For DNA extraction, 30 g of each of the five samples was combined and mixed thoroughly, providing a single sample.

The 2008, 2009, and 2010 samples were initially set up as three samples per site, A, B, and C, by dividing the square into thirds and sampling from one side of the square to its parallel side. Fifty grams of soil from samples A, B and C were combined and thoroughly mixed to produce a single sample for each site. This single sample was then used as inoculum for viable count plates and DNA extraction. All laboratory procedures were performed by attendees under supervision by the primary researcher.

Culture Techniques. Viable count plates were inoculated from 10-fold dilutions of 1 g of soil in sterile saline. Dilutions of 10^{-3}, 10^{-4}, and 10^{-5} were plated on ½ strength Difco™ R2A medium. The plates were incubated at room temperature for 2 weeks. Colony counts were obtained at the end of each week. The 2007 diluted samples were plated in triplicate. Duplicate plates were made for each dilution for the 2008, 2009, and 2010 samples.

DNA Extraction. Community genomic DNA was extracted from each sample using the UltraClean™ Soil DNA Isolation Kit (MoBio, Carlsbad, CA) according to the manufacturer's instructions. The quality of the genomic DNA was verified by electrophoresis on 0.1% analytical grade agarose gels stained with ethidium bromide (0.5 µg/ml). The bands were visualized using a GelDoc ™ XT 170-8171 (BioRad Laboratories, Hercules, CA).

16S rRNA Amplification. The polymerase chain reaction (PCR) was used to amplify the 1500 bp 16S rRNA gene. Primers were synthesized by Operon Biotechnologies, Inc. (Huntsville, AL). All PCR procedures were performed on an MJ Research PTC 100 thermal cycler (Global Medical Instrumentation, Inc., Ramsey, MN).

Prior to PCR, all samples were sheared 30 times using 16G needles and diluted 10-fold. The eubacterial 16S rDNA was amplified using universal forward primer fD1 and reverse primer rD1 (Table 4) [12]. The reaction mixture for PCR consisted of 1.5 mM $MgCl_2$, 200 µM of each dNTP, 1 µM of primers fD1 and rD1, 4 µl template DNA (5-127 ng/µl), 2.5 U/ml GoTaq® DNA polymerase, and 1x GoTaq® reaction buffer (Promega, Madison, WI) in a 40 µl final reaction volume. Purified *Escherichia coli* DH5-α (100 ng) genomic DNA was obtained following instructions for the QIAGEN Genomic DNA Isolation Kit (Valencia, CA) and used as a positive control. The PCR protocol consisted of denaturation at 95ºC for 2 min, 35 cycles of 95ºC for 15 s, 55ºC for 30 s, 72ºC for 2 min, followed by a final

extension at 72ºC of 6 min. The presence and approximate concentration of the

PCR products were verified by gel electrophoresis using 1% agarose gels.

Table 4. Primers used for amplification of the 1.5kb 16S rRNA gene

Domain	Primers	Coding	Reference
Eubacteria	fD1	AGAGTTTGATCCTGGCTCAG	Weisburg, et.al. [25]
	rD1	AAGGAGGTGATCCAGCC	
Archaea	AF25EF	CTGGTTGATCCTGCCAG	Robb, et.al. [22]
	AR1525R	GACGGGCGGTGTGTRCA	

The archaeal 16S rRNA gene was amplified using primers AF25EF and

AR1525 (Table 4). The reaction mixture consisted of 1.5 mM $MgCl_2$, 200 μM of

each dNTP, 1 μM of AF25EF and AR1525, 4 μl template DNA, 2.5 U/ml GoTaq®

DNA polymerase, and 1x GoTaq® reaction buffer (Promega, Madison, WI) in a

40 µl final reaction volume. *Haloarcula marismortui* strain DSM 3752 genomic

DNA was obtained from ATCC® (catalog number 43049-5™) and used as a

positive control. PCR protocol was 94ºC for 3 min, 38 cycles of 94ºC for 1 min,

55ºC for 1.5 min, 72ºC for 2 min followed by a final extension at 72ºC for 5 min.

The presence and approximate concentration of the PCR products were verified

by gel electrophoresis using 1% agarose gels.

Cloning and sequencing. PCR products were cloned into pGEM®-T Easy

plasmid vectors (Promega, Madison, WI) according to manufacturer's

instructions and used to transform competent JM109 *E.coli* cells (Promega, Madison, WI). Plasmids were purified using the Wizard Plus SV Minipreps Kit (Promega, Madison, WI). Isolated plasmids were stored at -20ºC and screened by restriction analysis using *EcoRI* (12u/µl). Plasmids containing the 1.5kb gene of eubacterial and archaeal DNA were sequenced by Sequetech (Mountain View, CA) using forward primer M13F for unidirectional sequencing of 1000 nucleotides.

 Phylogenetic Analysis. Sequences were visually screened for errors using BioEdit 7.0.9 [10], and vector sequences were edited using Vector Screening (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Bellerophon Chimera Detection programs (http://foo.maths.uq.edu.au/~huber/bellerophon.pl) [11] were utilized to determine the presence of chimera sequences. A level of identification between a sequence and existing database sequences was based upon ≥ 97% query coverage, an E value ≤ 0.0 and the highest maximum score calculated by the program [1].

 Taxonomic associations for all eubacterial and archaeal 16S rRNA gene sequences were determined using the Greengenes online full-length 16S rRNA gene database (http://greengenes.lbl.gov/) [8]. Sequences from each sample site were aligned using the Greengenes alignment tool, and then processed through the classification tool, which compared the clones to three different databases: Greengenes, Ribosomal Database Project (RDP) and the National Center for Biotechnology Information (NCBI) databases. The resulting files

grouped the 16S rRNA gene sequences into taxonomic units from Domain down to an operational taxonomic unit (OTU). The OTU designation does not necessarily correlate to the species level, but does indicate the most specific level of classification available. The OTU designations differed between the three databases, indicating that this nomenclature is not a recognized taxonomic label, but is only used to distinguish one phylotype from another. When a 16S rRNA gene sequence was classified to a species level, then other sequences which had the same OTU would also be the same species. If a 16S rRNA gene sequence was classified to the level of family, the OTU associated with that classification would indicate any other sequences which had the same OTU would also be of that family.

The resulting classifications were compared between the three databases for consistency at all taxonomic levels indicated in the analysis. The Greengenes and NCBI databases were most closely aligned in terms of higher level taxonomic assignments, particularly for the Archaea. Further analysis of the sequences for both microorganismal groups was based upon the Greengenes database results.

Diversity Analyses. Rarefaction curves were obtained using Analytic Rarefaction 1.3 by Steven Holland (http://www.uga.edu/~strata/software/), to represent the richness of the microbial community and determine the degree of species saturation. The Shannon-Wiener function was utilized to estimate diversity of Archaea and Eubacteria at both sites according to:

$$H = -\sum_{i=1}^{s} (p_i)(\ln p_i)$$

where **H** = information content of the sample (bits/individual) or index of species diversity, **s** = number of species, and p_i = proportion of total sample belonging to the i-th species [12].

Results

Vegetative Coverage. Each sample site was visually examined to determine the amount and type of vegetation present. The vegetation was divided into three groups: the herbal; small shrubs, and large shrubs or trees. The herbal vegetation consisted of grasses, often growing in clumps, flowering plants, and weeds, typically under 10cm high and covering an area 4-8cm in diameter. The coverage of vegetation varied with the sampling year, and reflected the different amounts of precipitation during the previous 12 months. The progression along the transect toward the driest sites presented an obvious visible decrease in plant coverage, regardless of the sampling year. With the exception of the wettest sampling site, Sand Hill Road, which was basic pasture land, the vegetation was predominately grasses, herbs, and scrub. Included in these groups of flora are saltbush and desert holly (*Atriplex* ssp.), creosote (*Larrea tridentate*), and sagebrush (*Artimisia tridentata*).

Because the actual 10x10m sampling area was selected to minimize the presence of vegetation, an estimation of the vegetative covering combined the immediate sample area and the surrounding 100m areas. The percent of vegetative cover at each site is indicated in Table 5 as a range, indicating the minimum and maximum over the four-year study period. During low rainfall seasons, the continuity of the grass/herbal coverage became separated by noticeable stretches of open soil.

Table 5. Vegetative coverage of sample sites by type and size of vegetation.

Site #	Name	% Vegetation coverage	Precipitation (cm)*
15	Sand Canyon	85-100	23
16	California City	60-75	15
17	Kramer Junction	50-70	12.7
18	Fields Road	40-60	12.7
19	Rasor Road	45-60	12.7
20	Black Tank	45-60	9
21	Henry Springs Road	25-45	8.9

*estimated from FRAP annual precipitation map

Black Tank and Kramer Junction sample sites had large, wide creosote bushes which were over 180cm tall and up to 140cm wide. These sites appear to be situated in the zones receiving 11.4 – 12.7 cm of precipitation yearly. By comparison, the sample sites at Fields and Henry Springs roads had 3 - 10 small shrubs, approximately 40cm wide by 30 cm tall. The sample site furthest east, Black Tank, supported more vegetation than the site immediately west, Henry

Springs Road. According to the FRAP maps, a continuation of the precipitation transect eastward toward Las Vegas, Nevada indicates an increase in annual precipitation, with the minimum average rainfall occurring just south of Baker, CA. Based upon the map and comparison of vegetation, sample site #21 on Henry Springs Road is considered to represent the driest site of the transect.

Soil Characteristics. The observable characteristics of the soil varied across the precipitation transcript. Sand Canyon soil, site 15, was very dark, moist, and captured amongst a network of fine roots from the herbaceous ground cover. The soil at site 16, 45 km east at the road to California City, was much lighter in color and consistency, with more sand, and small, distinct particles. Progression further east along the precipitation transect showed finer sand, and a general increase in the presence of a mineral crust overlying the sand and silt. The number and size of rocks did not follow any trend, being abundant at some sites and scarce at others. (Table 6)

Table 6. Soil and rock descriptions of sampling sites

Site	Soil type	Rock type	Crust (%)
15	rich, humic	none	0
16	sandy silt	angular quartz, feldspar	0
17	sandy silt	angular quartz, feldspar	100
18	sandy silt	feldspar, granite; very rocky	30
19	sandy silt	quartz, basalt; compacted dirt	sparse
20	sandy silt	lava, feldspar, sparse gravel	15
21	sandy silt	quartz, feldspar, 5% basalt	80

The rockiest sample site was Fields Road, where a mixture of feldspar, granite, and quartz was exposed on top of sandy soil. These rocks ranged from large pebbles (20-30mm) to small cobbles (70-100 mm). Basalt rocks up to 15cm in length were found at Rasor Road and Black Tank, indicating proximity to 10,000 year old lava flows. Quartz rocks with hypolithic cyanobacterial communities were found at Fields Road, Black Tank, and Henry Springs Road sample sites.

Initial chemical analyses of the soil at each sampling site were performed by Raphael Navarro-Gonzalez in 2007. From independently collected soil samples, Navarro-Gonzalez determined the ratio of organic nitrogen to total nitrogen for each sample site, as shown in Table 7 (private correspondence, R Navarro-Gonzalez). In 2009, similar measurements of the organic nitrogen

Table 7. Nitrogen content at sample sites

Site	Org N	Tot N	org/tot '09	org/tot '07
15	1310	1330	0.985	0.985
16	234	238	0.983	0.983
17	933	940	0.993	0.993
18	274	285	0.961	0.961
19	62.9	65.1	0.966	0.966
20	494	500	0.988	0.988
21	109	111	0.982	0.982

and total nitrogen were made by Basic Laboratories, Inc. (Redding, California) and the results from these determinations are also shown in Table 7. The ratios of organic nitrogen to total nitrogen at each sample site were the same for both years, indicating a relatively stable chemical environment.

Further chemical analysis of the soils from the sample sites was also performed by Basic Laboratories, Inc. (Redding, California). Four hundred grams of soil from each of the seven sample sites was analyzed for edaphic factors known to influence microbial growth in soil communities, such as moisture content, salt content, pH and percent organic carbon. The resulting chemical quantities at each sample site are in Table 8.

Table 8. Chemical analysis of soil samples

Sample Site	pH	Percent Moisture	% org Carbon	Sodium (mg/kg)	Chlorine (mg/kg)
15	7.18	18.5	1.34	1080	4.98
16	7.60	1.2	0.26	68	4.57
17	7.12	0.6	0.53	78	3.34
18	7.34	0.6	0.11	146	2.08
19	7.76	0.6	0.27	158	1.71
20	7.71	0.4	0.15	560	8.68
21	7.88	0.4	0.18	128	5.94

The observed decrease in percent moisture of the soil samples mirrors the FRAP annual precipitation values in the west to east direction of the transect. The decreased support for vegetation along the transect is due to the decreased

amounts of organic nitrogen and carbon. The one sample site which may be considered anomalous with regards to the salt and organics, site 15 - Rasor Road, is a popular, all-wheel terrain vehicle area, thus the soil chemistry is highly likely to be affected by anthropomorphic influences.

The pH levels along the transect indicate a tendency toward a more basic environment, as is shown in Figure 4. Values of pH vary due to vegetation, soil, and anthropomorphic differences, and may serve as a predictor of microbial community composition [14]. The pH values are from samples along the transect range from 7.12 to 7.88, a difference of 0.76. This small change in pH has been shown to affect the diversity of soil bacteria communities, and may affect the microbial communities studied [15].
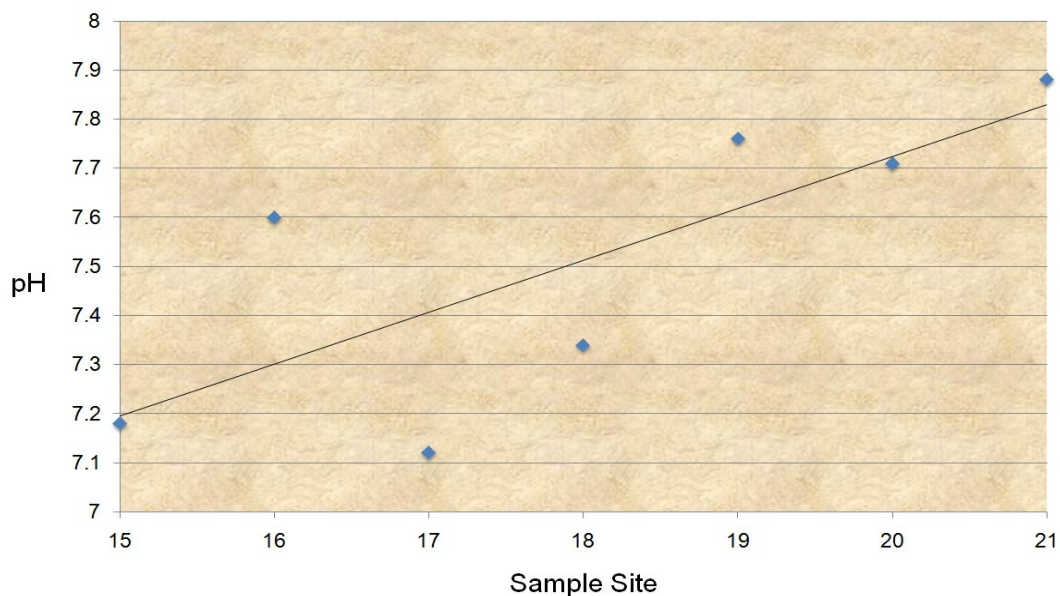
Figure 4. Scatter plot of pH values along precipitation transcript including a linear regression trend line.

Tables 6 and 7 reveal localized conditions along the precipitation transect. The organic nitrogen and carbon levels decrease along the length of the sample zone; however sites 17, 19, and 20, California City, Rasor Road, and Black Tank, show extreme divergence from this trend. The presence of sodium and chlorine also shows discontinuity from an overall trend due to the Black Tank results. These characteristics of the sample sites may contribute to differences in the microbial soil communities regardless of annual precipitation levels

Viable Plate Colony Count. Table 9 presents the colony forming units per gram (CFUs/g) of soil for four sampling years. The final column presents an average over the four-year period as a means of equalizing local effects of higher or lower than normal precipitation. The data indicates a decrease in the number of culturable microorganisms by 10 fold between the wettest and driest sites.

Table 9. Averaged colony forming units for statistically significant dilution plates for four years of sampling. Values are in terms of CFUs/g soil.

Site	2007	2008	2009	2010	Avg
15	4.4×10^6	1.5×10^8	1.5×10^7	4.2×10^7	5.29×10^7
16	2.9×10^5	1.7×10^7	2.3×10^6	2.7×10^7	1.16×10^7
17	2.5×10^5	1.5×10^7	3.2×10^7	2.6×10^7	1.83×10^7
18	2.6×10^5	1.3×10^7	3.3×10^7	3.4×10^6	1.24×10^7
19	1.3×10^5	1.5×10^7	1.5×10^7	2.1×10^6	8.06×10^6
20	NA	1.3×10^7	2.5×10^6	2.0×10^6	5.83×10^6
21	2.7×10^4	3.0×10^6	2.7×10^6	2.8×10^6	2.13×10^6

16S rRNA Gene Sequencing. Eubacterial 16S rRNA genes were sequenced for all seven sampling sites, but four of the sampling sites were chosen to represent the transect. Due to anthropomorphic interference at Rasor Road (site 19) this site was dropped from analysis. Soil chemistry and viable count plate similarities between sites 16 and 17, as well as 17 and 18, resulted in using sequence results from sites 16 and 18. Site 20 was dropped from analysis due to the observed increase in vegetation, indicating it was not as dry as site 21, as well as the similarity in pH, moisture content, percent organic carbon, and the organic /total nitrogen ratio.

The number of Eubacterial and Archaeal 16S rRNA genes sequenced for each remaining sample site is indicated in Table 19. The initial goal was to obtain over 100 eubacterial sequences per site, and 60 archaeal sequences. The resulting numbers indicate the maximum number of 16S rRNA genes positively classified. Several archaeal genes were classified as eubacterial sequences, particularly by the RDP database, which decreased the total number of archaeal sequences for all the sites.

Table 10. Number of 16S rRNA genes sequenced for transect

Site #	Site Name	# Eubacteria	# Archaea
15	Sand Canyon Road	107	9
16	California City Road	104	60
18	Fields Road	96	32
21	Henry Springs Road	108	45

Eubacterial 16S rRNA Gene Sequences, Phyla Classification. The sequences from the eubacterial soil community at the site of highest annual precipitation, Sand Canyon, were classified into six phyla, shown in Figure 5. The Proteobacteria were dominant at 89% of the total community. Numbers of 16S rRNA sequences were also identified for the Actinobacteria, Aquificae, Bacteriodetes, Firmicutes, and Thermotogae. Firmicutes were the next largest population at 6%, followed by Actinobacteria and Bacteriodetes at 2% each. Aquificae and Thermotogae were represented by a single sequence, or 1% of the total eubacterial population. Sequences from the Thermotogae were identified only at this site.
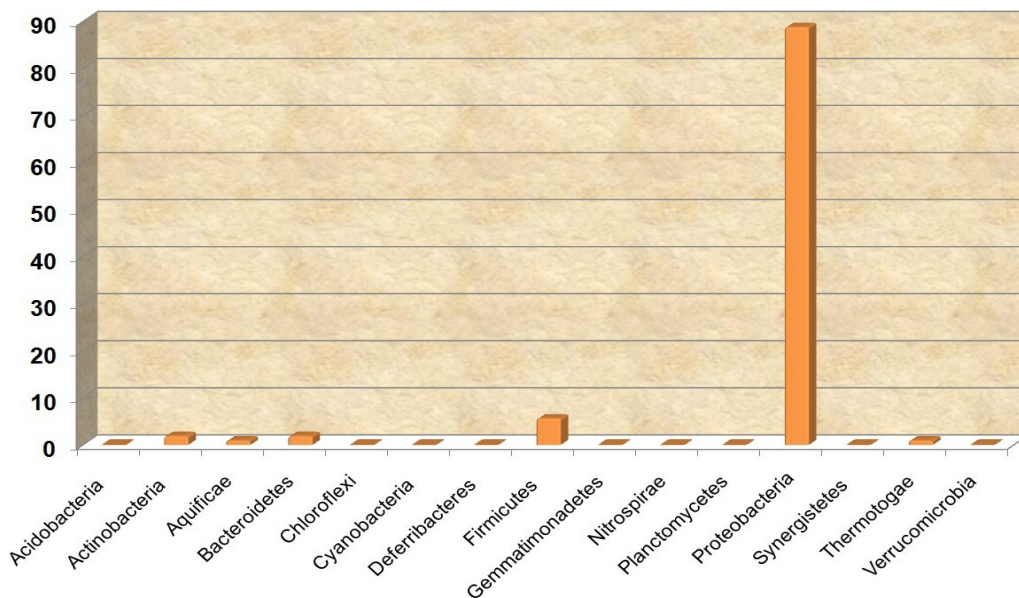
Figure 5. Relative abundance of 16S rRNA gene sequences at the Phylum taxonomic level from Sand Canyon, Site 15

Eubacterial 16S rRNA gene sequences from site 16, California City, showed an increase in the number of phyla. The Proteobacteria were still the dominant phylum, but only comprised 82% of the population. The number of sequences in Firmicutes decreased from 6% to 4%. New phyla at this site included the Acidobacteria, Cyanobacteria, Planctomycetes, and Verrucomicrobia. The number of sequences in Actinobacteria increased to 7. Figure 6 shows the relative abundance of phyla at this site. Planctomycetes were identified only at this sample site.
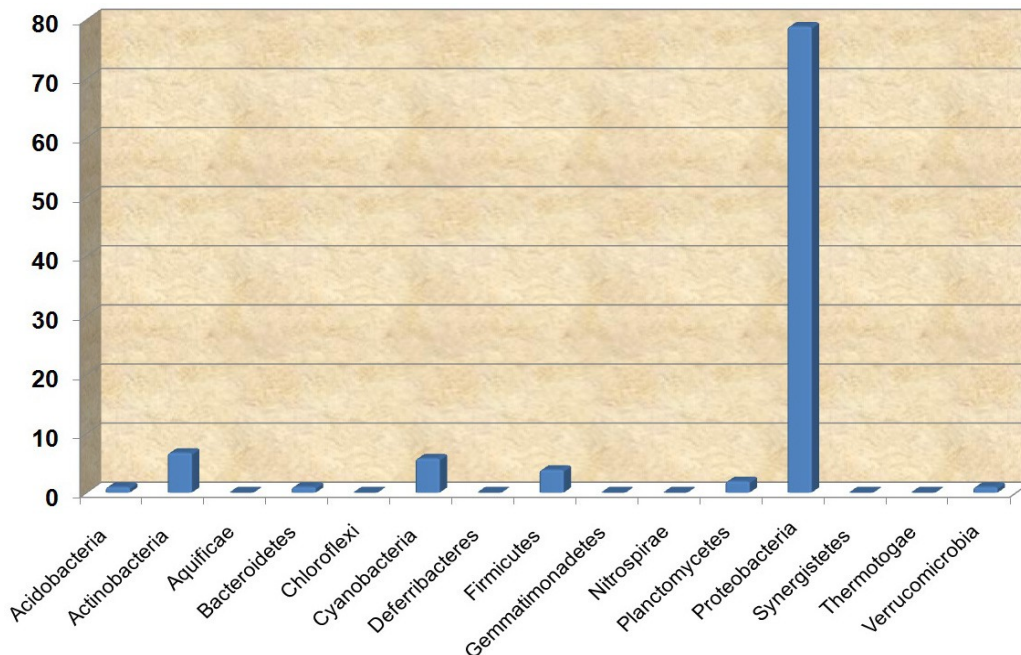
Figure 6. Relative abundance of 16S rRNA gene sequences at the Phylum taxonomic level from California City, Site 16

The 16S rRNA gene sequences from Fields Road showed a further decrease in the Proteobacteria, and an increase in the proportion of

Acidobacteria to 5%, Cyanobacteria to 8%, and Firmicutes up to 10%.

Deferribacteres and Synergistetes were two phyla represented only at Fields

Road, and accounted for 2% and 1% of the microbial community respectively.

Bacteriodetes, Planctomycetes, and Verrucomicrobia also were represented but

each at 1% of the microbial community. (Figure 7)
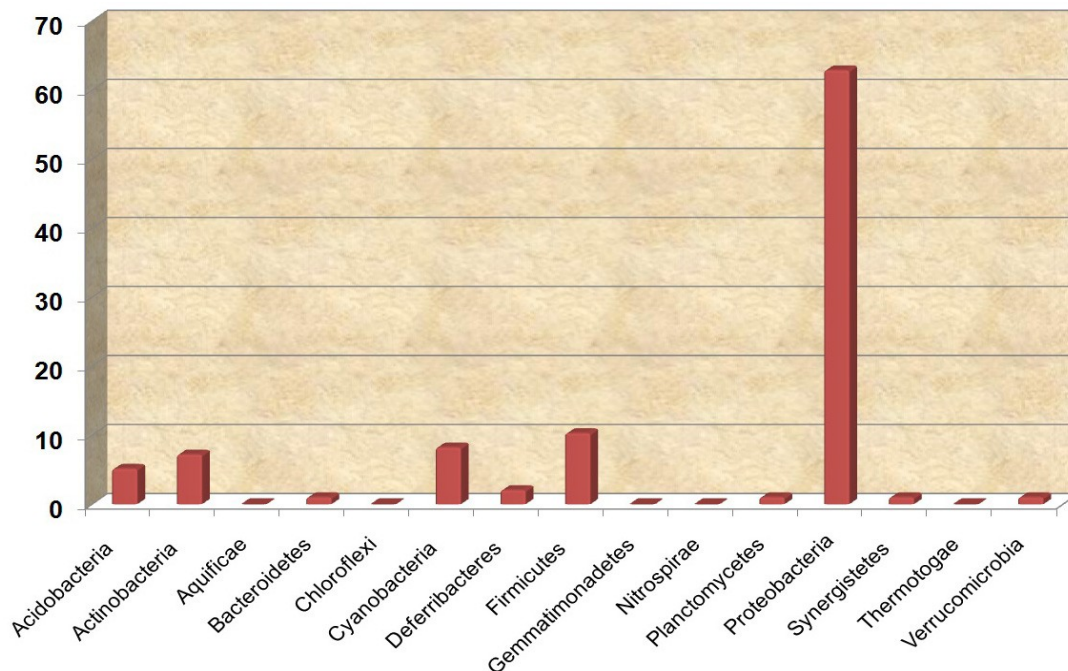
Figure 7. Relative abundance of 16S rRNA gene sequences at the Phylum taxonomic level from Fields Road, Site 18

The driest site of the precipitation transect, Henry Springs Road,

presented a more diverse eubacterial community. Single sequences from

Chloroflexi, Gemmatimonadetes, and Nitrospirae were present, representing a

total of 3% of the community. A continued decrease in the proportion of

Proteobacteria – down to 53%, Acidobacteria at 1%, and Actinobacteria

decreased to 4%. The proportion of Cyanobacteria increased to 31%, but Firmicutes decreased to 6%. Bacteriodetes and Aquificae were also represented with 1% of the microbial community each (Figure 8).
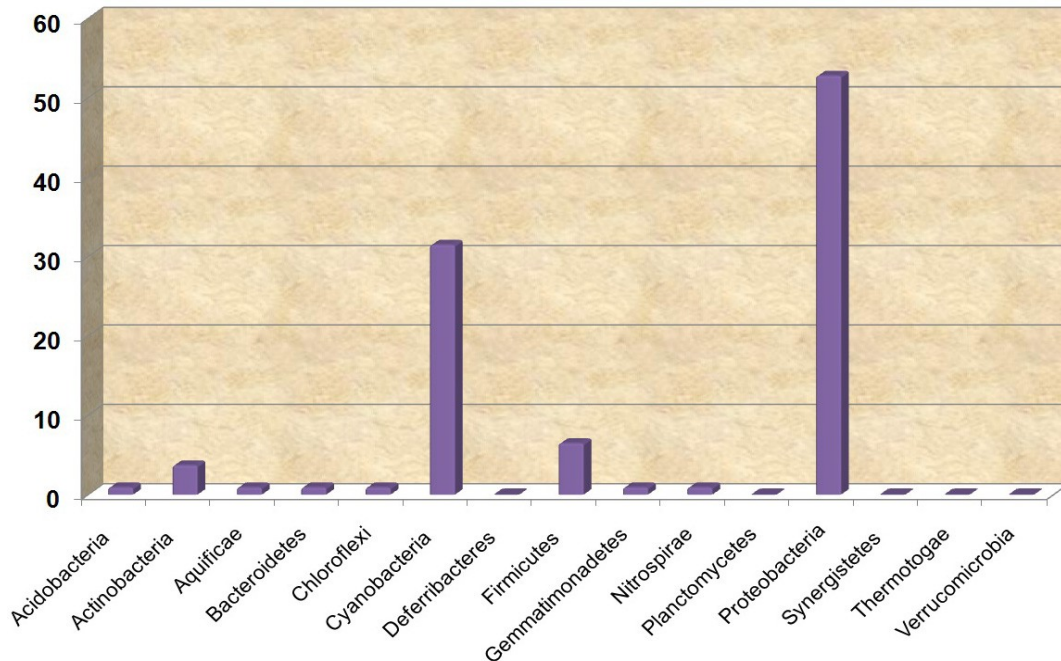
Figure 8. Relative abundance of 16S rRNA gene sequences at the Phylum taxonomic level from Henry Springs Road, Site 21

The trend in terms of the taxonomic classification of Eubacteria at the phyla level indicates that the diversity of the microbial communities increases as annual precipitation levels decrease. In particular, the Proteobacteria proportion of the community decreased from 89% to 53%, though it remains the dominant phylum. Of equal note, the Cyanobacterial proportion of the eubacterial community dramatically increased from 0% at Sand Canyon to 31% at Henry Springs Road.

Eubacterial 16S rRNA Gene Sequences, Class Classification. At the
taxonomic level of class several of the trends along the transect become more
obvious, and reflect more specifically what eubacterial groups are influenced by
different rainfall amounts. The further grouping of sequences along taxonomic
divisions indicates the increasing diversity, because distinctions can be made
about each group of sequences. Classification progression from phylum to class
indicated several taxonomic groups of which all the 16S rRNA gene sequences
from a phylum were grouped into a single class. An example of this is seen in
the phylum Bacteriodetes. All 5 sequences in this phylum were assigned to the
class Sphingobacteria, and thus are identical at this level of detail. The
Acidobacteria sequences were grouped into two classes, the Solibacteres and
"unclassified." Thus, further diversity analysis is not assisted by class distinction
for the Bacteriodetes, but it is for the Acidobacteria.

Insight into diversity at the class taxonomic level was based upon those
sequences from a phylum included two or more classes. Table 11 shows the
number of sequences per site associated with a class designation. The table
also indicates that members of the Alphaproteobacteria community decrease by
40% along the precipitation transect. Conversely, sequences in
Oscillatoriophycideae, the primary Cyanobacterial class, show an increase along
the precipitation transect.

Table 11. Number of eubacterial 16S rRNA gene sequences assigned to specific class designations. Phyla with one sequence per class are indicated.

Phylum	Class	Number of sequences at site:			
		15	16	18	21
Acidobacteria	Solibacteres	0	0	3	0
	Unclassified	0	1	2	1
Actinobacteria	Actinobacteria	2	5	5	3
	Rubrobacterales	0	2	2	0
	Solirubrobacterales	0	0	0	1
Aquificae	Aquificales	1	0	0	1
Bacteroidetes	Sphingobacteria	2	1	1	1
Chloroflexi	Chloroflexi	0	0	0	1
Cyanobacteria	Chloroplast	0	0	1	0
	Gloeobacterophycideae	0	1	0	0
	Nostocophycideae	0	0	0	16
	Oscillatoriophycideae	0	5	7	18
Deferribacteres	Deferribacteres (class)	0	0	2	0
Firmicutes	Bacilli	0	1	8	2
	Clostridia	6	3	2	5
Gemmatimonadetes	Gemmatimonadetes	0	0	0	1
Nitrospirae	Nitrospira	0	0	0	1
Planctomycetes	Planctomycea	0	2	1	0
Proteobacteria	Alphaproteobacteria	78	66	52	36
	Betaproteobacteria	10	10	4	13
	Deltaproteobacteria	4	6	4	4
	Gammaproteobacteria	2	0	1	4
	Unclassified	1	0	0	0
Synergistetes	Synergistia	0	0	1	0
Thermotogae	Thermotogae	1	0	0	0
Verrucomicrobia	Verrucomicrobiae	0	1	1	0

Also of note is the difference in the two classes of Firmicutes. Clostridium 16S rRNA gene sequences can be found at all sites, whereas Bacilli are found only at the drier sites. The Actinobacteria in the class Actinobacteria show a

preference for the intermediate precipitation sites, as do those from the class Rubrobacterales. The Acidobacteria also prefer the drier sites, though those from the class Solibacteres were only associated with Fields Road. While the Alphaproteobacteria class of the Proteobacteria showed proportional changes along the transect, the beta, delta, and gamma classes remained basically consistent.

Eubacterial 16S rRNA Gene Sequences, Family Classification. A comparison of the 16S rRNA gene sequences at the family taxonomic level produced further clarification of the organisms in flux along the precipitation transects. Three Alphaproteobacterial families, the Bradyrhizobiaceae, the Rhodospirillaceae and the Rhodobacteraceae, demonstrated a decrease in the proportion of the eubacterial community from wet to dry conditions: Bradyrhizobiaceae decreased from 34 to 15 sequences, Rhodospirillaceae, from 15 to 1 sequence, and Rhodobacteraceae decreased from 6 to 2 sequences. Figure 9 shows the trend for these families. Interestingly, two other alphaproteobacterial families demonstrated very different patterns. The proportion of sequences from the Methylobacteriaceae were consistent across the transect except at California City, where the number of sequences doubled. Only the Sphingomonadaceae showed preference for the dry sample sites, and preferred conditions at Fields Road – 4 sequences, to those at Henry Springs – 2 sequences.
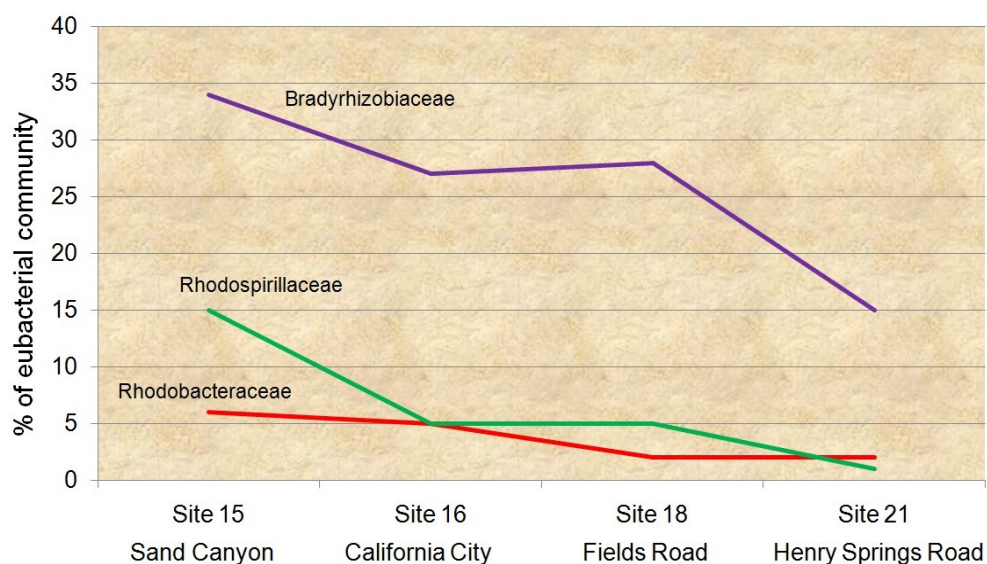
Figure 9. Comparisons of the proportion of major Alphaproteobacteria families which vary along the precipitation transect.

Other family classifications of interest are for the phyla Cyanobacteria, Firmicutes, and Betaproteobacteria. Figure 10 shows the trends in community proportion for two families of each phyla with the greatest variation across the transect. The cyanobacterial trends indicate the rapid rise of the Nostocaceae as the precipitation levels decrease, as well as the presence of 16S rRNA gene sequences from the Phormidiaceae only at the Henry Springs Road.

Figure 10 also demonstrates the opposing trends of Bacilli and Clostridia as their dominant families, Bacillaceae and Sulfobacillaceae, respond to decreasing levels of precipitation. Often associated with deserts, the Bacilli appeared to find Fields Road to be an optimal environment compared to Henry Springs Road.
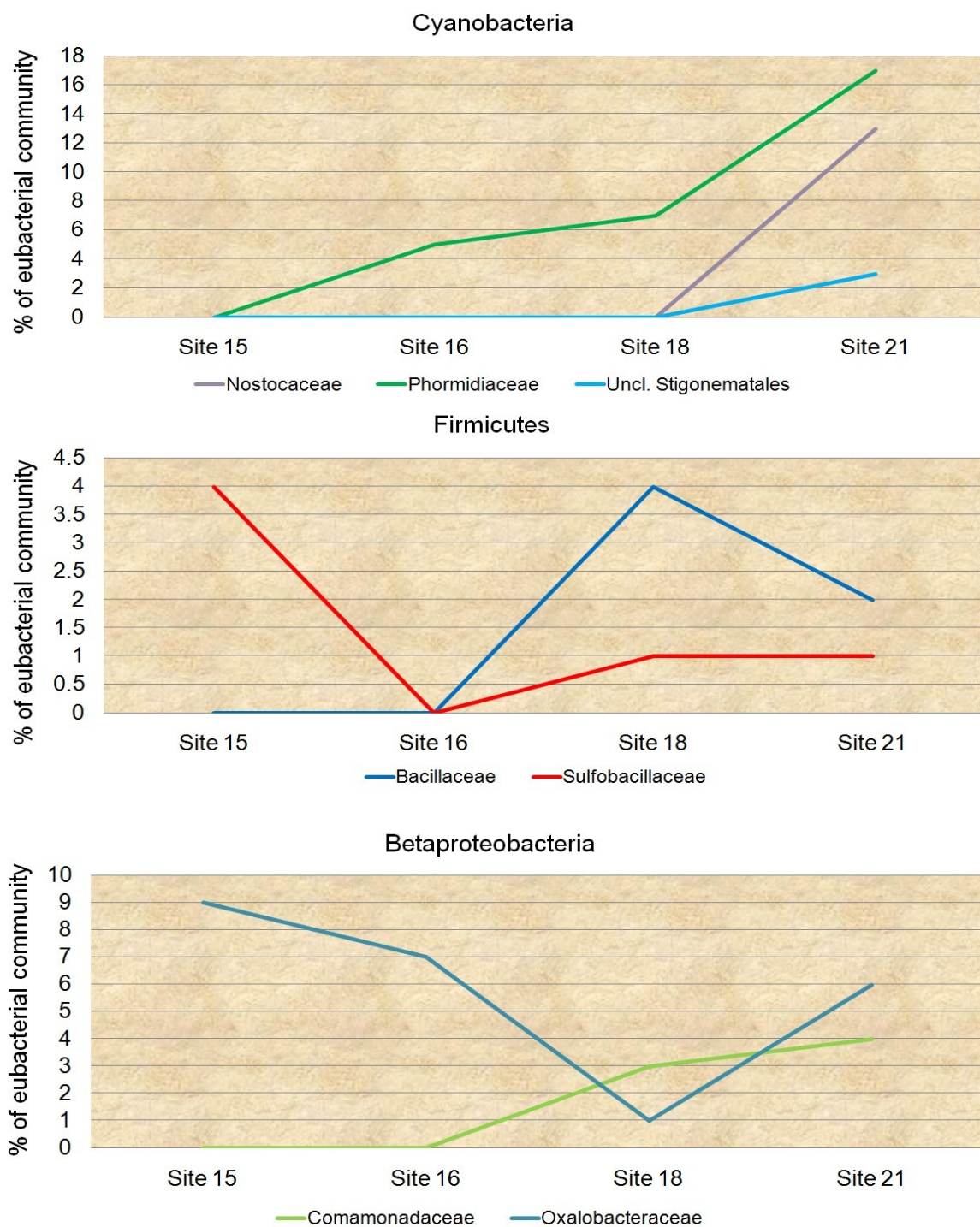
Figure 10. Family classification indicating interesting trends in eubacterial community proportions along the precipitation transect.

The third panel of Figure 10 shows opposing trends for the two dominant betaproteobacterial families. The Oxalobacteraceae appear to prefer wetter conditions of the western sample sites, whereas the Comamonadaceae appear in the drier sites. Whether these trends represent actual preferences for a specific soil chemistry and precipitation level or if there is simply less competition allowing the increase in some families is not discernable.

Eubacterial 16S rRNA Gene Sequence, Operational Taxonomic Unit Classification. Greengenes defines the operational taxonomic unit (OTU) as "the terminal level at which that taxonomy classifies the sequences." The term "that taxonomy" refers to the database selected: NCBI, RDP, or Greengenes databases. The OTU designations are thus database specific.

An example concerns two 16S rRNA gene sequences from Henry Springs Road, site 21. Both sequences, 21F5 and 21U2, were classified by all three databases as Domain Bacteria, Phylum Cyanobacteria. The Greengenes database placed both sequences in the class Nostocophycideae, NCBI did not assign a class, and RDP placed one sequence in the cyanobacterial class, and the other in no class. All three databases placed both sequences in the order Nostocales. Family assignments for sequence 21U2 were the same for all three databases, as Nostocaceae. Sequence 21F5 was also placed in Nostocaceae by Greengenes and NCBI but was classified as "Family" I by RDP. The genus name assigned by Greengenes and NCBI was Nodularia, but RDP put 21U2 in Nodularia and 21F5 in "Gp1." Species assignments were made only by

Greengenes for sequence 21F5, and the rest were unclassified. Greengenes

further assigned sequence 21F5, *Nodularia sphaerocarpa*, OTU_1430.

Sequence 21U2, *Nodularia* spp., was OTU_1429. NCBI classified these two

sequences as *Nodularia* unclassified and gave them both the designation

OTU_1214. RDP assigned sequence 21F5 to the genus *GP1* with an

unclassified species and OTU_1997. For the sequence 21U2, RDP agreed with

NCBI for all taxonomic classifications. (Table 12)

Table 12. Classification for two 16S rRNA gene sequences by three databases

Sequence	Designation	Greengenes	NCBI	RDP
21F5	Phylum	Cyanobacteria	Cyanobacteria	Cyanobacteria
	Class	Nostocophycideae	---------	Cyanobacteria
	Order	Nostocales	Nostocales	---------
	Family	Nostocaceae	Nostocaceae	Family I
	Genus	*Nodularia*	*Nodularia*	*Gp1*
	Species	*sphaerocarpa*	Unclassified	Unclassified
	OTU	OTU_1430	OTU_1214	OTU_1997
21U2	Phylum	Cyanobacteria	Cyanobacteria	Cyanobacteria
	Class	Nostocophycideae	---------	---------
	Order	Nostocales	Nostocales	Nostocales
	Family	Nostocaceae	Nostocaceae	Nostocaceae
	Genus	*Nodularia*	*Nodularia*	*Nodularia*
	Species	Unclassified	Unclassified	Unclassified
	OTU	OTU_1429	OTU_1214	OTU_1214

The use of OTU assignments allows further association at a classification,

beyond the taxonomic level of family. Its use is also beneficial in the examination

of the richness of the microbial communities. An increase or decrease observed

in the number of sequences grouped in the same OTU is as important as the appearance or disappearance of a higher taxonomic group.

The Greengenes classifier placed the 414 16S rRNA gene sequences into 157 different operational taxonomic units. OTU assignments may be compared in terms of taxonomic designations at all levels. This type of examination indicates diversity within taxonomic categories such as phylum and order, and can help illuminate characteristics of groups of sequences in terms of ability to adjust to different physical characteristics, such as precipitation or pH, which might affect microbial populations. Table 13 lists a phylum or class designation, the number of OTUs, and 16S rRNA gene sequences associated with that group of Eubacteria.

Table 13. Number of OTUs and 16S rRNA gene sequences in a bacterial taxon

Phylum	# of OTUs	# of 16S rRNA gene sequences
Acidobacteria	2	7
Actinobacteria	13	20
Aquificae	1	2
Bacteroidetes	4	5
Chloroflexi	1	1
Cyanobacteria	15	48
Deferribacteres	1	2
Bacilli	10	11
Clostridia	9	16
Gemmatimonadetes	1	1
Nitrospirae	1	1
Planctomycetes	2	3
Alphaproteobacteria	56	232
Betaproteobacteria	17	37

Table 13. (Continued)

Deltaproteobacteria	15	17
Gammaproteobacteria	5	8
Uncl. Proteobacteria	1	1
Synergistia	1	1
Thermotogae	1	1
Verrucomicrobiae	1	2
Total	157	411

The location of different OTUs along the precipitation transect provides a refinement of what populations in the microbial communities are changing. Of the 157 OTUs, 117, or 40% are associated with a single sample site. These OTUs represent 166, or 40% of the total number of 16S rRNA gene sequences. Grouped by the dominant taxonomic classifications, Table 14 lists the number of OTUs located at a single sampling site, and the associated number of sequences. As was previously mentioned, Sand Canyon had the highest percentage of Alphaproteobacteria sequences of all the sample sites. The OTU analysis indicates that 16 of the 56 alphaproteobacterial OTUs or 28% of all alphaproteobacterial diversity is associated with that site. At Henry Springs Road eleven OTUs were found, representing 69% of Cyanobacterial diversity. Fields Road had 7 of 10 OTUs assigned to the Bacilli, indicating that 70% of the diversity associated with Bacilli was found only at that sample site.

Table 14. Number of OTUs found at a single sample site and the number of actual 16S rRNA gene sequences represented.

Site 15 only	# of OTUs	# of Clones	Site 16 only	# of OTUs	# of Clones
Bacteriodetes	2	2	Actinobacteria	4	5
Clostridia	2	5	Bacteriodetes	1	1
α–Proteobacteria	16	23	Cyanobacteria	1	1
β–Proteobacteria	2	3	Bacilli	1	1
δ–Proteobacteria	4	4	Planctomycea	1	1
γ–Proteobacteria	1	1	α–Proteobacteria	7	7
Unclassified	1	1	β–Proteobacteria	5	5
Thermotogae	1	1	δ–Proteobacteria	4	5

Site 18 only	# of OTUs	# of Clones	Site 21 only	# of OTUs	# of Clones
Acidobacteria	1	3	Actinobacteria	3	4
Actinobacteria	3	4	Chloroflexi	1	1
Cyanobacteria	1	1	Cyanobacteria	11	33
Deferribacteres	1	2	Bacilli	2	2
Bacilli	7	8	Clostridia	4	4
α–Proteobacteria	6	6	Gemmatimonadetes	1	1
β–Proteobacteria	3	3	Nitrospirae	1	1
δ–Proteobacteria	3	4	α–proteobacteria	6	6
Synergistia	1	1	β–proteobacteria	4	9
			δ–proteobacteria	4	4
			γ–Proteobacteria	2	3

Seven OTUs were identified at all four sampling sites. Six of these were associated with the Alphaproteobacteria, and the seventh OTU was from the Betaproteobacteria. This group of OTUs included those with the highest number of actual 16S rRNA gene sequences, representing 143, or 34% of the total number of sequences. OTU_2503 had 62 total 16S rRNA gene sequences

which were primarily located at Sand Canyon and Fields Road. Significant numbers were also found at California City; however Henry Springs Road showed a definite decrease in the presence of this OTU. The second largest group of 16S rRNA gene sequences was for OTU_2508 with 30 sequences, which decreased in numbers along the transect. OTU_2552 had 20 associated 16S rRNA gene sequences, and was fairly evenly distributed except for an increase at California City. (Figure 11)

Figure 11. Upper graph: Number of OTUs per sample site for the most highly populated OTUs found at all sites along the precipitation transects. Lower graph: trend lines for each numbers of OTUs at each sample site.

The four remaining OTUs were found at all four sample sites represent fewer numbers of 16S rRNA gene sequences. Two of these OTUs, 2511 and

2786, increase in numbers at California City, then decrease, whereas the other two OTUs, 2697 and 3075 have their maximum number of sequences at Sand Canyon. The overall trend for each of these OTUs shows a decrease in numbers as the precipitation levels decrease. (Figure 12)
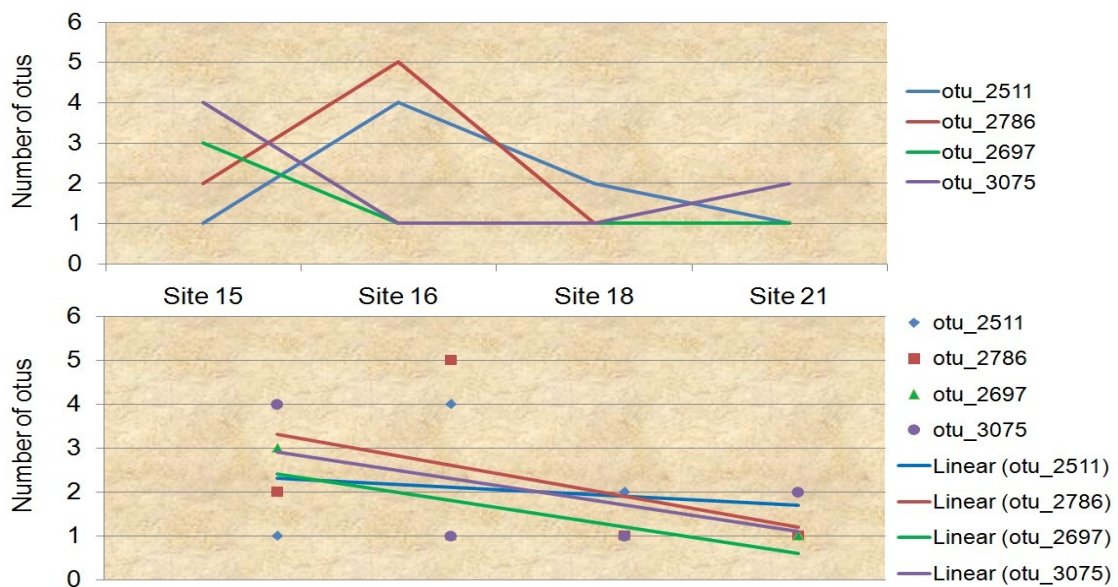
Figure 12. Upper graph: Minor OTUs associated with all four sample sites. Lower graph: trend lines for each numbers of OTUs at each sample site.

Seven OTUs were specifically associated with only the driest sites, and showed no definite trend in the number of 16S rRNA gene sequences. These groups contained 16 sequences, classified as Cyanobacteria, Firmicutes, Alphaproteobacteria, Acidobacteria, and Bacteroidetes.

At the western end of the transect, eight OTUs spanned from Sand Canyon to California City or Fields Road. These 24 16S rRNA gene sequences were from the Alpha- and Betaproteobacteria, Firmicutes, and the Actinobacteria.

Several of these OTUs demonstrated a decreasing population trend eastward along the transect.

The remaining OTUs spanned the transect in no apparent pattern. Eighteen OTUs represented 57 16S rRNA gene sequences, and were primarily associated with the Alphaproteobacteria. The Beta and Gammaproteobacteria were also represented in this group, as well as 2 OTUs from the Actinobacteria, and single OTUs from Aquificae, Cyanobacteria, Firmicutes, Planctomycetes and Verrucomicrobia

Archaeal 16S rRNA Gene Sequences. The analysis of the archaeal sequences for the sample sites of the precipitation transect was much less complex than for the Eubacteria. Sand Canyon did not yield a quantity of 16S rRNA gene sequences comparable to the number of sequences for California City, Fields Road, and Henry Springs Road. Thus, only the eastern most sites were used for analysis of archaeal diversity.

Greengenes classification placed all the archaeal 16S rRNA gene sequences into the phylum Euryarchaeota. Sequences were further classified into five classes: Archaeoglobi, Methanomicrobia, Methanopyri, Thermococci and Thermoplasmata. Figure 13 shows the proportion of each class at each sample site. Unlike the Eubacteria, the Archaea do not appear to show trends along the transect. The greatest diversity in the proportion of 16S rRNA gene sequences along the precipitation transect is at Fields Road, and thus suggests

that one or more factors other than precipitation affects the archaeal communities

in the desert.
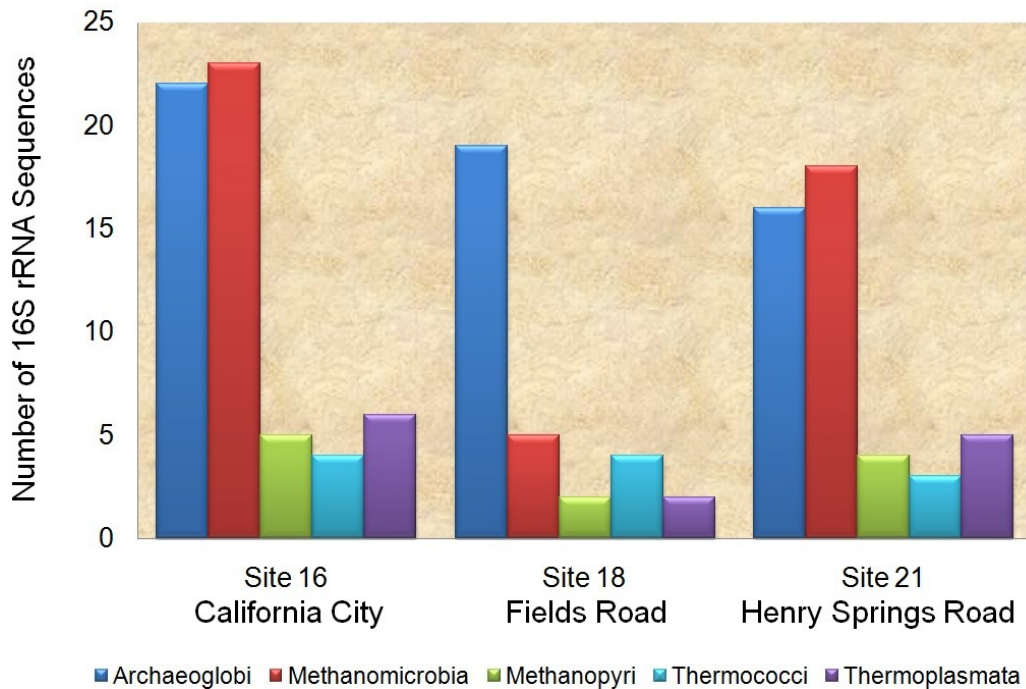
Figure 13. Taxonomic classes and corresponding number of archaeal 16S rRNA gene sequences

Further analysis of taxonomic levels introduced two families for the class

Thermococcus, but continued to group the rest of the sequences into single

families within the related class. The OTU assignments indicated that all 16S

rRNA gene sequences within a class or family are the same Archaean

microorganism.

Diversity Analysis. Rarefaction curves were produced for the Eubacteria

and Archaea based upon the OTU designations assigned by the Greengenes

classifier. (Figure 14) Eubacterial 16S rRNA gene sequences from all four

sample sites were combined to compare the overall coverage of the microbial

soil communities. None of the curves approach a plateau, indicating that the

number of sequences per site is not sufficient to fully represent the total number

of distinct bacterial organisms. The similar shape and slope of the four curves

also indicate that similar numbers of 16S rRNA gene sequences would be

needed at all sites to fully represent the richness of the bacterial communities.

An extrapolation of the curves suggests the need for another 70 to 100 16S

rRNA gene sequences to statistically encompass these communities.
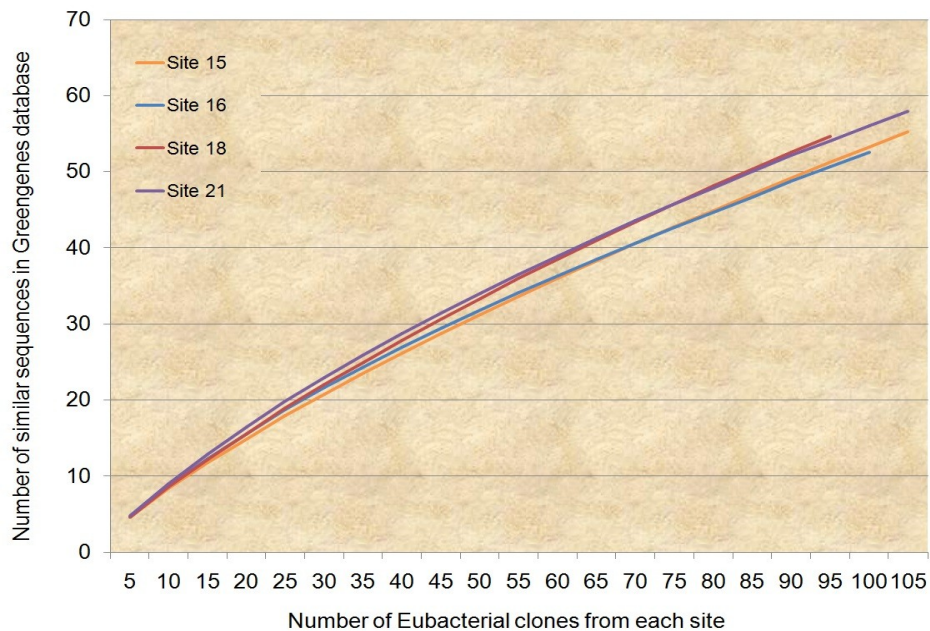
Figure 14.   Rarefaction curves for the Eubacterial 16S rRNA gene sequences
for the four sample sites of the precipitation transect.

Figure 15 shows the rarefaction curves for the Archaea sequenced along

the precipitation transect.   The number of 16S rRNA gene sequences for each

sample site appears to adequately represent the diversity of the Archaeal

communities sampled. Site 15 (Sand Canyon) was included in this analysis to

indicate the lack of sufficient sequences to properly assess this sample site.

Because there was so much similarity between the Archaea taxonomic

associations at sites 16, 18, and 21, the resulting rarefaction curve for these

three samples were identical. The curve for these sites does flatten at around 55

16S rRNA gene sequences, indicating that the sample size for California City,

Fields Road, and Henry Springs Road adequately represents the diversity

sampled.
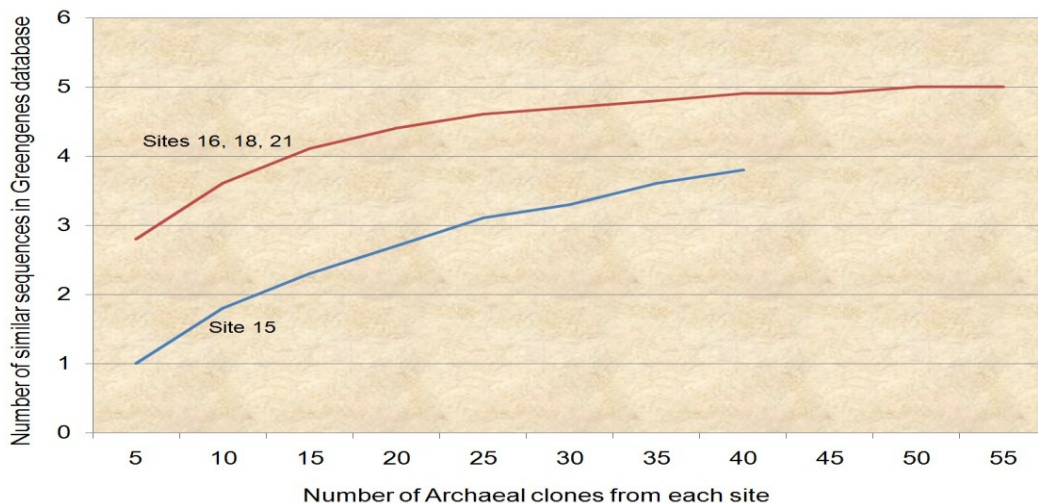
Figure 15. Rarefaction curves for the Archaeal 16S rRNA gene sequences for
the four sample sites of the precipitation transect.

In order to quantitatively determine the diversity of the microbial

communities sampled along the precipitation transect, the Shannon-Weiner

function was calculated. Based upon species richness and the evenness of the

species individuals, the function is used to assess the uncertainty of correctly

predicting the next species identify due to a random selection from a sample. The lower the diversity the closer H is to zero, thus increasing values of H indicate increasing diversity.

The analysis was based upon the classification of sequences to the level of OTU. The values for the Eubacteria showed a high level of diversity compared to that for the Archaea. For both groups of microorganisms, there was a notable decrease in diversity at Fields Road, followed by an increase in diversity at Henry Springs Road. The values for H are shown in Figures 16 and 17.
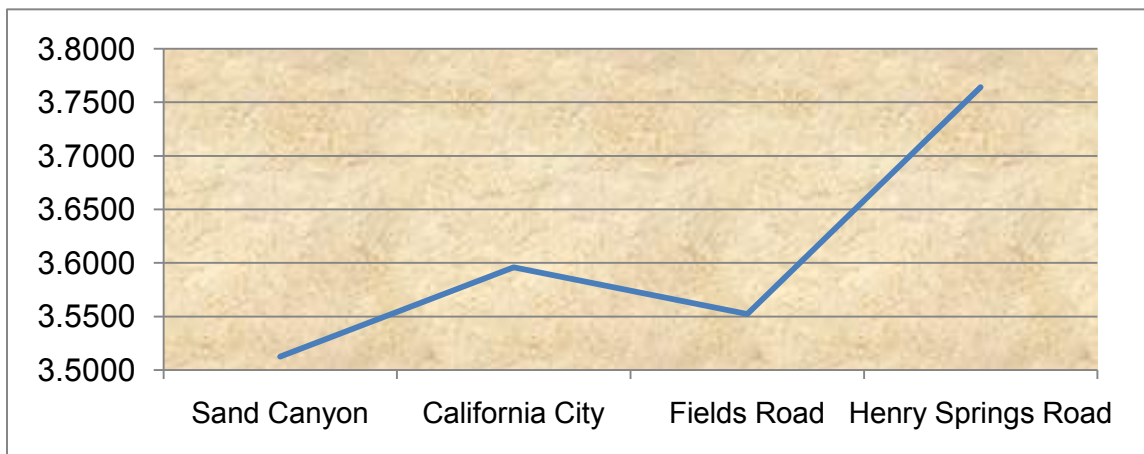
Figure 16. Shannon-Weiner function for Eubacteria 16S rRNA gene sequences
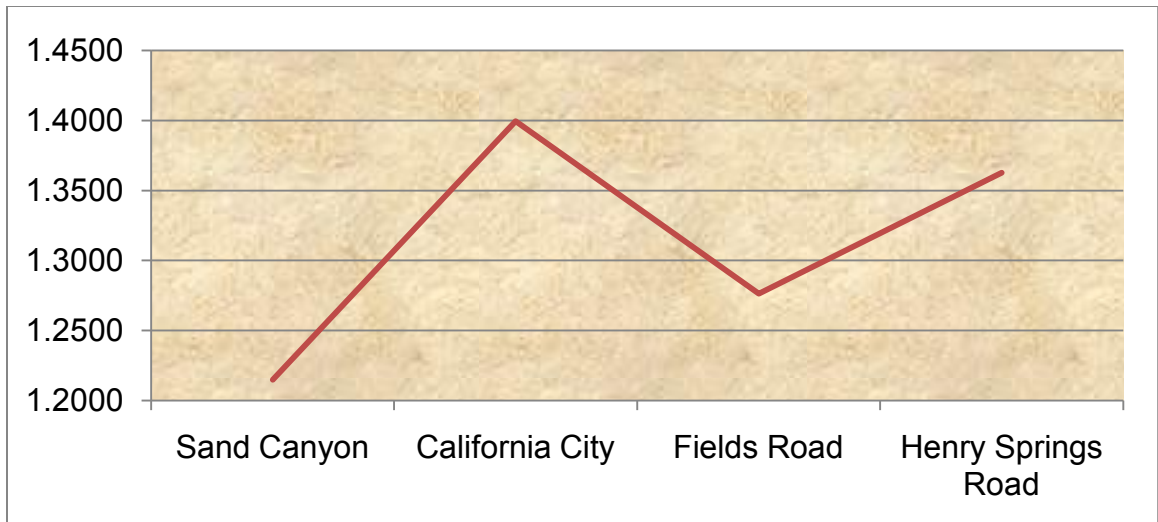
Figure 17. Shannon-Weiner function for Archaea 16S rRNA gene sequences

Discussion

Sample Site Considerations. Any discussion of microbial communities must take into account several considerations. One consideration is the size of the sampling area. This study encompassed a zone 225.5km west to east, 24 km north to south, and elevations from 403 – 1212 m. The National Geographic records the size of the Mojave Desert as 130,600km^2, and, covers elevations from the lowest and driest point in the United States - Badwater, California, at 146m below sea level, to over 1600m in the mountains surrounding Death Valley. Thus, the sampling zone represents only 4% of the Mojave Desert. The precipitation transect was selected based upon ease of access, reference points, or towns, which had precipitation records, and observed physical differences indicative of decreasing annual rainfall levels.

A second consideration for microbial diversity studies is the scale of the sample site. Microbes are quite sensitive to differences in temperature, soil types, soil moisture, pH, organic content on a scale of millimeters. The initial analysis included breaking the 10m^2 sample site into thirds and examining the differences across the site. Viable count plates recorded different CFUs/gm soil depending upon the segment of the site. Locations closest to plants also resulted in increased organic carbon content, thus affecting the microbial community in terms of richness and diversity.

Vegetation and Soil Characteristics. The contribution of available vegetation as a source of organic materials, and the chemical constituents of the soil cannot be ignored when discussing microbial populations in the soil. Many of the observed differences in types of vegetation and plant coverage are directly related to the amount of available liquid water. Though none of the sample sites were considered pristine, with the exception of Sand Canyon and Rasor Road, they had infrequent contribution of organic materials from anthropomorphic sources. Sand Canyon displayed indications of having been used as pasture lands, and Rasor Road is a known off-road vehicle excursion site. All sites were selected from 15 to 40m from a roadway, but prevailing winds possibly introducing chemical or mineral agents from automobiles, were not taken into consideration.

The types of vegetation observed along the transect was consistent with the exception of Sand Canyon, which displayed plants associated with pasture

lands. The obvious distinction in vegetation was the size of specific plants. Kramer Junction and Black Tank exhibited very large creosote bushes, on the order of 2m high and often grouped covering zones of 24m^2. The sagebrush was ubiquitous, though grew as smaller, individual plants as the sample sites became drier. Very little grass was observed at any of the sites beyond Sand Canyon, though herbal, flowering plants were a major part of the vegetative cover at Kramer Junction.

The soil chemistry reflected the vegetative coverage of the soil, thus reflecting the precipitation gradient, with a couple of examples. Considering the general trend of decreasing levels, the organic carbon levels were unusually high at Kramer Junction and Black Tank. This was also reflected in levels of organic nitrogen. These two sites are nestled against higher landforms. The Kramer Junction site is just south of an elevated area which 110m higher elevation. The presence of this hill may result in an increase in precipitation as air masses are lifted over it. A localized effect such as this would explain the increase in organic material as well as the large plant size and extensive herbal ground cover observed. Rasor Road is located between hills as well, though not as high as those north of Kramer Junction.

The levels of sodium and chlorine also displayed interesting variation. The Henry Springs Road site is on drainage for a dry lake bed. Due to occasional variation in the monsoonal precipitation, that site is often exposed to heavy runoff from precipitation events. Evaporative effects and winds over the

lake would affect the levels of salt at sites eastward. This would explain the increase in chlorine levels at this location. However, the sodium levels across the precipitation transect do not increase here. The sodium levels are highest at Sand Canyon, then drop to a minimum at California City, gradually increase along the intermediate sites, until reaching the dry zone of the transect. Black Tank soil shows a sharp increase in sodium levels, but Henry Springs Road sample site drops back to levels close to those of the intermediate sites. This sudden increase in sodium level at Black Tank is interesting because it is in a protected area, surrounded by volcanic hills. Possibly this is related to characteristics specific to the geology and geography of this site.

Viable Plate Counts and Precipitation Totals. Viable plate counts were performed in order to determine the relative size of microbial communities along the precipitation transect. With the understanding that the numbers of CFUs/g soil only represent the culturable members of the community, the comparison is a valid means of assessing differences in microbial communities between sites.

Because the viable plate counts were performed for each of the four years, the average was taken to normalize the counts susceptible to localized weather effects. The CFUs/g dry soil showed an overall decrease in the number of culturable microorganisms progressing west to east along the precipitation gradient. The 2007 and 2008 analyses showed the greatest difference between plate counts with a 100-fold decrease noted between Sand Canyon and Henry Springs Road. The CFUs/g dry soil calculations for 2009 and 2010, however,

indicated only a 10-fold difference. For 2007, the middle transect sites: California City, Fields Road, and Rasor Road, showed relatively small changes in CFUs/g soil, and presented an reasonable value between the two precipitation extremes. The following three years presented a similar relationship, though the overall numbers averaged 100-fold higher that in 2007. California City deviates from the trend in 2009, and Fields Road does the same in 2010, indicating decreased precipitation during the previous twelve months.

The variations in plate counts observed in 2009 do not appear correlated directly to the levels of organic carbon or nitrogen measured that same year. Otherwise, the CFUs/g dry soil would be highest at Kramer Junction than at any other site (with the exception of Sand Canyon.)   Nor do they appear related to levels of salt in the soil.

PH changes may affect microbial populations at the sample sites from year to year. Local precipitation levels directly impact plant growth and soil coverage, which directly impacts the pH. The current population data does not provide the level of detail necessary to determine further association.

If sites 17, 19, and 20 are removed (due to previously stated reasons), the CFUs/g soil pattern does not necessarily clarify the trend, however the average trend becomes very clear (Figure 18). The California City and Fields Road sites show a relatively flat curve or plateau for the number of culturable microbes directly related to the precipitation pattern used to establish the transect.
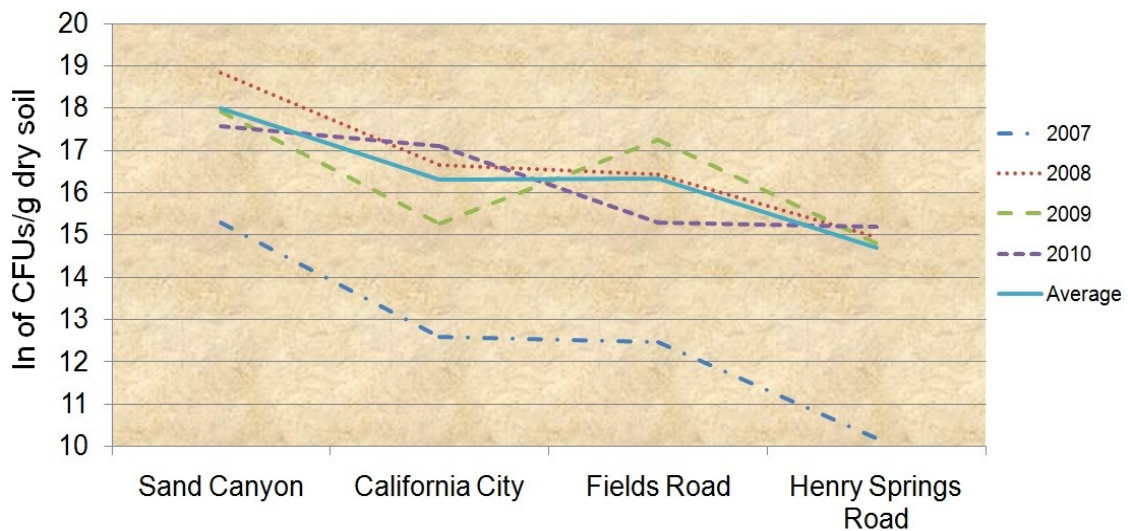
Figure 18. Trend in CFUs/g soil as ln(CFUs) vs. sample site.

Precipitation measurement devices were not deployed due to possible inadvertent or malicious interruption of the equipment. However, meteorological stations located across the Western United States are maintained by the Desert Research Institute (DRI) in Las Vegas, Nevada. Several DRI stations were located in close proximity to the transect and provided localized precipitation amounts throughout the 4-year study. Though south of the driest sampling sites, the Desert Studies Center (DSC) at Zzyzx, California, also maintains a weather station. This station and the Baker DRI station bracketed the Henry Springs Road site. Table 15 shows recorded precipitation totals for 12 months prior to each sampling expedition.

Table 15. Annual precipitation amounts (cm) at DRI weather stations and the DSC for 12-month period prior to each expedition.

Period	Tehachapi	Mojave	Barstow	Baker	Zzyzx
2006-7	16.12	1.35	3.81	5.51	2.62
2007-8	22.40	7.54	8.02	10.77	5.89
2008-9	22.73	5.21	10.31	6.78	3.12
2009-10	20.69	7.62	13.28	11.40	NA

The driest 12-month period was prior to the first sampling expedition in 2007. The wettest 12-month period is not as easily discerned due to decreases in CFUs/g soil calculated for California City, Fields Road and Black Tank for various years. There appear to be localized factors, such as the proximity of mountains, which effect storm movement and result in rainfall patterns counter to the decreasing precipitation trend observed over a sixty-year period (Figure 19).
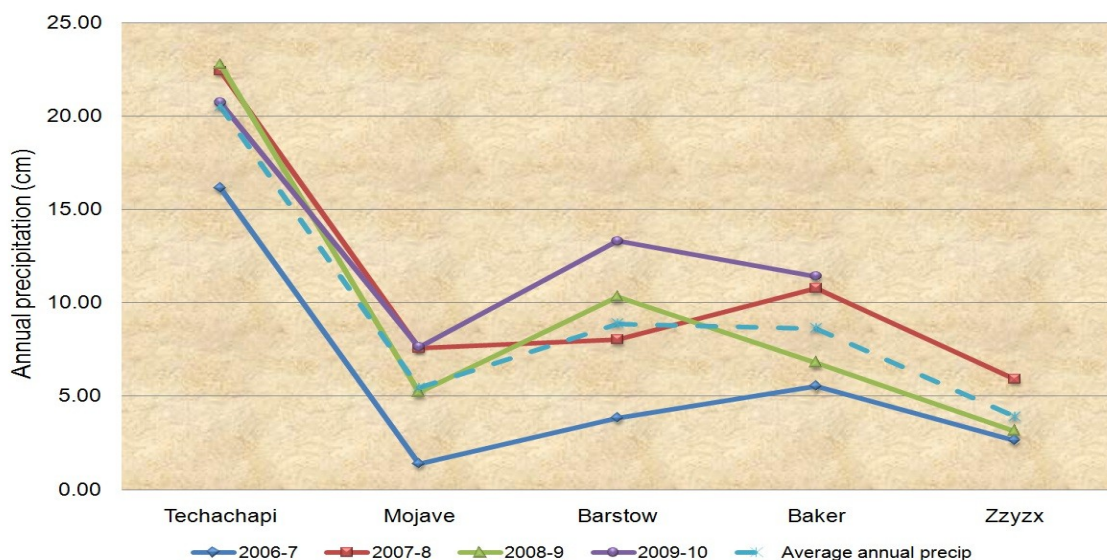
Figure 19. The recorded precipitation amounts for specified years along the precipitation transect.

The Mojave weather station consistently recorded precipitation amounts lower than the other DRI stations, perhaps as a result of the rain shadow effect of the Tehachapi Mountains. Orographic precipitation, where moist air is thrust upwards and water vapor condenses, then falls on the western side of mountains, usually results in the eastern side of the mountain range receiving very little precipitation. This is actually observed on the FRAP precipitation map as a small zone of decreased rainfall – 8.9 cm instead of the 11.4cm just east. The resulting low annual precipitation zone would indicate that CFU counts in this proximity might be unusually low compared to sample sites further east. This was not observed at the California City site. Three of the four years of viable counts established this site as having the largest culturable population west of the Tehachapi Mountains. Based upon the these results, the California City sample site, 23.4 km east of the DRI Mojave weather station, appears to be just outside the zone of low precipitation, but not so far from it that low counts, such as seen 2009, cannot occur.

The Henry Springs Road sample site is almost halfway between the Baker DRI and the Zzyzx weather stations, and thus probably receives annual precipitation amounts between the amounts recorded at the weather stations. This possibility is supported by the viable count plates showing this site to have the lowest CFUs/g soil averaged over the four year study period. Black Tank, a bit further east at the edge of a lava flow, has presented fewer CFUs/g soil for the

2009 and 2010 years, however it yielded ten times more for the 2008 sample year.

The number of culturable bacteria and the decrease in number is low moisture soils correlate with values determined by other desert studies. Rainey, et al. compared desert soil samples from the Sonoran Desert to the Atacama. The Sonoran Desert is considered an arid environment and receives an average of less than 20cm precipitation per year. The Atacama is a hyperarid desert and may go for several years without measurable rainfall. The relative humidity ranges from 30-50%, depending upon seasons. The Sonoran viable count plates produced 10^6 CFUs/g dry soil whereas the Atacama samples ranged from 2.2×10^4 to 1.8×10^5 CFUs/g dry soil [21].

Another desert environment, the Negev Highlands of Israel, was sampled beneath shrubs and in between vegetation during the four seasons of 2001. This temperate desert is very similar to the Mojave in terms of rainfall, with average precipitation of 9.8 cm, with fog contributing another 3.4cm precipitation per year. Temperatures range between 5-14°C in the winter and up to 32°C in the summer, and thus there is less fluctuation in temperature as in the Mojave. Plates cultured from the soil under the shrubs yielded 4.6×10^6 -1.07 $\times 10^7$ CFUs/g soil, and the intershrub areas recorded 6.56 - 8.75×10^6 CFUs/g dry soil. These values of culturable microbes have the same order of magnitude as from the Mojave, though all the Mojave sample sites were intershrub and were 10^5 CFUs/g soil for two years. In the Negev Highlands, the contribution of condensation from fog

may provide enough additional liquid water, particularly around the vegetation, to increase the culturable counts to those of a wet year in the Mojave [2].

Zdanowski and Węgleński made serial dilutions of soil from thirteen sample sites on King George Island, Antarctica, and plated them on full-strength NA medium. These sites were from a designated protected zone indicating restricted from anthropomorphic influences, and thus affected strictly by natural conditions of cold, no permanent snow cover, and organic material deposited from plants and animals. Eight of the sample sites were from exposed soil, while the remainder was taken from soil at the bottom of pools of water. The results for the soils averaged from 1.42×10^8 CFUs/g soil around the Ecology Glacier, and 4.8×10^6 CFUs/g dry soil on hills away from the glacier. The higher culturable counts adjacent to the glacier are likely due to proximity of liquid water when temperatures increase to above freezing [27].

Soil dilutions from areas in and around campsites at a subalpine lake in western Montana were plated on R2A medium to detect soil compaction and other anthropomorphic influences on microbial communities. The resulting plates indicated CFU values of 6.5×10^6 for campsites or human-affected areas, and 5.9×10^6 for the soil between campsites (less disturbed zones), indicating little anthropomorphic influence on campsite soil [26].

16S rRNA Sequence Analysis, Database Selection. The primary purpose of this research was to investigate the Eubacterial and Archaeal soil communities along a precipitation transect through the Mojave Desert. In order to analyze

16S rRNA sequence data and address this topic the decision was made to utilize the classification system provided by Greengenes rather than to use information from GenBank via BLAST. A comparison of BLAST results to Greengenes results present an interesting discrepancy in the purpose of the different databases. NCBI GenBank resides primarily as the research depository for all sequence information, regardless of taxonomic associations. Thus, when the sequences for Sand Canyon and California City were classified using BLAST, the majority of the sequences were associated with uncultured bacteria. Of the 107 sequences for Sand Canyon, 56% were in this category. Another 28% were uncultured, but associated with the Proteobacteria. Only 14% of the sequences were identified as a taxonomically distinct bacterium. A similar situation was observed for the California City sequences: 45% were uncultured bacteria, 14% were uncultured Proteobacteria and 28% were identified as a taxonomically unique bacterium. The maximum identity is a value calculated by the BLAST routine to provide a level of similarity between sequences. The standard values for taxonomic classification are ≥ 97% for the same species, ≥ 94% for the same genus. The maximum identity for the Sand Canyon sequences yielded 62% at ≥ 97% (species identification), another 30% ≥ 93% (genus assignment), and only 5% below 90%. The values for the California City sequences were 71%, 25%, and 2% below 90% maximum identity.

The databases utilized by Greengenes allowed for comparisons between NCBI, RDP, and the Greengenes repositories of sequence data which

associated each sequence with some level of taxonomic classification. These

offered slightly different interpretations of some of the sequences, resulting in

different classification. Table 16 compares the percentage of sequences

assigned to each phylum by the three databases for the four sample sites of

interest. By adding the minimum percentage of 16S rRNA gene sequences

designated by a database to a phylum, some level of agreement on classification

may be determined.

Table 16. Comparison of phylum assignments by three Greengenes databases for classification of sequences at two sites. Values are % of total sequences.

Phylum	Sand Canyon			Henry Springs Road		
	GG	RDP	NCBI	GG	RDP	NCBI
Acidobacteria	0	0	0	1	1	1
Actinobacteria	2	3	3	4	0	3
Aquificae	1	0	0	1	3	0
Bacteroidetes	2	0	2	1	1	1
Chloroflexi	0	0	0	1	1	1
Cyanobacteria	0	0	0	31	31	31
Deferribacteres	0	0	0	0	0	0
Deinococcus-Thermus	0	0	7	0	0	0
Firmicutes	6	7	7	6	7	8
Gemmatimonadetes	0	0	0	1	1	1
Nitrospirae	0	0	0	1	1	1
Planctomycetes	0	0	0	0	0	0
Proteobacteria	89	90	81	53	53	50
Synergistetes	0	0	0	0	0	0
Thermotogae	1	0	0	0	0	0
Verrucomicrobia	0	0	0	0	0	0
Unclassified	0	0	1	0	1	3

Eighty-nine percent of the Sand Canyon sequences were consistently classified in the same phyla regardless of database. This consensus was observed at the other three sample sites, with the Henry Springs Road sequences showing 92% classification agreement by the three databases. The differences in phylum assignments does not reflect a discernable pattern related to the databases, thus an explanation for RDP did not classify any sequences as Bacteriodetes and both Greengenes and NCBI did is not obvious.

Another interpretation of the database classification to the phylum level is to observe which phyla were fairly consistent across the databases. The Acidobacteria and the phyla associated with a single sample site, such as the Chloroflexi, the Gemmatimonadetes, Synergistetes, were grouped together by the three databases. The Cyanobacteria had 5 of 50 sequences, or 10% that were not consistently placed within this taxonomic unit. The Greengenes database placed two of these sequences in other phyla. The NCBI database had three of the 5 sequences separated into two different eubacterial phyla and actually identified one of these sequences as a eukaryotic organism. The RDP database placed two of these 5 sequences in Firmicutes and one in Bacteriodetes. Interestingly, the classification of these questionable sequences was only partially clarified by blasting them against GenBank. Table 17 indicates the results of all four databases concerning the sequences in question.

Table 17. 16S rRNA gene sequences of doubtful classification as determined by the Greengenes, NCBI, RDP and GenBank databases. ("Unc" → uncultured)

Seq #	GG	NCBI	RDP	GenBank
15M2	Firmicutes	Actinobacteria	Actinobacteria	Unc Bacterium
16I	Firmicutes	Cyanobacteria	Firmicutes	Unc Bacterium
16Q3	Cyanobacteria	Actinobacteria	Bacteroidetes	Unc Bacterium
16V4	Bacteroidetes	Cyanobacteria	Cyanobacteria	Cyanobacteria
16H2	Cyanobacteria	Firmicutes	Firmicutes	Unc Planctomycetes
18L	Cyanobacteria	Eukaryote	Cyanobacteria	Unc Bacterium
21Y2	Actinobacteria	Firmicutes	Firmicutes	Unc Bacterium

The confusion between classifications based upon database is not something resolvable at this time. The same situation arose when classifying Archaeal sequences. Using the same databases, NCBI and Greengenes tended to agree upon archaeal classifications more often than RDP or GenBank, which tended to associate the sequences amplified by confirmed archaeal primers as bacterial. Thus, the decision was made to use a single database for all the sequences and sampling sites, thereby gaining consistency across the project and establishing a basis for future analysis. The Greengenes database was selected for this analysis because it offered taxonomic specifications for class, order family, genus, and species, where applicable.

16S rRNA Sequence Analysis, Classification Parameters. The Greengenes alignment routine compares sequence information to its own rRNA gene database and produces an alignment of 7,682 characters. The user is asked to determine a minimum length of bases depending upon the length of the edited sequence length. The default is 1250 bases; however, 500 bases were

selected for this analysis due to resulting sequence lengths of approximately 700 bases for some of the sequences. There is also a selection for minimum percent identity. This value is not related to the classification of a sequence to a specific taxonomic level. Rather, it is a minimum association requested for comparison to the template sequence selected by the NAST (Near Alignment Space Termination) algorithm. The default setting for this value is 75%, which was used for this analysis. When that value was increased, the result was an increase in sequences which did not meet the minimum percent identity to the template sequence, so more sequences were dropped from the alignment and classification.

To test this distinction, alignments were run at the suggested 75% as well as 80, 85 and 90% minimum identity. For site 15 or Sand Canyon sequences, the difference between the 75, 80, and 85% identity resulted in the loss of a single sequence. When examined by BLAST, that sequence was classified as an uncultured bacterium but with only a 89% maximum identity. Running the alignment at 90% minimum percent identity resulted in the loss of eight more sequences. When these sequences were compared to GenBank, the resulting maximum identity ranged from 83 to 98%. These sequences were classified as uncultured bacteria though two were identified as members of the class Deltaproteobacteria. The results of this process using the sequences from the Henry Springs Road site were more dramatic. At each incremental 5% change in minimum percent identity from 75 to 85% dropped an additional sequence from

the analysis. When the minimum percent identity was increased to 90%, a total of twenty-nine sequences were lost.

The rarefaction curves for the eubacterial communities of the transect indicated that a total of 107 - 108 16S rRNA gene sequences for Sand Canyon and Henry Springs Road were not sufficient to adequately describe the existing communities. Thus, the loss of sequences due to higher levels of identity to a template sequence was not justifiable. Furthermore, because this exclusion from the alignment did not correspond to increasing the certainty of identification of the 16S rRNA gene sequences suggest that using the 75% level for maximum identity to a template sequence for the purpose of alignment was a satisfactory assignment.

16S rRNA Sequence Analysis, Eubacteria. The resulting classification provided by Greengenes for the 16S rRNA gene sequences from the four sample sites along the precipitation transect present are in agreement with the proportions of phyla along the transect.   The predominant phylum was the Proteobacteria, with the highest number of sequences associated with the class Alphaproteobacteria.   The numbers of Alphaproteobacteria decreased eastward along the precipitation gradient. Conversely, the Cyanobacteria were not observed at Sand Canyon, but became a part of the soil community and increased as precipitation levels decreased eastward along the transect, until it was within 7% of the population of alphaproteobacterial sequences. None of the

other phyla were present at levels near that of the Cyanobacteria or Alphaproteobacteria.

Research in other desert environments reveals a slightly different assessment of predominant bacterial phyla. The Eubacterial and Archaeal soil communities of the Tataouine Desert of Tunisia were examined using 16S rRNA gene analysis. The Tataouine Desert is considered an arid environment with an average precipitation of 11.5cm. Like the Mojave, temperatures range from 5 to 40°C, and vegetation is sparse. It differs from the Mojave because of the occurrence of Sirocco winds 10% of the year. Soil samples were taken from the top 5cm of soil and used to create a 16S rRNA gene library. The sequences were classified using GenBank and the results of the culturable bacteria indicated affiliations with the Actinobacteria, Proteobacteria, Firmicutes, and a single isolate labeled CFB group 1(distantly associated with *Flexibacter*). The molecular analysis of the 16S rRNA gene indicated that 83% of the 117 sequences tested belonged to the Proteobacteria (41%), Actinobacteria (25%), and Acidobacteria (17%). Of the remaining sequences, associations with Green Non-sulfur (GNS) bacteria, Deinococcus-Thermus, Planctomycetes, Verrucomicrobia and Firmicutes were present, and none were associated with Cyanobacteria.   The largest number of 16S rRNA gene sequences classified as Proteobacteria was actually associated with the Deltaproteobacteria. Of the 117 sequences, 78% were classified using GenBank and labeled uncultured bacterium [6].

The level of precipitation would associate the Tataouine study with the intermediate precipitation sample sites, specifically Fields Road. This site also showed the represented phyla to include Proteobacteria, Firmicutes, Acidobacteria, Planctomycetes, Verrucomicrobia, and Actinobacteria. Fields Road also included a sequence for Synergistetes, and 8 sequences for the Cyanobacteria. Neither of these phyla appears in the Tataouine study.

The arid soils of northern Arizona were the focus of a study comparing the pinyon-juniper woodlands of the Coconino National Forest to basaltic soils around Sunset Crater National Monument. Though not as hot as the Mojave or Sonoran deserts, the area is considered arid and subjected to many of the same weather patterns as the surrounding deserts. Soil samples were taken from two sites approximately 19 km apart at the time of year corresponding to maximum available moisture. Gene libraries were made from the 32 16S rRNA genes which were then identified by GenBank. Twenty sequences were associated with Proteobacteria, Gram Positive bacteria and 'flexibacteria', which come from a genus name, *Flexibacter*, in the phylum Bacteroidetes. The remaining sequences grouped into clades associated with uncultured soil bacteria. Four of these clades were clustered with Acidobacteria when classified on a maximum-likelihood phylogenetic tree. The remaining group of sequences fell between the Planctomycetes and the GNS bacteria. Compared to the Mojave sequences, the Proteobacteria, Bacteroidetes and Acidobacteria were identified, however, no sequences were categorized with the Cyanobacteria [13].

Numerous research projects on bacteria soil communities have been done in the extremely arid Atacama Desert. Drees et al. sampled the Punta Negra transect through the hyperarid zone of the Atacama. The 16S rRNA gene was amplified from extracted community DNA, then sequenced and analyzed. Two bacteria communities were identified, one associated with the limited plant growth along the transect, and the other from absolute desert – no vegetative growth. The sequences were classified using GenBank and resulted in the majority of 16S rRNA gene sequences being labeled as "uncultured bacterium clone".   Using parsimonious phylogenetic trees these sequences were grouped with the Acidobacteria, Gemmatimonadetes, and Planctomycetes. Compared to the Mojave study, the Acidobacteria and Gemmatimonadetes were found at the driest location [9].

A different analysis of the Atacama utilized phospholipid-fatty acid (PLFA) analysis as well as DNA extraction and amplification to examine the bacterial diversity of the soil. Ninety-four percent of the sequences were associated with the Actinobacteria, with the remainder of the sequences classified as Proteobacteria, Firmicutes and TM7 (an environmental bacterium associated with human diseases of the oral cavity). The PLFA analysis identified Actinobacteria, Proteobacteria, and Firmicutes as the dominant taxonomic groups [7].

Research on bacterial soil communities in arid environments has identified predominant taxonomic classifications as the Actinobacteria, the Firmicutes, and Proteobacteria. The Mojave data agrees with the predominance of the

Proteobacteria; however the populations of the Actinobacteria and Firmicutes were much lower than the population of Cyanobacteria. None of the related research has specified the existence of free-living Cyanobacteria as an important constituent of desert microbial communities. Because the majority of the related research incorporated sequence classification from GenBank, consistency between the Greengenes database and GenBank was examined. All of the cyanobacterial sequences from Henry Springs Road (site 21) were blasted against GenBank. The results indicated that 3 of the 34 sequences were not classified as Cyanobacteria by GenBank. Because this 8% difference is comparable to differences in assignment of sequences to phyla by the three Greengenes databases, and this difference does not indicate major discrepancies between the four databases.

Cyanobacterial microbes were detected in very cold arid environments. Neufeld and Mohn examined the microbial diversity of two sites in the Arctic tundra compared to forest soil from northern Canada. Samples from the top 10cm of soil were taken from 20x20m areas representative of the surrounding landscape. Ribosomal sequence tags and DGGE were used as template for amplification of the 16SrRNA gene. Sequences were classified by GenBank and identified the phylum Proteobacteria as the dominant group, as well as Actinobacteria, Acidobacteria, Firmicutes, Bacteriodetes, Verrucomicrobia, and Cyanobacteria. The proportion of cyanobacteria at all sample sites was highest

at the tundra sites, but very low overall compared to the other mentioned phyla (specific data was not available) [18].

Research in the Dry Valleys of Antarctica also produced evidence of Cyanobacteria in eubacterial soil communities. Smith, et al. sampled mineral soil from three locations: underneath a decaying seal carcass along the coast, a midslope area at 225m altitude, and a mountain pass at 584m. The16S rRNA genes were amplified from extracted DNA. Clone libraries were created and classified by GenBank to establish microbial populations by phyla designations. The coastal and mid-altitude sites were dominated by the Actinobacteria, Acidobacteria, and environmentally uncultured groups of bacteria. Chloroflexi, Bacteriodetes, and the Alpha and Betaproteobacteria were identified at the coastal site. The mid-latitude site showed the presence of Verrucomicrobia, Chloroflexi, Bacteriodetes and a group of unclassified bacteria. The high-altitude soil community was predominantly Cyanobacteria, with Bacteriodetes, Actinobacteria, and environmental uncultured bacterial groups present in lower proportion and minimal contribution from Verrucomicrobia, Acidobacteria, and unclassified bacteria. The explanation of the dominance of Cyanobacteria at the high-altitude site was the presence of hypolithic strata [23].

A different project examined soil communities which including Archaea and eukaryotic microbes. The sample area was McKelvey Valley, and was described as frost polygons against sandstone and quartz substrates. Ten samples were taken from soil. The top 2.5cm of loose soil was removed, and

then samples taken down to a depth related to the depth of hypolithic colonies. Samples were examined using quantitative PCR and restriction digests. The resulting clone libraries for the 16S and 18S rRNA genes were classified by GenBank. Results indicated the actual soil did not include Archaea, eukaryotic microbes or Cyanobacteria. The predominant phyla were Acidobacteria, Actinobacteria and unidentified bacteria, with Bacteriodetes, Chloroflexi, Deinococcus-Thermus, Gemmatimonadetes, and Betaproteobacteria making small contributions to the soil community. Cyanobacteria, algae, and fungi were associated with hypoliths, endoliths, and chasmoliths [20].

The samples from the Mojave Desert did not include sampling cyanobacterial crusts, though these established communities were present along the transect, particularly at Black Tank. There were also hypolithic quartz rocks found at Fields Road, Black Tank, and Henry Springs Road. There may be a limit to the availability of liquid water where the Cyanobacteria cease existing freely in the soil and can only survive in larger protective environments such as crack in rocks or mats.

The proportion of the eubacterial population represented by the Firmicutes was smaller than expected based upon other desert soil results. Because of the survivability of Bacillus spores, the low presence of this class was noted. In the previous discussion regarding the selection of a database for classification purposes, the comparison of the sequences which were of questionable assignment included several that were assigned to Firmicutes in difference to

one or the other database. The discrepancies were not removed by analysis using GenBank, because many of those sequences were labeled uncultured bacterium.

The relative low proportion of Actinobacteria was also of interest, though in many studies, the sequencing is performed on cultured organisms, rather than DNA extracted from the entire soil community. Culturing soil bacteria then sequencing those organisms would skew the resulting analysis toward those organisms which can be cultured, and would represent < 1% of the total soil community. Interestingly, several of the more recent studies based sequencing on DNA extraction from the soil samples, still indicates the Actinobacteria to be a predominant phylum.

16S rRNA Sequence Analysis, Archaea. The analysis of Archaeal sequences along the Mojave precipitation transect reveal a community comprised of the same groups of 5-6 organisms: Archaeoglobi, Methanomicrobia, Methanopyri, Thermococci and Thermoplasmata. Where the diversity of Eubacteria presented a definite increasing trend with decreasing precipitation, the Archaeal communities showed very little change related to precipitation or any of the other soil characteristics measured.

Early research on Archaea in soils from an agricultural research station near Madison, WI showed the predominant archaeal sequences to most resemble Crenarchaeota sequences associated with marine samples [4]. In 2002, Pesar and Widmer first isolated sequences from the Euryarchaeota in

forest soil, including the presence of *Thermoplasmales* which are a member of the class Thermoplasmatales, and were also identified along the Mojave precipitation transect [19].

Four semi-arid soils of northwestern New South Wales, Australia were sampled and sequencing produced equal percentages of 16S rRNA gene sequences from the Crenarchaeota and the Euryarchaeota. The diversity of the archaeal communities was highest for the sample from a cotton-wheat field, and did not differ significantly for the remaining soils from a park, a nature preserve and a national park. The latter two sites had been previously cleared and used for agriculture and grazing in the 1950s [17].

A lack of diversity in archaeal soil communities was observed in a comparison of biological soil crust (bcs) communities found in four North American deserts: the Chihuahuan, the Colorado Plateau, the Great Basin, and the Sonoran. The bcs is defined as aggregations of microorganisms on plant interfaces, and were observed in the Mojave. Two to five phylotypes of Archaeal sequences were identified from 12 bcs samples produced from DGGE analysis, demonstrating a rather low diversity similar to that of the Mojave precipitation gradient. To further investigate the low diversity, clone libraries were created from pooled samples previously used for DGGE. A total of 52 16S rRNA sequences were found to be of six phylotypes related to the Crenarchaeota [24].

Diversity Analysis of 16S rRNA Gene Sequence. The diversity of the microbial communities of the sample sites along the precipitation transect in the

Mojave Desert was analyzed both by taxonomic level increases, as well as by calculation of the Shannon-Weiner diversity function. The increase in diversity appears related to the effects of reduced rainfall. At Sand Canyon, the vegetation was uniform, and the Alphaproteobacteria dominated all other groups of Eubacteria. As the precipitation decreased, the vegetation types and numbers changed allowing for more niches for bacteria. At Henry Springs Road, the conditions favored the Cyanobacteria, which do not depend upon organic sources of carbon and nitrogen. The lack competition by Alphaproteobacteria also allowed the minority Eubacteria to establish themselves as part of the community. The Archaea which did not demonstrate as much of a change in their communities, apparently are less sensitive to precipitation related conditions.

The possibility of identifying a bacterial or archaeal organism which might be useful as an indicator organism for desertification does not present itself from this data. The original examination of the data suggested that a member of the Cyanobacteria might be an optimal candidate to reflect the level of desiccation and decreasing organic levels in soils. However, the lack of recognition by other desert microbial studies questions the primary requirement that the organism be a consistent constituent of the community and conditions in question. It is possible that the conditions in other arid and semi-arid environments, such as soil types, chemical composition, and weather extremes do not support cyanobacterial life in the soil. Further analysis of soil communities from deserts

with different conditions would be necessary to clarify the associations of such characteristics on Cyanobacteria.

Summary

The data from the Mojave Desert precipitation transect indicated an inverse relationship between the diversity of eubacterial soil communities and precipitation amounts. The viable counts gave an indication of overall decreasing numbers of culturable bacteria, but were not a factor for the examination of diversity. Further analysis of 16S rRNA gene sequences from the following three years of sampling will help determine if the trends observed for the Eubacteria and Archaea are consistent for the precipitation gradient, or reflect localized conditions. Clone libraries for the following three years may also clarify differences noted in taxonomic groups in the Mojave compared to other desert environments.

Two other techniques for diversity analysis will be utilized for the Mojave transect. Denaturing Gradient Gel Electrophoresis (DGGE) allows the comparison of Eubacteria and Archaea at the genus and possibly species level. This technique allows site to site comparisons of the two Domains and may suggest a relationship between them not yet observed.

Also of interest would be to assess community level physiological profiles (CLPP) of the eubacterial communities using Biolog GN sole-carbon-source

microplates. Analysis of the carbon-source utilization patterns offers a different

approach to diversity and may present interesting trends related to the

precipitation gradient.

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