A GEOBIOLOGICAL INVESTIGATION OF THE HYPERSALINE SEDIMENTS OF PILOT VALLEY, UTAH: A TERRESTRIAL ANALOG TO ANCIENT LAKE BASINS ON MARS

By
Kennda Lian Lynch
A thesis submitted to the Faculty and the Board of Trustees of the Colorado School of Mines in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Environmental Science and Engineering).

Golden, Colorado

Date__________________

Signed:_________________________________
   Kennda Lynch

Signed: _________________________________
   Dr. Junko Munakata Marr
   Thesis Advisor

Signed: _________________________________
   Dr. John R. Spear
   Thesis Advisor

Golden, Colorado

Date__________________

Signed: _________________________________
   Dr. John McCray
   Professor and Head
   Department of Civil and Environmental Engineering
Martian paleolake basins are prime habitability targets for future Mars rover missions as they are thought to be reasonable proxies for a Hadean-like origin-of-life environment. This is especially the case for the upcoming Mars 2020 mission as over half of the top landing sites being considered show evidence of lacustrine sediments. Many terrestrial paleolakes transitioned to modern day evaporite basins due to climate change and exhibit clay, sulfate, and chloride compositions similar to the aqueous minerals identified across the martian surface. These terrestrial systems are considered excellent analogs for habitability studies that will be useful for identifying and exploring lacustrine systems on Mars. This dissertation focuses on evaluating several aspects of martian paleolake basin habitability in a relevant analog environment, the Pilot Valley basin in northwestern Utah, in preparation for Mars 2020 and other future surface missions to the red planet.

Lacustrine sediments from the Pilot Valley basin were characterized using X-ray diffraction (XRD) and automated scanning electron microscopy (QEMSCAN) to gain contextual insight into the mineral assemblages deposited in terrestrial paleolakes and to use as ground truth for evaluating the efficacy of visible-near-infrared spectroscopy (VNIR) as a tool for the identification and characterization of martian paleolake surfaces. Results of this effort show that lacustrine sediments can be very complex and that current spectral reference libraries are not sufficient to interpret the spectral influence from complex mixed mineral matrices. Second, the microbial ecology was investigated in order to characterize the biological diversity within this understudied environment, assess the relationship, if any, between the microbial diversity and the mineralogical and geochemical variation present in the basin, and assess the influence of this relationship on biosignature preservation as a model for paleolake systems on the red planet. General results from this effort show that a novel ecosystem is present in Pilot Valley and community assembly is influenced by sediment grain size. Finally, the presence and distribution of naturally-occurring perchlorate was investigated along with the potential for perchlorate reducing organisms co-existing with naturally-occurring perchlorate as a model for potential metabolisms that could support a microbial
ecosystem in ancient martian lacustrine sediments. Results show that naturally-occurring perchlorate exists in the near surface sediments as does a small population of known perchlorate reducing organisms.
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“It takes a whole village to raise a child”
- African & Native American Proverb

“If we stand tall, we do so on the backs of those who came before us”
- Yoruba Proverb
DEDICATION

For my son, Gavin.
"It is good to renew one's wonder" said the philosopher.
"Space Travel has again made children of us all"
-Ray Bradbury, The Martian Chronicles.

1.1 Research Motivation: Life, the Universe, and Everything...

The possibility of life on other planets is a fundamental curiosity of the human species. Science fiction authors such as Ray Bradbury, H.G. Wells, and Edgar Rice Burroughs wrote of the possibility of alien life in our solar system and helped to fuel the imaginations of the early 20th century generations, who would later take humanity into space [Bradbury, 1950; Burroughs, 1917; Wells, 1898]. And indeed, upon the advent of
the space age, robotic pioneers such as Mariner 9, Viking, and Voyager (Figure 1.1) sped away from the Earth on missions of exploration to start turning science fiction into fact by exploring the surface of Mars and other planets in our solar system, and sending messages of greetings from Earth.

In the late 20th century, the Hubble telescope gave humanity a view into the origins of the universe, a tiny rover name Sojourner rolled along the surface of Mars, and a novel paper about a martian meteorite, written by a NASA science team, made the first definitive suggestion that life was possible elsewhere in the universe [McCauley et al., 1972; McKay et al., 1996]. All these events over the course of the past 50 years has led to the development of a new science, the science of astrobiology. In the 21st century, astrobiology has become an international driving force behind all current and future planetary missions and the inquiry into the possibility of life on other planets is no longer just within the purview of science fiction.

1.1.1 Astrobiology: Understanding the Origin, Evolution and Extent of Life in the Universe

Since its inception, NASA has had the focused goal of exploring the possibility of life on other planets. The 1950's and 60's were two defining decades for the rise of astrobiology as they saw the first origin of life experiments by Stanley Miller, the XRD research of DNA by Rosalind Franklin that led to the model of the DNA structure by Watson and Crick, and a race to the moon that was initiated but a Soviet satellite called Sputnik. With the signing of the National Aeronautics and Space Act, NASA and the field of Exobiology (later to be renamed as Astrobiology) was born with the goal of ensuring that the fundamental questions regarding the origin, evolution, distribution of life in the universe were at the core of every space mission. Exobiology would carry NASA, and the world, to the primary target for exobiology/astrobiology science: Mars.

1.1.2 Mars: The Search for Life on the Red Planet

Since the first description of "canals" on Mars by Percival Lowell, Mars has held a special bond as a sister planet to Earth [Lowell, 1906]. As our closest neighbor in the solar system, with similar day/night cycles, Mars is a vision of what Earth could be in a
few million years. Though the canals hypothesis propagated by Lowell as evidence that Mars was inhabited was eventually shot down by the return images of Mariner 4, 6, and 7, over the next century, Mars would show itself as a likely place for life to originate, thrive and leave its mark.

Mariner 4 was the first robotic vehicle to truly encounter Mars via flyby and send back the first images of the red planet surface [H R Anderson, 1965]. It was followed by Mariners 6 and 7 that provided more detailed images and studied the martian atmosphere. The final mission in the Mariner series was Mariner 9, which was the first orbiter at Mars and mapped 100% of the the surface, providing first views of the martian volcanoes, the polar caps, fluvial features, and Valles Marineris, some of the deepest canyons in the solar system. The Mariner program paved the way for the Viking missions that would be the defining missions for Mars exploration until the end of the 20th century.

The Viking program was the first astrobiology mission in human history. The mission objective of the Viking program literally read “[to] obtain scientific data which will significantly increase our knowledge of Mars, with particular emphasis on providing information relevant to life on the planet”. The Viking I & II landers settled on the surface of Mars within two months of each other in 1976 and proceeded to carry out the first life detection experiments on the red planet [Brown et al., 1978; Horowitz et al., 1977; Levin and Straat, 1977; Soffen, 1977]. The results of the experiments proved to be ambiguous and were cause for both the planet Mars and Exobiology science, in general, to take a back seat in NASA’s exploration plans until the end of the 20th century; though, the experimental results would be continuously revisited well into the 21st century [Glavin et al., 2001; Mancinelli, 1998; Ming et al., 2014; Navarro-González et al., 2006].

Mars would not be a focus of astrobiological research again until 1996 when a science team suggested the possibility of microscopic fossil evidence of life within the martian meteorite Allen-Hills 84001 [McKay et al., 1996]. The findings of this study would be a source of much scientific debate in the following decade and a significant motivator for the revival of exobiology as the new science of Astrobiology [Blumberg, 2003]. This was followed in 1997 by the Pathfinder mission that returned the first in situ
analysis of martian geology that suggested Mars may have had a wet past [Golombek et al., 1999]. The next decade of Mars exploration would be filled with remote sensing and surveying of the martian surface by the Mars Global Surveyor, Mars Odyssey, and the European Space Agency’s Mars Express that resulted in the global mapping of permanent ground ice, more detailed morphology of surface features, mineralogical surveys including detection of aqueous minerals, and evaluation of landing sites for the next generation of surface robotic missions [Boynton et al., 2002; Christensen, 2003; Christensen et al., 2004; Christensen et al., 2000; Christensen et al., 2003; Christensen et al., 2001; Golombek et al., 2005; Golombek et al., 2003; Grotzinger et al., 2014; Titus et al., 2003].

The Mars Exploration Rovers (MER) would reach the surface of the red planet by 2004 and would definitely confirm the past presence of liquid water on the martian surface [Klingelhofer et al., 2004; McLennan et al., 2005; Squyres et al., 2004]. This mission would be followed by the Mars Reconnaissance Orbiter (MRO) that would provide the most detailed remote sensing views of the martian terrain to date, and would vastly increase the mineralogical global survey of aqueous materials on the red planet [Murchie et al., 2009]. The Phoenix lander would discover evidence of the powerful oxidant perchlorate in the martian soils, causing a revisit to the Viking results, and the Mars Science Laboratory (MSL) rover, Curiosity, would find definitive evidence of ancient lake basins, nitrates and organics on the martian surface, thus confirming that Mars could have been a habitable planet at some point in its history [Catling et al., 2009; Freissinet et al., 2015; Grotzinger et al., 2014; Hecht et al., 2008; Navarro-González et al., 2010; Stern et al., 2015].

1.2 Project Overview and Approach

With the recent results of the MSL mission, it is clear that Mars was potentially a habitable planet and the focus of the scientific community has now turned to direct search of evidence of past life on Mars [Freissinet et al., 2015; Grotzinger et al., 2014; NRC, 2011; Stern et al., 2015]. A key focus of the Mars 2020 rover mission will be to seek out evidence of life in a relevant habitable Mars environment [John F. Mustard et al., 2013]. Given the results of the MSL mission, martian paleolake basins are prime
habitability targets for future Mars rover missions and over half of the sites being considered for the 2020 mission include access to lacustrine sediments. As such, it will be imperative to have a clear understanding of the habitability and biomarker preservation potential of paleolake systems and this can be accomplished by studying paleolake systems on Earth.

Terrestrial paleolake basins are mainly identified as modern day evaporite basins and they contain clay, sulfate, and chloride mineral compositions similar to the aqueous minerals identified across the Martian surface [Barbieri and Stivaletta, 2012; Currey, 1990; Douglas, 2004; Fornari et al., 2001]. They are known to harbor a diverse array of microbial life, and enhance the preservation of organic matter and fossils, hence they are excellent analogs for habitability studies that will be useful for identifying and exploring lacustrine systems on Mars [Barbieri et al., 2006; Douglas and Yang, 2002; Ventosa et al., 2008]. Also, as planetary analogs, these terrestrial environments provide the opportunity to study how this transition from humid to arid affects the mineralogy, geochemistry, and sedimentology of basin systems, and in addition, to study how climate change on Mars may have affected the habitability and preservation potential of Martian paleolakes. This dissertation focuses on several key aspects of habitability in preparation for Mars 2020 and other future surface missions to the red planet. The research conducted in this dissertation was done on samples collected from the Pilot Valley Basin of the Great Salt Lake Desert (GSLD) in northern Utah. Samples were also taken at the Bonneville basin of the GSLD and from the Great Salt Lake for a comparison study of microbial diversity as discussed in Chapter 3.

1.3 Research Questions

The overarching premise for conducting GSLD research is that a comprehensive understanding of the geological and biological characteristics of terrestrial paleolake basin systems will prove critical for understanding and interpreting the astrobiological aspects of martian paleolake basins. It will also aid in designing and implementing in situ investigations during future robotic and manned missions to Mars. The following objectives and hypotheses represent the initial information necessary to lay the foundation for long-term GSLD work. In addition, this proposed study has been divided
into an exploration-driven phase and a hypothesis-driven phase, with the defined hypotheses derived from preliminary results of the exploration phase. The exploration-driven objectives and the hypotheses are also re-visited in the concluding chapter.

1.3.1 Exploration-Driven Project Objectives

1. Define and characterize an appropriate study area within the GSLD (i.e. a self-contained sub-basin with minimal anthropogenic influence).
2. Characterize the geochemical & mineralogical diversity within the defined GSLD study area.
3. Characterize the biological diversity within the defined GSLD study area.
4. Evaluate the presence, quantity and distribution of the perchlorate anion within the defined GSLD study area.
5. Evaluate the presence and distribution of perchlorate-reducing bacteria within the defined GSLD study area.

1.3.2 Hypotheses

1. The Pilot Valley Basin is a suitable mineralogical analog for groundwater-filled, closed basin paleolakes on Mars.
2. The mineralogical and geochemical diversity of terrestrial paleolake basins will prove difficult to ascertain beyond major mineral classes using current planetary science exploration tools such as visible-near-infrared spectroscopy (VNIR) and in situ X-ray diffraction (as used by the CheMin tool on Curiosity).
3. The microbial diversity within the Pilot Valley basin will correlate with the mineralogical and geochemical variation along the defined study transect.
4. Microbial perchlorate reduction is a contributing mechanism of perchlorate removal within the Pilot Valley basin.

1.4 Research Tasks

The dissertation research tasks are listed below.

1.4.1 Task 1: Assess the Viability of Pilot Valley as a Mars analog

1. Evaluate the hydrology of the Pilot Valley Basin as an analog for the inferred groundwater-dominated hydrological cycle on early Mars.
2. Evaluate the mineralogy of the Pilot Valley Basin as an analog for the detected hydrated minerals and aqueous-altered minerals on Mars.
3. Investigate the capability of planetary remote sensing and *in situ* analysis techniques to detect the detailed mineralogy of Pilot Valley sediments.

### 1.4.2 Task 2: Evaluate the Microbial Diversity of the Pilot Valley Basin

1. Evaluate and compare the microbial diversity of Pilot Valley with the diversity of other regions of the GSLD and representative sediments of the Great Salt Lake.
2. Analyze the microbial diversity of Pilot Valley along the defined study transect and assess the correlation, if any, between microbial diversity and geochemical variation and spatial variation (vertical & horizontal).

### 1.4.3 Task 3 - Evaluate the Nature and Impact of Perchlorate and Perchlorate Reducing Bacteria Within Pilot Valley

1. Evaluate the presence, quantity and distribution of the perchlorate anion within Pilot Valley sediment and brine fluids.
2. Evaluate the presence, quantity and distribution of perchlorate-reducing bacteria within Pilot Valley sediment and brine fluids.

### 1.5 Organization of Dissertation

**Chapter 1: Introduction**

This chapter is an introduction to the research motivation behind this dissertation. This chapter also outlines objectives and organization of this dissertation.

**Chapter 2: Near-Infrared Spectroscopy of Lacustrine Sediments in the Great Salt Lake Desert: An Analog Study for Martian Paleolake Basins**

Chapter 2 addresses the first task of this dissertation, assessing the viability of the Pilot Valley field site as a Mars Analog research site. This chapter further summarizes the result of an analog Mars study conducted at Pilot Valley where the efficacy of visible-near infrared spectroscopy to characterize lacustrine sediments was
evaluated. This chapter is an original research article published in the Journal of Geophysical Research Planets [Lynch et al., 2015c].

Chapter 3: Lithology-Driven Community Assembly Within Hypersaline Paleolake Sediments Along a Geological Transect in the Great Salt Lake Desert, Utah.

Chapter 3 addressed the second task to evaluate the microbial diversity of the Pilot Valley Basin. This task also addresses the 3rd task as it allows us to assess the presence/absence of known perchlorate-reducing bacteria within the microbial community. This chapter is an original research article that is in preparation for submission to Environmental Microbiology.

Chapter 4 Biogeochemical Hunting: Investigating the nature and fate of naturally occurring perchlorate in the Pilot Valley Basin, Utah.

Chapter 4 addresses the third task to evaluate the nature and fate of naturally occurring perchlorate with respect to the presence/absence of perchlorate-reducing bacteria. This chapter is an original research article that is preparation for submission to Astrobiology.

Chapter 5: Conclusions and Future Work

Chapter 5 outlines the conclusions of the work done in this dissertation and outlines the areas of future work.
CHAPTER 2
NEAR-INFRARED SPECTROSCOPY OF LACUSTRINE SEDIMENTS IN THE GREAT SALT LAKE DESERT: AN ANALOG STUDY FOR MARTIAN PALEOLAKE BASINS

1A paper published in the Journal of Geophysical Research: Planets

Kennda L. Lynch2, Briony H. Horgan3, Junko Munakata-Marr, Jennifer Hanley4, Robin J. Schneider5, Kevin A. Rey6, John R. Spear, W. Andrew Jackson7, Scott M. Ritter6

2.1 Abstract

The identification and characterization of aqueous minerals within ancient lacustrine environments on Mars are a high priority for determining the past habitability of the red planet. Terrestrial analog studies are useful both for understanding the mineralogy of lacustrine sediments, how the mineralogy varies with location in a lacustrine environment, and for validating the use of certain techniques such as visible-near-infrared spectroscopy (VNIR). In this study, sediments from the Pilot Valley paleolake basin of the Great Salt Lake desert were characterized using VNIR as an analog for Martian paleolake basins. The spectra and subsequent interpretations were then compared to mineralogical characterization by ground truth methods, including X-ray diffraction (XRD), automated scanning electron microscopy (QEMSCAN), and several geochemical analysis techniques. In general, there is good agreement between

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2Graduate student, primary researcher/author and author for correspondence

3 Co-author provided access to VNIR equipment and contributed to data processing, analysis, and manuscript preparation.

4 Co-author provided access to chloride anion and oxyanion spectra and contributed to manuscript preparation

5 Co-author provided expert input to XRD analysis and contributed to manuscript preparation.

6 Co-authors provided field equipment, field support, access to field site and provided feedback to manuscript preparation

7 Co-author conducted chlorate and perchlorate analysis and provided feedback to manuscript preparation
VNIR and ground truth methods on the major classes of minerals present in the lake sediments and VNIR spectra can also easily discriminate between clay-dominated and salt-dominated lacustrine terrains within the paleolake basin. However, detection of more detailed mineralogy is difficult with VNIR spectra alone as some minerals can dominate the spectra even at very low abundances. At this site, the VNIR spectra are dominated by absorption bands that are most consistent with gypsum and smectites, though the ground truth methods reveal more diverse mineral assemblages that include a variety of sulfates, primary and secondary phyllosilicates, carbonates and chlorides. This study provides insight into the limitations regarding the use of VNIR in characterizing complex mineral assemblages inherent in lacustrine settings.

2.2 Introduction

Martian paleolake basins are prime habitability targets for future Mars rover missions [Grotzinger et al., 2014; John F. Mustard et al., 2013]. Life on Earth most likely began in the deep Hadean ocean, where there were significant geochemical gradients for energy, substrate catalysts for early biosynthesis, and sufficient protection from surface radiation and oxidation by superoxides [Abramov and Mojzsis, 2009; Russell et al., 2014]. While it is unclear whether or not Mars ever hosted a large ocean [Di Achille and Hynek, 2010], Martian paleolakes are an excellent proxy for a Hadean-like origin environment. The majority of terrestrial paleolakes transitioned to modern day evaporite basins due to climate change and exhibit clay, sulfate, and chloride compositions similar to the aqueous minerals identified across the Martian surface. On Earth, they are known to harbor a diverse array of microbial life, and enhance the preservation of organic matter and fossils. Therefore, these terrestrial systems are considered excellent analogs for habitability studies that will be useful for identifying and exploring lacustrine systems on Mars.

Terrestrial analog studies are especially important for interpreting mineralogy from spectroscopy on other planets as they provide examples of natural, complex mineral assemblages for planetary data analyses that otherwise would solely rely on comparisons to pure mineral end members and laboratory mixtures for data analyses [Baldridge et al., 2004; Cloutis et al., 2010; Ehlmann et al., 2012; Marcucci et al., 2013;
In this study, we compare mineralogical assemblages in lacustrine sediments from the Pilot Valley sub-basin of the Great Salt Lake Desert, derived from visible to near-infrared spectroscopy (VNIR) to those derived from ground truth methods such as X-ray diffraction (XRD) and automated scanning electron microscopy (QEMSCAN) in an effort to gain contextual insight of mineral assemblages deposited in closed basin paleolakes, and to evaluate the efficacy of VNIR spectroscopy as a tool for the identification and characterization of paleolake surfaces.

2.2.1 Background: Lacustrine Environments on Mars

The identification of numerous hydrous mineral-bearing deposits on the Martian surface supports the hypothesis that Mars once had an abundance of liquid water interacting with the crust during its early history [Bishop et al., 2008b; Ehlmann et al., 2009; Glotch et al., 2010; Mustard et al., 2008; Wray et al., 2009; Wray et al., 2011]. The geological context of some of these deposits suggest the presence of large volumes of water at the surface, based on their association with valley networks, outflow channels and, most significantly, basins that likely once hosted deep-water lakes [Grant et al., 2008; Wray et al., 2011].

Evidence for these deep-water paleolakes has been developing for several decades. Goldspiel & Squyres [1991] provided some of the earliest detailed investigations of Martian lake features using Viking and Mariner 9 imagery. They identified and mapped 36 potential paleolake basins based on the morphology of valley networks. Their estimates, based on fluvial transport of eroded material, show sediment basin fill depths from as low as 38 meters (125 feet) thick to as high as 780 meters (2559 feet) thick. Cabrol & Grin [1999] suggested the first classification system for Martian paleolakes located specifically in impact craters based on morphological data from global Viking orbital imagery, and developed the first catalog consisting of over 179 possible lacustrine systems. Fassett & Head [2008] expanded upon this catalog, utilizing imaging data from the High Resolution Stereo Camera (HRSC) on ESA’s Mars Express and topography data from Mars Orbiter Laser Altimeter (MOLA) from Mars Global Surveyor. Based on the water depth that would be required to overflow each of the 210 proposed lakes, the vast majority of targets in that study had maximum depths between 10 and 500 meters. They also calculated the lake volume-to-watershed area...
ratio and determined that a small portion of lakes had watershed areas that were too small to fill the lake volume, suggesting that these lakes relied on regional groundwater upwelling. At least some of these postulated deep-water paleolakes may have been periodically active throughout Martian geologic history. Age estimates based on crater counts by Cabrol and Grin suggest that the craters that host most of the paleolakes date to the mid-to-late Noachian, however they estimated that the age of the sedimentary deposits in most of the crater lakes extended into the early Amazonian [Cabrol and Grin, 1999; Fassett and Head, 2008; Grotzinger et al., 2014].

The extended age of the crater sediments and the noted evidence of groundwater influence on lake volume are both consistent with studies that have hypothesized that the Martian climate shifted from surface-dominated aqueous activity to groundwater-dominated aqueous activity during the late Noachian to Early Hesperian transition, but that occasional episodes of surface flow and/or groundwater upwelling continued into the early Amazonian [Buhler et al., 2011; Komatsu et al., 2009; Michalski et al., 2013; Wray et al., 2011]. During this hypothesized transition, the gradual waning of fluvial input to these proposed paleolakes (especially in craters) would have caused the lake systems to transition from deep lacustrine systems to groundwater dominated playa basins partially filled by lacustrine, and likely hypersaline, sediments. The Mars Science Laboratory Curiosity rover is currently investigating this hypothesis in Gale Crater, a Noachian crater partially filled by a 5 km mound of sediments [R B Anderson and Bell III, 2010; Milliken et al., 2010]. Curiosity landed on a large alluvial fan that feeds into one of the lowest elevations in the crater, where the rover identified clear evidence for long-term fluvial and shallow lacustrine activity [Grotzinger et al., 2014]. However, possible deltaic and shoreline landforms at higher elevations suggest that maximum lake depths in the crater may have been hundreds of km [Dietrich et al., 2014]. It has been hypothesized that the transition from clays to stratigraphically higher sulfates within the Gale Crater mound is related to Noachian-Hesperian climate change, but the relationship is between the mineralogy of the mound and the lacustrine history of Gale Crater is currently unclear.

On Earth, numerous examples of this climate-change-related lacustrine-to-playa transition are present, as many large paleolakes from the last ice age (primarily
freshwater/brackish lakes from the late Pleistocene/early Holocene Boundary) have gradually transitioned to modern day hypersaline playas. Well-studied examples include the Chott el Gharsa of Northern Africa, the Salar de Uyni of Bolivia, Death Valley in southern California, and the Great Salt Lake Desert in northwestern Utah [Barbieri and Stivaletta, 2012; Currey, 1990; Douglas, 2004; Fornari et al., 2001]. These studies have shown that during this transition from a wet to a dry environment, fresh water levels dropped, ions became more concentrated and as the developing brines reached saturation levels, carbonate, sulfate and chloride minerals would begin to form, respectively. Thus halophiles and other extremophilic microorganisms could have replaced microbial life that dominated the water column and sediments. These changing microbial communities have left behind mineral and morphological biosignatures as microbes influenced the precipitation of and/or become entrained in evaporite minerals [Barbieri et al., 2006; Douglas and Yang, 2002]. As planetary analogs, these terrestrial environments provide the opportunity to study how this transition from humid to arid affects the mineralogy, geochemistry, and sedimentology of closed basin systems, and in addition, to study how climate change on Mars may have affected the habitability and preservation potential of Martian paleolakes.

2.2.2 Motivation

Though several possible paleolakes have been identified morphologically on Mars, some difficulty remains in confirming that these environments are also mineralogically consistent with lacustrine sediments. VNIR spectrometers that have been used to investigate the mineralogy of possible paleolakes on Mars include the Compact Reconnaissance Imaging Spectrometer for Mars (CRISM) onboard the Mars Reconnaissance Orbiter [Murchie et al., 2007] and the Observatoire pour la Mineralogie, L'Eau, les Glaces et l'Activité (OMEGA) instrument onboard Mars Express [Bibring et al., 2004]. Analysis of CRISM spectra, coupled with observations with the Thermal Emission Imaging System (THEMIS) onboard Mars Odyssey [Christensen et al., 2001] has revealed both smectites and chlorides in association with low, flat intercrater plains and some crater basins in the southern highlands, and interbedded phyllosilicate-sulfate deposits have been detected in highland craters such as Columbus crater [Murchie et al., 2009; M. M. Osterloo et al., 2008]. However, more complex
mineralogies than these are generally expected in paleolake/playa systems, often including a combination of sulfates, phyllosilicates, carbonates, chlorides, other salts and ancillary minerals. The lack of detection of these complex mineral assemblages could be due to low spatial resolution (18-36 meters/pixel for CRISM and 0.3 to 5 km/pixel for OMEGA), burial of key minerals, spectral dominance by some minerals over others (non-linear mixing), or simply their absence [Baldridge et al., 2009; Clark, 1999]. For example, Osterloo et al. [2010] suggest that the lack of detection, using OMEGA, of phyllosilicate or sulfate deposits near chloride-bearing materials is possibly a result of low spatial resolution since these materials were detected in association with each other using CRISM in a separate study. Wray et al. [2011] hypothesize that the lack of chlorides within Columbus Crater and other intracrater deposits in Terra Sirenum may be due to burial by younger sediments, dissolution during later aqueous or diagenetic periods, or simply their absence. They do point out, however, that occurrence of an unknown poly-hydrated sulfate could also be a hydrated chloride salt, a consideration that is strongly supported by recent laboratory work on chloride hydrates [Hanley et al., 2014; Hanley et al., 2015].

Terrestrial analog studies have been used to supplement VNIR spectral interpretation of multiple environments on Mars (e.g. Ehlmann et al. [2012]; Marcucci et al. [2013]; Cloutis et al. [2010]). However, studies of paleolake minerals, specifically evaporites in terrestrial analogs are rare. Crowley [1991] conducted a comprehensive laboratory study of evaporite minerals that identified characteristic and diagnostic VNIR spectral features of many typical evaporite minerals. That study was followed by the first VNIR remote sensing investigation of evaporites in Death Valley, using data from the Airborne Visible/Infrared Imaging Spectrometer (AVIRIS), which indicated that some evaporite mineral assemblages were possible to identify and map (with noted limitations such as lack of reference spectra, mineral mixing effects, and low signal conditions) and that further study in other environments was needed [Crowley, 1993]. Kodikara et al. [2012] studied remote sensing of evaporites from Lake Magadi, Kenya, using spaceborne hyperspectral Hyperion imagery from the Earth Observing 1 (EO-1) satellite and cited similar difficulties in mapping of evaporite minerals as the Death Valley study. Stivaletta et al. [2013] conducted in situ VNIR analysis of evaporite deposits from
gypsiferous spring mounds in southern Tunisia. In their study, they determined that VNIR spectra did not reveal signatures attributed to any phyllosilicates or carbonates present in their samples due to low abundance and/or spectral masking due to the presence of gypsum. Though this analysis was extensive, their study focused on a specific type of evaporite material, as their site did not represent the diversity of mineral assemblages commonly found in paleolake basins. Thus, further work is needed to characterize the VNIR spectral properties of paleolake sediments and evaporites.

In this study, we present the Great Salt Lake Desert (GSLD) as a valuable terrestrial analog for studying the mineralogy and astrobiological potential of Martian paleolake basins, and in particular, on the efficacy of VNIR spectroscopy of paleolake surfaces as a tool for evaluating their geologic history and habitability potential. Thus, here we present the results of our comparison of the derived mineralogy of sediments from one region of the GSLD, the Pilot Valley Basin, using VNIR spectroscopy compared to quantitative laboratory methods.

2.3 Geologic Setting

This section depicts the detailed geological setting of the ancient Lake Bonneville Basin and the field site: Pilot Valley.

2.3.1 Ancient Lake Bonneville and the Modern Great Salt Lake Desert

The Great Salt Lake Desert (GSLD) is one of two remnants of the ancient Lake Bonneville (Figure 2.1). Bonneville was the largest of several North American paleolakes from the Pleistocene Epoch, covered about 51,000 square kilometers of western Utah and smaller sections of eastern Nevada and southern Idaho, and reached a maximum depth of ~300 meters. Bonneville formed as a freshwater lake from river inflow and direct precipitation. The lake began sometime between 30 and 32 ka and was sustained at various depths until about 14 ka, when lake levels began a sharp decline to the modern playa system of the GSLD basin and the Great Salt Lake. [DeRito and Madsen, 2008; Madsen et al., 2001; Spencer et al., 1984; Wilkerson, 2012].

The modern GSLD, located in the Basin and Range physiographic province of North America, extends from the western edge of the Great Salt Lake into the eastern edge of the Nevada state border and is bifurcated into north/south sections by
Interstate-80. The GSLD is classified as an arid desert with temperatures ranging from 44°C in the summer to 7°C in the winter and an average annual precipitation of less than 150 mm/yr. [Kottek et al., 2006; WRCC, 2013]. The desert basin formed within narrow grabens that were created by faulting that initiated in the middle of the Miocene (~ 15 Mya). Currently the average basin sediment fill is more than 1500 m thick. The composition of the fill is mostly sedimentary and evaporite deposits of fluvial or lacustrine origin, and the majority of the basin deposits stem from lake cycles that pre-date Lake Bonneville by up to 800 ka [Rey, 2012]. Though it looks like a singular basin, the GSLD encompasses multiple enclosed sub-basins, largest of which are the Bonneville Salt Flats, Pilot Valley and the Newfoundland basins [Jones et al., 2009], as shown in Figure 2.1. Typical of the Basin and Range Province, the subbasins are each

Figure 2.1. Map of the Great Salt Lake Desert and adjoining Great Salt Lake. The red line depicts Interstate 80 that bifurcates the Great Salt Lake Desert.
flanked by multiple mountain ranges that serve as the primary sources of water and sediment run-off into each sub-basin. Both the Newfoundland and Bonneville basins have undergone significant anthropogenic alteration, which has notably changed the brine chemistry and mineralogy of the Newfoundland Basin and has caused significant salt loss in the Bonneville basin [Jones et al., 2009; Mason and Kipp, 1997]. The more isolated Pilot Valley has remained relatively untouched, and thus is the focus of this investigation.

2.3.2 Field Setting – Pilot Valley

The Pilot Valley basin (Figure 2.2) has a long axis running from northeast to southwest, lies on the western edge of the GSLD and crosses over to the Nevada border on its most southwestern edge. The basin is approximately 33 km in length and 8-15 km wide with an average basin elevation of 1300 m above sea level (asl). The topographic center of the basin is located in the northwestern corner. The Pilot Mountain Range borders the basin on its western edge and the Silver Island Range borders the eastern edge as shown in Figure 2.2. The Silver Island Range reaches a peak elevation of approximately 2300 m asl at its highest point, Graham Peak, and is primarily composed of limestone, shale, and dolomite of Paleozoic age, with lesser amounts of Mesozoic granitoid rocks. The Pilot Range peaks at a considerably higher elevation, measuring over 3200 m asl at Pilot Peak, and is mainly composed of metamorphic quartzite, schist and phyllite, with smaller amounts of granitoid rocks scattered throughout the range as dikes and other small bodies. Both mountain ranges host extensive alluvial fan deposits adjacent to the basin boundary. On the western slope, the alluvial/basin boundary is rather abrupt as the playa sediments lie directly next to the alluvium. The transition between the playa sediments and the fans is more gradual on the eastern slope as the fans transition from mountain-front alluvial deposits to a thin alluvial covering on lacustrine pediment and local fan deposits interlaced with lacustrine deposits. The shorelines from the four major Bonneville Lake sequences are evident throughout the valley and appear as wave-cut terraces, in particular along the Silver Island Range [Lines, 1979; Miller et al., 1987; Rey, 2012].

As an endorheic basin, Pilot Valley has no permanent surface inflows or outflows, and the subsurface hydrology of the basin consists of three distinct aquifers. The first is
an alluvial fan aquifer, consisting of fresh to brackish water, present within the alluvial fans alongside each of the flanking mountain ranges. The second aquifer is a deep basin-fill brine aquifer that underlies the entire basin at a depth of ~30 m. The final aquifer is a shallow brine aquifer that encompasses the upper ~6 m of the basin sediment fill. Hydrological connectivity is very limited between the deep basin-fill aquifer and the shallow brine aquifer, although occasional loss of fluid from the shallow brine aquifer to the basin-fill aquifer and, conversely, occasional upwelling and recharge from the basin-fill aquifer to the shallow brine aquifer have both been suggested [Mason and Kipp, 1997]. Though the playa is recharged by some direct precipitation on the surface, the shallow-brine aquifer is primarily maintained by groundwater flow from mountain...
front recharge of the alluvial aquifer flanking the Silver Island Range [Carling et al., 2012]. The alluvial aquifer on the western playa flank is also maintained by mountain front recharge from the Pilot Range, but has minimal hydrological connectivity to the playa. Due to the frequency of recharge, the Pilot Valley sediments remain consistently moist throughout the basin, although they are clearly more saturated at the center of the basin where the water table is effectively just below the surface and can briefly flood after major rainstorms. The only loss mechanism from the Pilot Valley basin is capillary wicking and evaporation from the playa surface [Lines, 1979].

Pilot Valley exhibits classic closed-basin evaporite zonation due to differences in mineral solubilities that initially developed during the last regressive phase of Lake Bonneville, between 8,000 and 10,000 years ago [Eardley et al., 1957; C B Hunt, 1975; Lines, 1979]. The least soluble minerals, mainly carbonates, were deposited around the rim of the basin or are buried in the lower layers of the lacustrine sediments, in deposits that are described as a carbonate mud and occur as a soft, puffy surface or a hard compact surface. In the next zone, sulfates, specifically gypsum, intermix with the carbonate mud. Finally, within the hard "salt" pan at the topographic center of the basin, highly soluble chloride and Mg-sulfate salts are deposited. Extensive microbial mats are observed starting in the sulfate zone and propagating almost to the center of the basin. Due to continual reworking, mainly from episodic fluvial activity, all of the deposits are interbedded with mud layers originating from lower sedimentary deposits or clastic input [Lines, 1979].

The full stratigraphy of the 1600+ m of sediment in the Pilot Valley basin has been investigated by proxy through analysis of economic cores taken from the Bonneville Salt Flats. The lower segment of the Bonneville basin (> 300 m depth) is primarily filled with extrusive volcanic rocks of Tertiary age whereas the upper segment of the Bonneville basin is filled with sedimentary rocks of quaternary age [Lines, 1979]. A detailed analysis of the shallow stratigraphy in Pilot Valley conducted by Rey [2012] showed that of the upper 300 m of sediment fill, only the top 4 m represent the Lake Bonneville sequence. Hence, Pilot Valley is also an excellent model for multiple lake sequences within a single closed basin as similarly episodic and complex lake
sequences on Mars could have produced the observed lacustrine minerals during the tumultuous wet past.

2.4 Methods

This section outlines the sample collection and analysis methods for this study.

2.4.1 Field Sampling

Field samples were obtained during three separate field campaigns conducted in June and September 2010 (EX1 and EX2), and May 2012 (EX3). Our field sampling methodology was designed to assess the geochemical and mineralogical diversity along

![Figure 2.3. Field Sampling in Pilot Valley.](image)

(a) Core recovery tripod set-up. Arrow shows surface spectroscopy sample proximity to sample core. (b) Deep core sampling with the AMS extendible core sampler. (c) Shallow core sampling with generic garden bulb planter. (d) Recovered core. Plastic casing sleeve is cut away for core processing and sample collection. (e) Surface spectroscopy samples.
the expected evaporation gradient of the closed basin system. Therefore, sediment and aquifer fluid samples in all three campaigns were collected along the same defined horizontal transect from the basin rim to the topographic center of the basin, as shown in Figure 2.2. Deep cores (2.5 m) were obtained during EX3 using a recovery tripod (Figure 2.3a), and an AMS Extendible Core Sampler (Figure 2.3b), which retrieves 5 cm diameter cores up to 60 cm long. Shallow cores from EX1 and EX2 were obtained using a simple bulb planter, as shown in Figure 2.3c. This allowed extraction of ~50 cm cores. Surface samples for spectroscopy were also taken during EX3 using 5cm diameter PVC collectors. The collectors were driven into the playa surface and then dug out with a shovel to preserve the surface for spectral analysis, as shown in Figure 2.3e.

Table 2.1. Sample Identification & Depth

<table>
<thead>
<tr>
<th>Site ID</th>
<th>Core ID</th>
<th>Depth (cm)</th>
<th>Expedition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-45</td>
<td>EX1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0-45</td>
<td>EX2</td>
<td></td>
</tr>
<tr>
<td>PV-1</td>
<td>3(^b)</td>
<td>0-45</td>
<td>EX2</td>
</tr>
<tr>
<td></td>
<td>4A</td>
<td>0-10</td>
<td>EX3</td>
</tr>
<tr>
<td></td>
<td>4B</td>
<td>10-48</td>
<td>EX3</td>
</tr>
<tr>
<td>PV-2</td>
<td>A(^b)</td>
<td>0-13</td>
<td>EX3</td>
</tr>
<tr>
<td></td>
<td>B(^b)</td>
<td>13-22</td>
<td>EX3</td>
</tr>
<tr>
<td></td>
<td>C(^b)</td>
<td>22-28</td>
<td>EX3</td>
</tr>
<tr>
<td>PV-3</td>
<td>A(^b)</td>
<td>0-17</td>
<td>EX3</td>
</tr>
<tr>
<td></td>
<td>B(^b)</td>
<td>17-32</td>
<td>EX3</td>
</tr>
<tr>
<td>PV-4</td>
<td></td>
<td>0-45</td>
<td>EX3</td>
</tr>
<tr>
<td>PV-5</td>
<td>A</td>
<td>0-8</td>
<td>EX3</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>8-16</td>
<td>EX3</td>
</tr>
<tr>
<td></td>
<td>C(^b)</td>
<td>16-33</td>
<td>EX3</td>
</tr>
</tbody>
</table>

\(^a\)All surface spectroscopy samples were taken adjacent to all cores from EX3. Spectroscopy samples are referenced by Site ID through the remainder of the text. \(^b\)Samples selected for QEMSCAN Analysis. Core sections in PV-5 that did not undergo QEMSCAN analysis did not yield enough material in the clay fraction.
Although core depths during EX3 penetrated down to 2.5 meters, for the purpose of this particular study, only the upper ~50 cm were analyzed with all the techniques defined, under the premise that near future NASA in situ robotic missions conducting mineralogical analysis on Mars would not have the ability to access depths greater than a few tens of cm, though the 2018 ESA ExoMars drill is designed to reach up to 2 meters in depth of core recovery [Magnani et al., 2010; J.F. Mustard et al., 2013]. Sediments from coring samples were collected and stored on CO$_2$ ice in the field and frozen at -20°C in the lab. Surface spectroscopy samples were stored at room temperature prior to acquisition of spectra. Aquifer fluids were filtered to 0.2 µm and stored on H$_2$O ice in the field and refrigerated at -4°C in the lab. Deep core sample sediments from EX3 were separated into "sub-core" vertical layers based on a visual observation of lithology changes. All shallow cores and sub-cores were homogenized prior to sample collection. Table 2.1 lists all samples and their associated location and depth. The surface spectroscopy samples were obtained adjacent to the core samples and are simply identified henceforth by the site ID.

2.4.2 X-ray Diffraction

Qualitative XRD was conducted to determine the composition of salts, confirm the presence of minerals detected in QEMSCAN, and distinguish between chemically identical mineral phases seen along the transect. X-ray diffraction (XRD) measurements were made using a Scintag XDS 2000 theta/theta goniometer with 2.2 kW sealed copper X-ray source. Sediment samples were dried at room temperature for several days and then ground with an agate mortar and pestle. A subset of samples was used for phyllosilicate detection and the ≤3 µm fraction of sediment was extracted from each of those samples, washed of all salts and glycolated for ~36 hours prior to analysis. Measurements were made over a 2θ range of 2° to 70° at a continuous scan rate equated to 2° per minute for general scans, and over a 2θ range of 2° to 40° at 1° per minute for focused phyllosilicate scans. XRD patterns were collected and analyzed using the Scintag-DMSNT software. Qualitative analysis was done after K-α2 stripping and three-point boxcar smoothing of the patterns. Mineralogical fits were determined using the International Center for Diffraction Data (ICDD) embedded database and by comparing d-spacing to published values [Moore and Reynolds, 1997].
2.4.3 Automated Scanning Electron Microscopy

QEMSCAN was used in this study for specific identification of the phyllosilicates and carbonates in the core samples. QEMSCAN is an automated microanalysis technique that can provide mineralogical and petrographic composition of both thin sections of solid rocks and epoxy-mounted grains. To achieve instrument and economic efficiency, the ≤3 µm fraction was extracted from each of the sediment samples for analysis. Prior to sediment fractionation, samples were repeatedly washed with MilliQ water and then centrifuged at 6000 rpm for 3 minutes to remove all salt ions and allow for full dispersion of clay-sized particles. The samples were then centrifuged at 1000 rpm to suspend all particles that were ≤3 µm. The collected fluids were dried at 90°C overnight to remove the water and recover the clay-size fraction of sediment. Each sample was split into representative aliquots using a rotary micro-riffler. Sized graphite was added to mitigate particle agglomeration, preferred orientation and settling. Subsequently, all samples were mounted in a 30-mm block with epoxy-resin and left to cure. The blocks were ground and polished to obtain a flat surface for X-ray analysis and carbon coated to establish an electrically conductive surface. The samples were loaded into the QEMSCAN instrument equipped with a backscatter electron detector and four energy-dispersive X-ray (EDX) spectrometers. With an approximate beam size of 1µm, a beam stepping interval (i.e., spacing between acquisition points) of 5µm, an accelerating voltage of 25 keV, and a beam current of 5 nA, backscattered electrons (BSE) and X-rays were collected automatically. Minerals or phases were identified based on the BSE value and X-ray emission spectra. QEMSCAN determines mineralogical composition based on chemistry instead of structure. Hence two chemically similar yet structurally different minerals (e.g., calcite and aragonite, or illite and muscovite) cannot be differentiated in QEMSCAN. Bulk mineralogy was determined using the Intellection iDiscover™ analytical suite. The beam-surface interactions were modeled using Monte Carlo simulation and the mineral phases were assigned based on a local database. For this study, the QEMSCAN spectra and BSE values were collected and averaged for 5 random 3X3 mm areas across the sample surface.
2.4.4 Standard Geochemical Analysis (IC and ICP-OES)

To characterize the modern aquifer brines, major anions were measured using a Dionex ICS-90 ion chromatography system running an AS14A (4 x 250 mm) column. Major cations were also measured using a Perkin-Elmer Optima 5300 DV Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). All sediment samples were extracted for IC and ICP-OES analysis following the Florida Department of Environmental Protection method #NU-044-3.12. All fluid samples were filtered and prepared in the field as stated in Section 3.1. All ICP samples were acidified with trace-metal grade nitric acid as per standard procedure to ensure mobilization of all metal cations.

2.4.5 Chlorate and Perchlorate Analysis

Perchlorate (ClO$_4^-$) and chlorate (ClO$_3^-$) were measured by sequential ion chromatography-mass spectroscopy/mass spectroscopy (IC-MS/MS). Both anions were quantified using a Dionex LC 20 ion chromatography system consisting of GP50 pump, CD25 conductivity detector, AS40 automated sampler and Dionex IonPac AS16 (250 X 2 mm) analytical column. The IC system is coupled with an Applied Biosystems – MDS SCIEX API 2000™ triple quadrupole mass spectrometer equipped with a Turbo-IonSpray™ source. A hydroxide (NaOH) eluent at 0.3 mL min$^{-1}$ was followed by 90% acetonitrile (0.3 mL min$^{-1}$) as a post-column solvent. To overcome matrix effects, all samples were spiked with Cl$^{18}$O$_4^-$ or Cl$^{18}$O$_3^-$ internal standard. Samples were analyzed in batches of 8 including an analytical duplicate and spike. Samples with elevated Cl$^-$ (>10,000 mg/L) or SO$_4^-$ (>1000 mg/L) were either diluted prior to analysis or in some cases pre-cleaned using On-GuardTM II Ag or Ba cartridges (Dionex).

2.4.6 Visible and Near-infrared Spectroscopy (VNIR)

Visible and near infrared (VNIR) spectra of the EX3 surface samples were collected using a lab mounted FieldspecPro 3 Portable Spectroradiometer developed by Analytical Spectral Devices. The FieldspecPro 3 has a spectral range of 350-2500 nm and spectral resolutions of 3 nm in the 350-700 nm range and 10 nm in the 1400-2100 nm range. All spectra were acquired at incidence and emission angle of 0° and 30°, respectively. Three surface samples were taken from each sample site along the
transect and spectra were collected from three random spots on each sample. To mimic aeolian abrasion and the removal of loose surface fines, one surface sample from each collection site was abraded (Figure 2.4) using a commercial compressed gas duster (similar to those used for computer keyboards) and then spectra were collected again in the same manner as the non-abraded samples. Spectra were analyzed for the presence of absorption bands by using the calculated band depths shown in Table 2.2.

Table 2.2. Band Depth Spectral Indices used for VNIR analysis.

<table>
<thead>
<tr>
<th>Spectral Index</th>
<th>Left Shoulder</th>
<th>Center</th>
<th>Right Shoulder</th>
<th>Possible Corresponding Minerals</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD670</td>
<td>0.65</td>
<td>0.675</td>
<td>0.72</td>
<td>Oxygenic Photosynthetic Pigments&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BD1750</td>
<td>1.68</td>
<td>1.75</td>
<td>1.84</td>
<td>Gypsum, Kieserite</td>
</tr>
<tr>
<td>BD2210</td>
<td>2.14</td>
<td>2.21</td>
<td>2.24</td>
<td>Montmorillonite, Gypsum, Kaolinite, Illite/Muscovite, Silica</td>
</tr>
<tr>
<td>BD2310</td>
<td>2.27</td>
<td>2.31</td>
<td>2.33</td>
<td>Mg-Smectites, Dolomite, Aragonite, Illite/Muscovite</td>
</tr>
<tr>
<td>BD1940</td>
<td>1.85</td>
<td>1.94</td>
<td>2.07</td>
<td>Absorbed/Adsorbed Water, Hydrated Minerals</td>
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<tr>
<td>BD2400</td>
<td>2.35</td>
<td>2.4</td>
<td>2.5</td>
<td>Gypsum, Kieserite, other sulfates, Mg-chlorides, Mg-perchlorates, Mg-chlorates, Na-perchlorates, Illite/Muscovite</td>
</tr>
</tbody>
</table>

<sup>a</sup>From photosynthetic microorganisms

Band depth is defined as the percentage difference between the actual value of the spectrum at the band minimum and the value predicted by the continuum, which in this case is modeled as a linear fit between two points outside of the absorption band [Clark, 1981]. In most cases, band depths above 1-2% are considered detections. The band depth spectral indices calculated were specifically chosen so that they would not have overlap with other major absorption features. The one exception is the 2.27 μm shoulder of the 2.31 μm band depth spectral index, which could possibly overlap with the 2.27 μm gypsum absorption feature (Figure 2.5). However, our spectra do not show evidence for co-occurrence of these two features, so we do not anticipate any complications from this overlap in our analyses. Mineral identifications were made by visual comparison of spectral shape and band location with reference spectra from the
PDS Geoscience Spectral Library [Guinness et al., 1996], and the USGS Digital Spectral Library [Clark et al., 2007]. Since minerals in nature do not form as pure end-members, there are known effects such as grain size, mineral mixtures, structure, and composition that can complicate and limit mineral identifications in VNIR. Grain size effects are fairly well understood as larger grain sizes can produce deeper absorption bands, whereas the larger surface area of fine grains produces more reflectance and as a result they tend to dominate mixtures with various grain sizes [Clark, 1999; Crowley, 1991; Horgan et al., 2014]. Spectra can also be influenced by both areal and intimate mixtures of different minerals. Areal mixtures occur where materials are optically separated such that there is no scattering between components and the resulting spectra is simply a linear combination of the various materials. Intimate mixtures are composed of different minerals in close (intimate) contact, such as soil grains, that result in non-linear combinations of of the resulting spectra [Clark, 1999]. Spectral identification can also be complicated by mixtures of minerals with similar or overlapping absorption bands (Figure 2.5) as such we employed additional metrics to differentiate the identification of minerals with shared bands. Due to the limited penetration depth of VNIR spectroscopy, we recognize that the mineral phases identified are based on the composition of the upper few microns of each sample. Hence, there may not be total agreement between spectral analysis and bulk analysis with other techniques, though this is also the case for Mars.
2.5 Results

This section outlines the ground truth and VNIR results of this study.

2.5.1 Mineralogical Determination from Laboratory and Ground Truth Analysis

This section outlines the results of the ground truth measurements taken at Pilot Valley.

2.5.1.1 QEMSCAN

Semi-quantitative results were output by QEMSCAN software as a modal abundance in volume % (Figure 2.6a) and a calculated mass % based on an internal instrument density database (Table A1). A compositional map of the particles per sample area scanned was generated as shown in Figure 2.6b. The modal volume % and mass % values are only for the clay fraction (<3 μm) of Pilot Valley sediments. The key phyllosilicate groups identified in the upper 50 cm of Pilot Valley sediments are kaolinite, chlorite, and illite/muscovite. The data also indicate small traces of an illite-smectite-montmorillonite mixed layer clay. The key carbonates identified are calcite/aragonite, dolomite, and a small amount of a magnesium-iron-bearing calcite. The highest abundance of carbonates is seen at the center of the basin (PV-5) where
carbonates comprise almost 56% of the clay-fraction. Conversely, phyllosilicates are most abundant near the rim of the basin (PV-1), where they comprise over 73% of the clay-fraction. An inversion point occurs at PV-2, where both carbonates and phyllosilicates appear to have approximately equal abundances, as shown in Figure 2.6a. The ancillary minerals, such as quartz, K-feldspar and pyroxene, are seen throughout the sample transect. However, no clear gradient exists for these minerals. Salts were removed during sample preparation and therefore were not detected by QEMSCAN.

Figure 2.6. QEMSCAN output for Pilot Valley. (a) Figure shows the average modal mineral abundance of each sample site in volume %. (b) Compositional map of a 3x3 mm area scanned on sample PV-1. The colors in the compositional map correspond to the minerals in Figure 6a. The white area corresponds to the epoxy resin mount and is excluded from analysis.
Standard analysis of the samples from outer basin site PV-1 shows detection of halite, gypsum, muscovite, illite, quartz, calcite, dolomite, and a small amount of aragonite as shown in Table 2.3. Diagnostic peaks common to K-feldspars are also identified, but the specific phase cannot be determined. Scans of the glycolated PV-1 sediments reveal additional phyllosilicates – kaolinite, chlorite, and a small amount of what is likely an illite-smectite layered clay as shown in Figure 2.7a. Moving into the basin, standard scans of samples at PV-2 show clear detections of quartz, illite, muscovite, halite, and feldspar. Glycolated scans of the PV-2 sediments reveal the same diversity of phyllosilicates and carbonates as seen in PV-1 and small amounts of gypsum and halite are seen at various depths. The first considerable detections of gypsum occur at PV-3 as well as significant abundances of calcite and quartz as shown

<table>
<thead>
<tr>
<th></th>
<th>PV-1</th>
<th>PV-2</th>
<th>PV-3</th>
<th>PV-5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4A</td>
<td>4B</td>
<td>$\S1$-$3$</td>
<td>A</td>
</tr>
<tr>
<td>Gypsum</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓ ND</td>
</tr>
<tr>
<td>Halite</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>ND</td>
</tr>
<tr>
<td>Calcite</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Aragonite</td>
<td>ND</td>
<td>ND</td>
<td>✓</td>
<td>ND</td>
</tr>
<tr>
<td>Dolomite</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Smectite</td>
<td>✧</td>
<td>✧</td>
<td>✧</td>
<td>✧</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>✧</td>
<td>✧</td>
<td>✧</td>
<td>✧</td>
</tr>
<tr>
<td>Chlorite</td>
<td>✧</td>
<td>✧</td>
<td>✧</td>
<td>✧</td>
</tr>
<tr>
<td>Illite</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Muscovite</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Quartz</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Feldspar</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

The number of checkmarks corresponds to the intensity of peaks relative to the background noise in the XRD spectrogram and presents a qualitative estimation of abundance. ✔= Trace, ✔✔=Minor, ✔✔✔=Major. ND = no detection. ❌Salt concentrations interfered with detection of these minerals. § Selected samples scanned in raw form, then washed of salts, glycolated and re-scanned specifically for phyllosilicate detections and abundance estimates.

2.5.1.2 XRD

Standard analysis of the samples from outer basin site PV-1 shows detection of halite, gypsum, muscovite, illite, quartz, calcite, dolomite, and a small amount of aragonite as shown in Table 2.3. Diagnostic peaks common to K-feldspars are also identified, but the specific phase cannot be determined. Scans of the glycolated PV-1 sediments reveal additional phyllosilicates – kaolinite, chlorite, and a small amount of what is likely an illite-smectite layered clay as shown in Figure 2.7a. Moving into the basin, standard scans of samples at PV-2 show clear detections of quartz, illite, muscovite, halite, and feldspar. Glycolated scans of the PV-2 sediments reveal the same diversity of phyllosilicates and carbonates as seen in PV-1 and small amounts of gypsum and halite are seen at various depths. The first considerable detections of gypsum occur at PV-3 as well as significant abundances of calcite and quartz as shown
in Figure 2.7b. The shallow layers of PV-5 also show significant detections of gypsum with minor detections of halite, calcite and quartz. The bottom layer of PV-5 shows significant detections of halite, quartz, gypsum, illite, muscovite, and K-feldspar.
2.5.1.3 Geochemistry

Results from IC and ICP analysis indicate that Pilot Valley hosts a Na-K-Cl-SO$\textsubscript{4}$ enriched brine, as shown in Table 2.4, that maintains a circumneutral pH of 5.5-6. The lower calcium and magnesium concentrations are likely due to the formation of calcium- and magnesium-bearing carbonates and sulfates detected in the sediments by QEMSCAN and XRD. The high potassium composition of the aquifer fluids is consistent with the clastic potassium-feldspars identified by both XRD and QEMSCAN. The abundance of nitrate present in the Pilot Valley fluids is unusual when compared to other hypersaline systems [Oren, 2008], which suggests that some level of nitrification may be occurring. There is little difference in concentration across sample sites for all ions except for sulfate. A significant drop in sulfate concentration occurs from PV-2 to PV-3. This drop may signify the transition between the carbonate zone and the sulfate zone where gypsum precipitation would begin to occur, thus reducing the sulfate concentration; it could also indicate the transition to a more active microbial sulfate reduction zone. The Pilot Valley fluids were analyzed for perchlorate and it was not detected. However, low levels of chlorate and perchlorate were detected in the Pilot valley sediments as shown in Table 2.4.

<table>
<thead>
<tr>
<th>Sample Site</th>
<th>Ca (wt%)</th>
<th>K (wt%)</th>
<th>Mg (wt%)</th>
<th>Na (wt%)</th>
<th>Cl (wt%)</th>
<th>SO$\textsubscript{4}$ (wt%)</th>
<th>NO$\textsubscript{3}$ (mg/L)</th>
<th>$\textsuperscript{g}$ClO$\textsubscript{4}$ (ug/kg)</th>
<th>$\textsuperscript{g}$ClO$\textsubscript{3}$ (ug/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV-1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.1</td>
<td>1.35</td>
</tr>
<tr>
<td>PV-2</td>
<td>0.21</td>
<td>0.98</td>
<td>0.25</td>
<td>8.6</td>
<td>17.8</td>
<td>0.42</td>
<td>51</td>
<td>0.65</td>
<td>0.61</td>
</tr>
<tr>
<td>PV-3</td>
<td>0.26</td>
<td>1.4</td>
<td>0.31</td>
<td>8.8</td>
<td>19.2</td>
<td>0.27</td>
<td>67</td>
<td>0.39</td>
<td>0.63</td>
</tr>
<tr>
<td>PV-5</td>
<td>0.25</td>
<td>1.2</td>
<td>0.22</td>
<td>9.6</td>
<td>20.2</td>
<td>0.27</td>
<td>48</td>
<td>0.28</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Maximum allowable measurement error for both IC and ICP methods is 10%. ND = No data. $\textsuperscript{g}$Detected in Pilot Valley sediments only.
2.5.1.4 Summary of Ground Truth Mineralogy

Overall, both the QEMSCAN and XRD data indicate heterogeneous lacustrine sediments present in the upper layers of Pilot Valley. Both data sets are in agreement regarding the presence of major phyllosilicate groups and ancillary minerals and they collectively show dominance by the phyllosilicates in the outer basin with a shift to calcium sulfate and calcium carbonate dominance in the inner basin (Figures 2.6 and 2.7). XRD analysis also determined that both illite and muscovite are present at Pilot Valley. The quartz, plagioclase and K-feldspar and muscovite are clastic in origin from the surrounding mountain ranges [Miller et al., 1987; Rey, 2012] and the kaolinite, illite and smectite are likely the diagenetic products of these host minerals. The XRD data indicate that the main form of calcium carbonate detected is calcite with minor aragonite and dolomite in the upper sediment, which is consistent with the recent findings of Rey [2012]. The calcite and aragonite are authigenic in origin whereas the dolomite is most likely detrital. The major salt species detected by XRD are halite and gypsum. Given the brine chemistry of Pilot Valley, it is likely that other salts, such as magnesium sulfates, sylvite, polyhydrated chloride salts, and chlorine oxyanion salts, could form, though they were not definitively detected by any ground truth methods in this study.

2.5.2 Mineralogical Interpretation From VNIIR

This section outlines the detailed mineralogical interpretations of spectra at each sample site.

2.5.2.1 Spectra at Site PV-1

Site PV-1 is located on the eastern rim of the Pilot Valley basin and constitutes the first true sediments of the playa just inward from the inter-layered alluvial material and playa sediments. Spectra at PV-1 (Figure 2.8) show characteristic absorption bands near 1.45 μm and 1.94 μm due to both adsorbed water and hydrated minerals [G R Hunt, 1977]. Significant absorptions at 2.21 μm and 2.31 μm are likely due to Al-OH and Mg-OH combinations, respectively, though the band near 2.21 μm could also be attributed to a Cl-O combination, an S-O overtone, or an Si-OH combination [Bishop et al., 2008a; Cloutis et al., 2006; Hanley et al., 2014; Hanley et al., 2015; Rice et al., 2013]. These absorptions appear deeper in the abraded sample. A minor absorption at
1.19 μm is likely due to hydration, and a broad minor absorption at 1.75 μm may suggest the presence of a sulfate.

The 2.21 μm absorption band could be consistent with many minerals, but at PV-1 it is most consistent with an Al-bearing phyllosilicate such as kaolinite, mica, or montmorillonite. The narrow band at PV-1 is unlikely to be consistent with the Si-OH
overtone in silica, as these phases usually also exhibit an additional minor absorption at 2.26 \( \mu \text{m} \) that creates a characteristically broad absorption. Gypsum is also unlikely, as the gypsum signature in this region requires a second absorption at 2.27 \( \mu \text{m} \) to be diagnostic. It is also unlikely that this band is consistent with a Cl-O overtone that is indicative of chloride hydrate or chloride oxyanion salts as the 2.21 \( \mu \text{m} \) bands in these salts are either broad and shallow, or are present as shoulders on other absorptions. It should be noted, however, that relative absorption strengths of chloride hydrates and oxyanion salts have yet to be evaluated in controlled mixed mineral experiments.

The 2.31 \( \mu \text{m} \) band can be attributed to either Mg-OH or Fe(II)-OH bonds. However, because these spectra lack the characteristic red slope due to ferrous clays at shorter wavelengths, it is thus suggestive of several Mg-bearing minerals, including Mg-smectites, Mg-carbonates, and dolomite [Bishop et al., 2008a; Cloutis et al., 2006; Gaffey, 1986].

### 2.5.2.2 Spectra at Site PV-2

Spectra at PV-2 show significant absorptions at 2.21 \( \mu \text{m} \) and 2.31 \( \mu \text{m} \); like at PV-1, these are most likely consistent with Al-bearing and Mg-bearing phyllosilicates for the reasons outlined above in Section 2.4.2.1. In both PV-1 and PV-2, these absorption bands are deeper in the abraded samples. The 1.19 \( \mu \text{m} \) band is weaker in PV-2 than at PV-1, but the broad absorption at 1.75 \( \mu \text{m} \) is still present, and a broad absorption around 2.42 \( \mu \text{m} \) appears in some of the un-abraded samples, suggesting the presence of a local heterogeneously distributed mineral. Absorptions around 2.4 \( \mu \text{m} \) are usually attributed to the presence of sulfates but can also be due to Mg-bearing chloride salts, chlorine oxyanion salts, or micas such as illite and muscovite [Cloutis et al., 2006; Hanley et al., 2014; Hanley et al., 2015; G R Hunt, 1977]. This absorption could be due to gypsum but is more consistent with an Mg-sulfate, based on the lack of other diagnostic gypsum bands (as observed in samples discussed below). Furthermore, muscovite exhibits a corresponding absorption around 2.37 \( \mu \text{m} \) that is not seen in the spectra. Thus, we interpret this band to be due to Mg-sulfate, Mg-salt, or illite, and we conclude that there is no spectral evidence for the presence of muscovite. PV-2 also shows a minor absorption around 0.68 \( \mu \text{m} \) in the abraded sample that is likely
consistent with absorption by phototrophic microorganisms as discussed in section 2.5.4 [Wiggli et al., 1999].

2.5.2.3 Spectra at Site PV-3

PV-3 shows strong absorptions at 1.19 and 1.75 μm, as well as a diagnostic H₂O “triplet” at 1.45, 1.48, and 1.53 μm, and an S-O overtone doublet near 2.21 μm and 2.27 μm. All of these absorption bands together are consistent with gypsum [Cloutis et al., 2006]. A minor absorption at 2.31 μm persists in the abraded sample at PV-3, but it disappears entirely in the natural surface samples. The broad absorption around 2.42 μm is also present in PV-3 and the abraded sample also shows a stronger presumed phototrophic absorption at 0.68 μm than that seen in PV-2.

Figure 2.9. Trend plot of spectral variation along the transect. Spectra in black circles correspond to the band depths measured from spectra of the natural surface samples. Spectra in red circles correspond to band depths measured from spectra of the abraded samples. Dotted lines depict the average band depths and the vertical bars represent the calculated standard deviation for all measurements at each specific field site for each type of sample (natural vs. abraded). Star symbol represents spectral outlier shown in Figure 11 and discussed in section 5.5.
2.5.2.4 Spectra at Site PV-4

PV-4 shows the same characteristic absorptions consistent with gypsum, though not as strong as PV-3. The broad absorption around 2.42 µm occurs in both the natural and abraded samples, and the minor absorption at 0.68 µm is seen in the abraded sample. However, PV-4 displays more local heterogeneity than the other samples, with additional bands in some natural surface spectra at 1.78 µm, and one particularly interesting abraded surface spectrum with a host of additional bands near 1.17, 1.42, 1.72, 1.78, and 2.30 µm. Some of these bands could be consistent with bassanite, a partially dehydrated form of gypsum, while others could be consistent with Mg- or Ca-chlorate/chloride salts [Hanley et al., 2014; Hanley et al., 2015], as discussed in section 2.5.5.

2.5.2.5 Spectra at Site PV-5

Spectra at PV-5 show the strongest absorptions consistent with gypsum and the strongest absorptions around 2.42 µm. The strongest absorptions at 0.68 µm due to oxygenic photosynthesis are also seen in the abraded samples. The abraded sample at PV-5 also shows a significant absorption at 0.87 µm, which is most consistent with anoxygenic photosynthesis [Wiggli et al., 1999].

2.5.2.6 Spectral Trends

We have developed six spectral indices, identified in Table 2.2, to quantitatively analyze the presence and relative strength of key absorption bands present in our surface spectra. Using these spectral indices, we can assess spectral trends along the sample transect to refine our mineralogical assignments and compare these trends to those identified in QEMSCAN and XRD data. Figure 2.9 illustrates the mapping of the six target indices across the sample transect for both the natural surface spectra and the abraded surface spectra.

In Figure 2.9, the trend plot for the 1.75 µm band (BD1750) shows only shallow absorptions at PV-1 and PV-2 but very strong absorptions from PV-3 to PV-5. As BD1750 is principally diagnostic for gypsum [Cloutis et al., 2006], this most likely reflects gypsum abundance. The 1.94 µm band (BD1940) can either be caused by adsorbed/absorbed water in the sample or by structural water in hydrated minerals, especially hydrated sulfates like gypsum [G R Hunt, 1977]. Here, we are most likely
observing the influence of both wet samples (though stored at room temperature for an extended period prior to analysis, many samples retained water), and hydrated minerals. While the surface sediments get wetter closer to the center of the basin, the sharp increase in BD1940 at PV-3 (from ~30% to ~50% band depth) could also be due to increasing hydrated mineral abundance, namely gypsum. This is consistent with the sharp increase in BD1750 at this same location, which we interpret as due to high gypsum abundances.

The 2.4 μm band or shoulder (measured here by BD2400) is typically diagnostic of sulfates but can also be attributed to chloride salts and chlorine oxyanion salts, as stated in section 2.4.1.2. BD2400 is weak in PV-1 and PV-2 but is strong in PV-3 to PV-5, and it appears to generally correlate with BD1750, indicating gypsum. This suggests that gypsum is a major cause of the 2.4 μm absorption band; however, we cannot rule out a contribution from Mg-sulfates or Cl-bearing salts at this point.

The 2.21 μm band (BD2210) can be caused by Al-OH, Si-OH, and Cl-OH combinations, and as such, can indicate a variety of minerals, including Al-bearing phyllosilicates, gypsum, silica, and sodium perchlorate. In our spectra, BD2210 is detectable in PV-1 and PV-2 but also appears to correlate with BD1750 and BD2400 in the latter portion of the transect. This suggests that BD2210 is mainly influenced near the rim of the basin by the presence of an Al-bearing phyllosilicate, possibly kaolinite, illite, muscovite, or montmorillonite, whereas past PV-3, BD2210 is mainly influenced by the presence of gypsum.

The 2.31 μm band (BD2310) is indicative of Mg-smectites, Mg-carbonates, and dolomite [Bishop et al., 2008a; Cloutis et al., 2006; Gaffey, 1986]. Since our spectral region of study does not cover the additional diagnostic carbonate bands beyond 2.5 μm, we cannot distinguish between smectite and carbonate from spectra alone. BD2310 shows significant absorptions in PV-1 and PV-2 but disappears in PV-3 through PV-5, and it trends with the 2.21 μm band at PV-1 and PV-2, suggesting that the 2.21 and 2.31 μm bands may be caused by detrital minerals from a similar source exterior to the basin.

The 0.67 μm band is representative of oxygenic photosynthesis, and the spectra trend well with the observed microbial mats in the surface samples as well as with
Figure 2.10. Comparison plots of key spectral indices. Spectra in black correspond to natural surface samples. Spectra in red correspond to abraded samples. Star symbol represents spectral outlier shown in Figure 11 and discussed in section 5.5.
BD1750 and BD2210 from PV-3 to PV-5, suggesting a possible relationship between the microbial mats and the sulfate mineralogy at these sites.

2.5.2.7 Spectral Comparisons

To further evaluate the relationship between minerals influencing the VNIR spectra, several correlation plots were created to determine the relationship between four of the target spectral indices: BD2210, BD1750, BD2310, and BD2400. Figure 2.10 shows the correlation between the band depths across the entire site in the left column and specifically at PV-1 and PV-3 in the middle and right columns, respectively, which were largely representative of the inner vs. outer basin samples. In general, there is a strong positive correlation between BD2210, BD1750 and BD2400 (BD2210 vs. BD1750 not shown). As all three of these bands are associated with gypsum, this correlation suggests that gypsum dominates the signature in these spectra as opposed to phyllosilicates or carbonates. This correlation is even stronger in the abraded samples. However, this positive correlation is only apparent in the three inner basin sites, as represented here by PV-3. In PV-1, BD2210 does not exhibit a strong positive correlation with BD2400 or BD1750, suggesting that a mineral other than gypsum is causing the 2.21 \( \mu \)m band. The correlation plot of BD2310 offers some insight into this mineralogical difference between PV-1 and PV-3, as it shows a negative correlation with BD2210, BD2400, and BD1750 (not shown) across most of the spectra, but correlates positively with BD2210 in PV-1, again suggesting that a mineral other than gypsum is responsible for the 2.21 \( \mu \)m absorptions in the outer basin.

2.6 Discussion and Interpretations

This section discusses the interpretation of the VNIR spectra for each major aqueous mineral class. This section also attempts to reconcile the interpretation of the VNIR spectra with the ground truth data.

2.6.1 Sulfates

Gypsum is the only mineral that can be unambiguously identified in any of the given spectra and is also the only sulfate detected in XRD. The spectra are potentially consistent with the presence of minor Mg-sulfates but clear identification of mineral species is difficult and there is no evidence for Mg-sulfates in the XRD or QEMSCAN
data. The main spectral indices consistent with gypsum, BD1750 and BD2400 show very little detection of gypsum at PV-1 and PV-2 but a sharp increase in detection starting at PV-3. This apparent increase in gypsum from the VNIR spectra corresponds to the start of the main sulfate zone, where the majority of gypsum precipitation occurred during the formation of Pilot Valley basin, and much of the gypsum precipitated would have been buried over time due to continual deposition and reworking of the surface sediments [Lines, 1979].

2.6.2 Phyllosilicates

The spectra at PV-1 and PV-2 exhibit absorptions at 2.21 μm and 2.31 μm that could be consistent with a variety of Al- and Mg-bearing phyllosilicates. Kaolinite is present in the Pilot Valley sediments at a significant abundance of ~12 mass% at PV-2. Kaolinite has a strong doublet at ~2.16 μm and 2.21 μm, a hydration band between 1.38 μm and 1.40 μm, and additional minor bands at 2.45 μm and 2.5 μm [Bishop et al., 2008a], and thus could be responsible for the observed 2.21 μm band. Based on both the XRD and QEMSCAN data, gypsum, illite, and muscovite are also present at PV-1 and PV-2 and have absorptions at 2.21 μm [G R Hunt, 1977]. Gypsum does not appear to be spectrally dominant at PV-1 or PV-2, due to the lack of the spectral triplet around 1.4 μm and the minimal detection other key diagnostic bands. Illite has an absorption band at 2.34 μm and muscovite has a band at 2.35 μm. These two bands are nearly indistinguishable except at high resolution, but both minerals exhibit absorptions bands around 2.45 μm, with markedly different characteristic shapes. The 2.45 μm band in muscovite is very narrow, whereas the same band in illite tends to present as a very broad feature [Clark et al., 1990]. We do observe a broad absorption feature near 2.42 μm, which is inconsistent with muscovite but potentially consistent with illite. This interpretation is supported by the high abundance of illite at PV-1 and PV-2 detected in both the QEMSCAN and XRD results. Montmorillonite, identified as part of a mixed-layer clay by QEMSCAN, is also present at PV-1 and PV-2 and also exhibits similar absorption features to illite and muscovite, however the abundance is so low that it likely has minimal influence on the spectra. Thus, based on our analysis of VNIR spectra and constrained by our QEMSCAN and XRD data, we interpret the 2.21 μm band in the outer basin spectra to be due to a mixture of illite and kaolinite.
The only type of phyllosilicate detected by both XRD and QEMSCAN in this study that has a strong absorption near 2.31 µm is Mg-bearing smectite. The low abundance (<~1.6 mass % as estimated from QEMSCAN) of smectite detected at PV-1 and PV-2 by QEMSCAN could be contributing to the 2.31 µm absorption band, but it is unlikely that it is the only influence, given such a small abundance. The spectra are more likely primarily influenced by Mg-bearing carbonates such as dolomite or magnesite, which are discussed in the next section.

It is notable that we see no spectral evidence of muscovite or chlorite, two abundant minerals in the Pilot Valley basin. This could be due to burial of both minerals, and this is likely occurring as we move towards the center of the basin. However, chlorite is most abundant at PV-1 where it constitutes at least 28 mass % of the clay fraction material and muscovite constitutes at least 10 mass % of the clay fraction of material across the entire transect. So it is unlikely that both these minerals are simply not present in the surface materials in the outer basin. It is more likely that, in addition to burial, we are observing effects of spectral masking by other minerals with stronger absorption bands in the same region as these two minerals (Figure 2.5), namely illite and dolomite in PV-1 and PV-2 and gypsum in PV-3 through PV-5.

### 2.6.3 Carbonates

The detection of carbonates is a particularly important and yet complex issue for the detection of aqueous environments on the Martian surface. Calcium carbonates produce seven absorption bands in the typical VNIR range of 0.5 to 2.6 µm, as shown in Figure 2.8, with the strongest two bands centered near 2.3-2.33 µm and 2.50-2.53 µm [Gaffey, 1986; 1987]. Additional weak diagnostic bands centered near 3.4 - 3.5 µm and 3.9 µm have been used to confirm Martian carbonates detected with CRISM, as this instrument has the ability to detect absorptions out to 4.0 µm [Ehlmann et al., 2008].

This presents a limitation for terrestrial field-based spectrometers that are currently only available with ranges extending up to 2.5 µm (as was the case for this study); however, some discernible relationships can be determined for carbonate spectra.

PV-1, PV-2 and PV-3 all have an absorption band centered around 2.31 µm, which could be consistent with both dolomite and an Mg-carbonate such as magnesite, however there is no evidence of magnesite in the ground truth data. QEMSCAN results
show that dolomite content is 3.6 mass % at PV-1 and then averages of ~ 10 mass % across the rest of the transect. If the absorption feature is indeed due to dolomite then, ideally, this feature would be consistently evident across the transect (Supplementary Table 1). Instead, the 2.31 μm is discernible at PV-1 and PV-2, and in the abraded samples at PV-3, but the band disappears altogether in PV-4 and PV-5. Thus, this data could be interpreted to suggest that the 2.31 μm band is due to Mg-smectites, instead of dolomite. However, the 2.31 μm band could still be due to carbonates if the difference between the VNIR spectral detections and the QEMSCAN data is due to depth of the measurement combined with the high spectral contrast of gypsum compared to dolomite. Pilot Valley exhibits classical zonation of evaporites: in an idealized model of an evaporite basin, the carbonates are the first to precipitate in evaporite sequences and thus are concentrated around the rim and at the bottom of the basin and as the other salts precipitate, the carbonates near the topographic center would be subject to burial [C B Hunt, 1975; Lines, 1979]. Also, though we cannot see the full absorption at 2.5 μm, the slopes of the left edge of a possible 2.5 μm absorption features in PV-1 and PV-2 are consistent with the left-edge slope in dolomite. Further, although dolomite has a low abundance in the clay fraction, XRD data suggest a reasonable abundance of dolomite of larger than 3 μm grain size. Since larger grain sizes are known to result in deeper absorption bands, we would expect to see deep absorption bands from dolomite in comparison to smectite in this environment. It has also been documented that small amounts of smectite added to carbonates can influence the shape of the absorption feature [Bishop et al., 2013]. Thus, while the 2.31 μm absorption band in the outer basin spectra could be consistent strictly with smectite, the detection of a significant abundance of carbonates by QEMSCAN and XRD more strongly supports that the 2.31 μm band is due to the presence of dolomite combined with the possible influence from a small amount of Mg-smectite in the outer basin.

2.6.4 Microbial Mat

Absorption bands characteristic of oxygenic and anoxygenic photosynthesizing microbes are detected in many of the spectra in Pilot Valley. Absorptions at 0.68 μm are consistent with Photosystem II, which is the main light absorbing protein complex in photosynthesis and absorbs light at 680 nm whereas absorptions at 0.87 μm are
consistent with bacteriochlorophyll a, the main light absorbing complex in anoxygenic photosynthesis [Wiggli et al., 1999]. Figure 2.11 shows these spectral features, which are strongest at PV-3 and PV-5, compared to a reference microbial mat spectra. These spectra suggest that we are detecting a significant microbial population within the upper layers of Pilot Valley. Specifically, we are detecting a very thin microbial mat layer that is observed to lie just below the surface salts and sediments throughout the inner basin between PV-3 and PV-5, which is consistent with stronger absorptions in the abraded samples as more of the mat is exposed. Pyrosequencing of DNA extracted from Pilot
Valley sediments PV-3 and PV-5 confirms the presence of both cyanobacteria and purple photosynthetic bacteria (Lynch, unpublished data) within a highly diverse microbial community along the transect. Microbial populations such as those found at Pilot Valley might have existed during the Noachian/Hesperian transition on early Mars [Barbieri and Stivaletta, 2012]. Many studies have shown that hypersaline sediments and salt crusts can host, and subsequently preserve, a diverse array of microorganisms [Barbieri et al., 2006; Davila et al., 2013; Douglas, 2004; Sahl et al., 2008; Ventosa et al., 2008].

2.6.5 Other Salts

The spectra collected at Pilot Valley are generally dominated by gypsum salts. Halite is also an expected salt in Pilot Valley that, while featureless in VNIR spectra, was detected in XRD and to a small extent in QEMSCAN. Other salts are also expected to form in the Pilot Valley basin based on the brine chemistry observed. Detection of these potential salts using XRD is difficult due to the strong diffraction pattern of gypsum, but other indicators can be used to investigate the presence and influence of some of these salts on the VNIR spectra.

Based on the brine composition in Pilot Valley, it is possible that other salts, namely hydrated chloride salts and, to a lesser extent, hydrated chlorine oxyanion salts, could contribute to the spectra without necessarily being distinguishable from more abundant salts. Calcium-, iron-, and magnesium-bearing chloride hydrates share spectral absorptions with gypsum and Mg-sulfates at $1.19 \, \mu m$, $1.45 \, \mu m$, $1.96 \, \mu m$, and $2.42 \, \mu m$ [Hanley et al., 2014]. Magnesium is present in the basin brines at high enough concentration to support the formation of Mg-chloride-hydrate that could contribute to the spectra of PV-3 through PV-5.

Figure 2.12 depicts an anomalous spectrum from PV-4 that suggests the possible presence of such salts. The spectrum has major absorptions at 1.19, 1.45, 1.72, 1.75, and 1.94 $\mu m$. It also has minor absorptions at 1.49, 1.53, 1.78, and 1.98 $\mu m$ and has shoulder features at 2.30 and 2.42 $\mu m$. This spectrum is somewhat consistent with gypsum, and some of the shift in the bands could be due to gypsum’s partially dehydrated form, bassanite. However, many of the features are consistent with $\text{Mg(ClO}_3)_2 \cdot 6\text{H}_2\text{O}$ as well, which has major absorptions at 1.47, 1.75, 1.95, and 2.27 $\mu m$. 


and a minor absorption at 1.19 µm. Other hydrated chlorine salts with similar features cannot be ruled out if present in minor amounts. These include Mg-chloride hydrates, Ca-perchlorate hydrates and Ca-chloride hydrates as shown in Figure 2.12.

The absorptions we observe that are potentially consistent with Mg-sulfates, such as the 2.4 µm band, could also be attributed to hydrated chlorine oxyanion salts such a sodium perchlorate and associated hydrates, calcium perchlorate hydrates, and magnesium chlorate hydrate. No perchlorates or chlorates were found in the Pilot Valley brine fluids, however both anions were detected in the sediments suggesting that they exist in some salt/hydrate form. The concentrations of both of the salts is very
small (< 1 wt%), so direct spectroscopic detection of these salts is unlikely. However, VNIR analysis of hydrated chlorides and oxyanion chloride salts is fairly recent and so work remains to be done in understanding the strength of absorption of these salts when mixed with other mineral species. Hence, it is possible that a small amount of an oxyanion chloride salt could have a significant influence on VNIR spectra.

### 2.6.6 Summary Discussion of Spectral Interpretations

Because spectroscopy relies heavily on spectral libraries for interpretation and identification, terrestrial studies are crucial for providing context for complex mineral assemblages forming in natural environments. Overall, the spectra from this study indicate a transition from phyllosilicate-dominated sediments in the outer basin to gypsum/sulfate-dominated sediments in the inner basin, with general agreement between the spectral and ground truth analysis regarding the detection of the general classes of minerals: Al- and Mg-bearing phyllosilicates, sulfates, and carbonates. However, determining more detail beyond these general characteristics becomes a challenge as the analysis of the spectra indicates multiple influences from the minerals present in the heterogeneous sediments of Pilot Valley.

As stated previously in section 2.3.6, minerals in natural environments rarely form as a pure end members and therefore resulting spectra from natural samples are subject to effects from grain size, mineral mixing, spectral masking, structure, and composition [Clark, 1999], as is the case for this study. Gypsum, for example is a very strong absorber and tends to mask other minerals present within the same matrix as seen in our in VNIR data at PV-3 where gypsum dominates the spectrum, though other minerals are present in significant abundance based on XRD and QEMSCAN data. Grain size can also affect absorption bands as larger grains tend to create deeper absorption bands that can possibly dominate spectra and possibly mask other features. In addition intimate mixtures such as those in Pilot Valley can cause nonlinear combinations of end member spectra, resulting in the possible overemphasis of one mineral over another. Spectral contrast also becomes an issue in mixed mineral assemblages where multiple minerals, such as kaolinite, muscovite, illite, and chlorite, exhibit similar absorption bands. As this study has shown, it becomes very difficult to deduce the contribution from each candidate mineral based on VNIR spectra alone.
Though many studies have conducted on mineral mixtures between one or two specific mineral classes [Bishop et al., 2013; Cloutis et al., 1986; Horgan et al., 2014; Singer, 1981; Stack and Milliken, 2015; Sunshine and Pieters, 1993], more laboratory studies on highly complex mixed mineral assemblages, such as those seen in lacustrine settings, are warranted.

The biggest effect on mineral diversity that we observe in our spectra is geographic setting within the lacustrine basin. This is expected, as on Earth, closed basins will almost always present the evaporation sequence in a "bathtub-ring" fashion that identifies where key evaporite minerals would likely be found. In general, detrital materials (here mainly phyllosilicates) are more likely to be located near the rim of the basin whereas authigenic materials (here, mainly sulfates) are more likely concentrated in the interior of the basin, or buried. Further, burial (vertical variability) presents a particular problem for the detection of minerals such as carbonates that precipitate early in the evaporite sequence and are then subject to burial by other sediments.

2.6.7 Implications for Mars

Given the recent findings from the Mars Science Laboratory mission, lacustrine environments are increasingly important targets for the 2020 rover mission [Grotzinger et al., 2014]. As such, orbital investigations from CRISM will provide critical data input for determining sites with the highest habitability and preservation potential. Furthermore, the Mars 2020 instrument payload will include visible-to-near-IR spectroscopy analysis as an integral capability for obtaining contextual mineralogical information. This study has shown is that even with detailed laboratory analysis of sedimentary samples, understanding and interpreting the origin of near-infrared spectral signatures in complicated sedimentary settings is very challenging. While we can clearly identify the transition from alluvial to playa processes in the basin through near-infrared spectra the details of the mineralogy are difficult to specifically and confidently identify using this technique alone. Thus, while we can use orbital VNIR spectra to identify likely ancient lacustrine basins on Mars, understanding the details of the environment preserved in their sediments almost certainly requires in situ investigations with multiple-confirmatory techniques and the ability to investigate vertical variability, such as
the Mars 2020 suite. Further, the Pilot Valley Basin is an excellent analog site that could serve as a test bed for the 2020 science instruments.

2.7 Summary and Concluding Remarks

In this study, we have used lacustrine/playa sedimentary sequences from Pilot Valley Basin in the GSLD to investigate to characterize the complex mineral assemblages characteristic of closed-basin paleolakes and to evaluate the effectiveness of using VNIR in determining mineralogy. Below we present the following summary conclusions:

1. **Analysis of the VNIR spectra alone is insufficient** - Using XRD, QEMSCAN and aqueous geochemical analyses we have shown that the near-surface mineralogy of these sediments is dominated by a diverse assemblage of carbonates, phyllosilicates, quartz, feldspars, sulfates, and other salts. However, surface near-infrared spectra of these sediments are typically dominated by signatures most consistent with gypsum and smectite clays, two of the most common aqueous minerals detected on the Martian surface. Hence current orbital data have likely missed the vast majority of the mineralogical diversity that could be present in Martian paleolake basins. For orbital investigations, determining composition beyond major mineral classes is likely not possible for this type of geologic setting. This is relevant not only for current and future Mars missions, but also for other VNIR remote-sensing missions such as Ceres with Dawn and Europa clipper [Hanley et al., 2014]. For in-situ investigations, using multiple techniques alongside near-IR spectroscopy, such as those defined in the Mars 2020 instrument payload, is a more powerful method of determining contextual and fine scale mineralogy.

2. **More understanding of mineral mixing effects in lacustrine settings and other mineralogically complex geologic settings is required** - It is clear that more complex geologic environments likely exist on Mars. Hence, more detailed understanding of the mineral mixing effects of complex mineral mixtures is needed. Future work will include laboratory mixture studies of complex assemblages.
3. **Location matters** - The type of mineralogy expected in lacustrine environments, especially closed basins, is largely spatially driven. Therefore both orbital and in situ investigations will benefit from rim-to-center transect-driven investigations of the surface; though it is acknowledged that the large size of many Martian paleolake basins would make this type of analysis challenging, given the estimated traverse capabilities for the Mars 2020 and ExoMars rovers. Further, in situ analysis will benefit from vertical transect analysis as vertical variability is an important factor to consider in lacustrine settings.

4. **More understanding of chloride hydrates and chlorine oxyanion salts is required** - It is clear that Mars has a significant abundance of Cl-bearing minerals, many of which are salts. Though the spectra of many of these materials have recently been characterized [Hanley et al., 2014; Hanley et al., 2015], there is very little information regarding the near-IR properties of these materials in mixed mineral assemblages. This knowledge will also be relevant for the remote sensing studies of other planets such as Europa [Hanley et al., 2014]. Future work will include laboratory mixtures studies that include chloride hydrates and chlorine oxyanion salts to try to understand how these materials behave in mixed mineral assemblages.

2.8 **Paper Acknowledgements**

VNIR Spectra and QEMSCAN numerical data are available online in the supplementary material. The authors would like to thank Deanne Rogers and an anonymous reviewer for their thoughtful suggestions that significantly improved the clarity of this manuscript. We would like to thank Dr. Katharina Pfaff for training and support with QEMSCAN data analysis, Dr. Kathy Young for ENVI training and support, and Dr. Jim Bell for use of his VNIR laboratory. The authors also would like to thank Robert Lossing and Kelsey Zabrusky for their assistance with fieldwork. This research was supported by funding from the NASA Harriet Jenkins Pre-Doctoral Fellowship Program, the Edna Bailey Sussman Internship Program and the Bechtel K-5 Excellence in Education Initiative at the Colorado School of Mines.
CHAPTER 3
DISCRETE COMMUNITY ASSEMBLY WITHIN HYPERSALINE PALEOLAKE SEDIMENTS ALONG A GEOLOGICAL TRANSECT IN THE GREAT SALT LAKE DESERT, UTAH.

A paper in preparation for submittal to Environmental Microbiology

Kennda L. Lynch, Kevin Rey, Robin Schneider, Jennifer F. Biddle, Christopher Matthews, John Spear, and Junko Munakata-Marr

3.1 Abstract

Hypersaline paleolake sediments are understudied microbial ecosystems that harbor a breadth of microbial diversity and high biotechnological potential. Here we present the first documented study of the microbial ecology of the Pilot Valley Basin, a sub basin of ancient Lake Bonneville located in northwest Utah. The microbial ecology along a defined study transect was investigated in order to characterize the biological diversity within the playa environment and assess the relationship, if any, between the microbial diversity and the mineralogical and geochemical variation present in the basin. Results show that a novel ecosystem is present in Pilot Valley and community assembly is influenced by the lithological characteristic of grain size. Further, results of the study show that microorganisms of the phylum Gemmatimonadetes co-exist with some of the most halophilic archaea and bacteria, indicating a potential new characteristic of this relatively young phylum.

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8 This paper is formatted for Environmental Microbiology, hence methods are presented as the last section
9 Graduate student and primary researcher
10 Co-authors provided expert insight into geology and geochemistry at Pilot Valley
11 Co-author provided expert insight into bioinformatics and microbial ecology
12 Co-author conducted TC, TIC, TOC analysis as undergraduate research assistant
3.2 Introduction

Hypersaline ecosystems are globally distributed across a broad range of both aquatic and terrestrial environments such as soda lakes, saline lakes, hypersaline springs, solar salterns, saline flats, playas, and deep sea & oil-reservoir brines [Oren, 2006]. Hypersaline ecosystems are known for harboring diverse microbial communities with novel organisms, comprised of all three domains of life [Andrei et al., 2012; Cantrell et al., 2013; Dinsdale et al., 2008; Feazel et al., 2008; Hollister et al., 2010; Oren, 2008; Ventosa et al., 2008]. In addition to phylogenetic diversity, hypersaline systems bear a breadth of metabolic diversity with high biotechnological potential. Enzymes such as amylases produced by halotolerant microorganisms are highly sought after in the food and pharmaceutical industries where the extreme conditions of industrial processes

Figure 3.1. Field Site - Great Salt Lake Desert & Great Salt Lake. Inset - Pilot Valley Field site with sample site locations PV1 through PV5. Inset image obtained as an ASTER L1B image from site https://lpdaac.usgs.gov/ maintained by the NASA Land Processes Distributed Active Archive Center (LP DAAC), USGS/Earth Resources Observation and Science (EROS) Center, Sioux Falls, South Dakota. (2000). The data product for this image was provided by NASA.
tend to inhibit conventional enzymes. Halophilic exopolysaccharides are desired for their emulsifying and viscosifying properties and compatible solutes, such as ectoines, produced by halophilic organisms are useful as industrial stabilizers. Halophilic organisms also demonstrate the capability to metabolize a wide variety of environmental contaminants and are prime targets for bioremediation studies [Ventosa et al., 2008]. In general the majority of the microbial ecology and biogeochemical studies are focused on aquatic habitats, whereas hypersaline soils and sediments have been largely neglected [Bodaker et al., 2009; Harris et al., 2013; Oren, 2009]. This is especially true for hypersaline playas environments.

Hypersaline playas are remnants of ancient lake basins, commonly known as paleolakes. Across the globe, numerous large paleolakes from the last ice age (primarily freshwater/brackish lakes from the late Pleistocene/early Holocene Boundary) have gradually transitioned to modern day hypersaline playas such as the Chott el Gharsa of Northern Africa, the Salar de Uyni of Bolivia, Death Valley in southern California and, the specific focus of this study, the Lake Bonneville Basin in northwestern Utah [Barbieri and Stivaletta, 2012; Currey, 1990; Douglas, 2004; Fornari et al., 2001]. During this transition, the microbial life that dominated the water column and sediments was replaced by halotolerant and halophilic microorganisms as water levels dropped and ions became more concentrated. Many of these paleolake basins maintain closed groundwater systems that allow for continual wetting of the playa sediments and hence provide a supporting environment for modern day microbial ecosystems, yet investigation of the microbial ecosystem or the biogeochemical cycles that reside in these environments has been minimal [Hollister et al., 2010]. Given that many of the industrially relevant halophilic microbes were isolated from hypersaline soils or sediments and that global surveys of microbial communities indicate that sediment communities are among the most diverse of natural environments [Kanthi Kiran and Chandra, 2008; Lozupone and Knight, 2007; Ventosa et al., 2008], these communities should be examined in more detail. In addition, many of these basins contain hundreds of meters of lacustrine sediment that date back as far back as 800 ka B.P. [Rey, 2012]. As developing brines in these systems reached saturation levels, microbes could influence precipitation of minerals and/or become entrained in the resulting evaporites,
as such, these systems are excellent reservoirs of information for micropaleontology, paleobiogeochemistry, paleoecology, and paleoclimate studies [Barbieri et al., 2006; Douglas and Yang, 2002]. These systems are also excellent models for paleo-environments on Mars [Lynch et al., 2015c]. In this study we report the first documented analysis of the microbial ecology within the Pilot Valley sub-basin of the ancient lake Bonneville, the largest North American closed-basin paleolake of the Pleistocene Epoch. In addition, we report the results from our investigation of the biogeochemical relationship between microbial community assembly and the geochemical and lithological characteristics of these hypersaline lacustrine sediments.

3.3 Field Site Characterization

Ancient Lake Bonneville encompassed the majority of Northern Utah and the modern day remnants of the lake’s basin are the Great Salt Lake Desert (GSLD) and the Great Salt Lake (GSL). The Pilot Valley Basin is a sub-basin of the GSLD and is located on the western edge of Northern Utah (Figure 3.1). Pilot Valley is approximately 33 km in length and 8-15 km wide with the long axis running from northwest to southwest and the topographic center of the basin located in the northwestern corner [Lines, 1979]. Three main aquifers are associated with the basin: an alluvial fan aquifer, consisting of fresh to brackish water and present within the alluvial fans alongside each of the flanking mountain ranges; a deep basin-fill brine aquifer that underlies the entire basin at a depth of ~30 meters; and a shallow brine aquifer that encompasses the upper ~6 meters of the basin sediment fill [Mason and Kipp, 1997]. This study focuses on fluids and sediments from the shallow brine aquifer, which is maintained by ground water flow from mountain front recharge of the alluvial aquifer flanking the Silver Island Range [Carling et al., 2012]. It should be noted that hydrological connectivity between the deep basin-fill aquifer and the shallow brine aquifer is effectively non-existent [Mason and Kipp, 1997]. Due to the frequency of recharge, the Pilot Valley sediments remain consistently moist throughout the basin, although they are clearly more saturated at the center of the basin where the water table is effectively just below the surface. The only loss mechanism from the Pilot Valley basin is capillary wicking and evaporation from the playa surface [Lines, 1979].
Pilot Valley exhibits classic closed-basin evaporite zonation due to differences in mineral solubilities [C B Hunt, 1975]. Carbonates are deposited around the rim of the basin or buried in the lower layers of lacustrine sediments in deposits that are composed of detrital clays and some ancillary minerals. In the next zone, sulfates, specifically gypsum, are the dominant deposits that are intermixed with carbonates, quartz, and clays. At the topographic center of the basin, highly soluble chloride and Mg-sulfate salts are deposited. Extensive microbial mats are observed starting in the sulfate zone and propagating almost to the center of the basin (Figure B.1) [Lynch et al., 2015b]. Due to continual reworking, all of the deposits are interbedded with mud layers originating from lower sedimentary deposits or clastic input [Lines, 1979]. Additional detailed field site characterization and mineralogy can be found in Lynch et al. [2015c].

3.4 Results
A total of 28 samples were collected for community analysis (Table 3.1). Samples were collected for a comparison study from the Pilot Valley basin rim, from the Bonneville Salt Flats (BSF) basin rim, and from exposed lake sediments from the rim of the northern arm of the GSL. Four samples cores were collected and subsampled along a horizontal gradient from the Pilot Valley basin rim to the topographical center (Figure 3.1, Table 3.1).

3.4.1 Diversity of Pilot Valley in Comparison to Adjacent Basins
Samples sequenced from the rims of Pilot Valley, the Bonneville Salt Flats, and the Great Salt Lake yielded a total library of 55,754 high quality sequences post processing and quality control (Table B1). These sequences were clustered into 1769 operational taxonomic units with 97% similarity; rarefaction analysis shows reasonable sequencing coverage and sampling of the community (Figure B1). There were 5%, 1%, and 10% unassigned sequences in Pilot Valley, the BSF and the GSL respectively. In the archaeal domain across all three sample sites, the majority of the sequences (≥99%) mapped to phylum Euryarchaeota and the class/order/family Halobacteria/Halobacteriales/Halobacteriaceae. The only differentiation occurred at the genus level with > 30% of Pilot Valley sequences mapping to an unknown genus, BSF sequences mapping primarily to genus Halomina, and the GSL sequences mapping primarily to
genus *Haloarcula*. This suggests limited diversity within the archaeal domain between the sites. However there are significant differences in the bacterial domain. For Pilot Valley, the majority of the sequences, 34%, mapped to the phylum Gemmatimonadaetes, 30% to Bacteroidetes, and 11% to Proteobacteria (93% Gammaproteobacteria and 7% Deltaproteobacteria) (Figure B2). For both the BSF and the GSL (Figure B3 and B4), the majority of sequences mapped to Bacteroidetes phyla (65% and 58% respectively) and to the genus *Salinibacter* (38% and 51% respectively). The second largest group of sequences mapped to Proteobacteria (19% and 34% respectively) and specifically to the *Deltaproteobacteria* (18% and 34% respectively), home to the sulfate reducing bacteria (SRB); however, less than 1% SRB were detected.
in the Pilot Valley sample. Principal Coordinate Analysis (PCoA) of Bray-Curtis, weighted, and unweighted Unifrac metrics (Figure B5) shows that the GSL and the BSF are more related to each other than to Pilot Valley. These data indicate that Pilot Valley is compositionally unique in comparison to the BSF and the GSL. Most notable in Pilot Valley is the high relative abundance of the fairly new phylum Gemmatimonadetes and the apparent lack of SRB (< 1%), though sulfate is present in the basin rim, albeit an order of magnitude lower than its counterparts (Table B2).

3.4.2 Pilot Valley Geochemistry and Lithology

In addition to the mineralogical composition described in the literature [Lines, 1979; Lynch et al., 2015c; Rey, 2012], the Pilot Valley sediments and aquifer brines have a diverse array of mobile constituents (Tables B3, B4, B5, B6) with Na, Cl, K, Mg, Ca, and SO\textsubscript{4} present in the highest concentrations across the transect. The average free water content across the basin is 27% ±3.6, indicating that the aquifer fluids encompass and saturate most of the subsurface environment as described in the literature [Carling et al., 2012; Lines, 1979; Lynch et al., 2015c; Rey, 2012; Warren, 1999]. Though most of the analytes measured are fairly consistent from site to site, some major trends are notable across the sample transect. Soluble Ca and SO\textsubscript{4} are more abundant in the inner basin sediments at PV3 & PV5 and at the transition zone, PV2, compared to the outer basin at PV1 (One-Way ANOVA P ≤ 0.01; Kruskal-Wallis P ≤ 0.05) (Tables B3, B4). These results are reasonable given the endorheic nature of the Pilot Valley basin. During the final regressive phases of Lake Bonneville, carbonates would have deposited in the outer basin and the majority of sulfates would start to deposit in the transition zone near PV2 with continued deposition into the center of the basin [Lines, 1979; Lynch et al., 2015c]. In addition to the quantitative factors from geochemical, carbon, and water content analysis, grain size was determined qualitatively for the purposes of this study (Table 3.2).

Physical characteristics, namely grain size, of the sedimentary layers were determined qualitatively based on visualization of the samples cores from this study and previous work. Stratigraphic analysis of the upper 8 meters of sediment was conducted by Rey [2012] and five stratigraphic zones, representing different phases of Lake
Bonneville, were determined. Based on Rey’s analysis, the sediments of this study represent only two of the five stratigraphic zones: Unit I and Unit II. Unit I is identified as laminated silty and sandy sediments and represents the last transgressive phase of Lake Bonneville to the present day playa as the euhedral salt crystals found in north-central salt pan are also associated mainly with this unit. Unit II is identified as a transition from an olive gray marl to a yellow gray marl and represents the transition in lake levels from Bonneville to Provo. Three grain size classifications were identified that were consistent with material seen in both units (Table 3.2): fine clay, fine silt, and coarse.

3.4.3 Diversity of Pilot Valley Basin Sample Transect

Samples sequenced from the Pilot Valley transect yielded a total library of 190,806 high quality sequences post processing and quality control (Table B1). These sequences were clustered into 1336 operational taxonomic units with 97% similarity; rarefaction analysis shows that the sequencing effort captured the majority of the diversity across the transect (Figure B6).

3.4.3.1 Community Composition of Horizontal Transect

As discussed in Section 2.2, the archaeal domain at Pilot Valley is dominated by Halobacteria (99%) across all four sample locations. In the bacterial domain the

<table>
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<tr>
<th>Table 3.2. Cluster Groups</th>
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<tr>
<td><strong>Group 1</strong></td>
</tr>
<tr>
<td>PV1-1, PV1-2, PV2-2, PV2-3, PV2-4, PV2-5, PV5-4</td>
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<tr>
<td><strong>Group 2</strong></td>
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<tr>
<td>PV2-6, PV2-7, PV2-8, PV2-9, PV3-2, PV3-3</td>
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<td><strong>Group 3</strong></td>
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<tr>
<td>PV1-3, PV1-4, PV1-5, PV2-1, PV2-10, PV2-11, PV3-1, PV5-1, PV5-2, PV5-3</td>
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majority of sequences mapped to the phyla Proteobacteria (PV1=37%, PV2=50%, PV3=60%, PV5=58%), Gemmatimonadetes (PV1=34%, PV2=13%, PV3=4%, PV5=15%), and Bacteroidetes (PV1=13%, PV2=22%, PV3=19%, PV5=4%), though in PV3 the candidate phylum Acetothermia made up 6% of the population and in PV5 Acidobacteria made up 9% of the population (Figure 3.2). Almost no SRB (<1%) were seen at PV1 and PV5, however SRB were observed at PV2 (15%) and PV3 (12%). It should also be noted that though cyanobacteria are visibly evident in microbial mats dispersed throughout the surface of Pilot Valley basin between PV-2 and PV-5, the relative abundance of cyanobacterial sequences in the dataset is small. This is likely due the thin morphology and discontinuous distribution of the microbial mats, though known extraction bias may not have been fully accounted for during DNA extraction [Couradeau et al., 2011; Morin et al., 2010]. Beta diversity analysis of the depth-averaged community structure along the horizontal transect indicates that, despite
significant alpha diversity, there is no significant difference in the overall populations between the sample sites, suggesting minimal phylogenetic variability along the horizontal evaporative gradient, though there is variation in taxa abundance along the transect (Bray-Curtis $R^2=0.16$, $P_{\text{ADONIS}}=0.27$; weighted Unifrac $R^2=0.13$, $P_{\text{ADONIS}}=0.45$). The diversity of the vertical transect showed a much higher degree of phylogenetic variability as described in the next section.

3.4.3.2 Community Composition of Vertical Transect

In the vertical transect (i.e. the sub-core samples) the dominant members of the community shift abruptly into discrete layers (Figure 3.3a). PCoA and PERMANOVA analysis of the Bray-Curtis ($R^2=0.46$, $P_{\text{ADONIS}}=0.001$), Morisita-Horn ($R^2=0.52$, $P_{\text{ADONIS}}=0.001$), unweighted Unifrac ($R^2=0.25$, $P_{\text{ADONIS}}=0.001$) and weighted Unifrac ($R^2=0.61$, $P_{\text{ADONIS}}=0.001$) metrics show that the subsamples cluster into three distinct groups, with the exception of the unweighted Unifrac measurement where PV5-4 and PV2-5 cross from Group 1 to Group 2 (Figure B7, Table 3.2, Figure 3.3b). Within these three groups, the dominant taxa are evident down to the genus level. In Group 1 the dominant taxa are order/family Halobacteriales/Halobacteriaceae (20%) phylum/class Gemmatimonadetes/Gemm-4 (44%), genus Salinibacter (20%), and class Gammaproteobacteria (7%). Within the Group 1 Gammaproteobacteria the dominant clade is genus Halomonas at 50% (4% of total bacteria). In Group 2, the dominant taxa are phylum Bacteroidetes (35%), class Gammaproteobacteria (15%), phylum Acetothermia (16%), and order Halobacteriales (9%). Gemmatimonadetes is only 6% of total assigned sequences in Group 2, and within the Bacteroidetes, only 15% (5% of total assigned sequences) are genus Salinibacter. Within the Gammaproteobacteria, 63% (12% of total assigned sequences) are genus Halomonas. In Group 3, the dominant taxa are genus Halomonas (54%), genus Shewanella (12%), and phylum Acidobacteria (8%). The structure of the dominant taxa and the results of the beta diversity analysis both indicate that Group 1 and Group 2 are more similar to each other than to Group 3. The Unifrac metrics, in particular, may delineate the nature of this relationship as the unweighted metric suggests a close relatedness as far as the taxa present in both Groups 1 and 2, yet the difference between these two groups in
Figure 3.3. Phylum level relative abundances for Vertical Transect Study. a) Core sub-sections b) Community groups
weighted Unifrac is suggestive of an environmental influence in one or both groups that allows different taxa to thrive [Lozupone et al., 2007].

Mantel correlation results for both weighted and unweighted Unifrac metrics (Table B7) show little to no correlation between community structure and most of the quantitative environmental variables. Calcium, nitrate and sulfate show statistically significant positive correlation in the unweighted Unifrac metric, yet the weighted Unifrac shows almost no correlation for these environmental factors. This suggests that relative abundance of community members is not correlated to these factors, but that community structure and phylogenetic relatedness is. The only factor that is significant across both metrics is barium. It should be noted that all significant metrics are weak positive correlations as \( r < 0.5 \).

Canonical Correspondence Analysis (CCA) allows visualization of the relationship between environmental factors and community structure in more detail and is considered more sensitive in detecting gradient relationships than the Mantel statistic [Borcard et al., 2011; Legendre and Legendre, 2012; ter Braak and Verdonschot, 1995]. CCA plots the Pilot Valley transect microbial data constrained by 8 of the most correlated quantitative environmental variables and one qualitative variable (grain size), resulting in an eigenvalue for axis 1 of 0.428 (explaining 66.1% of the inertia; \( P=0.04 \)) and an eigenvalue for axis 2 of 0.20 (explaining 22.48% of the inertia; \( P=0.04 \)) (Figures 3.4 and 3.5; Appendix B). Figure 3.4 is the CCA plot with scaling factor type 1 that depicts the structure of the microbial communities among the sample sites with respect to the environmental constraints. The CCA plot confirms that the communities organize into the same three distinct groups as identified the PCoA plots (Table 2.2) and it shows what environmental variables are most related to each group. Group 1 is associated with high barium concentrations and is also qualitatively related to fine clay-sized particles. Group 2 is more spread out but mostly related to high total organic carbon, strontium, sulfate and calcium concentrations, and it is linked to coarse grains. Group 3 is related to increasing free water content and is loosely related to the fine silt grains. Figure 3.5 is the CCA plot with scaling factor 2, which shows how the most dominant phylum-level taxa are structured with respect to the environment constraints and each other. Both Gemmatimonadetes and Euryarchaeota, two very dominant phyla in Group
1, are likely to be found at high barium concentrations and in clay-sized sediments. It is notable that all archaea are associated with Group 1 and are correlated with the clay-sized sediments. Bacteroidetes, a dominant phylum in Group 1 and in Group 2, is also correlated with increasing barium concentrations and also increasing TOC concentrations, but it is not necessarily correlated strongly with the clay-sized sediments or coarse grains, which suggests its presence with both grain types along with Spirochaetes and the unassigned sequences. The more distance phyla OP1 and
Lentisphaerae are correlated with Group 2, but not clearly with Group 1, hence they cluster further away. All major phyla in Group 3 are correlated with high water content and correlated with silt-sized grains, though they are quite spread out. The primary cluster containing Firmicutes, Actinobacteria, Acidobacteria and Planctomycetes correlates more with nitrate and hydration. All other groups seem to spread out among the various analytes, suggesting that none of these variables are strongly correlated to Group 3.
3.5 Discussion

The discussion relates community composition to the physical factor of grain size and identifies a novel characteristic of the phylum Gemmatimonadetes.

3.5.1 Community Assembly Correlates with Grain Size

Twenty-two environmental variables were measured at Pilot Valley (Tables B3-B5 & Table 3.2) and of those, only the physical characteristic of grain size, seems to be an influential factor on community assembly based on some indicative factors. First and foremost, barium was determined to be a significant variable by both the Mantel test statistic and CCA. Barium is known to adsorb to clay minerals [Eylem et al., 1990; Gonneea and Paytan, 2006; Rutten and de Lange, 2002], hence increasing barium concentrations detected in the Pilot Valley sediments can reasonably be correlated with fine, clay-sized sediments as shown in the CCA analysis.

Nitrate was identified as a factor in both the Mantel correlation and CCA. Nitrate is highly mobile in water and often highly utilized in microbial systems. Nitrate is only known to accumulate in sediments with minimal water flow and/or minimal nitrate-related microbial activity [Lybrand et al., 2013]. In the case of Group 1 in Pilot Valley, the sub-core samples with the highest nitrate concentrations are correlated with having clay-sized particles, lower water content, and low total organic carbon (Figure 3.4).

The high sulfate and calcium concentrations associated with Group 2 may also correlate with grain size. Group 2 is correlated with large coarse grains that are also defined as large euhedral evaporite crystals as discussed previously. These grains may be mostly composed of gypsum and/or other sulfate salts, hence the elevated calcium and sulfate concentrations may be artifacts of sample preparation for IC and ICP-OES analyses as these minerals would have dissolved to some degree during the sample extraction process (Appendix B). In addition, these coarse grains may likely allow for greater water movement through the groundwater system and continual precipitation and re-dissolution of minerals. A possible indicator of an increase in groundwater flow may be the significant presence of SRB in the Group 2 layers. It has been suggested by previous studies that the inhibition of sulfate reduction in finer sediments may be due to pore space exclusion of SRB as a result of smaller sediment grain sizes [Bartlett et al., 2010]. This mechanical restriction may be a factor in a
reduced abundance of SRB in the Group 1 and Group 3 grain types, but given the significant presence of other similarly sized microorganisms such as the *Halobacteria*, it is likely that the concentration of hydrogen sulfide plays a more influential role. It is well known that sulfate reduction is inhibited by high hydrogen sulfide concentrations [Icgen and Harrison, 2006; McMahon and Daugulis, 2008; Okabe et al., 1995; Reis et al., 1991; Reis et al., 1992]. Hence, it is plausible that H$_2$S concentrations in the Group 1 and Group 3 (fine clay and fine sediment respectively) pore water spaces are high enough to inhibit sulfate reduction such that SRB cannot thrive in those lithological zones [Roychoudhury et al., 2003].

Though water content was not identified as significant in the Mantel correlation, it becomes a relevant factor in the CCA plot (see Supplemental Information for detail on CCA analysis and interpretation). This may also be a result of the grain size, which drives other soil/sediment properties that affect water content [Brutsaert, 2005].

Finally, the dominant taxa in Group 1 are *Halobacteria*, *Salinibacter* and Gemmatimonadetes, and they all correlate with increasing barium concentrations. Both *Halobacteria* and *Salinibacter* require high salt concentrations on the order of 15-30% to thrive, and Gemmatimonadetes is considered a desiccation-resistant phylum [Andrei et al., 2012; DeBruyn et al., 2011; Fawaz, 2013; Oren, 1994; 2013]. The presence of these three taxa in Group 1 makes a reasonable case for high salinity pore water in the clay-sized sediment layers of the basin. Since direct salinity measurements of the pore water was not possible, ionic strength was calculated as an indicator of pore water salinity in each sample. Other drivers of community assembly are possible as we could not measure all key environmental variables in this study; for example, we were unable to measure H$_2$S at the time of sample collection. Hence further study of this system would benefit from measurement of additional environmental parameters.

3.5.2 Gemmatimonadetes as an possible indicator of water activity

One of the more interesting aspects of the Pilot Valley microbial community is the high abundance of the phylum Gemmatimonadetes. Originally identified as candidate group BD by Hugenholtz et al. [2001] and as candidate group KS-B by Madrid et al. [2001], Gemmatimonadetes was identified as a new phylum by Zhang et al. [2003] after this group isolated the first cultured representative *Gemmatimonas aurantiaca*. The
members of this phylum represent an average of 2 percent of soil microbial communities and this phylum is considered one of the top 9 phyla found in soils [DeBruyn et al., 2011]. This enigmatic phylum is also known for possessing novel carotenoids and photosynthetic capabilities thus suggesting a very unique evolutionary history [Takaichi et al., 2010; Zeng et al., 2014]. The key putative characteristic of this phylum is that members are desiccation resistant. Both Debruyn [2011] and Fawaz [2013] provide compelling evidence for tolerance to low moisture conditions (as low as 8.7%) in Gemmatimonadetes across multiple environments. However, in our study the overall average moisture content of the sediments is 27% with a standard deviation of 4% (median = 27%, min = 22%, max = 38%) and the average moisture content of Group 1 (the lowest moisture content group according to the CCA) is 24% and the average moisture content of the highest group, Group 3 is 28%. With only a 4% difference in water content, Group 1 has the maximum relative abundance of Gemmatimonadetes at 44% and Group 3 has the minimum at 2%. As previously discussed, Gemmatimonadetes in Pilot Valley were always identified with the extreme halophilic taxa Halobateria and Salinibacter. Gemmatimonadetes has not been identified as a halophile let alone a halotolerant organism to date, so this association is very curious. Our study provides the first evidence that organisms in the phylum Gemmatimonadetes are not necessarily simply tolerant to low moisture, but are tolerant to low water activity. Water moisture is defined as the amount of water in % volume or % weight present in a given system, whereas water activity is defined as the amount of water available for chemical reactions. Given the results of our study in comparison to previous work, low water activity tolerance is a reasonable and logical explanation for the behavior exhibited by this phylum, potentially defining a new category of extremophilic microorganism. Verification of this proposed low water activity tolerance will be carried out through geochemical modeling and laboratory experiments, but is outside the scope of this study.

3.6 Summary and Concluding Remarks

Pilot Valley presents an interesting study for community assembly in sedimentary systems. In this study we have identified grain size as a potential influential factor in
the community structure, which has implications from both a general microbial ecology perspective and from an astrobiological perspective. First and foremost, we have shown that the microbial community is more diverse vertically than horizontally, i.e. the evaporitic zonation seen on the surface has little influence on community assembly overall. Secondly, previous studies have suggested that microbial activity in clay sediments effectively ceases due to pore space limitations. However, many of these studies were conducted prior to advances in high-throughput sequencing of environmental samples, only evaluated limited metabolic or culture evidence of microbial activity, or did not measure bioactivity at all [Albrechtsen and Winding, 1992; Bartlett et al., 2010; Fredrickson et al., 1997; Li et al., 2015; C Zhang et al., 1998]. In this study we have found that, though sulfate-reducing bacteria are likely inhibited from metabolic activity in finer grain and clay-sized sediments, a diverse population of microbes are present in this limited environment. Finally, we have potentially unlocked another clue regarding the environmental capability of the phylum Gemmatimonadetes.

From an astrobiological perspective, active evaporite mineral formation within a sedimentary system is considered an ideal environment for the preservation and subsequent detection of biosignatures [Summons et al., 2011]. In Pilot Valley there exists a system that may be actively entraining microbes and as such presents as a model to learn how to analyze such types of samples. Secondly, grain size effects on community structure observed in this study could impact what actually gets preserved in which sedimentary layers. It is likely that preservation would be higher in the clay-sized sedimentary layers than in the secondary mineral layers as continual groundwater flow could potentially cause re-dissolution of minerals and destruction of preserved biomarkers in those layers.

In closing, Pilot Valley is a fascinating natural lab for studying subsurface microbial ecology in extreme environments. Below we present some future work that should be conducted at Pilot Valley:

1. Determination of other key environmental parameters - As stated previously, study of this ecosystem would benefit from further detailed measurement of other environmental factors (such as $\text{H}_2\text{S}$, acetate, and hydrogen, etc.) to
allow for a full picture of all geochemical parameters that may influence this system.

2. Detailed understanding of the functional/metabolic influence on community assembly - This study has provided a window into the phylogenetic diversity of hypersaline sediments; more detailed understanding of the metabolic diversity of such environments would provide further understanding of community structure.

3. Detailed evaluation of the relationship between grain size, salinity, solute concentration, and water activity - subsurface hydrological systems are very complex as many of the geochemical and physical parameters are interconnected. Further research at Pilot Valley would benefit from more detailed hydrological analysis, such as reactive transport modeling, of the ground water interaction with the subsurface sediments and effect on the biogeochemistry of the Pilot Valley system [Beisman et al., 2015].

3.7 Methods

This section outlines the detailed methods for field sampling of the Pilot Valley basin. It also outlines the detailed methods of all geochemical and biological analyses in this chapter.

3.7.1 Field Sampling & Geochemical Analysis

Field samples were obtained during two field campaigns, June 2010 (EX1) and May 2012 (EX3). Sediment and aquifer fluid samples from Pilot Valley were collected along the same defined horizontal transect from the basin rim to the topographic center of the basin (Figure 1). Sample cores were obtained using an AMS Extendible Core Sampler and recovery tripod, which retrieves 5 cm diameter cores up to 60 cm long at a time. Sediments from coring samples were collected and stored on CO$_2$ ice in the field. Geological samples were stored at -20 °C and DNA samples were stored at -80 °C when returned to the lab. Aquifer fluids were filtered to 0.2 µm and stored on H$_2$O ice in the field and refrigerated at -4 °C in the lab. Cation/anion compositions and TIC/TOC, and average hydration were determined for all core samples (Tables B1-B3). Average Free water content of the sediments was determined by weight. Cation and anion
compositions of brine aquifer fluids were determined at each sample site as well (Table B4). More details are provided in Appendix B. Mineralogical analysis of each sample site was previously measured [Lynch et al., 2015c].

3.7.2 DNA Extraction, PCR-amplification, and Next Generation Sequencing

Bulk DNA was extracted using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories). DNA was extracted from ~25 mg of each sample in triplicates, following the standard protocol with 2 specific modifications that allowed for optimal recovery, concentration, and purification of the DNA. The first modification was at the clean up step of the protocol as the Pilot Valley samples contained compounds that inhibited PCR. Hence, the humic removal step that involved solution C3 was repeated twice in order to ensure purity of the final DNA extraction. The second modification was at the last step of the protocol where DNA from each replicate was concentrated onto the same single spin filter, eluted into a single tube and stored at -20°C. DNA was amplified with barcoded primer set 515/927r-modified and using a methods as described by Osburn et al. [2011]. The resulting amplicons were shipped to Selah genomics (Columbia, SC) for 454-titanium sequencing (Roche Applied Sciences).

3.7.3 Processing, Quality Control and Statistical Analysis of 16S rRNA

The resulting SSU rRNA sequences were processed in QIIME 1.8 [Caporaso et al., 2010]. Sequences and barcodes were demultiplexed using the split_libraries.py script with the default parameters. They were then denoised using the low-level interface to the QIIME denoiser. Sequences were chimera checked using USEARCH 6.1 and then clustered into operational taxonomic units using UCLUST at 97% similarity. OTU's were picked de novo using UCLUST with default parameters. Representative sequences were aligned using PyNAST and the Greengenes 13_8 aligned reference database. Taxonomy was assigned using Uclust and the Greengenes 13_8 97% similarity taxonomy reference database. Statistical analyzes were conducted using QIIME and PAST software packages [Caporaso et al., 2010; Hammer et al., 2001].

3.8 Acknowledgements

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Bechtel K-5 Excellence in Education Initiative, and the National Science Foundation Grant NSF IOS 1318843. All DNA sequence data related to this study can be obtained through the European Nucleotide Archive (ENA) via accession number PRJEB11779. The authors would like to thank Dr. Chase Williamson and Dr. Lisa Gallagher for training and support on 454 sequencing preparation. The authors would also like to thank Dr. Jackson Z. Lee, Dr. William Orsi, and Joseph Russell for thoughtful discussions on bioinformatics, multivariate statistics, and coding.
4.1 Abstract
The presence of perchlorate on the red planet suggests a possible energy resource for microorganisms. Perchlorate-reducing microorganisms have been well documented in several environments on earth with the exception of environments that contain naturally-occurring perchlorate. Here we present the results of field and laboratory studies that characterize the presence and distribution of naturally-occurring perchlorate in the Pilot Valley basin, Utah, and investigate the potential for perchlorate-reducing organisms to co-exist with naturally-occurring perchlorate as a model for potential microbial ecosystem in ancient martian lacustrine sediments. Results show that naturally-occurring perchlorate exists in the near surface sediments as does a small population of known perchlorate-reducing organisms.

4.2 Introduction
Perchlorate (ClO$_4^-$) is a highly oxidizing anion that is known to block the uptake of iodine by the thyroid gland in mammals and as such is a drinking water contaminant of increasing international concern [Coates and Achenbach, 2004; Dyke et al., 2007; Gu and Coates, 2006; Logan, 1998; Shi et al., 2007; Stroo and Ward, 2009; Urbansky, 1998; 2002]. Though the main source of perchlorate is generally from the synthetic compound that is utilized in a wide range of industrial applications, perchlorate also occurs naturally as it is generated in the atmosphere and is mainly found to accumulate...
in a variety of arid environments across the planet including the Pilot Valley Basin of the Great Salt Lake Desert in Utah [Bao and Gu, 2004; Catling et al., 2010; Duncan et al., 2005; Jackson et al., 2010; Jackson et al., 2012; Lybrand et al., 2013; Lynch et al., 2015c; Orris et al., 2003; Urbansky et al., 2001]. Starting with the discovery of perchlorate with the Phoenix lander mission on Mars in 2008, perchlorate has also been found to be a potentially ubiquitous salt anion present in the martian soil and as such, it may be an important metabolic constituent that could support life on the red planet [Hecht et al., 2009; Ming et al., 2014].

With a redox potential on par with oxygen (1.287 V) perchlorate is a powerful oxidant that can serve as an terminal electron donor in microbial metabolism. Microbial reduction of perchlorate has been known and documented for over 70 years, and more than 40+ isolates of dissimilatory perchlorate reducing bacteria (DPRB) have been documented [Bardiya and Bae, 2011]. DPRB are seemingly ubiquitous and phylogenetically diverse in nature as they have been isolated from a variety of environments including gold mine drainage sites, contaminated and pristine groundwater sites, wastewater sludge, natural swamps, and from deep sea sediments. They even occur across the domains of life as there is evidence of perchlorate reducing archaea [Liebensteiner et al., 2013; Nerenberg, 2013; Oren et al., 2014] Oren et al., 2014]. Hence, we will use the phrase perchlorate reducing microorganisms (PCRM). However, to-date, little evidence of PCRM co-occurring with naturally occurring perchlorate has been documented [Liebensteiner et al., 2015]. Finding these two entities together in a relevant analog environment would then make for an excellent model for this kind of ecosystem on Mars. Furthermore this finding would allow further study into the evolutionary history of perchlorate reduction in the natural environment. In this study we present evidence of PCRM co-existing with naturally occurring perchlorate in the Pilot Valley basin of the Great Salt Lake Desert as a model for potential microbial metabolisms that could occur in ancient paleolake basins on Mars.

4.3 Field Site Description and Study Motivation

Pilot Valley is a closed basin paleolake that consists of hypersaline fluvial and/ or lacustrine deposits, hosts a shallow brine aquifer that encompasses the upper 6 m of
basin fill and maintained through subsurface groundwater flow by mountain-front recharge from the adjacent Silver Island Mountain Range, and contains extensive microbial mats and putative microbial induced sedimentary structures (MISS) [Carling et al., 2012; Lines, 1979; Lynch et al., 2015b].

Sediment and aquifer fluid samples were collected from the Pilot Valley basin for geochemical analysis, along a defined study transect (Figure 4.1), as part of an ongoing geobiological investigation of this Mars analog environment [Lynch et al., 2015a; Lynch...
et al., 2015c] and perchlorate and chlorate anion concentrations were measured as a part of this study (Table 4.1). A declining concentration trend in the Pilot Valley sediments from the rim of the basin to the topographic center of the basin was seen in the perchlorate data set and confirmed through ordinary least squares regression analysis (Figure 4.2). In addition, no perchlorate was detected in the Pilot Valley aquifer fluids, thus suggesting a loss mechanism for the perchlorate. Given the recalcitrant nature of the perchlorate anion, a logical hypothesis for the trend is microbial reduction of perchlorate, though no evidence has been documented of naturally occurring perchlorate co-existing with PCRM in arid or hypersaline sediments where naturally-occurring perchlorate is most likely to accumulate [Kounaves et al., 2010; Liebensteiner et al., 2015; Lybrand et al., 2013; Rajagopalan et al., 2006; Rao et al., 2007]. The goal of this study was to evaluate presence of PCRM as a potential sink to the naturally occurring perchlorate detected in the basin.

### 4.4 Material and Methods

This section outlines the field sampling and experimental methods for this chapter. This section also outlines the methods for next generation sequencing of enrichment samples.

#### 4.4.1 Field Sampling and Enrichment

The four samples (SLD113, SLD114, SLD115 and SLD124) used for the experiments in this study were taken from the putative MISS structures discovered at

Table 4.1. Pilot Valley Average Sediment Oxyanions*

<table>
<thead>
<tr>
<th>Site ID</th>
<th>ClO3- μg/kg</th>
<th>ClO4- μg/kg</th>
<th>ClO3- SD</th>
<th>ClO4- SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV-1</td>
<td>1.35</td>
<td>0.73</td>
<td>0.71</td>
<td>0.13</td>
</tr>
<tr>
<td>PV-2</td>
<td>0.61</td>
<td>0.65</td>
<td>0.33</td>
<td>0.24</td>
</tr>
<tr>
<td>PV-3</td>
<td>0.65</td>
<td>0.39</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>PV-5</td>
<td>0.68</td>
<td>0.28</td>
<td>0.57</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Data obtained from previous studies. [Lynch et al., 2015a; Lynch et al., 2015c]
site PV-4 (Figure 4.1). This site has the greatest concentration and diversity of MISS features, hence it was likely to have a high biomass concentration and community diversity. The samples for this study include two different morphologies: laminated leveling structures and roll-up mats, as described by Noffke [2010] (Figure 4.3). Upon collection, the samples were placed in vacuum-sealed bags and stored at 4°C. This study was designed to allow the enrichment of perchlorate reducing microorganisms in a near-native environment. A minimal enrichment medium was prepared in accordance with Coates and Jackson [2009] and sodium perchlorate was added to create final media at 0.1% and 100 ppm sodium perchlorate-concentration. The 0.1% and 100ppm microcosm experiments were set up in quadruplicate and triplicate respectively. For the 0.1% experiment, cross-sectioned subsamples were
removed from each field sample in 20 & 40 gram amounts and added to 120 ml serum vials with 100 ml of media. For the 100 ppm experiment, cross-sectioned subsamples were removed from each field sample in 6 gram amounts and added to 120 ml serum vials with 60 ml of media to create a media to substrate ratio of 10:1. All serum vials were sparged for ~10 minutes with gas mixture of 80% nitrogen and 20% CO₂. The vials were then incubated at room temperature over a span of 371 days (November 10, 2013 to November 16, 2014) and a span of 285 days (February 15, 2014 to November 23, 2014) for the 0.1% experiment and 100 ppm experiment respectively. Microcosms were subject to light versus dark treatments as shown in Table 4.2. Media blanks were
preparing and incubating with the sample replicates for each experiment. Three time points were taken at 25 days, 97 days and 371 days for the 0.1% experiment. One time point was taken at day 285 for the 100 ppm experiment. At each time point, ~20 ml of fluid was removed for geochemical analysis and 1-2 ml of fluid/sediment mix was removed for DNA analysis. For the 0.1% experiment, 20 ml of fresh perchlorate media was added to each microcosm (blanks included) at time point #1 (Day 25). Headspace gas volume was also measured as an anecdotal measure of microbial activity. Geochemical samples were 0.2 micron filtered and stored at 4°C and samples for DNA analysis were stored at -80°C.

### 4.4.2 DNA Extraction & Quantification

Bulk DNA was extracted using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories). DNA was extracted from ~250 μl of each sample in triplicates, following the standard protocol with a modification to remove additional humic substances and a modification to combines the DNA from each replicate on the same single spin filter in order to concentrate the DNA. DNA was quantified using the Life Science Qubit 2.0 fluorometer.

<table>
<thead>
<tr>
<th>Table 4.2. Samples &amp; Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0.1 % Experiment</strong></td>
</tr>
<tr>
<td>SLD 113: A, B, C, D</td>
</tr>
<tr>
<td>SLD 114: A, B, C, D</td>
</tr>
<tr>
<td>SLD 115: A, B, C, D</td>
</tr>
<tr>
<td>SLD 124: A, B, C, D</td>
</tr>
<tr>
<td>SLD Blank 1</td>
</tr>
<tr>
<td>SLD Blank 2</td>
</tr>
<tr>
<td><strong>100 ppm Experiment</strong></td>
</tr>
<tr>
<td>SLD 113: I, J, K</td>
</tr>
<tr>
<td>SLD 114: I, J, K</td>
</tr>
<tr>
<td>SLD 115: I, J, K</td>
</tr>
<tr>
<td>SLD 124: I, J, K</td>
</tr>
<tr>
<td>SLD Blank</td>
</tr>
</tbody>
</table>

All C and K replicates were subjected to light treatments. All others were subjected to dark treatment.
4.4.3 DNA Sequencing and Analysis

The extracted DNA from the 6 most concentrated extractions (SLD 115A, SLD 115C, SLD 124A-D) from Day 95 (this sample set will be referred to the seq-six sample set from henceforth) was amplified in triplicate using a Roche Light Cycler 480 II (Roche Life Sciences) without any sequencing adaptors and using a 515F forward primer (5’ GTGYCAGCMGCCGCGGTAA 3’) and a 12bp golay adapted 806R reverse primer (5’XXXXXXXXXXXXXXCCGGACTACHVGGGTWTCTAAT 3’) [Jasper et al., 2014]. The samples were amplified in 25 ul reactions and contained per reaction: Phusion Master Mix (New England BioLabs, Inc), 3% final volume DMSO, 0.4x final concentration SYBR Green, and 200nM of each primer. The lightcycler program for amplification was 94°C for 3 min; ~30 cycles: 94°C for 45 s, 50°C for 10, 72°C for 90 s. The program was terminated when all samples had amplified (~27 cycles). The samples were pooled and purified using the Agencourt AMPure XP system and quantified using a Life Sciences Qubit 2.0 Fluorometer. Normalized amplicons were sequenced on the Illumina MiSeq platform using the NEBNext Ultra DNA Library Prep Kit and a MiSeq Reagent Kits v2 2x250 500 cycle kit. The resulting sequences were processed in QIIME 1.8 [Caporaso et al., 2010] starting with the joined_paired_ends.py script to stitch together the paired reads with minimum 100 bp overlap. Stitched sequences were then oriented and parsed of barcodes using the extract_barcodes.py script. The sequences and barcodes were demultiplexed using the split_libraries_fastq.py script with the default parameters with the exception of the --barcode_type 12 to suppress error correction of barcodes. OTU's were picked de novo using Uclust with default parameters. Chimeras were filtered out using ChimeraSlayer and the Greengenes 13_8 aligned reference database. Representative sequences were aligned using PyNAST and the Greengenes 13_8 aligned reference database. Taxonomy was assigned using Uclust and the Greengenes 13_8 97% similarity taxonomy reference database. The Illumina sequence data are available via the ENA database, accession number PRJEB11780.

4.4.4 Quantitative PCR

Quantitative PCR analyses of the chlorite dismutase (cld) gene and the perchlorate reductase (pcrA) gene were conducted on the seq-six sample set from Day
371 using a LightCycler 480 II instrument (Roche Life Sciences). The qPCR standard was generated from a culture of *Dechloromonas agitata*. The *cld* gene was amplified using the primer set UCD-238F/UCD-646R [Bender et al., 2004] (UCD-238F 5'-TYGAVAARCAAYAGGAAYGT-3' and UCD-646R 5'-GAGTGGTAVARYTTGCTT-3'). The *pcrA* gene was amplified using the primer set pcrA320F/pcrA598R [Nozawa-Inoue et al., 2008] pcrA320F 5'-GCAGCCACGACTACATGTYGGNC-3' and pcrA598R 5'-GGTGGTGGGCTGGCAGGCTC-3'). The qPCR formulation for both gene sets contained, per 20 μl reaction: 10 μl Perfecta SYBR Green Supermix (Quanta Biosciences), 6 μl nuclease free water, 1 μl each template (10 μM μl-1 each), and 2 μl template DNA. The thermocycler programs for both primer sets were as described by De Long et al. [2010].

### 4.4.5 Perchlorate and Chloride Analysis

Perchlorate (ClO$_4^-$) was measured by sequential ion chromatography-mass spectroscopy/mass spectroscopy (IC-MS/MS). The anion was quantified using a Dionex LC 20 ion chromatography system consisting of GP50 pump, CD25 conductivity detector, AS40 automated sampler and Dionex IonPac AS16 (250 X 2 mm) analytical column. The IC system is coupled with an Applied Biosystems – MDS SCIEX API 2000TM triple quadrupole mass spectrometer equipped with a Turbo-IonSpray™ source. A hydroxide (NaOH) eluent at 0.3 mL min-1 was followed by 90% acetonitrile (0.3 mL min-1) as a post-column solvent. To overcome matrix effects, all samples were spiked with Cl$^{18}$O$_4^-$ or Cl$^{18}$O$_3^-$ internal standard. Samples were analyzed in batches of 8 including an analytical duplicate and spike. Samples with elevated Cl (>10,000 mg/L) or

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Sampling Time Point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 25</td>
</tr>
<tr>
<td>Blank 1</td>
<td>490</td>
</tr>
<tr>
<td>Blank 2</td>
<td>230</td>
</tr>
</tbody>
</table>

Table 4.3. Chloride Measurements of Sample Blanks: 0.1% sodium perchlorate
SO₄ (>1000 mg/L) were either diluted prior to analysis or in some cases pre-cleaned using On-Guard™ II Ag or Ba cartridges (Dionex). Statistical analysis of perchlorate results was conducted using the statistical tools in KaleidaGraph (Synergy Software) and PAST [Hammer et al., 2001].

4.5 Results

This results section is as follows and presents the results of both the 0.1% and 100 ppm study. This section also presents the results of next-generation sequencing of a limited subset of enrichment samples.

4.5.1 Perchlorate Utilization

Figures 4.4 and 4.5 show the results of the PCRM microcosm experiments. All sample treatments show a significant reduction in perchlorate across both experiments (0.1 %: one-way ANOVA p<0.0001; 100 ppm paired t-test p<0.001). Pairwise comparisons in the 0.1% experiment also show significant differences between all sample time points (Turkey's HSD p<0.0001) with the exception of between Day 0 and Day 25. The greatest change in perchlorate concentrations occurred in the replicates for SLD 124 in both experiments. No significant difference was seen between the light-treated and the dark-treated microcosms.

The decrease in perchlorate in the blank controls in the 0.1% experiment is significant. This variation in the blank controls suggests some form of contaminating microbe present in the media. To rule out contamination and subsequent microbial processing of the perchlorate in the blank controls, the chloride concentrations were measured on all blanks at all time-points (Table 4.3). Contamination by PCRM, would significantly increase chloride concentrations in the blanks, though this change would not be seen in the microcosms due to the high chloride concentration present in the Pilot Valley sediments. For the 0.1% experiment, there is little to no variation in the chloride concentration across time points. This result is verified in the 100 ppm experiment (Figure 4.5) and hence, we conclude that the blanks remained uncompromised throughout the experiment. Each sample shows a steep drop with similar slope between day 25 and day 97, but after that the slopes vary between day 97 and day 371.
4.5.2 Microbial Diversity

Samples sequenced from the enrichment study yielded 30,5337 quality sequences after processing and quality control (Table 4.4). These sequences were clustered into 7653 OTU’s; alpha rarefaction analysis (Figure 4.6) shows reasonable sample coverage and highly diverse, samples, though this may be an artifact of OTU-picking methods. The abundant phylum level taxa are consistent across all samples (Figure 4.7). Firmicutes and Bacteroidetes are the two most abundant taxa followed by
Proteobacteria and a consistent population of unassigned sequences (~9%). Beta diversity (Figure 4.8) shows that the enrichment communities in SLD 115 A and C are more related to each than to SLD 124A-D, and vice versa, across all metrics tested: Bray-Curtis, Morisita-Horn, unweighted Unifrac, and weighted Unifrac. SLD 124 A-D
shows some variable relatedness with the Bray-Curtis and Morisita-Horn metrics both indicating the same pairing relationships (A&C and B&D), whereas, in the weighted and

Table 4.4. Summary Sequencing Statistics*

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<th>Total Raw Sequences</th>
<th>Total QC Sequences</th>
<th>Mean Sequence Length</th>
<th>Sequence Number</th>
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<td>30,537</td>
<td>282</td>
<td>1425 11664 3959 5089 3275 7653</td>
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</tbody>
</table>

*Sequencing statistics based on 6 ECRP enrichments from the Day 97 time point: SLD 115A, SLD 115C, and SLD 124A-D.

Figure 4.6. Rarefaction Plots of Enrichments of Seq-six Sample Set. Red = SLD 115A, Blue = SLD 115C, Orange = SLD 124A, Green = SLD 124B, Purple = SLD 124C, Yellow = 124D.
unweighted Unifrac metrics, SLD 124A and SLD 124B respectively occur as dissimilar from the other SLD 124 samples. Given the similar abundance of the dominant taxa across all the samples sites, it is likely that these differences are due more to the rare biosphere taxa or due to an artifact from the OTU picking method.

Taxa known to contain PCRM are present in the enrichments. Several well-known taxa, and a few newly defined, are seen at the genus level: Azospira, Dechloromonas, Shewanella, Pseudomonas, Sulfurospirillum, Haloarcula, Acinetobacter, Arcobacter, Sporomusa, and Marinobacter. Though these taxa are present, combined they constitute only ~1% of sequences. At these low abundances it is difficult to ascertain if the taxonomic assignment is valid given the short reads produced by Illumina next generation sequencing, though there have been studies that show that assignment down to the genus level, with the methods used in this chapter, can be accurate for a significant percentage of a sequence population from high throughput sequencing.

Figure 4.7. Phylum Level Relative Abundances for 0.1% Perchlorate Enrichment Experiments. Sample DNA obtained from Day 97 seq-six sample set.
In any case, caution will be preserved and assignments below family level will be considered suspect. At the order level there are 6 major taxa that contain PCRM: *Halobacteriales, Rhodocyclales, Campylobacterales, Pseudomonadales, Altermonadales*, and *Clostridiales*. Together, these taxa make up over 19% of all sequences with order *Clostridiales* being the largest (93%).

[Bokulich et al., 2015; Claesson et al., 2010].
4.5.3 Detection of Perchlorate Reductase and Chlorite Dismutase

qPCR shows the presence of both the \textit{cld} gene and the \textit{pcrA} gene in all of the SLD 124 replicates (Table 4.5). The highest copy number of the \textit{cld} gene is seen is SLD 124A and the lowest copy number is in SLD 124D. The copy number concentration of the \textit{pcrA} gene is fairly consistent across all four SLD 124 samples. No evidence of either gene is seen in SLD 115A or SLD 115C. In order to estimate what percentage of the enrichment culture consists of PCRM based on each gene assay, the following calculation obtained from De Long et al. [2010] was used, which assumes that both genes occur as a single copy in each PCRM:

\[
P = \frac{Q}{M \times C} \times 100
\]

Where \(P\) is the percent PCRM. \(Q\) is the \textit{cld} or \textit{pcrA} copy number per qPCR reaction (this assumes that both genes occur as a single copy in each PCRM). \(M\) is the mass of genomic DNA per qPCR reaction. \(C\) is a constant: 9.13X10^{11} \text{ bp/ng DNA} and \(G\) is the estimated average bacterial genome size: 4.501X10^{6} \text{ bp} [De Long et al., 2010]. The \textit{cld} assay estimates the abundance of PCRM at ~ 0.5-1% (Table 4.5) whereas the \textit{pcrA} assay only estimates the abundance of PCRM at ~0.01-0.02%, though it should be noted that the \textit{pcrA} qPCR assay may undercount the \textit{pcrA} gene [De Long et al., 2010] [De Long et al., 2012].

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>\textit{cld} gene (copy #/ml) std error</th>
<th>%PCRM</th>
<th>\textit{pcrA} gene (copy #/ml) std error</th>
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<td>N/A</td>
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<tr>
<td>SLD 115C</td>
<td>BDL</td>
<td>N/A</td>
<td>BDL</td>
<td>N/A</td>
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<td>8.44E+06 ±4.30E+05</td>
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<tr>
<td>SLD 124D</td>
<td>1.26E+06 ±1.90E+04</td>
<td>0.56</td>
<td>2.41E+04 ±9.40E+03</td>
<td>0.011</td>
</tr>
<tr>
<td>SLD Blank 1</td>
<td>BDL</td>
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<td>BDL</td>
<td>N/A</td>
</tr>
<tr>
<td>SLD Blank 2</td>
<td>BDL</td>
<td>N/A</td>
<td>BDL</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 4.5. Quantitative PCR Results
4.6 Discussion

The cld qPCR assay correlates with the sequencing results, thereby substantiating the estimate of the population of PCRM in the SLD 124 enrichments and the drawdown of perchlorate seen in the enrichment experiments. Perchlorate utilization is seen in both SLD 115 and SLD 124, however perchlorate reduction genes were detected only in SLD 124 samples. This could suggest that there was little to no growth from microorganisms in SLD 115, that there is a different gene system and a different pathway for perchlorate reduction, as has been seen in newly discovered strains of PCRM [Liebensteiner et al., 2013], or that there are significant PCR inhibitors within the SLD 115 samples that prevented qPCR amplification. Given that the sequencing results show approximately the same population of perchlorate reducers present in the SLD 115 samples as in SLD 124 samples, and the correlation between the qPCR estimate and the sequencing relative abundance, it is likely that SLD 115 qPCR assays were simply inhibited.

The results of this work may help to explain the variation seen in the sediment samples from the horizontal transect as the decrease of perchlorate concentration across the sample transect from the rim to the center is statistically significant (Table 4.1, Figure 4.2). This trend follows the increase in hydration of the basin sediments and the increased presence of microbial mats along the transect [Lynch et al., 2015b]. Given the mobility of perchlorate, a reasonable explanation for loss of perchlorate would be transport of the perchlorate into the subsurface aquifer. However, though the surface of the Pilot Valley basin floods on occasion, due to the nature of the basin hydrology there is no downward transmission of that floodwater and only upward transmission of evaporating brine fluid [Rey, 2015]. Hence, given that the nature of the basin hydrology prevents downward transmission of water, that the perchlorate detected on the surface of the basin was most likely atmospherically deposited [Catling et al., 2010], that surface microbial biomass (microbial mats) appears to increase along the transect, and that the only natural mechanism for perchlorate reduction is microbial metabolism, then the presence of the small community of PCRM in the basin is suggestive of perchlorate reduction activity in the near surface sediments of Pilot Valley. It should be noted that though indicative, these results are not definitive and further work is needed to
determine the extent of perchlorate reduction activity, especially since, prior to this work, there has been no documented evidence of perchlorate reducing microorganisms co-existing with naturally occurring perchlorate in hypersaline sediments; though there are known species of halotolerant and halophilic microorganisms that possess the capability [Okeke et al., 2002; Oren et al., 2014]. Furthermore, this study does not explain the lack of perchlorate in the deeper aquifer fluids and further study is warranted on this subject as well. From an astrobiological perspective, the results of this study present compelling evidence for the possibility of perchlorate-based ecosystems within early martian habitability zones [Davila et al., 2010; Dohm et al., 2011; Fairen et al., 2009; Grotzinger et al., 2014; Ming et al., 2014].

4.7 Summary and Concluding Remarks

In this study we have evaluated the co-existence of naturally occurring perchlorate and perchlorate reducing bacteria in hypersaline paleolake sediments as a model for microbial ecosystems in lake basins on early Mars. Below we present the following summary of conclusions and recommendations:

1. Naturally occurring perchlorate and PCRM coexist at Pilot Valley and can co-exist in hypersaline environments - This study has shown that naturally occurring perchlorate and PCRM can be found together in a hypersaline sedimentary environment and hence the Pilot Valley basin could serve as a model environment for early mars habitability. However, this evidence does not provide any insight into whether the PCRM are actively using the perchlorate. Therefore additional studies focused on metabolic activity should be conducted.

2. Additional study of the PCRM community is needed - Though the preliminary sequencing of the perchlorate enrichments elucidated evidence of a small PCRM community, this result needs to be validated through repeat experiments.

3. More study of the subsurface hydrology of the Pilot Valley basin in necessary - The absence of perchlorate in the brine aquifer fluids is not explained by evidence in this study and is a compelling mystery that should be
in order to accomplish this, more understanding of the subsurface hydrological system is needed.

4.8 Acknowledgements

We would like to thank Zackary Jones for assistance with Illumina sequence protocols and analysis. This research was supported by funding from the NASA Harriet Jenkins Pre-Doctoral Fellowship Program, the Edna Bailey Sussman Internship Program and the Bechtel K-5 Excellence in Education Initiative at the Colorado School of Mines. All DNA sequence data related to this study can be obtained through the European Nucleotide Archive (ENA) via accession number PRJEB11780.
CHAPTER 5
CONCLUSIONS AND FUTURE WORK

The results of the work include a comprehensive characterization of the Pilot Valley Basin, a first use of the basin as a Mars analog site, and unveiling of a compelling study site for terrestrial microbial ecology. Hence, the overarching objective of this dissertation has been accomplished as the results herein will lay the groundwork for more detailed habitability studies in preparation for Mars 2020 and for more detailed studies of microbial ecology of hypersaline sediments. This chapter presents a summary of the results of this study followed by suggested future work in the Pilot Valley Basin.

5.1 Summary of Conclusions that Address the Hypotheses

This section lists the hypotheses discussed in Chapter 1 and presents a summary of results for each study and whether the results prove or disprove the hypothesis.

5.1.1 The Pilot Valley Basin is a suitable mineralogical analog for groundwater-filled, closed basin paleolakes on Mars

The majority of the GSLD is comprised of three primary sub-basins, the Newfoundland basin, the Bonneville Salt Flats and Pilot Valley. Based on literature review of the GSLD /Lake Bonneville system, this study determined that both the Newfoundland and Bonneville basins have been subjected to significant anthropogenic activity and as a result have been altered from their nature state. Whereas Pilot Valley has remained relatively pristine, hence it is the chosen focus of this ongoing project.

Pilot Valley presents as complex mineralogical system. The Pilot Valley sediments are comprised of a diversity of phyllosilicates (kaolinite, smectite, chlorite, illite and muscovite), sulfates (mainly gypsum), carbonates (calcite, aragonite and dolomite), and ancillary minerals (quartz, k-felspar, plagioclase and minor amounts of pyrite). Several of these minerals have also been found on Mars. The active closed-basin ground-water system coupled with the mineralogical diversity makes Pilot Valley an excellent analog for paleolake basins in Mars.
5.1.2 The mineralogical and geochemical diversity of paleolake basins will prove difficult to ascertain using current planetary science exploration tools such as Visible-Near Infrared Spectroscopy (VNIR) and in situ X-ray Diffraction (CheMin).

A comprehensive evaluation of the efficacy of Visible-Near Infrared Spectroscopy as a detailed in situ exploration tool to evaluate lacustrine sediment has shown this hypothesis to be correct in that analysis of complex sediments with VNIR alone is insufficient. VNIR in its current state with current spectral libraries is sufficient to determine the major class of minerals and major transitions in composition. However, for in-situ investigations, contextual and fine scale mineralogy will benefit from inclusion of more mixed-mineral spectra in reference libraries, evaluation of oxyanion spectra, and mutual confirmation of mineralogy from other in situ instruments.

5.1.3 The microbial diversity within the Pilot Valley basin will correlate with the mineralogical and geochemical variation along the defined study transect.

Detailed evaluation of the phylogenetic diversity of the microbial community present in Pilot valley has shown this hypothesis to be, for the most part, incorrect. There is no clear correlation of community assembly with the geochemical or mineralogical variation along the horizontal basin transect. Instead, notably, diversity within the vertical transect is likely driven by grain size and possible geochemically driven.

5.1.4 Microbial perchlorate reduction is a potential contributing mechanism of perchlorate removal within the Pilot Valley basin.

Based on enrichment experiments, qPCR analysis and Illumina sequencing of SSU rRNA, there is evidence of a small PCRM community present in the near surface sediments of the Pilot Valley basin. Multiple lines of indirect evidence indicate that this community actively reduces perchlorate in the basin: the statistically significant decrease in perchlorate concentration along the study transect, the unique hydrology of the basin coupled with the mobility of perchlorate, the atmospheric origin of natural perchlorate, and the recalcitrant nature of perchlorate. Hence, it is likely that this hypothesis is correct, however more work is needed to definitely prove this hypothesis.
5.2 Recommendations for Future Work

Though many questions were answered as a result of this study, several new questions are raised as a result of this work. Hence, presented here are some recommendations for future work:

1. **Laboratory study of mineral mixtures and the effect of oxyanion salts on mixed VNIR spectra** - A logical next step to prepare for potential analysis of lacustrine sediments during the Mars 2020 mission would be to conduct comprehensive laboratory analysis of control mixtures of relevant lacustrine minerals in order to determine the effect of content variation on VNIR spectra. These mixture experiments would benefit from the inclusion of oxyanion salts as there is no documented study of effect on VNIR spectra from the mixing of oxyanion salts with other minerals.

2. **Simulated outcrop analysis using Pilot Valley basin cores** - Many aqueous features on Mars have been excavated through impacts or aeolian weathering and leaving outcrops of sedimentary sequences exposed. VNIR spectral analysis would benefit from study of various lacustrine outcrops. In the case of modern, unaltered sediments, this can be accomplished by spectral analysis of basin sample cores, which represent the sedimentary sequence that would be expected in an outcrop.

3. **Detailed geochemical and lithological analysis of the Pilot Valley sediments** - The results of this study have indicated that microbial community assembly is driven by physical grain size and possibly by geochemistry, however there are several geochemical measurements that have yet to be made and the lithological analysis would benefit from detailed quantitative input of grain size, porosity, permeability and other subsurface hydrological factors.

4. **Reactive transport modeling of the Pilot Valley basin aquifer** - In addition to detailed measurement of subsurface this work would benefit from reactive transport modeling of the subsurface. Research has shown that fluid transport in the Pilot Valley basin does not exhibit expected hydrological behavior [Rey, 2015], hence reactive transport modeling will aid in the
understanding the subsurface fluid flow and the interaction between the biosphere and the lithosphere.

5. **Metagenomic analysis of the Pilot Valley Microbial Community** - Through this work, we now have an initial idea of microbial community present at Pilot Valley. However it is still very unclear as to how the community is structured metabolically. Hence a metagenome will provide some insight into the breadth of metabolic diversity and perhaps provide additional clues to community structure and interaction with the sedimentary environment.

6. **Single Cell Sequencing for Gemmatimonadetes and PCRM** - In addition to metagenomic analysis of the entire microbial community at Pilot valley, single cell genomic sequencing would be beneficial for target genome analysis of both the organisms of phylum Gemmatimonadetes that are present in the basin and for the PCRM that are present. For the Gemmatimonadetes, the would allow further insight into the metabolic capability of organisms in this phyla, and it would also allow a window into the different type of proteins available for managing the putative water activity tolerance seen. For the PCRM, this would be beneficial, after targeted isolation of perchlorate reducing bacteria, to elucidate any new strains of microorganisms with perchlorate reducing capability in this environment. This would also beneficial for discovering if there is different metabolic machinery present for perchlorate reduction.

7. **Repeated perchlorate utilization experiments & isolation of PCRM** - This study would benefit from further perchlorate utilization experiments with fresh sediments from PV4 and from other parts of the transect. These isolation experiments should include tighter constraints on starting media composition (including and especially perchlorate concentration), variation in salinity as a treatment, more sampling over the enrichment timeframe, and transfers for confirmed isolation of PCRM.

8. **RT-qPCR of perchlorate reduction genes from RNA isolated from Pilot Valley transect sediments** - To verify perchlorate reduction activity, RNA should be extracted from Pilot Valley sediments along the defined study transect and
RT-qPCR should be conducted to evaluate if perchlorate reducers are actively using this metabolism.
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<td>Back Scatter Electrons</td>
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<tr>
<td>TOC</td>
<td>Total Organic Carbon</td>
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<td>Visible and Near Infrared Microscopy</td>
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XRD  X-Ray Diffraction
### APPENDIX A

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<th>PV2-2 (%)</th>
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<th>PV3-1 (%)</th>
<th>PV4-1 (%)</th>
<th>PV5-C (%)</th>
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<td>Volume %</td>
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<td>0.28 (0.06)</td>
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<td>0.09 (0.02)</td>
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<td>10.4 (1.08)</td>
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*Measured volume % and calculated mass %, both with standard deviation, of target minerals in the clay fraction (<3 micron) of Pilot Valley sediments. Both measured volume % and calculated mass % of each sample are averaged from five random 3 X 3mm spots scanned on each sample. Average volume % and average mass % are averaged across all samples per core analyzed by QEMSCAN.
APPENDIX B

SUPPORTING INFORMATION

FOR

MICROBIAL DIVERSITY OF HYPERSALINE PALEOLAKE SEDIMENTS ALONG A GEOLOGICAL TRANSECT IN THE GREAT SALT LAKE DESERT, UTAH.

1. SI Materials & Methods
2. SI Figures and Tables
   Table B1. Summary 454 Sequencing Statistics
   Table B2. Summary Anions for Comparison Study
   Table B3. Detailed Pilot Valley Geochemistry 1 - Sediment Cations
   Table B4. Detailed Pilot Valley Geochemistry 2 - Sediment Anions
   Table B5. Detailed Pilot Valley Geochemistry 3 - Hydration and Carbon
   Table B6. Pilot Valley Aquifer Brine Chemistry
   Table B7. Mantel Correlations for Transect Study
   Figure B1. Rarefaction Plots of for Field Site Comparison Study
   Figure B2. Krona Plot of Comparison microbial Diversity at Pilot Valley
   Figure B3. Krona Plot of Comparison Microbial Diversity of Bonneville Salt Flats
   Figure B4. Krona Plot of Comparison Microbial Diversity of Great Salt Lake
   Figure B5. PCoA Plots for Comparison Study of a) Bray-Curtis, b) un-weighted Unifrac, and c) weighted Unifrac beta diversity metrics.
   Figure B6. Rarefaction Plots for Pilot Valley Transect Study
   Figure B7. PCoA Plots for Transect Study of a) Bray-Curtis, b) Morisita-Horn, c) un-weighted Unifrac, and c) weighted Unifrac beta diversity metrics.
   Figure B8. Images of Grain Types
SI - Material and Methods

1. Field Sampling & Geochemical Analysis

1.1. Field Sampling

Each 60 cm core was collected using the AMS Extendable Core Sampler and contained using plastic sleeves. The sleeves were cut using a hand-held circular saw so that the entire core was exposed for evaluation and sampling. DNA and geological samples were taken at each point where the lithology of the sediments clearly changed.

1.2. IC and ICP OES Analysis

Pilot Valley fluid samples for IC analysis were diluted 10 and 100-fold prior to analysis. fluid samples for ICP-OES, were also diluted 10 and 100-fold and acidified with trace-metal grade nitric acid. All sediment samples were extracted for IC and ICP-OES following the Florida Department of Environmental Protection Method #NU-044-3.13 (note: the current version to-date is NU-044-3.17). Resulting extracts were diluted 10 and 100-fold and extracts for IC analysis were also acidified with trace-metal grade nitric acid. All IC samples were analyzed for major anions using a Dionex ICS-90 ion chromatography system with a AS14A (4x 250 mm) column. All ICP-OES samples were analyzed for major cations using a Perkin-Elmer Optima 5300 DV Inductively Coupled Plasma Optical Emission Spectrometer.

1.3. Total Carbon Analysis

Sediment samples were weighed, dried overnight at 100 °C, and ground with a mortar & pestle in preparation for carbon analysis. Total inorganic, total organic, and total carbon analyses were conducted using a UIC Carbon Analyzer System. Total inorganic carbon was determined using a UIC CM5130 Acidification Module. ~10 mg of sample was placed in a heated glass vial and 2 ml of 2% H₂SO₄ was added to dissolve the inorganic carbon and release it as CO₂ gas. The effluent was collected and quantified by a UIC CM5014 Coulometer for total inorganic carbon by % weight. Total carbon was determined using a UIC CM5300 Furnace Module. ~20-40 mg of sample was placed in a ceramic crucible and inserted into the oven. The sample was heated to ~935 °C so that all organic and inorganic carbon was incinerated to release CO₂ gas. The effluent was also collected and quantified by a UIC CM5014 Coulometer for total carbon by % weight. Total organic carbon was calculated from TC and TIC measurements.
Table B1. Summary 454 Sequencing Statistics

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<th>Total QC Seqs</th>
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<td>Transect</td>
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*Raw sequences after "split_library.py" command script to parse sequences from shared 454 plates.

Table B2. Anion Data for Comparison Sites\(^a\)

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\(^a\)There is no cation data available for these samples. BDL = Below Detection Limit
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<th>Na wt %</th>
<th>K wt %</th>
<th>Mg wt %</th>
<th>Fe mg/kg</th>
<th>Ba mg/kg</th>
<th>Li mg/kg</th>
<th>Sr mg/kg</th>
<th>V mg/kg</th>
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Table B4. Detailed Pilot Valley Geochemistry 2 - Sediment Anions

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<th>F mg/kg</th>
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Table B6. Pilot Valley Geochemistry 4 - Aquifer Brine Chemistry$^a$

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$^a$There is no data for Sample Site PV-1 as the bore hole could not reach the water table at the rim of the basin.
Table B7. Mantel Correlations

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Mantel test statistic and P-value based on 999 permutations for each variable. *P ≤ 0.05, **P ≤ 0.01
Supplementary Figures

Figure B1. Rarefaction Plots for Field Site Comparison Study. 1a) Observed Species. 1) Faith's Phylogenetic Diversity [Faith and Baker, 2006].
Figure B2. Krona Plot of Comparison Microbial Diversity at Pilot Valley
Figure B3. Krona Plot of Comparison Microbial Diversity of Bonneville Salt Flats

Figure B4. Krona Plot of Comparison Microbial Diversity of Great Salt Lake
Figure B5. PCoA Plots of a) Bray-Curtis, b) un-weighted Unifrac, and c) weighted Unifrac beta diversity metrics. Blue = Pilot Valley, Red = Bonneville Salt Flats, Orange = Great Salt Lake.
Figure B6. Rarefaction Plot of Samples Coverage for Transect Study. 4a) Observed Species 4b) Faiths Phylogenetic Diversity [Faith and Baker, 2006].
Figure B7. PCoA Plots for Transect Study. a) Bray-Curtis, b) Morisita Horn, c) Unweighted Unifrac, and d) Weighted Unifrac beta diversity metrics on core subsamples across Pilot Valley Transect. Sample Sites are identified by color: Red = PV-1, Blue = PV-2, Orange = PV-3, Green = PV-5.
Figure B8. Images of Grain Types. A) Fine Clay B) Coarse C) Fine Silt
Appendix References

Faith, D. P., and A. M. Baker (2006), Phylogenetic diversity (PD) and biodiversity conservation: some bioinformatics challenges, Evolutionary Bioinformatics Online, 2, 121-128.