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Bacterial Diversity and Ecology of Two Cryptoendolithic Habitats in the Grand Staircase Escalante National Monument, Utah, USA

Sukhpreet Kaur
Clemson University

BACTERIAL DIVERSITY AND ECOLOGY OF TWO CRYPTOENDOLITHIC HABITATS IN THE GRAND STAIRCASE ESCALANTE NATIONAL MONUMENT, UTAH, USA

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Sukhpreet Kaur
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Dr. Harry D. Kurtz, Jr., Committee Chair
Dr. Barbara J. Campbell
Dr. J. Michael Henson
Dr. David L. Freedman

ABSTRACT

Sandstone outcrops in the Grand Staircase Escalante National Monument, Utah are host to cryptoendolithic communities dominated by cyanobacteria. These communities produce extracellular polymeric substances that not only aid their survival, but also support other heterotrophic bacteria. Developing a better understanding of the role of these cryptoendolithic communities requires a deeper knowledge of the microbial diversity present. We analyzed the cryptoendolithic bacterial communities in the Jurassic Navajo Sandstone samples collected from several microgeological features associated with a large sandstone dome. These communities clustered into distinctive groups that correlated with topography, suggesting that moisture availability plays an important role in shaping the community structure in this microhabitat. Comparisons of diversity between these distinctive groups showed that a core bacterial community exists in this habitat. The overall bacterial community structure was dominated by Cyanobacteria, Proteobacteria, Bacteroidetes and Actinobacteria. Cyanobacteria were chiefly represented by *Leptolyngbya* in this habitat while the genus *Acidiphilium* was particularly abundant in the class Alphaproteobacteria. In contrast, the major inhabitants of the cryptoendolithic communities in the Entrada Sandstones are Cyanobacteria followed by Proteobacteria, Actinobacteria, Bacteroidetes and Deinococcus-Thermus. Cyanobacteria were chiefly represented by *Chroococcidiopsis* in this habitat while the genus *Truepera* belonging to the phyla Deinococcus-Thermus had a notable presence. The differences in the bacterial composition of the Navajo and Entrada cryptoendolithic habitats are attributed to the varying sand grain size and distribution patterns which affects porosity and moisture

availability in the underlying substrate. The number of unclassified OTUs found in both the cryptoendolithic habitats suggests that abundant, unexplored microbial diversity exists in this microecosystem, identifying a conservation value for these communities. EPS produced by both the natural and laboratory grown cryptoendolithic communities are capable of binding divalent metal cations (Fe, Mn, Mg, Cu, Zn), all essential cofactors for oxygenic photosynthesis. Based upon these data, we conclude that the EPSs produced by these cryptoendolithic communities act as a biofilter to bind and concentrate essential metal ions, to provide a source of key nutrients for cellular metabolism. This study sets the premise for elaborating on the ecological functions of cryptoendolithic communities in sandstones.

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TABLE OF CONTENTS

LIST OF TABLES

List of Tables (Continued)

Table Page

LIST OF FIGURES

List of Figures (Continued)

Figure Page

List of Figures (Continued)

Figure Page

Chapter 1

Literature review

Living on the edge: cryptoendolithic bacterial communities in desert sandstones

Background

Deserts are the most extensive terrestrial biome, covering approximately one-third of the global land surface (Redfield et al., 2002; Garcia-Pichel, 2003; Laity, 2009). Water availability is one of the major constraints for the existence of life in deserts. The water deficit in deserts is reflected by the precipitation to potential evapotranspiration ratio (P/ETP) ratio known as the aridity index (AI) as per the criterion set by the United Nations Environment Program (UNEP, 1992). Based on the Aridity index, deserts have been divided into four zones, sub-humid AI = 0.5–< 0.65; semi-arid AI = 0.2–< 0.5; arid AI = 0.05–< 0.2 and hyper-arid AI < 0.05, as shown in Figure 1.1 (UNEP, 1992; Chan et al., 2012; Pointing and Belnap, 2012).

Over fifty percent of all desert landscapes are covered by desert pavement, which refers to surfaces composed of pebbles or rocks embedded in and covering the soil surface (Laity, 2009). Deserts are typically low-energy systems with coarse alkaline sands deficient in organic matter and nutrients in addition to high heat, diurnal temperature fluctuations, scanty rainfall, low moisture content in the air, winds and shifting sands, desiccating conditions and intense solar irradiation (Friedmann, 1980; Budel and Wessels, 1991; Belnap, 2003; Blackhurst et al., 2005; Thomas and Dougill, 2006; Gorbushina, 2007; Chan et al., 2012). Despite the fact that desert landscape does not seem conducive for life, a number of microhabitats harbor microorganisms that have

adapted to withstand the harsh environmental conditions. These microhabitats include rocks as well as soil ecosystems, which will be discussed in the following sections.

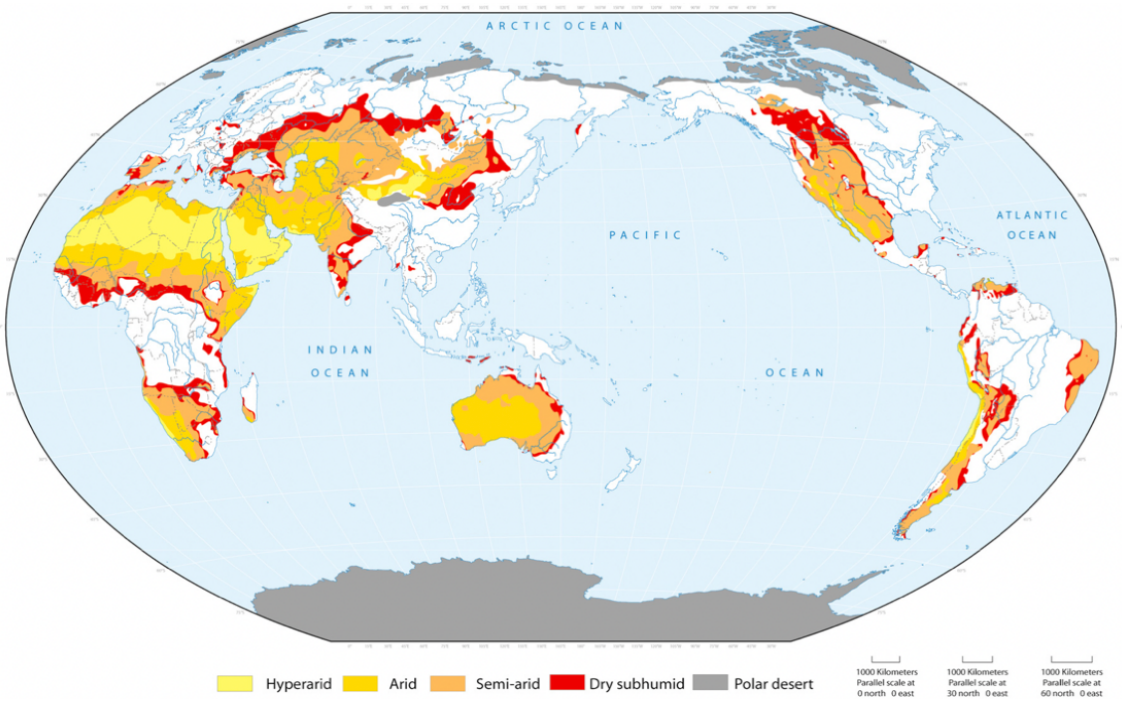

Figure 1.1 Distribution of deserts based on the Aridity Index on a global scale. Re-printed from Chan et al., 2012- Reproduced with permission from Wiley publishing group.

Geological setting: The Grand Staircase Escalante National Monument, USA

The Grand Staircase Escalante National Monument (GSENM) located in southern Utah is a part of the Colorado Plateau physiographic province of the USA (Figure 1.2, Panel A). The Colorado Plateau is a rugged area composed of canyons, plateaus and cliffs derived from diverse geologic formations (Doelling et al., 2000). The GSENM is located at the center of the Colorado Plateau encompassing a large area of semi-arid to arid lands.

Portions of the GSENM are dominated by large sandstone domes and other barren rock outcrops with expanses of rocky flats interspersed between elevated features (Figure 1.2, Panel B). This is especially prevalent in the arid Escalante Canyons region of the monument.

B.

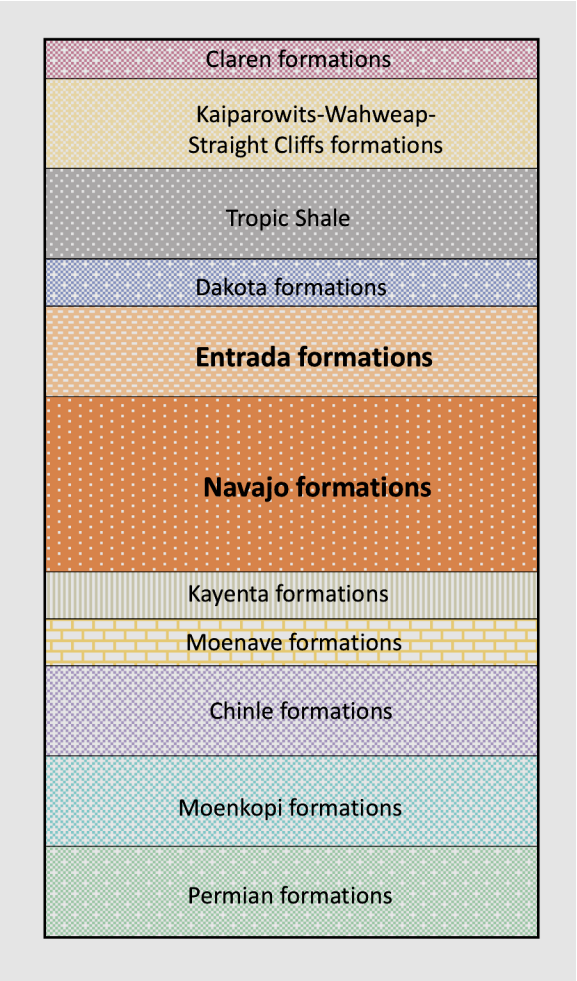

Figure 1.2 (A). The geological setting: The Grand Staircase Escalante National Monument located in southern Utah (B). The stratigraphy of the Jurassic Navajo and Entrada Sandstones in the GSENM (Adapted from Doelling et al., 2000).

The erosion of the Colorado Plateau has resulted in the sculpting of a series of multi-hued, steep, narrow canyons and slickrock outcrops in the Escalante Canyons section of this region (Doelling et al., 2000). The prominent features of this region are lithified eolian deposits arising from large to regionally extensive ergs, that ultimately formed the Jurassic Navajo and Entrada Sandstone units. Hematite and strata bound layers occur in

both the Jurassic Navajo and Entrada sandstone formations (Chan et al., 2000). The Jurassic Navajo Sandstone is one of the largest ergs known to have existed in the geological record (Beitler et al., 2005). It is a massive, cross bedded sandstone that was deposited by wind in a vast desert which covered most of the Colorado Plateau (Doelling et al., 2000). The Entrada Sandstones are relatively friable sandstones forming earthy slopes and ridges. While both the Jurassic Navajo and Entrada Sandstones are eolian deposits, they differ in grain size and geological characteristics as listed in Table 1.1 (Foos, 1999).

Table 1.1 Description of the two stratigraphic sandstones in this study, the Jurassic Navajo and Entrada Sandstone units.

Characteristics	Navajo Sandstone	Entrada Sandstone
Age	Jurassic	Jurassic
Thickness (Ft.)	250-550	60- 250
Coloration	Pale orange	Reddish brown to pale orange
Grain size	fine to medium grained	fine grained
Sorting	well sorted	poorly sorted
Features	cliffs and hummocky knobs	cliffs

Lithic microhabitats in arid landscapes

Microbes are ubiquitous on Earth's surface, with the possible exception of cooling lava flows. Rocks constitute an important refugium for diverse microbial life forms, especially in deserts. In Geobiology, rock-dwelling microbes have been broadly classified as endoliths, epiliths and hypoliths. Microbes that colonize the exposed outer surface of the

rocks are epiliths while those colonizing the underside of rocks are known as hypoliths (Antony et al., 2012). Endoliths are microorganisms that colonize the inside of the rock matrix and can be divided into three types depending on the mode of entry into the rocks. Microbes that actively bore into the rocks are euendoliths, those living in crevices and fissures are chasmoendoliths, while the ones colonizing the pre-existing pore spaces are cryptoendoliths (Bell and Sommerfield, 1986; Bell et al., 1988; Bell, 1993; Hirsch et al., 1995; Cockell and Herrera, 2008; Blackhurst et al., 2005; Antony et al., 2012). The endolithic habitat provides protection from multiple environmental stressors in deserts, creating suitable conditions for growth of microorganisms. However, the microclimate of rocks in hot deserts can be harsh enough to inhibit or discourage the existence of microorganisms on rock surfaces i.e. epilithic microbial growth (Bell, 1993; Ferris and Lowson 1996; Walker and pace 2007). Indigenous microorganisms penetrate and live inside rocks where they are afforded a degree of protection from desiccation, intense sunlight, physical abrasion and extreme temperature fluctuations (Bell, 1993). The focus of this review is cryptoendolithic microorganisms, which are one of the clearest examples of how a microbial community is able to avoid climate extremes (Weirzchos et al., 2012). The colonization of a cryptic niche holds advantages over exposed niches in terms of thermal and moisture buffering as well as physical stability. Cryptoendolithic microbes have been studied in many rocky desert environments: the Colorado Plateau (Bell, 1993), Antarctica (Friedmann and Weed, 2006; Friedmann, 1982), the Atacama Desert (Weirzchos et al., 2006), Southern Tunisia (Stivaletta and Barbieri, 2009) and South Africa (Budel et al., 2004). Typically, these communities are located within first few

millimeters of the host rock surface, regardless of the global location, as the communities are supported by photosynthetic microorganisms (Omelon et al., 2006). An advantage of the enclosed endolithic microenvironment is that the bacteria are directly in contact with the host rock, which allows the best possible situation for the manipulation of local chemistry and derivation of micronutrients (Walker and Pace, 2007). The weathering of rocks is a natural phenomenon, caused by prevailing physical conditions such as temperature changes, wind erosion, dehydration and rewetting, rain as well as by microbial activities, leading to the formation of soil (Saiz-Jimenez et al., 1990; Hirsch et al., 1995; Omelon et al., 2006). Microbial communities flourishing in soil systems tend to form a framework collectively known as biological soil crusts.

Microbes in desert soils: Biological soil crusts

One of the most studied microhabitats in deserts is a system known as biological soil crusts. Biological soil crusts are basically surface bound assemblages of microorganisms dominated by cyanobacteria that consolidate soils into millimeter to centimeter thick crusts also known as cryptogamic, cyanobacterial or microbiotic crusts (Johansen, 1993; Evans and Johansen, 1999; Garcia-Pichel et al., 2003; Maestre et al., 2011). Cyanobacterial crusts cover about 70% or more of soils in arid landscapes, playing an integral role in the functioning of desert ecosystems (Garcia-Pichel et al., 2001; Belnap and Lange, 2002; Redfield et al., 2002; Belnap et al., 2004; Belnap and Weber, 2013). There is an extensive literature reporting biological soil crusts as important contributors to soil fertility, moisture and stability of the region (Johansen, 1993; Garcia-Pichel and

Belnap, 1996; Garcia-Pichel et al., 2001; Belnap, 2002; Redfield et al., 2002; Belnap et al., 2004; Belnap and Weber, 2013). These crusts are critically important in the carbon and nitrogen cycling in desert ecosystems (Belnap, 2003). They facilitate the formation of soil aggregates and the microbial mats enhance water retention and nutrient acquisition, increasing the productivity of the soil ecosystem (Belnap, 1993; Mazor et al., 1996; Redfield et al., 2002; Garcia-Pichel et al., 2003; Schwinning et al., 2008; Flemming and Wingender, 2010). Microbial mats in desert soils have been used as model systems to study the linkage between diversity and function resulting in better understanding of the soil ecosystem at several spatial scales (Bowker et al., 2010; Maestre et al., 2011). However, it is important to realize that these soils receive a considerable input from the parent material (rocks), so it is essential to have a fundamental understanding of the diversity and physiological functioning of the rock-inhabiting microorganisms. The parent rock outcrops are subject to exfoliation of rock flakes, especially in the case of sandstones, because of physical conditions such as high winds, and freeze-thaw events. During these events, microbes living in the rocks may be exposed to the outer environment and may land on nearby soils via wind dispersal, potentially contributing to the growth of biological soil crusts.

Cryptoendolithic habitat: hidden microbiota in desert rocks

The cryptic mode of microbial colonization is a stress avoidance strategy where the rock habitat provides physical and environmental protection to microbial life. In the hostile environmental conditions of deserts, the ability to utilize available energy and nutrients

varies from zero to high after precipitation events. The changing environmental conditions in deserts force rock inhabitants into long periods of stress-induced dormancy that are suddenly interrupted by the intermittent return of growth permissive conditions. Only microorganisms capable of surviving under oligotrophic conditions while tolerating multiple fluctuating stressors are the ones that can establish themselves under these extreme environmental conditions (Gorbushina and Broughton, 2009). In hot deserts, the most favorable combination of water availability and moderate temperature occurs in the early morning hours after dew formation. Later in the day, the temperatures rise, and relative humidity drops dramatically. This combination of high temperatures and extreme aridity imposes a severe environmental stressor that precludes the survival of eukaryotic organisms. The rapid fluctuations between favorable humid and unfavorable dry conditions results in the hot desert endolithic ecosystems being dominated by prokaryotes (Friedman, 1980). To avoid or reduce exposure to these stressors, prokaryotes have adapted to living in the pores of sandstones as cryptoendolithic microbial communities. These communities are macroscopically visible as a greenish band in the rock interior at a depth of up to a few millimeters (Figure 1.3).

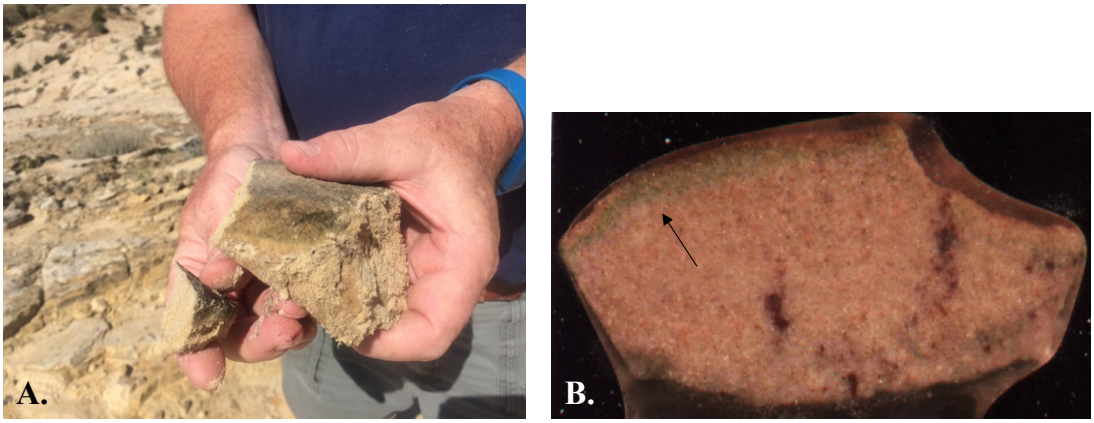

Figure 1.3 Cryptic microorganisms indicated by (A). black greenish color on the surface of sandstones (B). green layer a few millimeters beneath the surface of sandstones.

Cryptoendolithic microbial communities are amongst one of the most resistant assemblages of life forms existing in desert ecosystems. They have been found in hot and cold deserts such as the Negev Desert in Israel and the Dry Valleys of Antarctica (Friedmann et al., 1967; Friedmann and Ocampo, 1976). One of the characteristics of cryptoendoliths is their ability to grow and photosynthesize at low water potentials while being resistant to drying events. In the event of *in situ* weathering of bedrock, it is likely that the cryptoendolithic communities may act as a reservoir of microbial biomass that gets dispersed to other habitats under dry windy conditions or precipitation events. Alternatively, if liquid water is available, exposed cryptoendoliths may be drawn back into the rock matrix, thereby maintaining a stable cryptoendolithic zone as the rock weathers in its natural course (Bell, 1993). Cryptoendolithic microorganisms in hot deserts are most commonly found in crystalline fine-grained sandstones as well as in

coarse-grained quartzites and limestones (Bell, 1993; Ferris and Lowson, 1997; Cary et al., 2010).

Desert rocks, particularly those with a porous structure such as sandstones, serve as an ideal growth substrate for cryptoendolithic communities (Friedmann, 1980; Bell and Sommerfield, 1986; Bell, 1993; Ferris and Lowson, 1997). The porosity of the sandstones allows water to seep into the rocks and sand crystals permit light to penetrate the rock surface enabling the photosynthetic microorganisms in the cryptoendolithic communities to survive (Bell et al., 1986; Bell, 1993). Sandstones also act as thermal collectors as winds cool the surface of the rock but not the rock matrix leading to a greater thermal load for the cryptoendoliths (Bell, 1993). Interestingly, this increased thermal load turns out to be an advantage for the cryptoendolithic microorganisms as it leads to frequent dew formation on the rock surfaces and this water may be taken in by capillary action as the primary source of water by these microbes (Friedmann and Galun, 1974; Friedmann and Ocampo, 1976; Evans and Johansen, 1999; Walker and Pace, 2007; Antony et al., 2012). The Jurassic Navajo and Entrada Sandstones in the GSENM include the cryptoendolithic habitats analyzed in this study.

Cryptoendolithic microorganisms – community structure and ecology

Cryptoendolithic microorganisms in oligotrophic sandstones form distinct green layers about 0.5-5-millimeter beneath the sandstone surface, receiving enough sunlight to allow photosynthetic processes (Ferris and Lowson, 1997; Budel et al., 2004; Omelon et al., 2006; Gorbushina, 2007; Walker and Pace, 2007). Numerous studies have suggested that

the dominant photosynthetic microorganisms in the cryptoendolithic microbial communities of sandstones are cyanobacteria (Bell, 1993; Hirsch et al., 1995; Blackhurst et al., 2005; Hammes et al., 2013). This observation is supported by the fact that cyanobacteria have a broad eco-physiological tolerance to cope with extreme hydrological and thermal stressors (Stahl, 1995). Cyanobacteria are the main contributors to flow of carbon and energy as well as fixing nitrogen in these cryptoendolithic microbial communities (Bell and Sommerfield, 1986; de la Torre et al., 2003). Apart from being the pioneer photoautotrophic colonizers of bare rocks in deserts, cyanobacteria have also been shown to play a role in shaping the sandstone landscapes in the geological past (Budel et al., 2004; Blackhurst et al., 2005). The most frequently reported cryptoendolithic cyanobacteria in the sandstones of hot deserts belongs to the genus *Chroococcidiopsis,* which reproduces via baeocytes, and is observable by phase contrast and scanning electron microscopy (Palmer and Friedmann, 1990; Saiz-Jimenez et al., 1990; Casamatta et al., 2002; Wynn-Williams, 2002; Budel et al., 2004). The phyla that are most common heterotrophs in cryptoendolithic communities include Proteobacteria, Bacteroidetes, Actinobacteria and Acidobacteria (Makhalanyane et al., 2015; Lee et al., 2016; Lacap-Bugler et al., 2017). The close physical relationship between cyanobacteria and heterotrophic bacteria likely results in nutrient cycling within endolithic communities. At the same time, reactions between organic matter and metal oxyhydroxides are important in rendering otherwise insoluble metals, such as iron and manganese, soluble and mobile, making these nutrients accessible to the microbes growing within the rock habitats (Ferris and Lowson, 1997).

Previous studies have confirmed that cryptoendolithic communities are indeed analogous to microbial mats or biofilms (Bell, 1993; Kurtz Jr. et al., 2005). Known survival strategies of these microbial biofilms to extreme environmental conditions include production of hygroscopic extracellular polymeric substances (EPS), which supports the growth of other heterotrophic bacteria forming an intermixed ecosystem (Hirsch et al., 1995; Omelon et al., 2006). A notable feature of the microbial consortia embedded in a matrix of EPS is that they overcome environmental stresses more efficiently than what a microorganism can do alone by itself, suggestive of possible synergistic interactions (Gorbushina, 2007). EPS serve many ecological functions, including protection from desiccation through the retention of water, moderating temperature fluctuations, increasing nutrient availability and hardening the surface of sandstone outcrops protecting the sandstone against wind and water erosion (Seibert et al., 1996; Kurtz Jr. and Netoff, 2001; Omelon et al., 2006; Belnap, 2012; Casamatta et al., 2002; Mager, 2010). EPS has also been known to reduce water loss via evaporation potentially helping the cryptoendoliths survive better and providing an effective, long-term carbon sink (Belnap, 2003; Brostoff et al., 2005; Thomas and Dougill, 2006). Cyanobacterial mats can endure repeated cycles of desiccation and rewetting, resuming their metabolic activities within minutes of rehydration (Evans and Johansen, 1999; Gorbushina, 2007; Walker and Pace, 2007). Futhermore, upon rewetting the EPS swells and few coccoidal cyanobacterial cells may be expelled from the main aggregate forming a mechanism for continual colonization of cryptoendoliths in these sandstones (Bell et al., 1986; Budel et al., 2004).

Cryptoendolithic microorganisms form stratified communities (de la Torre et al., 2003) with high physiological diversity along with spatial separation in layers with distinct activities within the community (Seibert et al., 1996). Validating the diversity of microbial mats is challenging because of the differences over spatial scales and the wide variety of environments in which they occur (Redfield et al., 2002). Another reason is that not all microorganisms can be isolated from the microbial consortia based on their dependence on interactions with other resident organisms that is required for their survival. Moreover, the effects of species evenness and richness, upon biogeochemical function are less frequently studied because of the complexity of these model systems. The cryptoendolithic microbial diversity within these sandstones was studied using microscopic and molecular techniques in the past (Bell, 1993; Kurtz et al., 2005; Hammes et al., 2013).

More recently, the advent of Next Generation Sequencing (NGS) technologies has enabled scientists to explore microbial diversity at greater depths. Illumina MiSeq sequencing has become popular since it can generate multi-million reads with increased sequencing length to meet the data demands of environmental microbial ecology studies and reduced costs (Mizrahi-Man and Gilad, 2013; Caporaso et al., 2011). The characterization of bacterial communities using 16S RNA gene sequences is a standard approach in microbial ecology and allows for the identification of populations in low abundance that may account for functional diversity and ecosystem stability (Birtel et al., 2015).

Analysis of life under extreme conditions: importance and implications

Cryptoendolithic microorganisms provide valuable insights into how the biosphere influences the desert terrestrial environment and their importance is highlighted in Figure 1.4. Firstly, this study will setup the baseline community structure for the cryptoendolithic communities in the sandstones outcrops of the GSENM. It will be helpful to analyze the effects of environmental changes either because of natural causes or human activities that may potentially influence the cryptoendolithic community structure over a period of time. Secondly, temperate environments such as the semi-arid and arid deserts of the GSENM, are repositories of biota that live under water and nutrient-limited conditions. Thirdly, the first step in understanding the functioning of microbial communities in any ecosystem is gaining adequate knowledge of the constituents of the microbial communities. This study aims to determine the cryptoendolithic community structure in the Jurassic Navajo and Entrada Sandstones which sets the premise for elaborating the functioning of these communities in this microecosystem. GSENM is a rich national and cultural heritage in the United States and having knowledge about the cryptoendolithic communities will be one step towards the conservation of our natural desert ecosystems. Last but not the least, cryptoendoliths have evolved to exploit extreme environments on Earth, where they play key roles in ecosystem function, as pioneer species and primary producers essentially terraforming these ecosystems (Friedman and Weed, 1978; Lester et al., 2007; Ohad et al., 2005). Initial colonization of dry, nutrient poor environments, in many cases, begins to alter the area to allow it to be used by less tolerant biota, marking the boundary in between a

landscape with productive soils and a desert (Belnap et al., 2004; Omelon, 2008; Mager and Thomas, 2011). Liquid water is the quintessential ecological requirement for all life on Earth to grow or reproduce. Based on this simple fact, the search for life beyond the Earth is often characterized by a "follow the water" strategy, it is important to understand the limits of life on Earth to be able to get an insight on life beyond our planet (Tracy et al., 2010; Stivaletta, 2011). The study of cryptoendolithic microbial life gives insights into the ability of life to exist under extreme conditions on Earth, and therefore the potential for life elsewhere (Ascaso and Weirzchos, 2002). From a microbial ecologist's point of view, these model systems could be helpful to study the complex interactions between microbes and minerals that drives the biosphere (Walker and Pace, 2007). Cryptoendolithic habitats in arid landscapes may serve as model systems for studies on interactions of biology and geology (Geobiology: Ehrlich 1997; Walker and Pace, 2007), biogeochemical cycles (Holloway and Dahlgren, 1999; Hammes et al., 2013), the origins and evolution of life and the search for life elsewhere in this universe (Beitler et al., 2004; Chan et al., 2004; Potter et al., 2011; Chan et al., 2012).

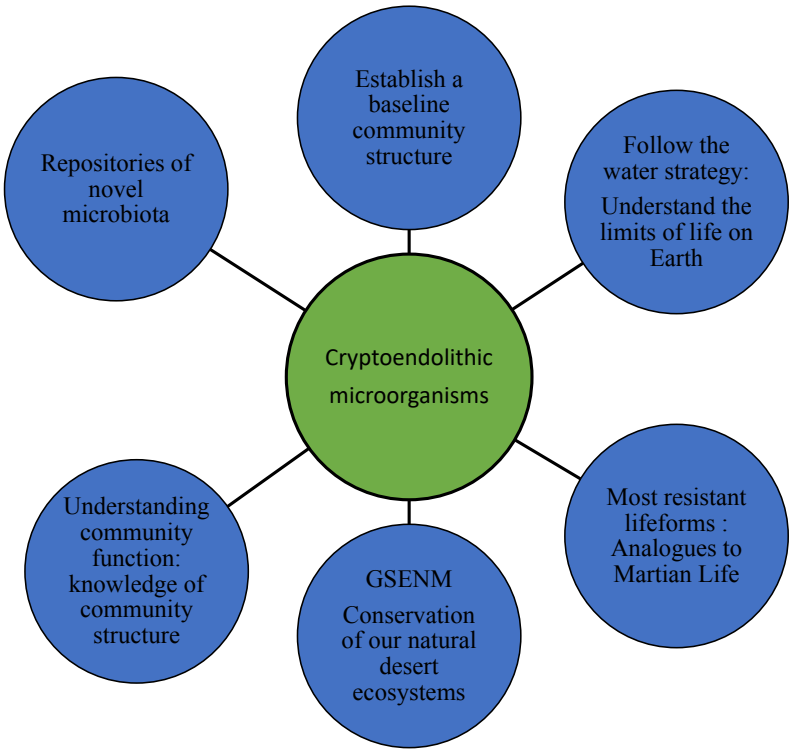

Figure 1.4 Importance of the study of cryptoendolithic microorganisms in desert ecosystems.

Hypothesis and Objectives:

Based upon the previous research of Kurtz and colleagues, I hypothesize that the cryptoendolithic bacterial communities in the Jurassic Navajo Sandstone are highly diverse, with members potentially involved in microscale iron cycling. The overall diversity of these communities may be influenced by the local topographic features and moisture availability regimes. Considering the heterogeneity of geological features in the GSENM, I also hypothesize that the cryptoendolithic bacterial community structures in the Jurassic Navajo and Entrada Sandstones are distinctive, with exclusive core bacterial

communities that are shared within each habitat. Lastly, I hypothesize that the extracellular polysaccharides (EPSs) produced by cryptoendolithic communities are capable of binding not just ferrous iron, but other divalent cations making them available to the underlying microorganisms for basic life processes.

The following objectives were set to test these hypotheses.

Objective 1: Characterize the cryptoendolithic bacterial communities existing in the Jurassic Navajo Sandstone samples, associated with varying topographic features of the rock outcrop.

Objective 2: Use Next Generation Sequencing to determine the dominant taxa associated with the cryptoendolithic bacterial communities in the Entrada Sandstone and compare the microbial diversity between the two geological substrates: Entrada and Jurassic Navajo Sandstones.

Objective 3: Investigate the presence and availability of selected metal cations in uncolonized and colonized (cryptoendolithic communities) portions of both Jurassic Navajo and Entrada sandstones. Measure the cation binding profile of EPSs isolated from laboratory grown cryptoendolithic microbial biofilms and semi-pure cultures of cyanobacteria.

The results for each objective are presented in the chapters that follow.

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Chapter 2

Core bacterial community composition of a cryptoendolithic ecosystem in the Grand Staircase-Escalante National Monument, Utah, USA

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Sukhpreet Kaur
Department of Biological Sciences
Clemson University
Clemson, SC 29634

Corresponding Author
Harry D. Kurtz, Jr.
Department of Biological Sciences
Clemson University
Clemson, SC 29634
Phone: 864-656-6915
FAX: 864-6565-0435
Email: hkurtz@clemson.edu

Running Title – Cryptoendoliths in the Jurassic Navajo sandstone

Originality-Significance Statement: This is the first report describing the core cryptoendolithic bacterial community composition in the Jurassic Navajo Sandstone of the Grand Staircase Escalante Monument, Utah, USA. Community structure was found to be independent of inorganic nutrient content, and dependent upon topography. *Acidiphilium* spp. were found to be abundant in these samples, potentially explaining the presence of high amounts of ferrous iron previously detected in this ecosystem.

ABSTRACT

Cryptoendolithic bacterial communities in the Jurassic Navajo Sandstones play an important ecological role in this ecosystem. Developing a better understanding of the role of these cryptoendolithic communities required a deeper knowledge of the microbial diversity present. We analyzed the bacterial diversity in eight sandstones samples from several micro-geological features associated with a large sandstone dome. Cryptoendolithic bacterial diversity clustered into three distinct groups which correlated with topography, suggesting the duration of water retention might be a factor. Comparisons of diversity between each cluster showed that a core bacterial community exists in this habitat. The overall bacterial community structure was dominated by *Cyanobacteria*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria*. The most prevalent genera in Cyanobacteria were *Leptolyngbya*, *Chroococcidiopsis* and unclassified Cyanobacteria accounting for the bulk of cyanobacterial sequences. Within the *Proteobacteria*, Alphaproteobacteria were the largest class detected, with members of the Acetobacteraceae, particularly the genus *Acidiphilium*, being the most abundant. *Acidiphilium* spp. are capable of aerobic ferric iron reduction under moderately acidic conditions, explaining the high levels of iron (II) in this system. This study highlights the extent of unexplored bacterial diversity in this habitat system and sets the premise for elaborating on the ecological function of cryptoendolithic communities.

Keywords: GSENM; cryptoendolithic bacterial communities; amplicon sequencing, *Cyanobacteria*; *Acidiphilium spp.*

INTRODUCTION

Deserts constitute the most extensive terrestrial biome and cover about thirty percent of the United States land area (Housman et al., 2006; Pointing and Belnap, 2012). The Grand Staircase-Escalante National Monument (GSENM) located in southern Utah, USA, encompasses a large area of semi-arid and arid regions with diverse geological features (Doelling et al., 2000). Throughout the GSENM, large sandstone domes and other barren rock outcrops dominate the landscape with expanses of rocky flats interspersed between elevated features. This is especially prevalent in the arid Escalante Canyons region of the monument. Among the different rock formations in GSENM, the eolian Jurassic Navajo sandstone unit in known for patterns of pink to red coloration resulting from iron removal throughout its burial history (Beitler et al., 2005; Potter et al., 2013). Some areas of the Jurassic Navajo sandstone have been bleached of their color which has been mainly attributed to paleo-geochemical processes (Chan et al., 2000; Beitler et al., 2003; Potter and Chan, 2011). Previous studies suggest that microorganisms are at least partially involved in this ongoing process of iron bleaching (Loope et al., 2010; Hammes et al., 2013).

The Jurassic Navajo Sandstone unit is a friable, poorly cemented sandstone that is highly porous (Kurtz Jr. and Netoff, 2001). Moisture availability is a major constraint affecting microbial diversity and activity in arid environments (Potts and Friedmann, 1981; Bhatnagar and Bhatnagar, 2005; Laity, 2009). The irregular system of pores in sandstones provides a protective network for microorganisms, creating a place for condensation and retention of water, while allowing light to penetrate the upper sandstone

surface (Friedmann, 1980; Bell, 1993; Walker and Pace, 2007). In hot deserts, the combined effects of temperature and aridity along with lack of nutrients in sandstones leads to unique adaptations in desert microbiota (Gorbushina, 2007; Makhalanyane et al., 2015).

One survival adaptation of bacterial communities is to reside within the pore spaces of desert sandstones as cryptoendoliths. This ecological niche provides microorganisms physical stability and protection from extreme environmental conditions in hot deserts (Friedmann and Ocampo, 1976; Wierzchos et al., 2012; Yung et al., 2014; Archer et al., 2017). Cryptoendolithic communities produce extracellular polymeric substances (EPS) under moist conditions as another survival adaptation to retain water, entrap nutrients and reduce temperature fluctuations (Kurtz Jr., 2002; Büdel et al., 2004; Omelon et al., 2006; Gorbushina and Broughton, 2009; Antony et al., 2012; Lacap-Bugler et al., 2017). Cryptoendolithic biomass acts to stabilize the friable sandstone surface through the production of EPS and in some cases by filamentous cell growth (Kurtz Jr. and Cox, 2010), protecting the sandstone surfaces from erosional processes, resulting in diverse micro-scale features, such as rock visors and undercut ripples in the sandstones (Kurtz Jr. and Netoff, 2001). Such micro-geomorphological features have a direct effect on water and nutrient availability based on infiltration, run-off, erosion and water accumulation (Li et al., 2010). This geologic heterogeneity increases the potential for heterogeneous communities being assembled in the cryptoendolithic habitat.

Cyanobacteria are the primary producers within the cryptoendolithic communities, providing energy in the form of fixed carbon by photosynthesis and

supporting the growth of heterotrophic microorganisms (Casamatta et al., 2002; de la Torre et al., 2003; Archer et al., 2017). Cryptoendolithic bacterial communities are involved in nutrient cycling within the oligotrophic rock substratum (Kurtz Jr. et al., 2005, Hammes et al., 2013). Microbial diversity within these sandstones has previously been studied using microscopic and molecular techniques (Bell, 1993; Kurtz et al., 2005; Hammes et al., 2013). While these analyses have provided some data on the structure of these cryptoendolithic communities, the data are insufficient to accurately describe these communities. To address the lack of depth with respect to cryptoendolithic community structure in the Jurassic Navajo sandstones, we used Illumina Miseq sequencing technology to acquire the requisite data.

In this study, we examine the bacterial diversity of cryptoendolithic communities associated with the Jurassic Navajo sandstone of the GSENM. Considering the heterogeneity of geological features around this land form, we expect that the sub-communities would differ from each other and that from these data, a core bacterial community could be derived. Additionally, we expect to find taxa having members known to participate in nutrient cycling. Below, we provide data to support these hypotheses.

MATERIALS AND METHODS

Site characterization and sampling procedure

All samples were obtained from the Jurassic Navajo Sandstone, an eolian sandstone unit located in the Harris Wash area of the GSENM in southern Utah, USA (Hammes et al., 2013). Eight sandstone samples were collected from sites around a sandstone dome

having different topographic features (Table 2.1, Figure 2.1). The HW in the samples denotes the Harris Wash area followed by the site number and the year of sampling. Three samples, HW01_04, HW01_05 and HW07_05, were from rock surfaces that were slightly sloped and without eolian sediments. HW07_04 was obtained from a rock slope (Figure 2.2). HW03_04 and HW04_05 were from rock surfaces near sediment deposits near the base of a sandstone dome. Two samples, HW06_04 and HW04_04 were obtained from an alcove that is a wind-eroded depression in a small cliff (Figure 2.2). Samples were obtained using a chisel to remove the upper 5-10 mm of sandstone surface from an area of approximately 50 cm^2 and placed into sterile sample bags. All the sandstone samples were stored in the dark at room temperature as dry samples until further processing.

Chemical analysis of the sandstones

Sandstone color was analyzed by comparison with the Munsell soil color charts (Munsell color company, 1975) assigning each moist sandstone sample the nearest integer unit of hue, value and chroma (Escadafal et al., 1989). The pH of the sandstone samples was measured with an Accumet Research AR 25 dual channel pH/ion meter (Fischer Scientific Ltd, USA) using the slurry technique, by mixing 1 g of crushed sandstone with 2.5 ml of deionized water and allowing the samples to settle (Lee et al., 2012). Nitrate, ammonium, nitrite, sulfate and ferrous ion levels were measured using colorimetric assays following methods that have been described earlier (Carter, 1971; Gerhardt, 1994; Kartal et al., 2006; Souza et al., 2012). Phosphate levels were determined using the

Malachite Green Phosphate Assay Kit (Cayman Chemical, USA) based on a colorimetric assay (D'Angelo et al., 2001).

DNA extraction and Illumina 16S rRNA amplicon sequencing

Total genomic DNA was extracted from approximately 500 mg of each sandstone sample using the PowerSoil® DNA Isolation Kit (Mo Bio Laboratories Inc., USA) following the manufacturer's instructions. Ten nanograms of DNA from each sample was used to amplify the V4 region of the 16S rRNA gene following the methods as listed in Miseq wet-lab standard operating procedures (Kozich et al., 2013). The amplified PCR products were then submitted to the Duke Genome Sequencing and Analysis Core facility for Illumina Miseq sequencing.

Bioinformatics

Illumina sequence reads were processed using the Mothur software package version 1.39.1 (Schloss et al., 2009). Contiguous sequences (contigs) were created by merging the forward and reverse sequences using mothur pipeline. The Ribosomal Database Project (RDP) pipeline was used to trim the ends of the sequences to 255 base pairs so that all the sequences started and ended at the same coordinates (Cole et al., 2009). All further analysis was done using mothur following the Miseq standard operating procedures (Kozich et al., 2013). Processed sequences were screened for chimeras using the UCHIME algorithm within mothur (Edgar et al., 2011). All sequences were classified using the Bayesian classifier against the SILVA database (Pruesse et al., 2007) and

clustered into operational taxonomic units (OTUs) using average neighbor joining method at 97% identity followed by taxonomy assignment.

To account for differences in the number of sequences for each sample, the dataset was rarefied by subsampling to the smallest sample dataset with 13,041 sequences using mothur (Schloss et al., 2009). Chao1 richness indicators and inverse Simpson diversity indices were used to assess bacterial richness and evenness. Dendrograms were constructed to describe the similarity between the sandstone samples at phyla hierarchical level, based on thetayc coefficients using the mothur pipeline (Kozich et al., 2013). Principal coordinate analysis plots were constructed using an eigenvector-based approach using thetayc calculator to examine the bacterial community OTU relatedness between the sandstone samples, the more related samples tend to be clustered together. The variability between the clusters was tested using the Analysis of molecular variance (AMOVA) statistical method in the mothur pipeline (Schloss et al., 2009). The OTUs responsible for the spatial separation of microbial communities along the two axes of the PCoA plot were measured by the correlation of the relative abundance of each OTU with the two axes using the non-parametric Spearman correlation method. The cumulative diversity within each cluster was compared to obtain a core bacterial community that was shared between the clusters using Venn diagram analysis. To test for any correlation between the relative OTU abundance data for each sandstone sample and physiochemical parameters, we constructed the Canonical Correspondence Analysis plot using the eigenanalysis algorithm in the Paleontological Statistics Software, Version 3.19 (Hammer et al., 2001; Legendre and Legendre, 2012).

Sequence data availability

Fastq files containing the raw data from this study were submitted to the NCBI sequence read archive (www.ncbi.nlm. nih.gov/sra) and can be accessed by the Bio project number PRJNA292826.

RESULTS

Chemical analysis of the sandstone samples

The pH of all the sandstone samples was fairly constant ranging between 6.4 - 6.8 (Table 2.2). Nitrite seemed to be completely absent in two samples, namely HW07_04 and HW04_04, while in the other samples it ranged between 0.4 - 5.4 nanomoles/gram dry weight of sandstone. Nitrate levels ranged between 21 - 660 nanomoles/gram dry weight of sandstone, while ammonium levels ranged between 212 - 4000 nanomoles/gram dry weight of sandstone. Phosphate and sulfate levels measured were within 112 - 472 and 0.4 - 23.9 nanomoles/gram dry weight of sandstone, respectively. Ferrous iron concentrations ranged between 98 - 280 nanomoles/gram dry weight of sandstone. The sandstone color was in the spectrum of reddish brown-pink to pale red. The Munsell color notation for moist sandstone samples showed that sandstone color was fairly uniform in all the sandstone samples, the hue was 2.5YR-5YR, the value and chroma ranged between 4/8 – 8/4 as shown in Table 2. The canonical correspondence analysis (CCA) plot showed no correlation between the relative OTU abundance and the physiochemical parameters of the sandstone samples (data not shown).

Overview of the total bacterial diversity in cryptoendolithic communities

Sequences from the eight sandstone samples were pooled and processed together, resulting in a total of 152,451 high quality sequences with an average length of 253 bases. A total of 2487 OTUs were generated after clustering at a 97% similarity index. Relative abundances of the taxa observed in each sandstone sample were calculated using the number of sequences obtained for each taxon against the total number of sequences obtained for that particular sandstone sample. Phyla with greater than 0.1% sequence abundance were analyzed, resulting in 12 distinct phyla observed amongst all sandstone samples (Figure 2.3).

Based on the relative abundance of phyla in all sandstone samples, 35% of the total sequences were assigned to *Cyanobacteria* making it the most abundant phylum (Table 2.S1). *Cyanobacteria* were prominent in all samples except the sandstone samples collected from alcove region, HW04_04 and HW06_04 (Figure 2.3). The most abundant OTUs belonged to the genus *Leptolyngbya* followed by unclassified cyanobacteria and *Mastigocladopsis* (Table 2.S2).

The second most abundant phylum was *Proteobacteria* represented by 28.5% of the total bacterial sequences obtained from all sandstone samples (Table 2.S1). Within the phylum *Proteobacteria*, 95% sequences belonged to the class Alphaproteobacteria. The genus *Acidiphilium,* a member of the family Acetobacteraceae was the most abundant OTU in this class (Table 2.S2).

Actinobacteria was the third most abundant phylum with 7.8% of the total sequences followed by *Bacteroidetes* with 7.4% of the total sequences. When considering

the sequences with greater than 0.1% relative abundance, all of the sequences assigned to *Actinobacteria* were binned to unclassified families and genera. *Bacteroidetes* had the highest number of bacteria in class Sphingobacteriia, family Chitinophagaceae and genus *Segetibacter*. About 1.5-5.5% of total sequences were assigned to other phyla that included *Chloroflexi, Deinococcus-Thermus, Verrucomicrobia, Planctomycetes and Gemmatimonadetes* (Figure 2.3). For the all sandstone samples analyzed, approximately 4.1% of the total sequences were binned to unclassified phyla.

Comparing the bacterial diversity between sandstone samples at phyla level based on the dendrogram suggests that slick rocks HW01_04, HW01_05 and HW07_05 are more related to each other while HW03_04, HW04_05 (slick rock associated with sediments) formed another clade (Figure 2.3). HW04_04, HW07_04 (alcove and slick rock slope sample) are more related while HW06_04 seems to differ from the rest of the sandstone samples (Figure 2.3). *Proteobacteria* were the most abundant in HW04_04 while *Actinobacteria* were the most abundant in HW06_04 and HW7_04 samples (Table 2.S1). *Bacteroidetes* were present in greater numbers in the alcove samples (HW04_04 and HW06_04) compared to the other sandstone samples. *Deinococcus-Thermus* and *Chloroflexi* were notably present in higher numbers in HW06_04 while *Verrucomicrobia* numbers declined to less than 0.1% (Table 2.S1). *Planctomycetes* and *Verrucomicrobia* were present in greater numbers in the slick rock samples compared to the alcove samples. Unclassified phyla accounted for 12.5% of the sequences obtained from HW07_04, the sandstone sample collected from a slope (Table 2.S1).

Analysis of Cyanobacterial community structure

Based on the relative abundance of sequences at the order hierarchy level, Cyanobacteria_Subsection III was the most dominant comprising 13% sequences, with unclassified Cyanobacteria accounting for 8.6% of total sequences. Cyanobacteria_Subsection II was the third most abundant order followed by Cyanobacteria_Subsection I, each spanning 6.64% and 5.29% of the total sequences, respectively. At the family level, 34% of the total sequences were assigned to Cyanobacteria_Subsection III_FamilyI, unclassified Cyanobacteria, Cyanobacteria_Subsection II _FamilyII and Cyanobacteria_SubsectionI_FamilyI. Cumulatively, at the genus level, *Leptolyngbya* was the most abundant genus occupying 9.4 % sequences followed by unclassified cyanobacteria, *Chroococcidiopsis, Mastigocladopsis* and *Cyanobacteria_SubsectionIII_Family_unclassified* occupying 8.7%, 6.6%, 5.3% and 3.7% of the total sequences respectively. Other genera included *Nostoc*, *Synechococcus* and *Microcoleus* each representing less than 1% of the overall cyanobacterial diversity detected in all sandstone samples analyzed.

Broadly, *Leptolyngbya* and *Chroococcidiopsis* were the most prevalent genera in all sandstone samples tested except HW04_04 in which the former was completely absent (Figure 2.4). *Synechococcus* had less than 0.1% relative abundance in all samples except one of the alcove samples, HW06_04 where it occupied 32% of the total cyanobacterial sequences at genera level. *Cyanobacteria_SubsectionIII_Family_unclassified* occupied 52% of the total cyanobacterial sequences in the slick rock slope sample, HW07_04 while unclassified cyanobacteria spanned 59.2% of the cyanobacterial sequences in one

alcove sample, HW04_04. *Mastigocladopsis* was present in relatively higher numbers in HW03_04 and HW04_05 which were stone samples taken near eolian sediment deposits while this genus was completely absent in one of the alcove samples, HW06_04 (Figure 2.4).

Bacterial species richness and diversity

Richness and diversity indices were calculated for all the sandstone samples based on the number of observed OTUs after all the data were rarefied to normalize the dataset. The resulting observed number of species, Inverse Simpson diversity index and Chao richness indices, indicated that the slick rock HW03_04 had the greatest amount of richness and diversity while HW06_04, the sample from the alcove, the least (Table 2.3). Rarefaction curves based on a 97% similarity showed a considerable difference between the sandstone samples, with HW03_04 showing highest diversity, while HW06_04 exhibited the lowest diversity (data not shown). Based on the principal coordinate analysis (PCoA) plot, the samples separated into three distinctive clusters representing cryptoendolithic communities that clustered based on the topography of the sampling sites for the sandstones (Figure 2.5A). Cluster 1 communities were associated with rock features that were not associated with steep slopes or sediment deposits. Cluster 2 comprised rock communities that were found near eolian sediment deposits and soils. Cluster 3 communities were associated with steep slopes. The p-values obtained using AMOVA test for statistical comparisons of all three clusters, cluster 1 and 2, cluster 1 and 3, cluster 2 and 3 were 0.09, 0.023 and 0.064 respectively. Therefore, the three clusters were

significantly different from each other with a p-value <0.01. The vectors of correlation indicated that *Bacteroidetes* and *Actinobacteria* were more prominent in cluster 3 while *Cyanobacteria* were more dominant in cluster 1 and cluster 2 samples (Figure 2.5B).

Core bacterial communities in cryptoendoliths

There was a considerable amount of heterogeneity observed between seemingly similar sites. The total OTUs from all the sandstone samples within each cluster were grouped such that each cluster was representative of a group of cryptoendolithic communities associable with varying topography. Comparing the three clusters, there were 284 OTUs in common, suggesting that a core community exists within the cryptoendolithic habitat (Figure 2.6). Within this shared group of OTUs, *Cyanobacteria* were the dominant representatives, while *Proteobacteria*, unclassified Bacteria, *Actinobacteria* and *Bacteroidetes* were the next most prevalent phyla (Table 2.S3). *Leptolyngbya* was the major representative of the *Cyanobacteria* along with *Chroococcidiopsis, Mastigocladopsis* and unclassified Cyanobacteria. Alphaproteobacteria were widely represented by a number of genera including *Acidiphilium*, unclassified *Acetobacteraceae,* unclassified *Sphingomonadeles*, *Sphingomonas*, unclassified *Rhizobiales*, *Belnapia* and unclassified *Methylobacteriaceae* (Table 2.S3). More than half of the shared OTUs were assigned to unclassified genera.

DISCUSSION

The Jurassic Navajo Sandstone is one of the most porous and permeable sandstone formations found within the GSENM (Chan et al., 2004) making it a suitable habitat for

cryptoendolithic microbes (Kurtz Jr. and Netoff, 2001; Kurtz Jr., 2002; Kurtz Jr. et al., 2005). Abundance and diversity of cryptoendoliths has been correlated to sandstone color in the past (Bell et. al., 1988; Bell, 1993). In this study, sandstone color variations did not seem to have any correlation with the diversity data obtained via next generation sequencing, nor did minor differences in the availability of inorganic nitrogen species, phosphate, ferrous iron or sulfate. pH did not vary considerably among the sandstone samples. Based upon these data, we conclude that these factors have little to no effect on community structure.

Next generation sequencing of the sandstone samples revealed the presence of 12 distinct phyla having more than 0.1% abundance in each sample indicating that this micro ecosystem is quite diverse. This result was somewhat surprising as a highly diverse community was not expected under these environmental conditions. The number of unclassified sequences strongly suggests that these cryptoendolithic communities harbor a significant number of novel organisms for which there is no data available.

The sequencing data affirms that *Cyanobacteria* is the dominant phylum in cryptoendolithic habitats (Kurtz Jr. and Netoff, 2001; Kurtz Jr. et al., 2005; Hammes et al., 2013; Lee et al., 2016). Previous studies have reported *Chroococcidiopsis* to be the predominant cyanobacteria observed in arid lithic systems based on morphological characterization (Friedmann, 1980; Büdel and Wessels, 1991; Bell, 1993; Wessels and Büdel, 1995; Casamatta et al., 2002; Bhatnagar and Bhatnagar, 2005; Pointing and Belnap, 2012). However, our data show that *Leptolyngbya* was the most abundant cyanobacterium in this microecosystem followed by unclassified cyanobacteria and

Chroococcidiopsis. While the overall data indicate that *Leptolyngbya* was the most prevalent genus of cyanobacteria present, there was considerable heterogeneity in cyanobacterial diversity between stone samples.

Bacterial diversity analysis indicated three distinctive clusters of cryptoendolithic communities that correlated to topography of the sampling sites. From this, we infer that separation of the communities is most likely due to water availability, specifically the duration of time water is present. Cluster 1 communities were only exposed to limited water that may penetrate the pore spaces during a precipitation event with excess precipitation moving downslope as runoff. Cluster 2 communities were potentially exposed to water for longer periods of time as the nearby sediment deposits and soils would tend to hold water in place, making water available via capillary action. Cluster 3 communities were exposed to water as it percolated downslope from higher elevations through pores and cracks in the sandstone. The vectors of correlation indicated that the microbial communities with longer water availability tended to have more *Bacteroidetes* and *Actinobacteria* while *Cyanobacteria* were dominant under less favorable conditions with limited water availability. From this analysis, we conclude that water availability is one of the primary forces affecting community structure. This conclusion is in concurrence with current thought regarding the effects of episodic events such as precipitation on microbial communities (Neilson and Ball, 2015; Meslier et al., 2018).

The sandstone samples from alcoves, HW04_04 and HW06_04 had the least microbial diversity as compared to other samples, including sample HW07_04, which clustered with these samples. This suggests that the presence of water for an extended

period of time is enough to allow a small group to out compete other microbes within the slick-rock core. The two alcove samples had considerably higher percentage of *Bacteroidetes*, and *Deinococcus-Thermus* outcompeting the cyanobacterial members. Given the diversity within the *Bacteroidetes* phylum and the lack of information associated with the unclassified OTUs, it is not possible to draw a specific conclusion regarding the underlying driving factors resulting in these shifts in community structure. Previous studies suggest that reduction in temperature and water stress brings a marked shift in endolithic community (Bell et al., 1988). HW07_04, a slick rock slope sample and HW06_06, an alcove sample harbored *Actinomycetes* in slightly more abundance than other sandstones and also exhibited the highest percentage of bacteria assigned to unclassified bacteria among all the samples.

When we examined the diversity shared between the clusters obtained via PCoA plot analysis, we found an overlapping core of 284 OTUs that represented the core microbial community ubiquitous in sandstone samples collected during different years and topographic locations. *Cyanobacteria* were the most abundant in terms of the cyanobacterial OTUs being observed the maximum number of times amongst all the shared diversity. Nearly half of the shared OTUs belong to unclassified genera, indicating the extent of unexplored diversity in this microbial ecosystem. Thirty percent of the shared diversity belonged to Alphaproteobacteria with Acetobacteraceae being the dominant members in this phylum. In context of the overall community, these bacteria are subsisting on the exudates of the dominant cyanobacteria. Given that the genus *Acidiphilium* comprises a large proportion of the Acetobacteraceae, with members of this

genus known to be capable of aerobic iron reduction, we hypothesize that these bacteria are integral members of these cryptoendolithic communities whose role is to reduce ferric iron to ferrous iron (Bridge and Johnson, 2000; Bilgin et al., 2004). The dominant cyanobacteria are slow growing and desiccation resistant due to their ability to produce organic rich extracellular polymeric substances (Ferris and Lowson, 1997). These extracellular polymers can be subsequently metabolized by heterotrophic bacteria, lowering the ambient pH values through the production of low molecular weight organic acids and respiratory carbon dioxide (Ferris and Lowson, 1997; Gorbushina, 2007). This basic set of metabolic processes set the conditions required for the aerobic reduction of iron by *Acidiphilium spp.*, which will return the pH back to neutrality (Küsel et al., 1999; Bilgin et al., 2004). Under these conditions, the reduced iron would be captured by the EPS produced by members of these communities (Hammes et al., 2013). These observations allow us to outline a hypothetical ecological cycle where the cyanobacteria produce EPS and other metabolites that support a robust heterotrophic community. General metabolic processes cause a localized lowering of pH, which in combination with the metabolites produced by *Cyanobacteria*, supports the growth of *Acidiphilium sp.* which reduces ferric iron to ferrous iron, making it more readily available to the larger community.

Comparing these communities to other desert communities, we see that with only a few exceptions, namely the Negev Desert and the Atacama Desert (Friedmann et al., 1967; Bell, 1993; Connon et al., 2007; Wierzchos et al., 2012; Crits-Christoph et al., 2013), the microbial diversity is quite similar (Friedmann, 1980; Bell et al., 1986; de la

Torre et al., 2003; Antony et al., 2012; Lacap-Bugler et al., 2017). In comparison with the community structure of local desert soils, we find that the overall structure is comparable, with *Cyanobacteria* and *Proteobacteria* numbers being higher on a relative basis (Garcia-Pichel et al., 2001; Garcia-Pichel et al., 2003; Lee et al., 2016). However, we also note that while the structure is similar, the presence of certain phyla such as the *Acidobacteria, Verrucomicrobia* and *Planctomycetes* are more pronounced in the soil environments (Nagy et al., 2005; Gundlapally and Garcia-Pichel, 2006). This similarity in community structure is not unexpected as the proximity of the soil and cryptoendolithic communities logically suggests that one of these two distinct ecosystems influences the assembly of the other. Wind dispersal of dust and sand in semi-arid regions has the potential to move not only sediments, but also bacterial cells from one habitat to the other. Thus, we cannot say with certainty which community influences the assembly of the other. However, the differences in diversity between the cryptoendolithic community and the soil community can be attributed to more moderate conditions within the soil, specifically higher nutrient levels, longer availability of water and the presence of reduced carbon. While wind dispersal of cells cannot be attributed to colonization of newly deposited sediments or fresh stone surfaces in a directional manner, water dispersal of cells is most likely from the cryptoendolithic community to the soil and sediment communities. The sandstone outcrops sampled in this study have very little sediment cover and are generally higher in elevation than the surrounding soils. Thus, when precipitation runs down slope, cells and sediment will be carried from the cryptoendolithic communities to the local soils and sediment deposits.

This research provides evidence that unexplored cryptoendolithic bacterial diversity exists in the Jurassic Navajo sandstones, therefore identifying a conservation value for these desert communities. The cyanobacterial diversity in cryptoendolithic communities varies with location, potentially reflecting their adaptation to available moisture regimes in this semi-arid ecosystem. Further studies are required to expand the understanding of the biological functions of the cryptoendolithic communities in sandstones.

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TABLES AND FIGURES

Table 2.1 Sampling sites of the eight Jurassic Navajo sandstones analyzed in this study.

Site	Sandstone	Sampling year	Location	Notes
1	HW01_04	2004	37° 41' 10.02" N; 111° 18' 41.70" W	Slick rock
2	HW03_04	2004	37° 41' 10.02" N; 111° 18' 41.70" W	Associated with sediments
3	HW04_04	2004	37° 41' 17.73" N; 111° 18' 56.74" W	Alcove area
4	HW06_04	2004	37° 41' 17.73" N; 111° 18' 56.74" W	Alcove area
5	HW07_04	2004	37° 41' 06.69" N; 111° 19' 11.64" W	Slick rock from slope
6	HW01_05	2005	37° 40' 42.36" N; 111° 18' 29.28" W	Slick rock
7	HW04_05	2005	37° 40' 38.80" N; 111° 18' 11.23" W	Associated with sediments
8	HW07_05	2005	37° 40' 08.49" N; 111° 19' 37.81" W	Slick rock

Table 2.2 Physiochemical analysis of the Jurassic Navajo sandstone samples.

Sandstones	Nitrate*	Nitrite*	Ammonium*	Sulfate*	Phosphate*	Ferrous Iron*	Munsell Color (wet)	pH
HW01_04	232.94	2	950.77	0.38	228.70	149.39	2.5 YR 8/4	6.36
HW03_04	291.76	0.4	966.15	6.86	196.73	170.26	2.5 YR 5/4	6.57
HW04_04	366.27	0	4007.18	9.56	472.14	165.04	2.5 YR 5/6	6.36
HW06_04	21.18	2.6	381.54	23.88	456.06	143.59	2.5 YR 4/8	6.52
HW07_04	134.90	2	1627.69	6.59	317.27	279.54	5 YR 5/3	6.49
HW01_05	397.65	5.4	212.31	21.44	332.51	122.14	2.5 YR 7/4	6.80
HW04_05	350.59	2.2	458.46	1.73	160.43	98.38	2.5 YR 8/3	6.51
HW07_05	660.39	0	268.72	6.32	126.96	153.74	2.5 YR 7/2	6.69

* The units are nanomoles per gram of dry weight of crushed sandstone.

Table 2.3 Bacterial diversity metrics based on the16S rRNA gene analysis of the Jurassic Navajo sandstones collected from the GSENM, Utah. Community richness (Chao1 richness estimate), coverage of sampling (in percentage), sobs and evenness (Inverse Simpson diversity index) were calculated in mothur at 97% similarity after normalizing samples to 13041 sequences.

Sandstone	Reads	OTUs	Coverage	Sobs	Invsimpson	Chao
HW01_04	20552	653	98.38	551.80	16.14	832.76
HW01_05	21250	424	99.03	355.73	14.10	561.33
HW03_04	13462	750	98.21	742.66	43.06	1007.20
HW04_04	16243	496	99.08	470.14	33.73	575.92
HW04_05	22732	657	98.35	528.70	10.18	827.89
HW06_04	13041	292	99.59	292.00	22.00	326.07
HW07_04	25046	778	98.40	651.52	22.75	841.60
HW07_05	20125	489	99.06	439.71	27.43	534.93

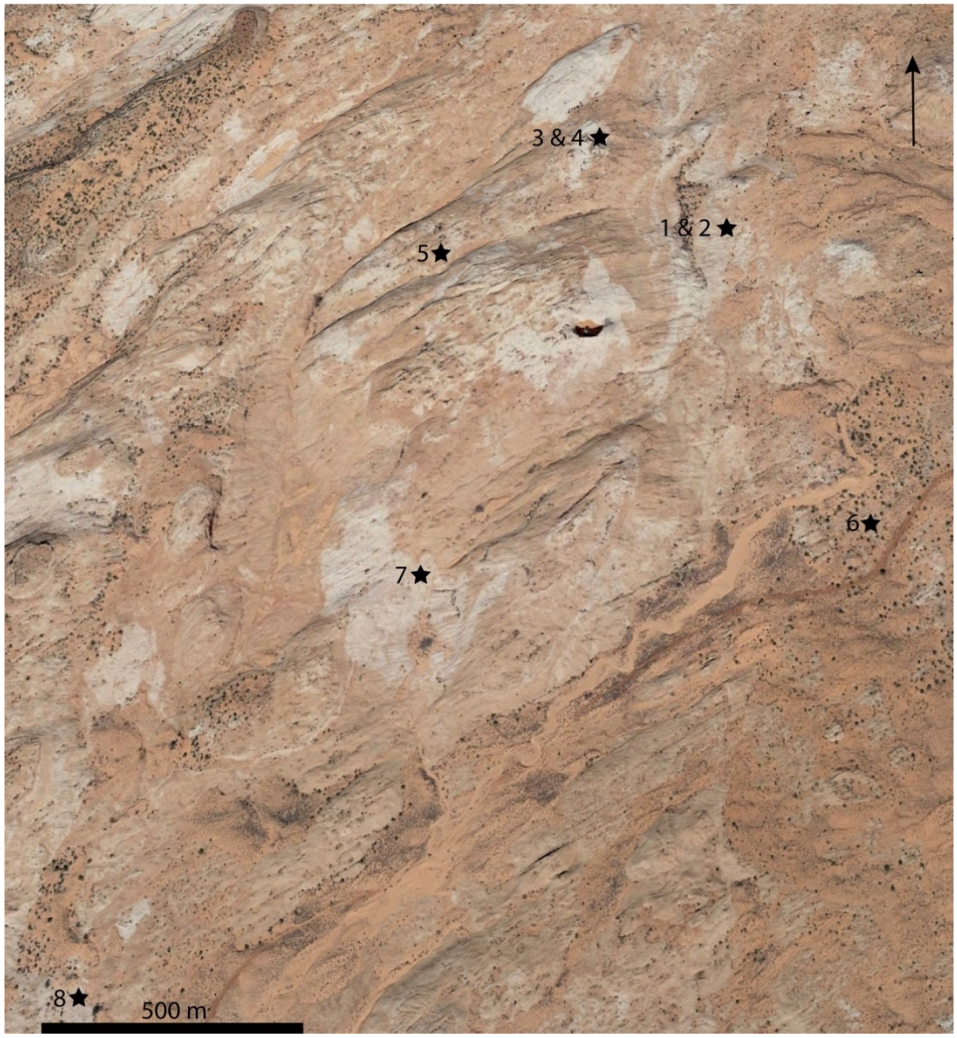


Figure 2.1 Map of sampling sites in the Harris wash area of the GSENM obtained through Google Earth. Jurassic Navajo Sandstones corresponding to sampling sites and the GPS coordinates are shown in Table 2.1. Scale bar in the lower right corner equals 500 m. Arrow indicates north.

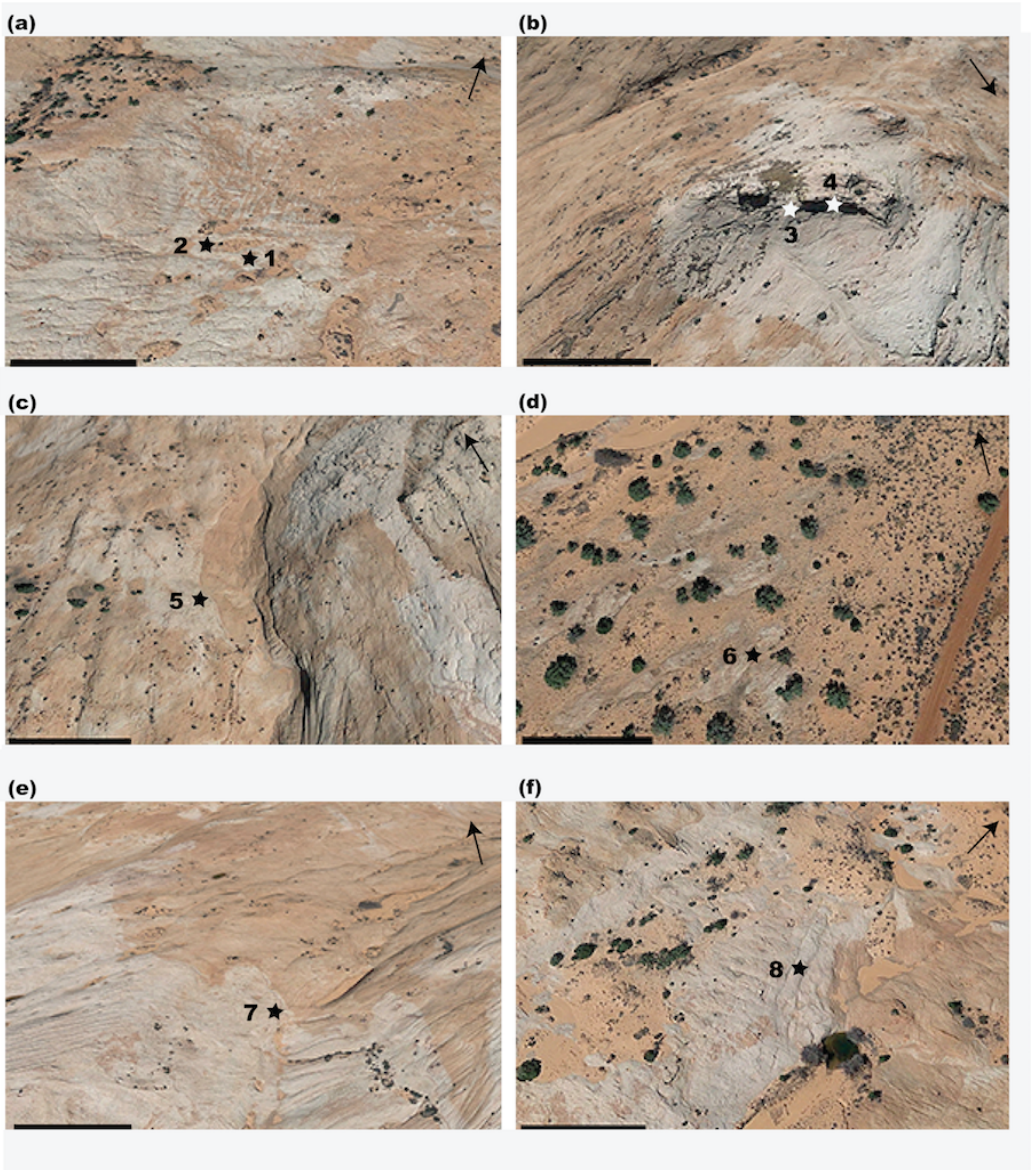


Figure 2.2 Closeup view of the sampling sites obtained through Google Earth, representing a) Slickrock HW01_04, Slickrock HW03_04 associated with sediments b) Samples HW04_04 and HW06_04 from an alcove area represented by the darker colored region c) HW07_04 slickrock obtained from a slope d) Slickrock HW01_05 e) HW04_05 slickrock associated with sediments f) Slickrock HW07_05. Scale bars in the bottom left corner represents 35 m. The arrows indicate north direction.

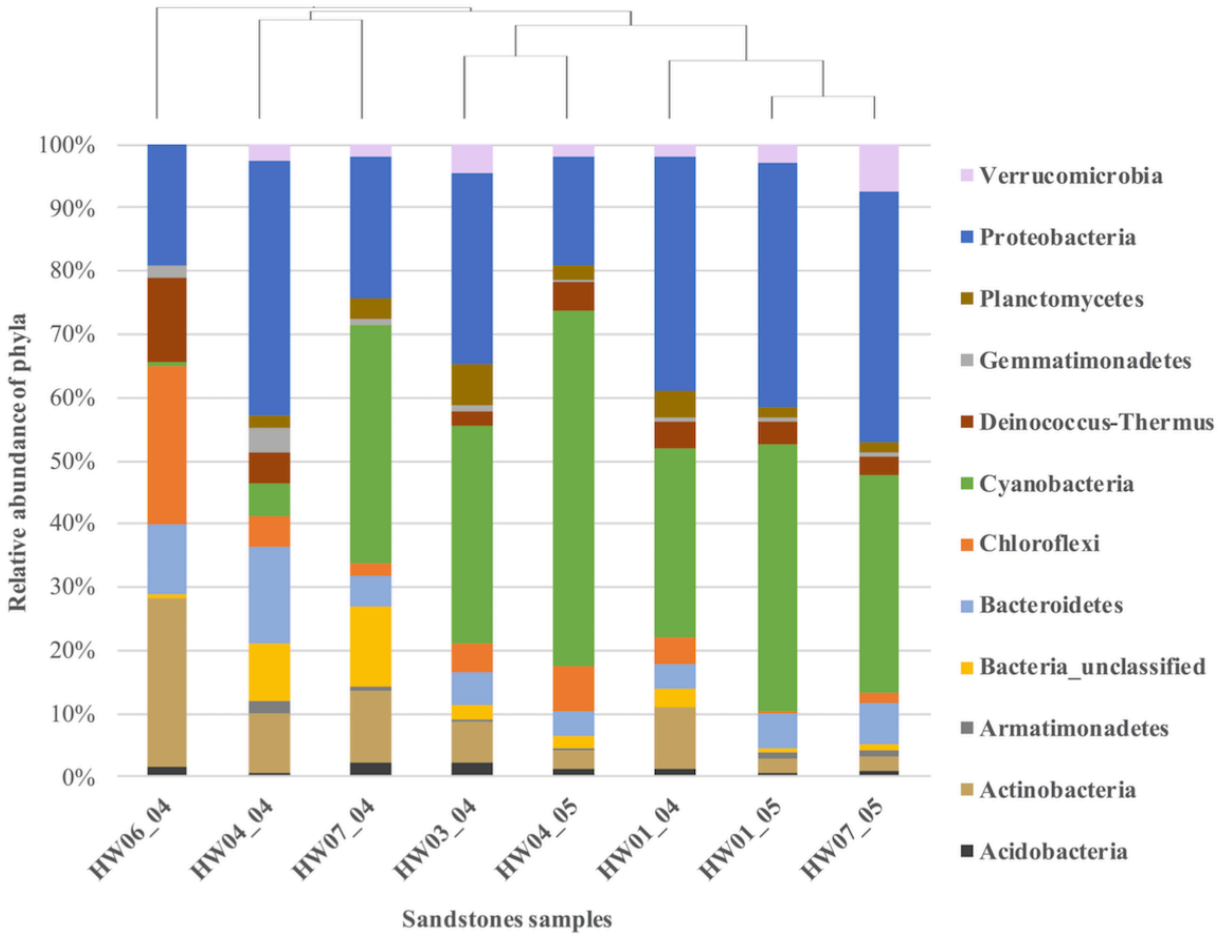


Figure 2.3 Relative abundances of the major phyla identified with >0.1% sequence abundance in cryptoendolithic bacterial communities of the Jurassic Navajo Sandstones. Taxa are arranged in order as they appear on the stacked bar graph with each rectangle representing the relative percentage abundance of a phylum in a particular sandstone sample.

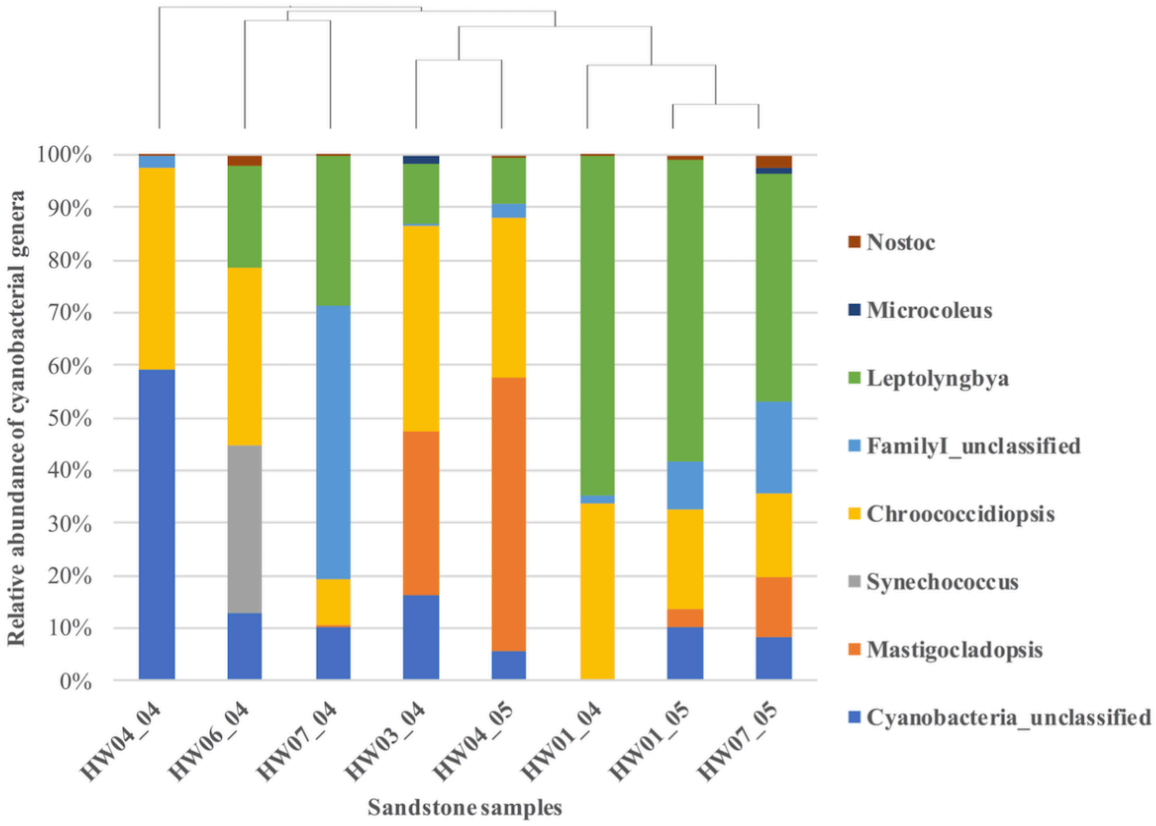
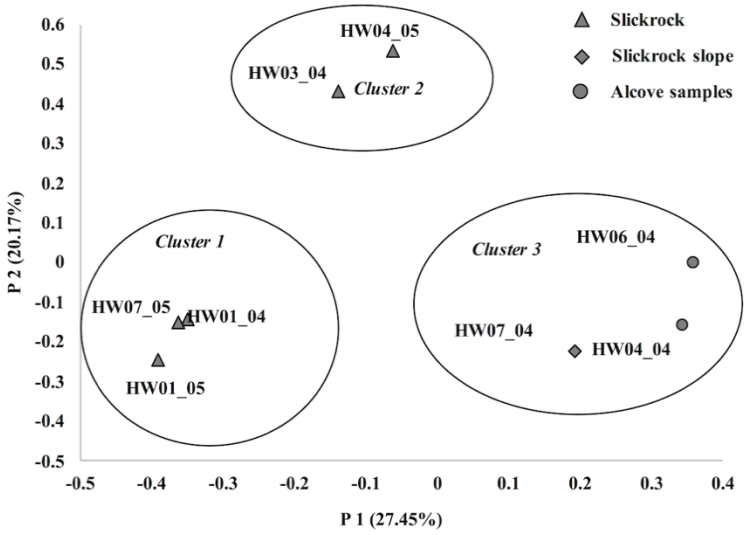


Figure 2.4 Comparison of the relative abundance of cryptoendolithic cyanobacterial communities in Jurassic Navajo sandstones. Taxa are arranged in order as they appear on the stacked bar graph with each rectangle representing the relative percentage of cyanobacterial genera in a particular sandstone sample.

A.



B.

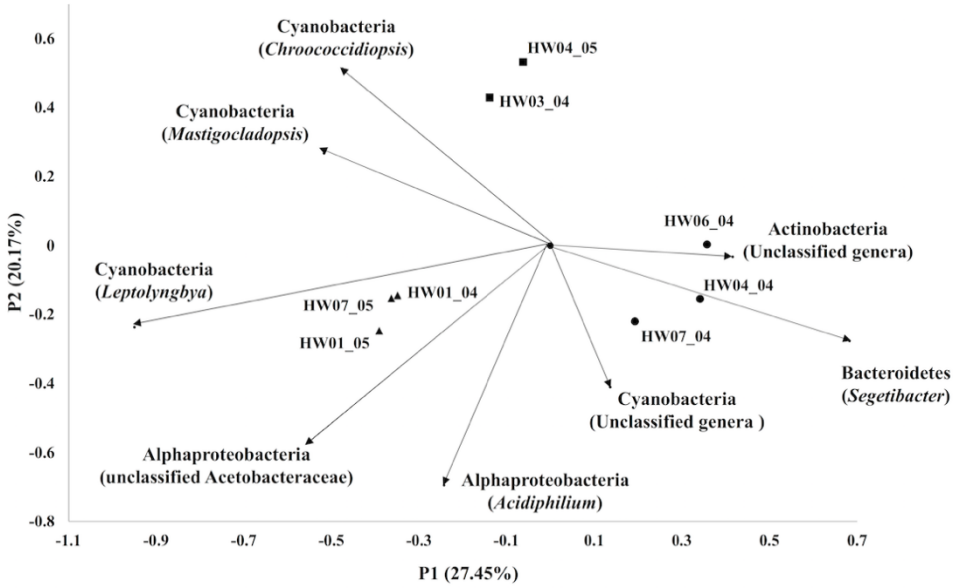


Figure 2.5 (A) Principal coordinate analysis plot comparing the cryptoendolithic bacterial communities amongst Jurassic Navajo sandstone samples. The p-values obtained using AMOVA test for statistical comparisons of all three clusters were <0.001. **(B)** Vectors of correlation values from OTUs that explain the clustering of the microbial communities.

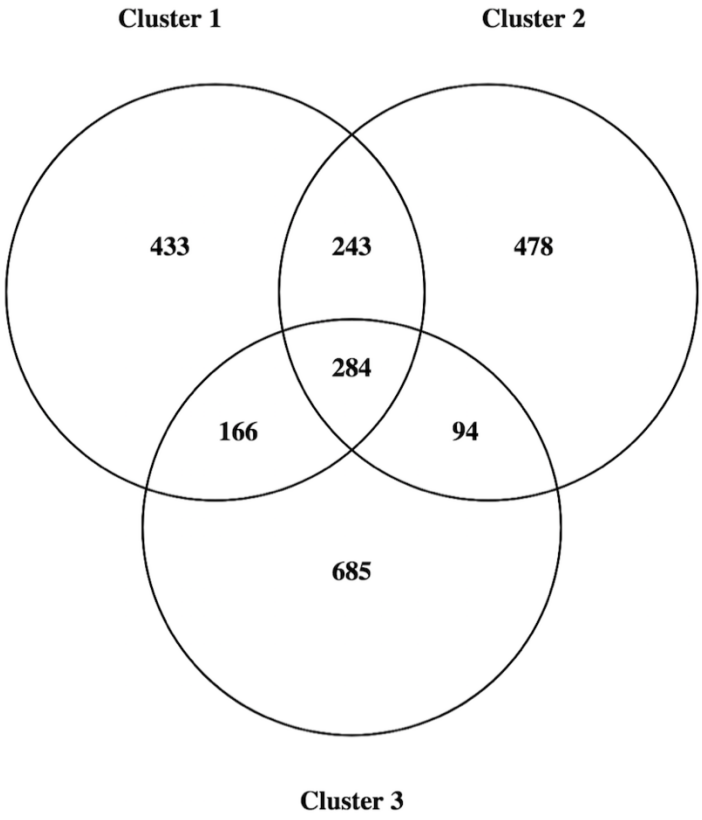


Figure 2.6 Venn diagram representing the shared OTUs between bacterial communities that indicates the core community existing in the cryptoendoliths in sandstones samples.

Supplementary data

Table 2.S1 List of the relative sequence abundance for the major phyla obtained from the dataset. Sequence abundances greater than 0.15 % was considered while minor taxa with less than 0.15 % abundance were removed.

	HW01_04	HW01_05	HW03_04	HW04_04	HW04_05	HW06_04	HW07_04	HW07_05
Acidobacteria	1.38	0.54	2.24	0.49	1.16	1.53	2.31	0.96
Actinobacteria	9.61	2.40	6.64	9.48	3.09	26.49	11.26	2.32
Armatimonadetes	0.07	1.01	0.25	1.93	0.12	0.22	0.63	0.95
Bacteria_unclassified	2.80	0.60	2.16	8.96	2.03	0.44	12.56	1.02
Bacteroidetes	3.88	5.33	5.10	15.45	3.88	11.00	4.80	6.42
Chloroflexi	4.24	0.39	4.75	4.70	7.31	25.21	2.06	1.54
Cyanobacteria	29.91	42.39	34.45	5.18	55.95	0.36	37.42	34.49
Deinococcus-Thermus	4.15	3.35	2.21	4.72	4.66	13.33	0.27	2.65
Gemmatimonadetes	0.58	0.89	0.78	4.09	0.37	1.95	0.94	0.68
Planctomycetes	4.27	1.39	6.55	1.72	2.16	0.21	3.23	1.70
Proteobacteria	37.06	38.85	30.19	40.07	17.26	18.86	22.12	39.40
Verrucomicrobia	1.97	2.84	4.61	2.63	1.90	0.07	2.02	7.49

Table 2.S2 Taxonomy and abundance of the OTUs observed in Jurassic Navajo sandstones with different topographic features.

Table 2.S3 Taxonomic profiling and distribution of the core microbial community comprising of 284 OTUs, based on the Venn diagram computed using mothur as the analysis tool.

Note- Table 2.S2 and Table 2.S3 are included as attached excel sheets.

Chapter 3

Cryptoendolithic bacterial diversity in the Entrada and Jurassic Navajo Sandstones of the Colorado Plateau

ABSTRACT

The Jurassic Navajo and Entrada Sandstones are prominent geological features of the Grand Staircase Escalante National Monument in Utah, USA. The diversity and abundance of cryptoendolithic bacterial communities in the Entrada Sandstone was examined using Illumina Miseq sequencing of the 16S rRNA gene. Cyanobacteria are the dominant inhabitants followed by Proteobacteria, Actinobacteria, Bacteroidetes and Deinococcus-Thermus. The genus *Truepera* was the primary representative of the phylum Deinococcus-Thermus. We also present data on the comparison of the cryptoendolithic communities between the Jurassic Navajo and Entrada Sandstones. Although both the communities appear similar superficially, variations in the physical and chemical properties of rock substrates, such as pore sizes, mineral composition and porosity influence the bacterial composition in these habitats. The Jurassic Navajo communities were dominated by *Leptolyngbya* and other genera of filamentous cyanobacteria. The Entrada Sandstones were dominated by coccoidal forms of cyanobacteria, specifically the genus *Chroococcidiopsis*. These differences were correlated to the greater pore size in the fine to medium-grained Jurassic Navajo sandstone compared to the smaller pore size in the fine-grained Entrada Sandstone. Despite the differences, there exists a core of eleven OTUs from the Cyanobacteria,

Proteobacteria and Actinomycetes shared between both the two cryptoendolithic habitats. These results show that the communities found in the Jurassic Navajo and Entrada Sandstones are distinct communities with little overlap between them.

INTRODUCTION

Barren rock outcrops are a prominent feature of the drylands region of Colorado Plateau in the western USA (Moucha et al., 2009). Erosion of the Colorado Plateau has resulted in the sculpting of a series of multi-hued, steep, narrow canyons and slickrock outcrops in the Escalante Canyons section of this region, that includes the Grand Staircase Escalante National Monument (GSENM; Doelling et al., 2000). One of the prominent features in the GSENM are eolian deposits resulting in large to regionally extensive ergs such as the Jurassic Navajo and Entrada Sandstone units (Chan et al., 2000). The Navajo is a tan-colored, fine to medium grained sandstone while the Entrada is mostly fine grained, red colored sandstone forming earthy slopes and ridges (Doelling et al., 2000). One common feature in these sandstone outcrops is the hidden microbiota residing within the pore spaces of these sandstones, collectively known as the cryptoendolithic communities. This study aims to assess the effects of varying underlying substrate i.e. sandstones on the composition of cryptoendolithic communities.

Rock surfaces in arid landscapes are exposed to multiple environmental stressors including high temperatures, solar irradiation, insufficient water, desiccation and re-hydration events coupled with limited nutrients (Bell, 1993; Ferris and Lowson, 1997; Kurtz Jr. and Netoff, 2001; Gorbushina, 2007). Under these circumstances, living within

the pore spaces of sandstones is a survival strategy employed by cryptoendolithic communities, forming microbial biofilms a few millimeters beneath the surface of sandstones (Kurtz Jr., 2002). The cryptoendolithic environment moderates temperature fluctuations, providing protection from ultraviolet light and enhancing access to moisture, creating suitable conditions for microbial growth (Seibert et al., 1996; Kurtz Jr. and Netoff, 2001; Omelon et al., 2006; Pointing and Belnap, 2012).

Generally, rock-associated microorganisms are ecological communities characterized by variable compositions of microorganisms that co-occur within a defined habitat (Walker and Pace, 2007). Biogeographical characteristics such as the physical and chemical properties of rock types, mineralogy, porosity and permeability, capacity for moisture uptake and retention, and access to nutrients influence the specific microbial composition of endolithic communities (Omelon et al., 2007; Walker and Pace, 2007). Although they might appear similar superficially, variations in the local microenvironmental conditions can influence colonization by recruiting distinctly different microbial assemblages. Physical characteristics such as pore size and structure, are expected to influence moisture properties of rock substrates which could further potentially affect the microbial composition (Walker and Pace, 2007; Camara et al., 2014).

Cryptoendolithic communities in deserts are highly specialized communities dominated by cyanobacteria that support diverse heterotrophic assemblages (Kurtz Jr., 2002; Smith et al., 2014;). The photosynthetic zone is limited by the light penetration, which is also affected by physical properties such as rock color, mineralogy and structure

(Walker and Pace, 2007). We hypothesize that the varying petrographic properties of the Jurassic Navajo and Entrada sandstones could influence the cyanobacterial community composition in these two cryptoendolithic habitats. Cyanobacteria are central to the existence of cryptoendolithic communities as they contribute to the physical stability of the microbial mats besides providing fixed nitrogen and photosynthetically fixed carbon to the other microbes within the community. Different cyanobacteria have varying metabolic capabilities and physiological tolerances to the extreme environmental conditions in arid lands (Redfield et al., 2002). Thus, the knowledge of cyanobacterial diversity is important in understanding the functioning of cryptoendolithic communities in this microecosystem and arid land productivity on a long-term scale.

Here, we present data describing the cryptoendolithic bacterial diversity in the Entrada Sandstones, followed by a comparison with the cryptoendolithic communities in the Jurassic Navajo sandstone. Based on the sandstone substrate, microbial diversity is expected to display distinct patterns that reflect ecosystem function. We also hypothesize that although the cryptoendolithic communities vary between the Jurassic Navajo and Entrada Sandstones, a shared bacterial community exists within the two cryptoendolithic habitats.

METHODS AND MATERIALS

Study sites and sample collection

Four samples of Entrada Sandstone (HW10_04, HW11_04, HW12_04, HW13_04) were obtained from the Harris Wash area in the GSENM (Figure 3.1). The HW in the samples

denotes the Harris wash site followed by the site number and the year of sampling. The sampling locations of the Jurassic Navajo samples (HW01_04, HW03_04, HW07_04, HW01_05, HW04_05) were reported in Kaur and Kurtz (in press). All samples were acquired by chipping approximately 50 cm^2 of sandstone, 5-10 mm in thickness from the indicated rock outcrops. Samples were placed into sterile Nasco Whirl-Pak bags and transported to the laboratory where they were stored under dark, dry conditions at room temperature.

Physiochemical analysis of the Sandstone samples

The physiochemical properties of the Entrada sandstones, including pH, sandstone color, levels of nitrate, ammonium, nitrite, sulfate, phosphate and ferrous iron were measured using methods previously described (Kaur and Kurtz, in press).

DNA extraction and Illumina Miseq sequencing analysis

Total genomic DNA was extracted from approximately 500 mg of each sandstone sample using the PowerSoil® DNA Isolation Kit (Mo Bio Laboratories Inc., USA). One nanogram of DNA from each sample and previously designed dual indices were used for amplification of the V4 region of the 16S rRNA gene (Kozich et al., 2013; https://github.com/SchlossLab/MiSeq_WetLab_SOP/blob/master/MiSeq_WetLab_SOP_v4.md). PCR amplifications were performed using C1000 Touch™ Thermal cycler (Bio-Rad laboratories, Hercules, USA) using thermal cycling conditions as follows: an initial denaturation at 95°C for 2 min, and 35 cycles at 95°C for 20 sec, 55°C for 15 sec and

72°C for 5 min, with a final extension at 72°C for 10 min. Following amplification, 2 µL of each PCR product was used to confirm successful amplification using agarose gel (1%) electrophoresis. Amplicon quantities were normalized with the SequalPrep™ Normalization Plate Kit, 96 well (Invitrogen, Carlsbad, CA, USA) and quantified using Qubit™ DNA HS assay (Life Technologies, Austin, TX, USA). The normalized amplicons were pooled, and the library was run on an Illumina Miseq paired end (2 X 250) platform at Clemson University. The sequencing data were analyzed using Mothur software package version 1.39.5 (Schloss et al., 2009), following the methods described earlier by Kaur and Kurtz (in press). Raw sequence results are available through GenBank BioProject PRJNA292826.

RESULTS

Physiochemical analysis of the Entrada Sandstones

The physiochemical attributes of the Entrada Sandstone were analyzed while those of the Jurassic Navajo Sandstones have been previously reported (Kaur and Kurtz, in press). The pH values did not vary much within the Navajo sandstones as reported earlier (pH 6.36-6.80) while the Entrada sandstones exhibited a wider pH range (pH 6.57-8.01) with a shift towards the alkaline spectrum (Table 3.1). Comparatively, there were higher levels of sulfates, phosphates and nitrates and lower levels of ammonium and ferrous iron in the Entrada Sandstone compared to the Jurassic Navajo Sandstone (Table 2.2).

Cryptoendolithic bacterial diversity in the Entrada Sandstones

Illumina Miseq amplicon sequencing of Entrada samples (HW10_04, HW11_04, HW12_04 and HW13_04) resulted in a total of 1,221 operational taxonomic units (OTUs) at a 97% similarity index. Phyla with greater than 0.1 % sequence abundance were analyzed, resulting in 12 distinct phyla observed in the Entrada Sandstones (Figure 3.2). Based on the relative percentage abundance, 52% of the total sequences were assigned to Cyanobacteria making them the most abundant inhabitants of the cryptoendolithic communities in the Entrada Sandstone. The most abundant OTUs belonged to Cyanobacteria, including genera *Chroococcidiopsis*, unclassified genera, *Microcoleus* and *Mastigocladopsis* (Supplementary Table 3.S1). Proteobacteria was the second most abundant phylum, occupying 15% of the total sequences. Within Proteobacteria, the orders Rhizobiales and Sphingomonadales, which are members of the Alphaproteobacteria, were most prevalent. The majority of the sequences were not classified to the family or genus level. The third most abundant phylum was Actinobacteria, occupying 11% of the total sequences obtained. The genus *Blastococcus* belonging to the phylum Actinobacteria was present in high numbers in HW12_04 while it had little to no presence in the other three sandstones (Supplementary Table 3.S1). The phyla Chloroflexi, Bacteroidetes, Deinococcus-Thermus, Acidobacteria, Verrucomicrobia, Planctomycetes, Gemmatimonadetes and unclassified phyla, all had less than 5% relative abundance (Figure 3.2). The phylum Deinococcus-Thermus was primarily represented by the genus *Truepera*, detected in all the analyzed sandstone

samples (Supplementary Table 3.S1). The other listed phyla were composed of unclassified genera.

The CCA plot revealed that higher nitrate levels in HW12_04 sample corresponded to greater abundance of Actinomycetes and higher pH corresponded to more *Mastigocladopsis* in sample HW10-04 (Supplementary Figure 3.S1). There seemed to be no correlation between the other physiochemical parameters tested and the relative OTU abundance observed in the Entrada samples.

We found that 57 OTUs were shared in all the Entrada sandstones analyzed (Figure 3.3, Supplementary Table 3.S2). This core bacterial community includes members of the Cyanobacteria (17 OTUs), Proteobacteria (16 OTUs), Actinomycetes (11 OTUs), Deinococcus-Thermus (4 OTUs), Chloroflexi (4 OTUs), Gemmatimonadetes (3 OTUs) and Planctomycetes (1 OTU). Half of the shared OTUs were assigned to unclassified genera (Supplementary Table 3.S2).

Comparative analysis of the cryptoendolithic bacterial diversity in the Jurassic Navajo and Entrada Sandstones

The cryptoendolithic bacterial diversity in the Jurassic Navajo Sandstones (HW01_04, HW03_04, HW07_04, HW01_05, HW04_05) as reported previously (Kaur and Kurtz, in press) was compared to the bacterial diversity obtained for the Entrada samples (HW10_04, HW11_04, HW12_04 and HW13_04) in this study. When the data from the two communities were combined, a total of 2,812 operational taxonomic units (OTUs) were obtained at a 97% similarity index. Based on the relative abundance of sequences

with greater than 0.1 % incidence, 12 distinct phyla were obtained, and the abundance data was averaged individually for both the sandstones (Figure 3.4). Cyanobacteria is the most abundant phylum in the entire dataset (Supplementary Table 3.S3), followed by Proteobacteria, Actinobacteria, Bacteroidetes, Chloroflexi and unclassified bacterial phylum. Other phyla that are consistently present in both the Entrada and Navajo samples, include Deinococcus-Thermus, Planctomycetes, Verrucomicrobia, Gemmatimonadetes and Acidobacteria. At the phylum level, the two community structures seemed somewhat similar, with Cyanobacteria being the most dominant followed by Proteobacteria, Actinomycetes and Bacteroidetes (Figure 3.4). Actinomycetes have a greater presence in the Entrada as compared to the Jurassic Navajo Sandstone while Proteobacteria are more dominant in the latter (Supplementary Table 3.S3). Based on relative sequence abundances, the top ten most prevalent genera in the bacterial communities of the two cryptoendolithic habitats were analyzed. As expected, the most abundant genera were unclassified Cyanobacteria, *Chroococcidiopsis*, *Leptolyngbya* and *Mastigocladopsis* in decreasing order, all belonging to phylum Cyanobacteria (Supplementary Table 3.S4). The next most abundant genera included unclassified Acetobacteraceae (Alphaproteobacteria), *Segetibacter* (Bacteroidetes) and *Acidiphilium* (Alphaproteobacteria). *Acidiphilium* and *Segetibacter* were more prevalent in the Jurassic Navajo Sandstone (Supplementary Table 3.S4). Unclassified sequences at each hierarchical level reveal the extent of unexplored bacterial diversity in cryptoendolithic microbial communities (Table 3.2). We found a greater incidence of unclassified sequences at the genus level as compared to the phylum level (Table 3.2).

The Chao richness and Inverse Simpson estimates indicate that cryptoendolithic community structure in both Navajo and Entrada sandstone communities is variable within the two sandstone systems, making it difficult to generalize diversity patterns (Table 3.3). Beta diversity was examined after subsampling to the smallest dataset obtained for one of the Entrada sandstone samples, HW13_04. Among all the samples analyzed, one of the Entrada Sandstone, HW11_04 had the highest bacterial diversity while another Entrada sample, HW12_04 had the least diversity (Table 3.3). The Principal Coordinate plot analysis (PCoA) plot revealed a clear delineation between the two cryptoendolithic habitats. The p-values obtained using AMOVA test for statistical comparisons of the two clusters was 0.006, indicating that are significantly different from each other. The Entrada samples cluster together while the Navajo sandstones form another group which was relatively more scattered (Figure 3.5). The cyanobacterial genera within the Entrada and Navajo cryptoendolithic communities were analyzed. Both the communities were dominated by different clades of Cyanobacteria (Figure 3.6; Supplementary Table 3.S5). The Entrada Sandstones were dominated by unclassified Cyanobacteria and *Chroococcidiopsis* genera while the Jurassic Navajo Sandstones were dominated by *Leptolyngbya*, *Mastigocladopsis,* unclassified *Cyanobacteria_FamilyI* genera along with *Chroococcidiopsis* (Figure 3.6). Other notable cyanobacterial genera included *Microcoleus*, *Nostoc* and *Synechococcus*, all of which were present in greater numbers in the Entrada Sandstone (Supplementary Table 3.S5). The Entrada Sandstones were dominated by coccoidal forms of cyanobacteria whereas the Jurassic Navajo Sandstones were dominated mainly by filamentous forms of cyanobacteria.

While the two cryptoendolithic habitats are quite diverse, the core bacterial communities obtained for each habitat were compared to see if there was a core bacterial community consistently present within both the communities. The core community of the Navajo sandstones was 63 OTUs while that in the Entrada Sandstones comprised 57 OTUs. Comparing the two community structures, it was found that 11 OTUs were common between the cryptoendolithic communities of the Navajo and Entrada sandstones (Figure 3.7). The core community shared between the two habitats includes Cyanobacteria (5 OTUs), Proteobacteria (3 OTUs) and Actinomycetes (2 OTUs). Cyanobacterial members included *Chroococcidiopsis* and unclassified genera, Proteobacteria includes *Sphingomonas* and unclassified genera while both the OTUs representing Actinomycetes belonged to unclassified genera.

DISCUSSION

This study presents data on the cryptoendolithic bacterial diversity in the Entrada Sandstones followed by a comparison with the cryptoendolithic diversity in Jurassic Navajo Sandstones described earlier (Kaur and Kurtz, in press). The diversity data presented indicates that Cyanobacteria represent nearly half of the bacterial diversity in the pores of the Entrada Sandstones. The most prevalent cyanobacterial genera detected was *Chroococcidiopsis,* which is in accordance with previous studies reporting *Chroococcidiopsis* as the primary photosynthesizer in arid lithic systems (Friedmann, 1980; Bell 1993). We suggest that the composition of a cryptoendolithic bacterial community is a result of the diversified local physiochemical conditions making it

endemic to a specific site. The most resistant genera in desert lithic habitats are *Deinococcus* and *Blastococcus*, which can thrive in conditions of low availability of water and nutrients besides tolerating ultraviolet light and ionizing radiations (Mohammadipanah and Wink, 2016; Sghaier et al., 2016). Members of the genus *Blastococcus* have been isolated from sandstone monuments and many of them originated from extreme cryptoendolithic environments, however, their physiology is still poorly characterized (Urzi et al., 2001; Salazar et al., 2006, Chouaia et al., 2012). Uncultured members of the genus *Truepera* have been previously detected in the Arctic endolithic habitats, rock samples in the Mars Desert Research Station (MDRS) in Utah and coastal desert rocks (Rainey et al., 2007; Direito et al., 2011; Choe et al., 2018). All members of the phylum Deinococcus-Thermus are extremely radiation resistant (Daly, 2009; Cox and Battista, 2005) which explains their presence in this micro-ecosystem that is exposed to intense solar radiations. Although there are variations in the community structure between the Entrada Sandstones, a shared bacterial community prevails which can be attributed to similar local environmental conditions.

The cryptoendolithic bacterial diversity in the Jurassic Navajo Sandstones was described earlier and there was no correlation of diversity with the physiochemical properties of the sandstones (Kaur and Kurtz, in press). The higher amounts of sulfates, phosphates and nitrates measured in the Entrada Sandstones compared to the Navajo reflects the varying mineralogy's of the sandstones (Table 3.1). Higher nitrate levels in one Entrada sample, HW12_04, corresponded to increased abundance of Actinomycetes in that sample (Figure 3.S1) and higher pH in another sample corresponded to an

increased presence of *Mastigocladopsis.* The other physiochemical parameters had little to no correlation with the cryptoendolithic bacterial community structure in the Entrada Sandstones.

The overlap of only 11 OTUs between the two communities indicates that the two habitats place distinctly different constraints onto the microbial communities that assemble on the sandstones. An explanation for this variation are the differences in sand grain sizes between the Jurassic Navajo and Entrada Sandstones, resulting in different pore sizes available for microbial colonization. In this context, the Jurassic Navajo Sandstone with larger sand grains has larger pores, while the Entrada Sandstone has smaller pores. Microbes colonizing the Jurassic Navajo Sandstone would easily enter the stone and be afforded protection from solar radiation, while microbes colonizing the Entrada Sandstone would be excluded from deeply penetrating the sandstone and would be exposed to greater solar irradiation. This could explain the higher prevalence of radiation tolerant microbes such as *Truepera* and *Blastococcus*. Overall, these results are consistent with previous studies suggesting that rock microbial composition is influenced by physical and chemical properties of rock substrates, such as pore structure, mineral composition and permeability, as well as environmental factors such as climatic exposure, nutrient sources and water availability (Friedmann et al., 1993; Omelon et al, 2006). A relatively large percentage of sequences failed to be assigned to known taxonomic units in both the Jurassic Navajo and Entrada Sandstones (Table 3.2), suggesting the presence of many potentially novel bacteria within the cryptoendolithic communities of sandstones in the GSENM.

Cyanobacteria occupy at least a third of the cryptoendolithic niche in both the Jurassic Navajo and Entrada sandstones. The Jurassic Navajo Sandstones were dominated by filamentous cyanobacteria, namely *Leptolyngbya* and *Mastigocladopsis* genera while the Entrada Sandstones were dominated by coccoidal cyanobacteria that were members of the genus *Chroococcidiopsis* along with unclassified cyanobacterial genera (Supplementary Table 3.S3). These differences are most likely attributable to the structure of the sandstones, with the Jurassic Navajo Sandstones being a fine to medium grained sandstone, while the Entrada Sandstones being a fine-grained sandstone (Peterson and Pipiringos, 1979). Differences in porosity would affect the ability of cryptoendolithic cyanobacteria to penetrate the sandstone. Previous studies reported a 20.6% porosity for the Jurassic Navajo sandstones (Kurtz and Netoff, 2001) and a 19% porosity index for the Entrada Sandstones (Lama and Vutukuri, 1978; Schultz et al., 2003). Larger pore size in the Jurassic Navajo implies greater loss of water through capillary action while also allowing the filamentous cyanobacteria to form a biofilm-like matrix beneath the surface of the sandstone. Conversely, restricted pore sizes in the Entrada Sandstone correlated with relatively longer lasting moisture availability besides harboring coccoidal forms of cyanobacteria.

Comparing the beta diversity using principle coordinates analysis, both the cryptoendolithic communities segregated into separate clusters. Based on the data obtained in this study, we deduce that the separation of the Jurassic Navajo and Entrada Sandstone cryptoendolithic communities was mainly attributed to the differences in the dominant cyanobacterial genera, that have been discussed earlier (Figure 3.4). The

cryptoendolithic communities within the Jurassic Navajo Sandstone were relatively more scattered on the PCoA plot and it has been reported that moisture availability is one of the major drivers in shaping the community structure in this microecosystem (Kaur and Kurtz, in press). These findings present evidence that difference in textural sandstone properties, including the porosity, influences the cryptoendolithic bacterial composition in the Jurassic Navajo and Entrada Sandstones.

Cryptoendolithic microorganisms are a major focus of many investigations of life in harsh environments and studies with astrobiological implications (Friedmann 1980; Friedmann et al. 1993; Stivaletta, 2011; Wierzchos et al. 2012). The data presented in this paper expands on our knowledge of the cryptoendolithic bacterial composition in the Entrada sandstones in the GSENM. It will potentially aid in future studies focused on understanding the microbial functioning in this micro-ecosystem.

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TABLES AND FIGURES

Table 3.1 Physiochemical analysis of the Entrada Sandstone samples.

Table 3.2 Ratio of unclassified sequences at different taxonomic levels in the Entrada and Navajo sandstone samples.

Table 3.3 Cryptoendolithic bacterial diversity metrics based on 16S rRNA gene analysis of the Jurassic Navajo and Entrada Sandstone. Community richness (Chao1 richness estimate), coverage of sampling (in percentage), sobs and evenness (Inverse Simpson diversity index) were calculated in mothur at 97% similarity after normalizing samples to 8988 sequences.

Figure 3.1 (A) Preliminary site map for the Entrada Sandstones. Pins show the locations of the sampling sites for the Entrada Sandstones. (B) Stratigraphy of the two geologic rock types in this study, the Jurassic Navajo and Entrada sandstones.

Figure 3.2 Relative abundances of the major phyla identified with >0.1% sequence abundance in cryptoendolithic bacterial communities of the Entrada Sandstones. Taxa are arranged in order as they appear on the stacked bar graph with each rectangle representing the relative percentage abundance of a phylum in a particular sandstone sample.

Figure 3.3. Core bacterial community structure in the analyzed Entrada sandstone samples, represented by 57 OTUs. The taxonomy file for the shared OTUs is presented in supplementary table 3.S2.

Figure 3.4 Comparative analysis of the cryptoendolithic bacterial communities within the Entrada and Jurassic Navajo Sandstone based on averaged relative percentage sequence abundances.

Figure 3.5 Principal coordinate plot analysis showing the delineation between the Entrada and Navajo cryptoendolithic communities. The p-value obtained using AMOVA test for statistical comparisons of both the communities was 0.006.

Figure 3.6 Comparison of the relative abundance of cyanobacterial genera in the cryptoendolithic communities of the Jurassic Navajo and Entrada samples based on averaged relative percentage sequence abundances.

Figure 3.7 The core bacterial communities shared between the cryptoendolithic habitats in the Jurassic Navajo and Entrada Sandstones.

SUPPLEMENTARY DATA

Table 3.S1 Cryptoendolithic bacterial diversity in the Entrada samples in terms of most abundant OTUs – attached excel file

Table 3.S2 Core bacterial community within the Entrada Sandstones represented by 57 shared OTUs – attached excel file

Table 3.S3 Averaged relative abundance of sequences at phyla level in the Entrada and Navajo Sandstones analyzed in this study. The values represent the averaged percentage abundance ± standard error.

Table 3.S4 Top ten most abundant bacterial genera in the Entrada and the Jurassic Navajo Sandstone based on relative abundance of sequences.

Table 3.S5 Averaged relative abundance of cyanobacterial genera within the cryptoendolithic communities in all the Entrada and Navajo sandstones. Values represent the averaged percentage abundance ± standard error.

Figure 3.S1 Canonical correspondence plot displaying the correlation between physiochemical parameters and the relative OTU abundance in the Entrada sandstone samples.

Table 3.1 Physiochemical analysis of the Entrada Sandstone samples.

Parameter	HW11_04	HW13_04	HW12_04	HW10_04
Nitrate	188.65 ± 0.005	363.53 ± 0.004	8548.74 ± 0.252	320.53 ± 0.002
Ammonium	1393.33 ± 0.03	343.33 ± 0.004	301.67 ± 0.012	572.5 ± 0.00
Sulfate	127.73 ± 0.006	159.50 ± 0.035	281.69 ± 0.015	64.19 ± 0.009
Phosphate	862.31 ± 0.05	839.87 ± 0.06	350.77 ± 0.10	879.62 ± 0.04
Ferrous Iron	30.08 ± 0.15	27.33 ± 0.01	69.17 ± 0.06	23.41 ± 0.05
Munsell Color (wet)	2.5 YR 4/6	5 YR 4/4	2.5 YR 3/4	5 YR 4/6
pH	6.57	7.6	7.02	8.01

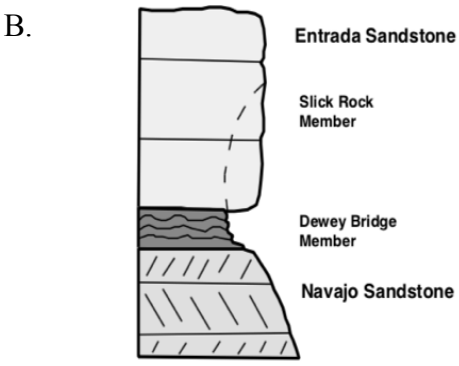
*Values for nitrate, ammonium, sulfate, ferrous iron and phosphate represent nanomoles per gram of sandstone sample.

Table 3.2 Ratio of unclassified sequences at different taxonomic levels in Entrada and Navajo sandstone samples.

	Relative abundance (%)		
Taxonomy	Total	Entrada	Navajo
Phylum	3.05	1.45	3.93
Class	4.51	3.70	5.31
Order	22.68	30.48	13.85
Family	30.61	40.06	21.35
Genus	48.94	51.09	43.87

Table 3.3 Cryptoendolithic bacterial diversity metrics based on the 16S rRNA gene analysis of the Jurassic Navajo and Entrada Sandstone. Community richness (Chao1 richness estimate), coverage of sampling (in percentage), sobs and evenness (Inverse Simpson diversity index) were calculated in mothur at 97% similarity after normalizing samples to 8988 sequences.

Sandstone	Reads	OTU's	Coverage (%)	Sobs	Invsimpson	Chao
HW11_04	11735	726	97.38	663.00	76.29	921.17
HW13_04	8988	482	98.03	482.00	26.21	737.34
HW12_04	12503	193	99.44	175.71	4.45	243.21
HW10_04	9518	606	97.71	593.91	28.09	834.86
HW01_04	41102	665	98.15	462.12	14.82	649.52
HW03_04	26926	760	98.03	625.39	36.03	771.22
HW07_04	25061	773	97.67	569.34	22.60	791.13
HW04_05	45462	666	98.12	440.05	10.06	635.63
HW01_05	42502	420	98.95	296.03	14.06	405.05



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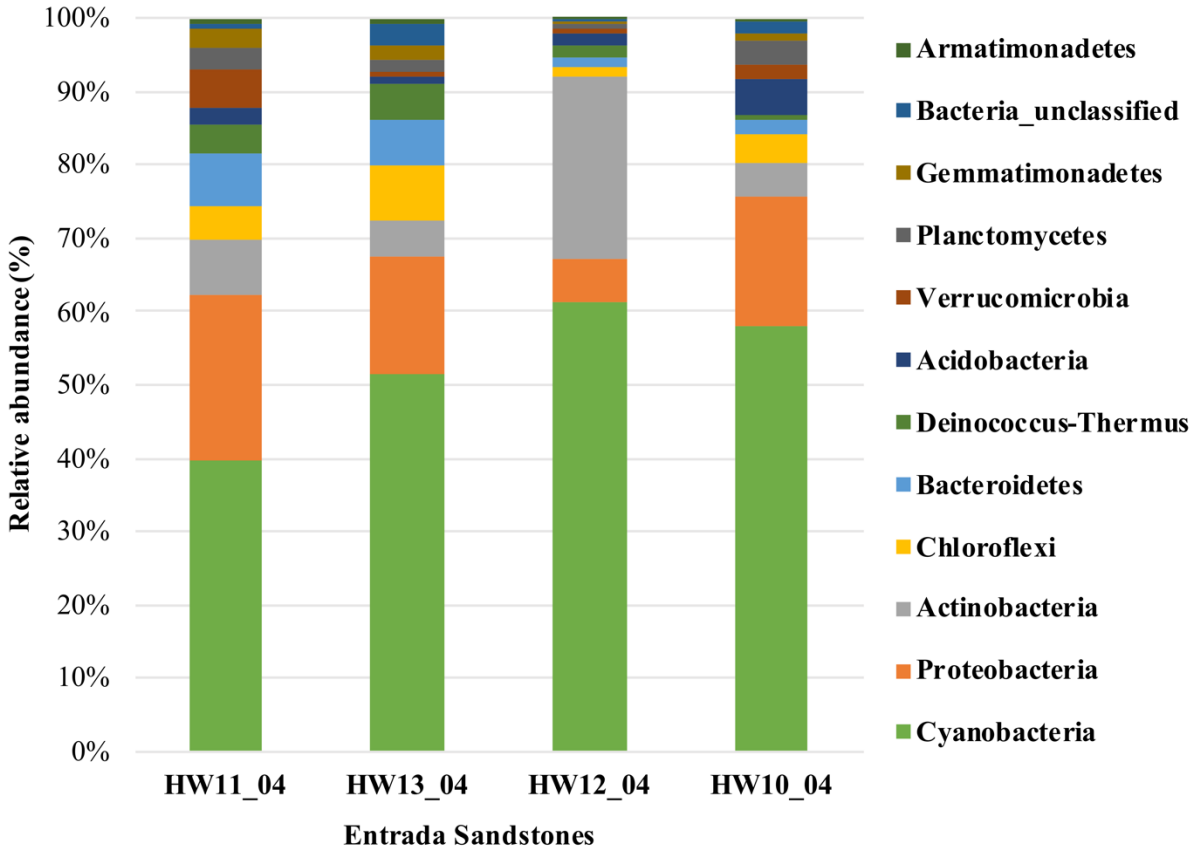

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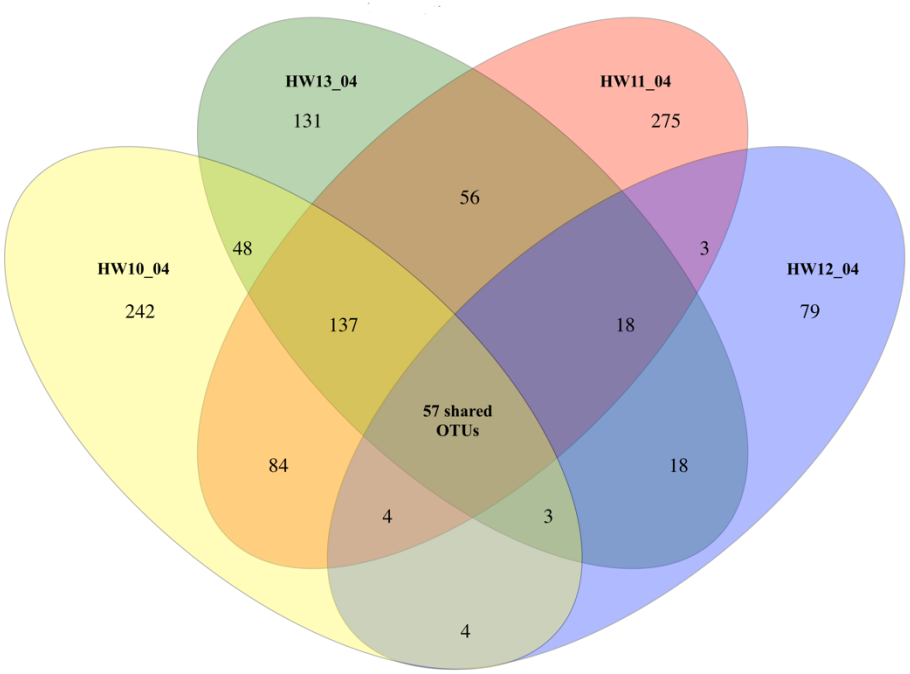

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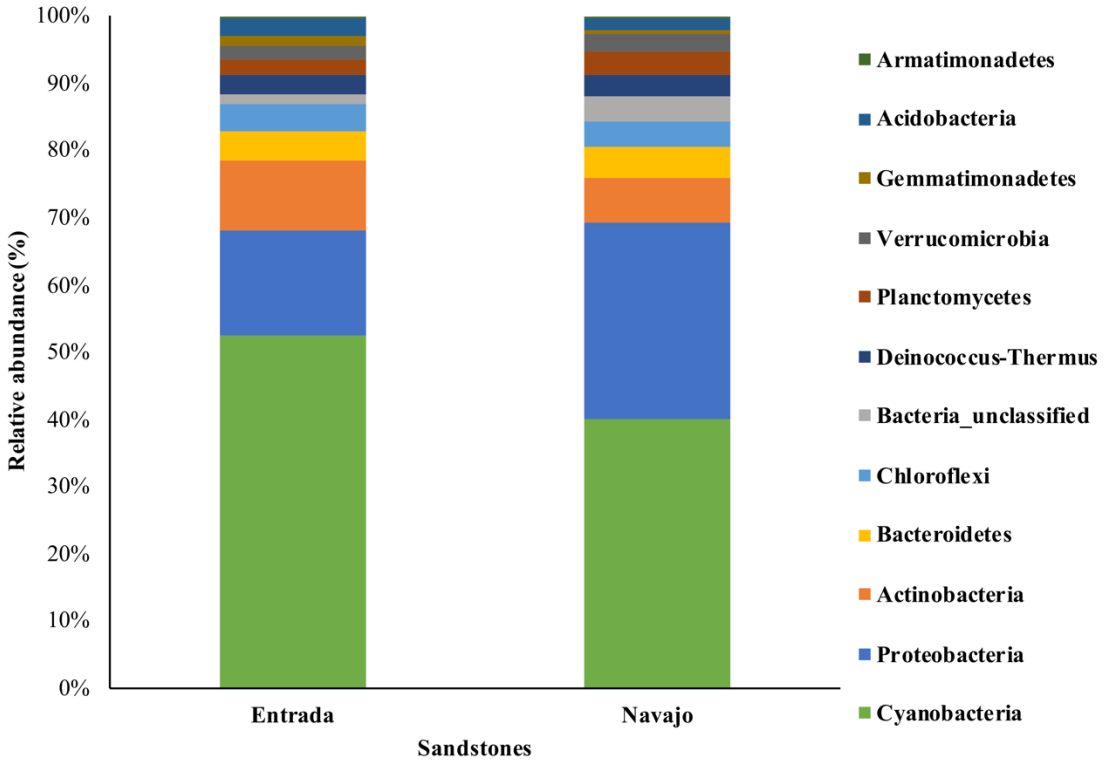

Figure 3.4 Comparative analysis of the cryptoendolithic bacterial communities within the Entrada and Jurassic Navajo Sandstone based on averaged relative percentage sequence abundances. Data for Navajo Sandstones obtained from Kaur and Kurtz (in press).

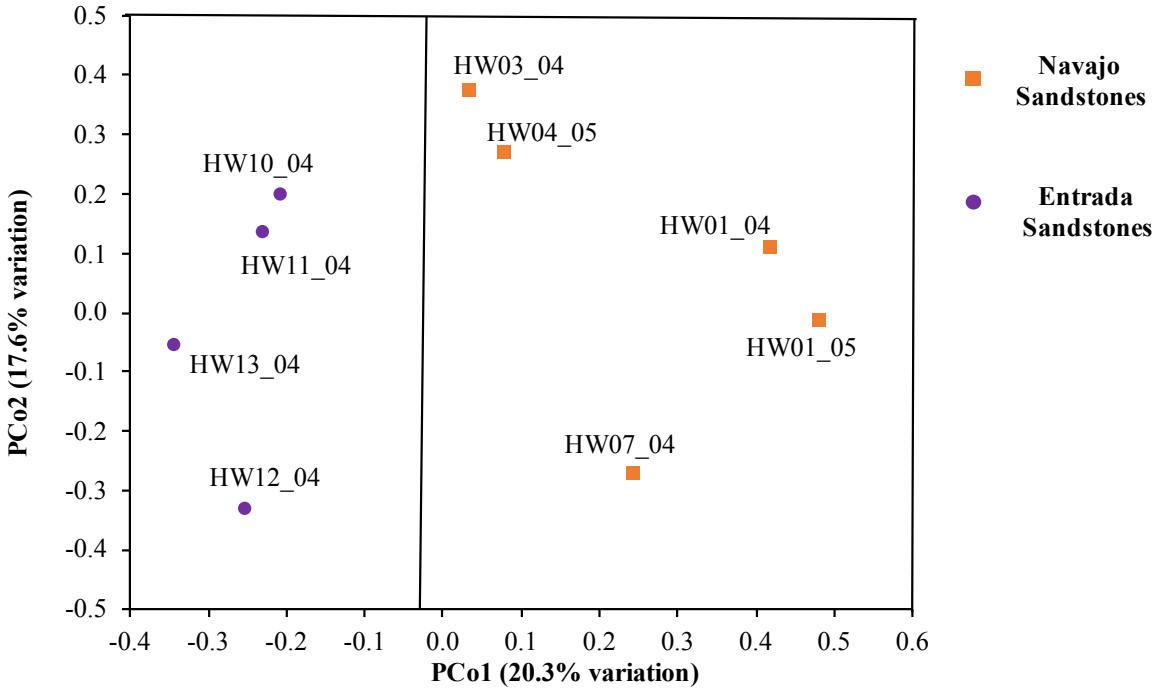

Figure 3.5 Principal coordinate plot analysis showing the delineation amongst Entrada and Navajo cryptoendolithic communities. The p-value obtained using AMOVA test for statistical comparisons between bacterial communities of the two sandstone types was 0.006. Data for Navajo Sandstones obtained from Kaur and Kurtz (in press).

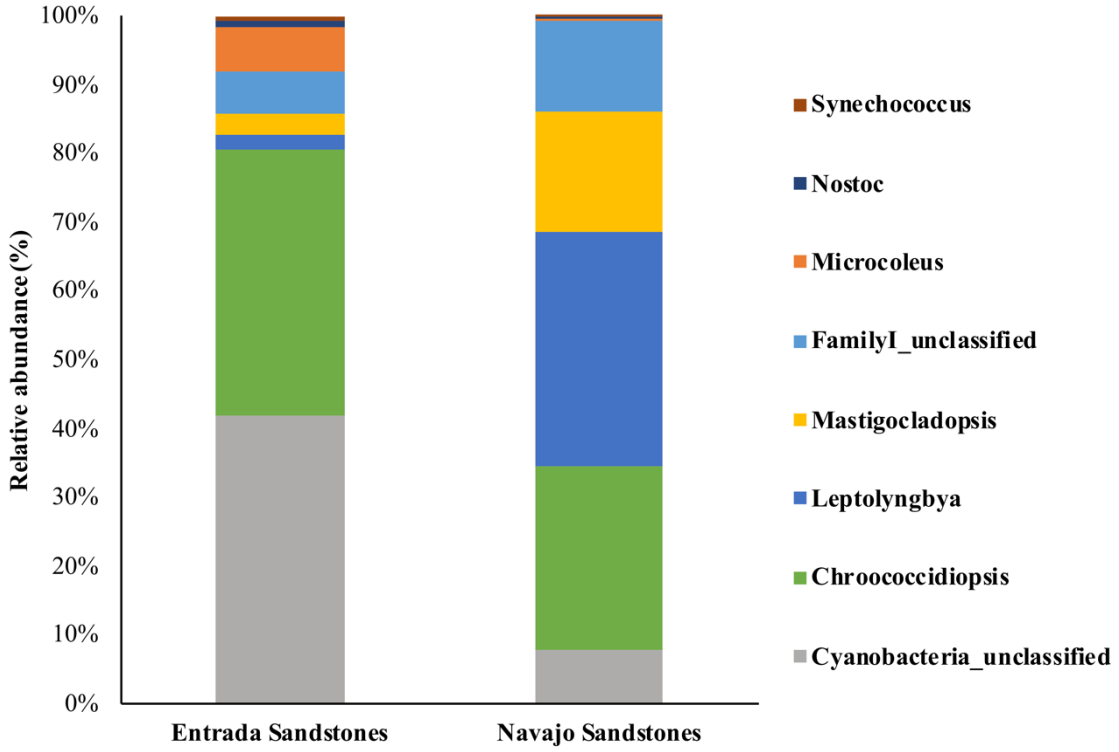

Figure 3.6 Comparison of the relative abundance of cyanobacterial genera in the cryptoendolithic communities of the Jurassic Navajo and Entrada samples based on averaged relative percentage sequence abundances. Data for Navajo Sandstones obtained from Kaur and Kurtz (in press).

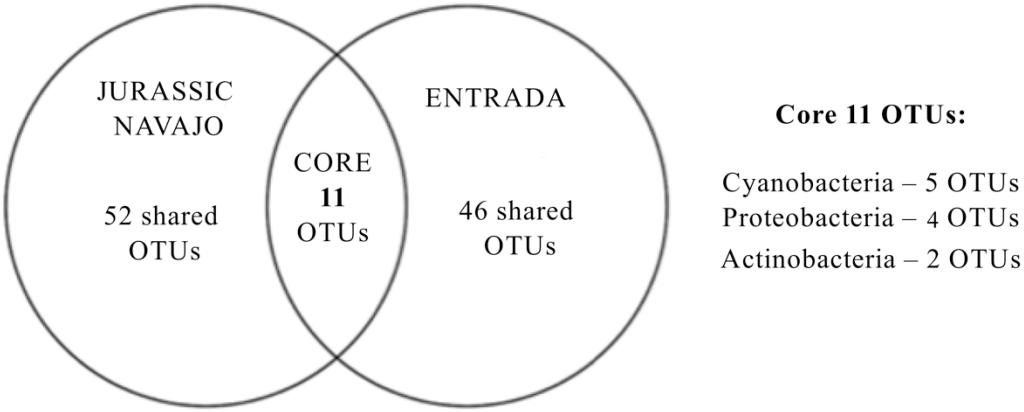

Figure 3.7 The core bacterial community structure shared between the cryptoendolithic habitats in the Jurassic Navajo and Entrada Sandstones. Data for Navajo Sandstones obtained from Kaur and Kurtz (in press).

Supplementary data

Table 3.S3 Averaged relative abundance of sequences at phyla level in the Entrada and Navajo Sandstones analyzed in this study. The values represent the averaged percentage abundance ± standard error. Data for Navajo Sandstones obtained from Kaur and Kurtz (in press).

Phyla	Relative percentage abundance	
	Entrada Sandstones	Navajo Sandstones
Cyanobacteria	52.40 ± 4.7	40.03 ± 4.5
Proteobacteria	15.47 ± 3.5	29.01 ± 4.2
Actinobacteria	10.42 ± 4.7	6.60 ± 1.7
Bacteroidetes	4.18 ± 1.5	4.60 ± 0.3
Chloroflexi	4.30 ± 1.2	3.76 ± 1.2
Bacteria_unclassified	1.45 ± 0.6	3.92 ± 2.1
Deinococcus-Thermus	2.89 ± 1.0	2.97 ± 0.8
Planctomycetes	2.15 ± 0.7	3.52 ± 0.9
Verrucomicrobia	2.09 ± 1.0	2.67 ± 0.5
Gemmatimonadetes	1.51 ± 0.5	0.71 ± 0.1
Acidobacteria	2.46 ± 0.8	1.53 ± 0.3
Armatimonadetes	0.41 ± 0.1	0.47 ± 0.2

Table 3.S4 Top ten most abundant bacterial genera in the Entrada and the Jurassic Navajo Sandstone based on relative percentage abundance of sequences. Data for Navajo Sandstones obtained from Kaur and Kurtz (in press).

Genus	Entrada Sandstones				Jurassic Navajo Sandstones				
	HW11_04	HW13_04	HW12_04	HW10_04	HW01_04	HW03_04	HW07_04	HW04_05	HW01_05
Cyanobacteria_unclassified	10.82	32.89	12.97	30.71	0.04	4.55	3.68	3.06	4.16
Chroococcidiopsis	11.39	17.50	45.34	9.66	9.94	14.45	3.23	17.03	8.07
Leptolyngbya	2.62	0.45	0.00	0.41	19.18	3.95	10.73	4.56	24.32
Mastigocladopsis	0.57	0.20	0.00	6.35	0.01	10.61	0.14	29.11	1.57
Acetobacteraceae_unclassified	1.11	0.61	0.11	0.13	2.13	1.91	4.36	2.04	17.02
Segetibacter	2.90	0.72	0.00	0.13	1.58	2.56	1.23	0.56	2.66
Acidiphilium	0.14	0.13	0.15	0.01	0.32	0.15	4.04	0.73	7.60
Bacteria_unclassified	0.91	3.14	0.29	1.45	2.60	2.08	12.42	1.89	0.62
Cyanobacteria_FamilyI_unclassified	6.71	0.22	0.00	3.52	0.54	0.07	19.08	1.76	2.14
Alphaproteobacteria_unclassified	1.31	0.62	0.00	1.30	12.35	4.29	0.01	0.71	0.08

Table 3.S5 Averaged relative percentage abundance of cyanobacterial genera within the cryptoendolithic communities in all the Entrada and Navajo sandstones. Values represent the averaged percentage abundance ± standard error. Data for Navajo Sandstones obtained from Kaur and Kurtz (in press).

Cyanobacterial genera	Entrada Sandstones	Navajo Sandstones
Cyanobacteria_unclassified	41.40 ± 10.13	7.69 ± 2.26
Chroococcidiopsis	38.58 ± 12.63	26.66 ± 5.82
Leptolyngbya	2.05 ± 1.54	33.96 ± 11.53
Mastigocladopsis	3.20 ± 2.60	17.40 ± 10.39
FamilyI_unclassified	5.96 ± 4.02	13.21 ± 9.77
Microcoleus	6.56 ± 3.82	0.37 ± 0.34
Nostoc	0.66 ± 0.61	0.29 ± 0.18
Synechococcus	0.83 ± 0.83	0.01 ± 0.01

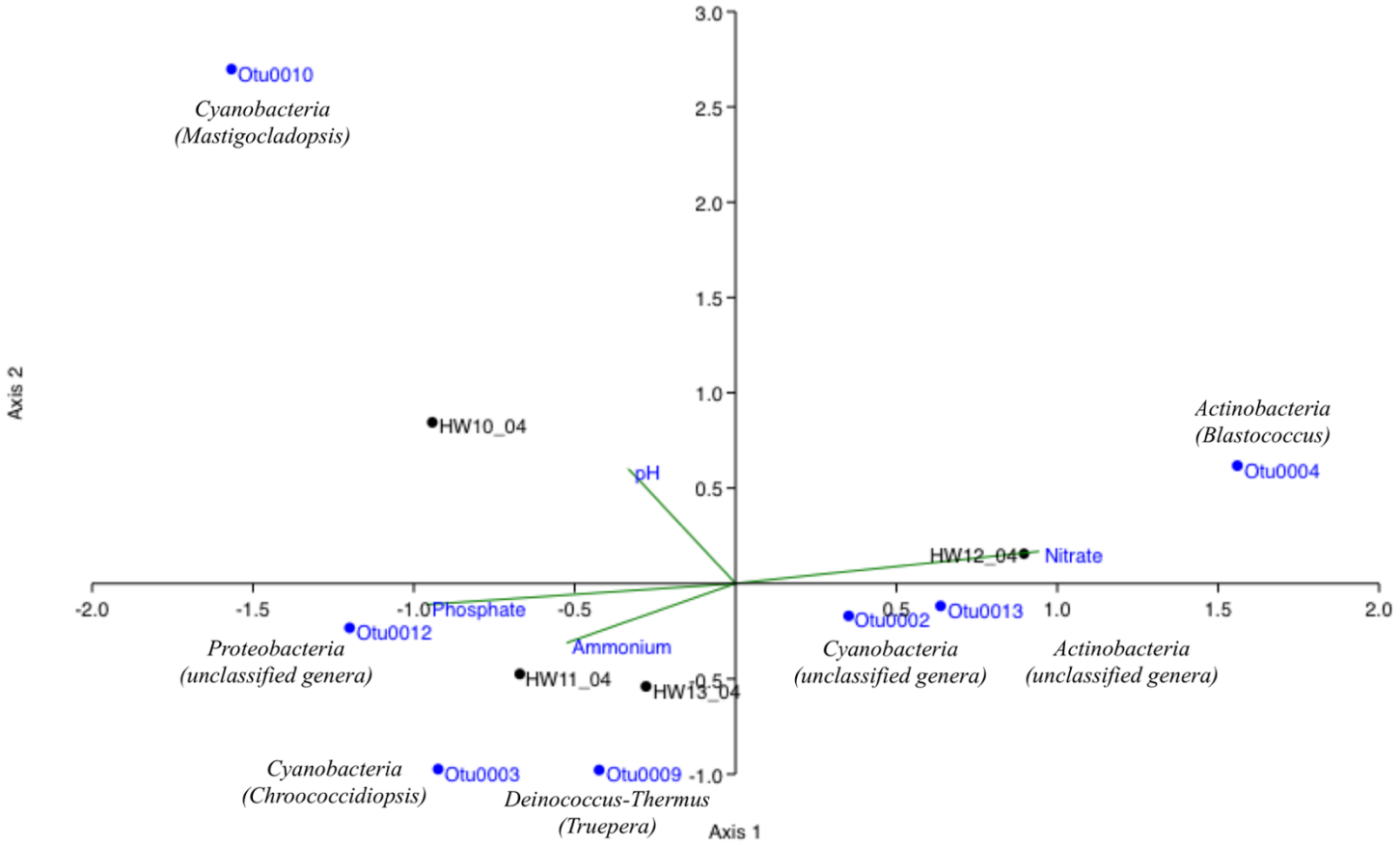

Figure 3.S1 Canonical correspondence plot displaying the correlation between physiochemical parameters and the relative OTU abundance in the Entrada sandstone samples.

Chapter 4

Extracellular Polysaccharides Produced by Cryptoendolithic Communities Concentrate Metals in An Oligotrophic Environment

ABSTRACT

In the Navajo sandstone formations of the Colorado Plateau, a phenomenon known as "Iron-bleaching" has been studied previously. This process is largely attributed to paleo geochemical processes resulting in the lightening of the sandstone. However, this process may still be occurring due to the activity of the cryptoendolithic communities found in the sandstones of the region. EPS produced by cryptoendolithic communities in both the Jurassic Navajo and Entrada sandstones is capable of binding ferrous iron in the range of 15-67 ng Fe^{2+} per gram sandstone. Using ICP-AES, we investigated the metal binding capacities of EPS isolated from laboratory-based communities, both as microbial mats including soluble and biofilm fractions and semi-purified cyanobacterial cultures. The biofilm associated EPS extracted from the microbial mats was more efficient at binding metal cations than the soluble counterpart. EPS isolated from the liquid cyanobacterial cultures exhibited the highest metal binding capacity. The metal binding preference of both the natural and laboratory-based communities is in the following order: Mg >Mn >Fe> Cu > Zn. We conclude that EPS produced by cryptoendolithic communities act like a bio-filter concentrating metal cations, making them available to the microbial consortia embedded in the EPS matrix.

Keywords: Cryptoendolithic communities, Sandstones, EPS, metal cations, ICP-AES

INTRODUCTION

Cryptoendolithic bacterial communities are ubiquitous in the porous sandstone outcrops of the Grand Staircase-Escalante National Monument (GSENM), which is known for its diverse geographical and geological features (Doelling et al., 2000). These features include the Jurassic Navajo and Entrada Sandstone units, the former being the largest erg that exists in the geological record (Potter and Chan 2011; Beitler et al., 2005). The Jurassic Navajo and Entrada Sandstones are both eolian deposits with hematite and strata bound layers (Chan et al., 2000). The Jurassic Navajo is a tan colored, fine to medium-grained, massive sandstone with abundant iron concretions (Chan et al., 2012). While the Entrada is mostly fine grained, red colored sandstone that forms earthy slopes and ridges (Doelling et al., 2000). Past studies have noted color variations in the Jurassic Navajo Sandstones from regional red to white bleaching patterns, mainly associated with paleo-geochemical processes that removed iron (III) from the hematite cladding of the sand grains within the stone (Chan and Parry, 2002; Beitler et al., 2003; Potter and Chan, 2011). There have also been reports attributing this bleaching phenomenon to be partially microbial in nature (Loope et al., 2010; Weber et al., 2012).

Cryptoendolithic habitats are often detected as a distinct green biofilm that forms a few millimeters beneath the surface of a sandstone. This biofilm is indicative of cryptoendolithic communities that are dominated by cyanobacteria, the primary photoautotrophs in hot desert ecosystems (Bell et al., 1988; Bell, 1993; Wierzchos et al., 2012; Antony et al., 2012; Hammes et al., 2013; Kaur and Kurtz, in review). These

communities survive in extreme environments through the production of extracellular polysaccharides (EPS) that not only provides physical stability, but also retains water and concentrates nutrients (Omelon et al., 2006; Seibert et al., 1996; Belnap, 2012; Casamatta et al., 2002; Mager, 2010). Previous research demonstrated that EPS produced by cryptoendolithic communities trapped ferrous iron and maybe contributing to the continued iron bleaching of the Jurassic Navajo Sandstones (Hammes et al., 2013). The functionality of EPS has not been studied extensively because of its complex and variable chemical composition depending on the rock type, environmental conditions and nutrient levels (Stuart et al., 2016). EPSs are heterogenous polymers that are mainly composed of high molecular weight polysaccharides that form the framework of microbial biofilms (Mager, 2010; Hokputsa et al., 2003). In a cryptoendolithic habitat, cyanobacteria are the major contributors of carbon and nitrogen input supporting the growth of heterotrophic bacteria (Palmer and Freidman, 1990; Wynn-Williams, 2000; Wessels and Budel, 1995). However, the cryptoendolithic environment does not relieve a microbial community from stresses resulting from nutrient limitation in rocks. In oligotrophic environments, electron sources and sinks are critical to build microbial communities (Azua-Bustos et al., 2012) and minerals within sandstones are excellent candidates for this requirement.

Metals are basic micronutrients required for fundamental biochemical processes such as respiration and photosynthesis for all living organisms (Huertas et al., 2014). Specifically, cyanobacteria require several metal ions in abundance for photosynthetic processes. These ions include Fe^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, and Zn^{2+}, which are essential cofactors involved in oxygenic photosynthesis (Giner-Lamia et al., 2016; Shcolnick and

Keren, 2006). Copper is a required cofactor for plastocyanin, which is part of the thylakoid lumen electron transport system (Shcolnick and Keren, 2006). Manganese is specifically required to form the Mn cluster at the donor site of photosystem II which catalyzes the water splitting reaction (Shcolnick and Keren, 2006; Huertas et al., 2014). Magnesium is ligated at the center of the chlorophyll ring and is by far the most abundant metal ion in the thylakoid membrane (Shcolnick and Keren, 2006; Huertas et al., 2014). Unlike other essential metal ions which are required in trace amounts, Mg^{2+} is a macronutrient for photosynthetic microbes (Shcolnick and Keren, 2006). Zinc is required by the carbonic anhydrases (Huertas et al., 2014). Lastly, Fe^{2+} is a limiting factor for microbial life in arid ecosystems (Shcolnick and Keren, 2006, Huertas et al., 2014). Iron is a cofactor for all three-photosynthetic electron transfer chain supercomplexes in cyanobacteria (Huertas et al., 2014). Although iron is abundant in the earth's crust, it is not present in a readily available form to microbiota as in the presence of oxygen and neutral pH, iron oxidizes and precipitates as Fe^{3+} oxides (Shcolnick and Keren, 2006). Overall, the quota of metal ions required by photosynthetic microorganisms exceeds that of other prokaryotes because of the high demand imposed by the photosynthetic machinery (Huertas et al., 2014; Shcolnick and Keren, 2006).

In this paper, we present data on selected metal ions that were complexed by natural communities associated with Jurassic Navajo and Entrada Sandstones as well as the content of metals found in uncolonized sandstone samples. EPSs produced by microcosms derived from Jurassic Navajo Sandstone based communities were tested for their capacity to bind essential metal ions. EPS production is dependent on the

cyanobacterial strain as well as the culture conditions (De Philippis and Vincenzini, 1998). In order to reduce EPS variability upon subsequent batch transfers of the culture, we also analyzed the metal content sequestered by the EPS produced by partially purified cyanobacterial cultures. Based upon the evidence, we hypothesize that the EPS produced by cryptoendolithic communities act like a biofilter to bind and concentrate multiple metal ions, not just ferrous iron, providing a ready source of key nutrients for cellular metabolism.

MATERIALS AND METHODS

Site description and sample collection

Two Jurassic Navajo (HW03_04, HW07_04) and two Entrada (HW13_04 and HW12_04) Sandstones samples were obtained from the Harris Wash area in the Grand Staircase-Escalante National Monument (GSENM) in 2004 (Figure 4.1). The GPS coordinates for the Navajo samples HW03_04 and HW07_04 are 37° 41' 10.02" N; 111° 18' 41.70" W and 37° 41' 06.69" N; 111° 19' 11.64" W respectively. The GPS coordinates for the Entrada samples HW13_04 and HW12_04 are 37° 37' 46.86" N; 111° 20' 59.52" W and 37° 37' 49.98" N; 111° 20' 48.78" W respectively. Eolian sand sediments were obtained from a wind-formed deposit in the Harris Wash area of the GSENM and used as substratum for microcosm setup in the laboratory. The upper 5-10 mm of sandstone surface was chipped from an area of approximately 50 cm^2 and collected in sterile sample bags (Nasco Whirlpak) which were stored at room temperature in the dark as dry samples.

Determination of metal content in sandstones

The availability of essential metals (Mg, Cu, Zn, Mn and Fe) in four sandstone samples, HW03_04, HW07_04, HW13_04 and HW12_04 was measured, using Inductively Coupled plasma Atomic Emission spectroscopy (ICP-AES). For each sandstone, a portion of biofilm associated sandstone was scraped from the sample surface. Approximately 250 mg of each sandstone colonized with cryptoendolithic growth was mixed with 1.0 ml of 1N HCl, in triplicates. Stone not associated with the cryptoendolithic community was separated for each sandstone. Approximately 250 mg of each sandstone sample lacking visible microbial growth was mixed with 1.0 ml reverse osmosis purified water (17.0 MΩ cm^{-1}; RO H_2O) and 1.0 ml of 1N HCl respectively, in triplicates.

All sandstone samples were then briefly vortexed and centrifuged at 8000 xg for 10 min. The supernatants were collected, and the pH adjusted to 2-3 before submission for ICP-AES analysis at the Clemson Agricultural Service laboratory. All concentrations were normalized to ng metal per gram of sandstone sample. The experimental setup for the metal content determination in sandstones has been outlined in Figure 4.2 (A).

Microcosm setup

Fifty grams of eolian sand sediments were autoclaved and mixed with 150 ml of BG-11 freshwater broth (Rippka, 1988) in Roux flasks to setup microcosms as described earlier (Kurtz et al., 2005). The Roux flasks were inoculated with crushed sandstones containing cryptoendolithic microorganisms to setup microcosms: Samples A, B and C. The Roux

flasks were incubated at room temperature, with 17,000 lumens of light provided on a day-night cycle (16 hours and 8 hours respectively). Microbial biofilms were allowed to grow with water added periodically to keep the sand surface moist. After a 6-month incubation, mature microbial biofilms were harvested for EPS isolation and characterization.

Purification of Cyanobacterial cultures and whole cell culture setup

Cyanobacterial growth from mature microcosms (Samples A, B and C) was streaked on to BG-11 freshwater plates incorporated with 50 μg/ml cycloheximide to prevent fungal growth. The plates were incubated at room temperature for 8-10 weeks under the day-night cycles described above. Isolated colonies were subsequently transferred onto fresh BG-11 plates to obtain partially purified cyanobacterial cultures. These cultures were used to inoculate whole cell cyanobacterial cultures in 500 ml BG-11 broth in 1000 ml flasks and incubated as stated earlier for microcosms with intermittent shaking. After incubation for 3-4 months, the whole cell cyanobacterial cultures were centrifuged at 10,000 xg for 20 min. The whole cell supernatants (WCS) obtained from all three cultures (A-WCS, B-WCS and C-WCS) were stored at 4 °C for EPS extraction.

Extraction of extracellular polymeric substances (EPS)

The mature microbial biofilms (Samples A, B and C) and supernatant from whole cell cyanobacterial cultures (A-WCS, B-WCS and C-WCS) were harvested for EPS extraction. EPS from mature microbial biofilms was partly soluble in nature (Soluble

EPS) and partly embedded within the microbial biofilm matrix (Biofilm EPS). The microbial biofilms were suspended as a slurry and centrifuged at 4,000 xg for 15 min. The supernatant was decanted and used as the starting material for the extraction of the soluble form of EPS. The pelleted microbial biofilm was used to extract the biofilm associated EPS. The supernatants from whole cell cyanobacterial cultures (A-WCS, B-WCS and C-WCS) were used as the starting material for EPS extractions while the pellets were excluded since the amount of biomass obtained was insufficient for extraction of EPS. EPS extractions were performed using methods described earlier (Hammes et al., 2013). Six EPS extracts from three microbial mats each having a soluble and biofilm associated fraction and three EPS extracts from the whole cell supernatants were obtained. All EPS extracts were dialyzed overnight against RO water using cellulose ester dialysis membranes (Spectra/Por® Biotech) with a molecular cutoff of 2000 Da. These fractions were then stored at -20 °C for further analysis. The polysaccharide content of the EPS extracts was quantified using the anthrone assay, with glucose as the standard (Brinks et al., 1960).

Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) analysis

Before setting up the ICP experiment, each EPS extract was confined in dialysis tubing (Spectra/Por® Biotech) with a molecular cutoff of 2000 Daltons and pretreated with 0.1N HCl solution for 30 min in order to remove any metal ions bound to the EPS (Micheletti et al., 2008). The dialysis tubing containing acidified EPS was then transferred to 2000 ml RO H_2O and dialyzed for 2 hours. The dialysis tubes were transferred to 2000 ml of fresh RO H_2O dialyzed overnight at room temperature with stirring.

Stock solutions of 40 mg l^{-1} Fe^{2+}, Cu^{2+}, Mn^{2+}, Mg^{2+} and Zn^{2+} were prepared, for each metal ion, using $FeCl_2.4H_2O$, $CuCl_2.2H_2O$, $MnCl_2.4H_2O$, $MgCl_2.6H_2O$ and $ZnCl_2$. All working solutions were prepared by diluting each stock solution with an appropriate volume of RO H_2O to the desired concentration. The pH of the solutions were adjusted to 5.0 with concentrated hydrochloric acid to avoid any possible hydroxide precipitation (Micheletti et al., 2008).

Ferrous iron binding capacities of the EPS extracts were tested using ICP-AES by adding the acid pretreated EPS extract and $FeCl_2.4H_2O$ in a dialysis tubing for a final concentration of 10 mg l^{-1} of Fe^{2+} in the reaction mixture. The ability of the EPS extracts to bind multiple metal ions simultaneously was tested by adding each acid pretreated EPS fraction and metal chloride solutions to a dialysis tubing for a final concentration of 10 mg l^{-1} of each metal ion in the solution. The dialysis bags were sealed and placed in 1000 ml beakers containing 800 ml of RO H_2O that was stirred at room temperature. After 2 hours, the old dialysate was discarded and replaced with fresh RO H_2O and stirred overnight at room temperature. Acid-pretreated EPS extracts and untreated EPS extracts without any metal ions added, dialyzed using the same protocol stated above, were used as controls. Based on the variable EPS yield from different microcosms, 2.5 mg of the soluble EPS and 0.35 mg each of the biofilm- associated and the whole cell supernatant EPS were used to set up the respective reaction mixtures for ICP-AES analysis. Another set of controls were 10 mg l^{-1} solutions of each Fe^{2+}, Cu^{2+}, Mn^{2+}, Mg^{2+} and Zn^{2+} prepared by diluting stock solutions, dialyzed using the same procedure as for the reaction mixtures in the experimental setup. The contents of the dialysis bags were acidified to pH

1.5 with concentrated hydrochloric acid and metal concentrations were determined using ICP-AES. All assays were run in triplicate. All concentrations were normalized to ng metal per mg of EPS extract. The basic ICP-AES setup for all the EPS extracts has been outlined in Figure 4.2 (B).

RESULTS

Sandstones and metal availability

Metal cations extracted with water were used to represent the readily available pool of metal cations in the sandstones while the acid extracted fractions were the metals that are tightly bound to the sandstone grains. The biofilm associated fractions of metal cations represent the pool of metal ions that were bound and sequestered by the cryptoendolithic microbial communities. The concentrations of the aforementioned fractions of essential metal cations, namely Fe^{2+}, Cu^{2+}, Mn^{2+}, Mg^{2+} and Zn^{2+}, were compared between the Jurassic Navajo and Entrada Sandstones as shown in Table 4.1. The amount of freely available iron was greater in the Jurassic Navajo Sandstones (HW03_04, HW07_04) as compared to the Entrada Sandstones (HW12_04, HW13_04). However, natural microbial communities in the pores of Entrada Sandstones bound about 34–67 ng iron (II) per gram of sandstone sample, which was considerably higher than the amount of iron (II) complexed by the microorganisms in the Jurassic Navajo Sandstones analyzed in this study (Table 4.1). Conversely, magnesium (II) was present in higher amounts in the Entrada compared to the Navajo Sandstones, in terms of the readily available and tightly bound Mg in the sandstones as well as the amount sequestered by the cryptoendolithic

microorganisms in the biofilm fraction. There was a smaller pool of readily available copper, zinc and manganese ions compared to magnesium and iron present in both the Jurassic Navajo and Entrada Sandstones. Mn and Mg were found to be highly concentrated in the biofilms when compared to the available metals within the underlying substratum for both the Jurassic Navajo and Entrada Sandstone communities (Table 4.1). In both communities, the metal concentrations were as follows: Mg > Mn ≧ Fe > Cu > Zn.

Cryptoendoliths: EPS and metal binding capacities

The ferrous iron binding capacities of the exopolysaccharides obtained from the biofilm, soluble fraction of microbial biofilms, namely samples A, B and C and the whole cell cyanobacterial supernatant (A-WCS, B-WCS, C-WCS) were averaged, respectively as shown in Figure 4.3. The biofilm associated EPS fraction from mature microbial biofilms bound at least 4-7 times more ferrous iron than the soluble fraction of EPS (Table 4.2). The whole cell cyanobacterial supernatant bound 2-4 more ferrous iron than the biofilm fraction of EPS extracted from microbial mats (Figure 4.3). The ferrous iron binding capacity of all the EPS extracts decreased in the presence of the other essential metal cations, viz., Mg^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+} (Table 4.2). EPS from the whole cell cyanobacterial supernatant bound the most ferrous iron while the soluble EPS extracted from the mature microbial biofilms had the least ferrous iron binding potential (Figure 4.3).

The amount of metals, namely Mn^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+} and Fe^{2+}, bound by the biofilm, soluble EPS fractions of microbial biofilms, namely samples A, B and C and the whole cell cyanobacterial supernatant (A-WCS, B-WCS, C-WCS) were averaged, respectively as shown in Figure 4.4. The biofilm associated EPSs were more efficient at binding these metal ions compared to the soluble EPSs (Figure 4.4). The whole cell supernatant (WCS) EPSs exhibited highest metal binding properties for each of the metals analyzed in this study (Figure 4.4). Consistent with the metal data obtained from the native communities, the laboratory grown microcosms (both microbial mats and whole cell cyanobacterial cultures) sequester metal cations in the decreasing order of Mg >Mn >Fe> Cu > Zn (Figure 4.4).

DISCUSSION

The cryptoendolithic communities thriving in the oligotrophic sandstones of GSENM serve as a model system to investigate the survival adaptations of microbes in arid ecosystems. While cyanobacteria are known to be the dominant phototrophs in cryptoendolithic communities (Hammes et al., 2013; Archer et al., 2017; Lee et al., 2016), the community composition of cryptoendolithic communities in Jurassic Navajo and Entrada Sandstones varies between the two different geological substrates (Fig 3.4, Fig 3.6).

Data on readily available metals (the water extractable fractions) in the sandstones revealed that iron is less available in the Entrada Sandstone, corresponding to higher magnesium levels when compared to Navajo Sandstones, where iron is present in greater

amounts with a lower Mg content. This is in accordance with previous studies reporting that cyanobacteria compensate for limiting Fe with abundant Mg (Shcolnick and Keren, 2006). Iron limited cyanobacteria produce a photosynthetic antenna complex that facilitates excitation energy transfer to photosystem I, in effect compensating for limited Fe with greater Mg levels (Kouril et al., 2005).

The biofilm associated content of metals was higher than those of the water or acid extractable metal content of the underlying substratum. This suggests that the cryptoendolithic communities concentrate metal ions into a pool which can then be utilized by microorganisms for key cellular processes. Mg is a macronutrient unlike Mn, Cu, Fe and Zn which are required in trace amounts, which may explain the high concentrations of Mg in the biofilm associated fractions of the sandstones. Significant amounts of manganese were sequestered in the biofilm fractions of all sandstones. This may help explain the high levels of manganese in desert varnish, which is a dark, thin rock coating found on some desert rocks (Hungate et al., 1987; Kuhlman et al., 2006; Esposito et al., 2015; Dorn and Oberlander, 1981; Dorn, 1991; Perry and Adams, 1978, Perry and Kolb, 2004).

Previous studies in our lab demonstrated the capability of EPS produced by cryptoendolithic bacteria in Jurassic Navajo Sandstones to trap ferrous iron (Hammes et al., 2013). Interestingly, we also found the presence of *Acidiphilium* spp. in these cryptoendolithic communities (Kaur and Kurtz, in review). Some members of this genus are capable of coupling the reduction of ferric iron to ferrous form by oxidation of glucose under aerobic conditions (Kusel et al., 1999). This reduction of Fe^{3+} to Fe^{2+}

results in mobilization of iron, which can then be bound by the EPS and subsequently used by microbes for metabolic purposes. It is important to consider that besides ferrous iron, EPS has the potential to interact with other metal ions, especially those required by microorganisms for their metabolic activity. The process of metal binding to EPS may be noninteractive, synergistic or competitive, depending on the metal ions and characteristics of the polysaccharide (Ozturk et al., 2014). In this study, all the EPS extracts exhibited a decreased capacity to bind iron in the presence of other essential metals. This suggests that metal ions compete interactively for available ligand binding sites on EPS. The decrease in ferrous iron binding can result either from direct competition between metal cations for the EPS binding site or the modification of EPS conformation by interaction with a preferred metal cation, which hinders the access of ferrous iron to the binding sites. We also found that the whole cell cyanobacterial supernatant bound 2-4 more ferrous iron than the biofilm fraction of EPS extracted from microbial mats, suggesting that cyanobacteria are the major producers of EPSs that have the property of sequestering ferrous iron.

It is known that some EPSs have the ability to trap and concentrate nutrients (Mager and Thomas, 2011; Mazor et al., 1996). The biofilm associated fractions of EPS were more efficient in binding essential metal ions, namely, Cu^{2+}, Zn^{2+}, Fe^{2+}, Mg^{2+} and Mn^{2+} than the soluble EPS fraction. This is in contrast with previous studies where soluble EPS or released exopolysaccharides bound more metal cations than the biofilm associated EPS fractions (Sun et al., 2009). These results suggest that in this ecosystem, soluble EPS is scavenging available ions from the environment, ultimately transferring

those ions to the tightly bound cell associated EPS, where the ions would be readily available for uptake by cryptoendolithic microorganisms.

Overall, the whole cell cyanobacterial EPS fractions had considerably higher metal binding properties when compared to both the soluble and biofilm fractions of the cryptoendolithic microbial communities. Cyanobacterial EPS possess typical features that distinguish them from the polymers produced by other bacteria. They exhibit a strong anionic character due to the presence of uronic acids and are complex hetero-polysaccharides that are highly variable in nature (De Philippis et al., 2011; De Philippis and Vincenzini, 1998; Klock et al., 2007). The overall negative charge of cyanobacterial EPS may be essential for sequestering metal cations that are essential for cell growth but are present at low concentrations in the underlying sandstone substrate (Pereira et al., 2011; Cassier-Chauvat and Chauvat, 2014). This explains the ability of cyanobacterial whole cell supernatant to bind considerably greater amounts of essential metal cations compared to the EPS produced by the cryptoendolithic community as a whole. The amount of the essential metal cations bound by EPS is higher than the amount of these freely available metals detected in the substratum i.e. sandstones. This supports our hypothesis that EPS binds and concentrates essential metal ions, making them more accessible to the underlying cryptoendolithic microbial community.

CONCLUSIONS

This study provides data on the ability of cryptoendolithic communities to bind and sequester metal ions required for basic life processes through the production of EPS. The

ability of whole cell cyanobacterial supernatant to bind metals more efficiently than the biofilm or soluble fractions of cryptoendolithic communities suggests that cyanobacteria are the major producers of the EPS required for this process. We conclude that cryptoendolithic communities produce EPSs that act as a biofilter to concentrate metal ions, making them available to the microbial consortia embedded in the matrix of EPS.

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TABLE AND FIGURES

Table 4.1 Average metal concentrations detected in Navajo sandstones (HW03_04 and HW07_04) and Entrada sandstones (HW13_04 and HW12_04). Values represent ng metal / gram sandstone sample analyzed ± Standard error. Standard error values were zero when rounded off to two significant digits.

Table 4.2 Ferrous iron binding ability of EPS in a single metal system compared to the ferrous iron binding in the presence of multiple essential cations. All the values represented indicated ng of ferrous iron per mg of EPS analyzed along with the standard errors.

Figure 4.1 A) Location of the study area, GSENM in southern Utah (M=Moab, SLC=Salt Lake City, SG=Saint George), B) Sampling sites for the Jurassic Navajo Sandstones, 1 and 2 represent HW03_04 and HW07_04 respectively, C) Sampling sites for the Entrada Sandstones, 3 and 4 represent HW12_04 and HW13_04 respectively.

Figure 4.2 A) Experimental setup for the metal content determination in the sandstones. B) Experimental setup for analyzing the amount of metals sequestered by EPS extracts.

Figure 4.3 Ferrous iron binding capacities of EPS extracted from microcosm microbial mats (Soluble and Biofilm fractions) and whole cell cyanobacterial cultures (WCS).

Figure 4.4 Comparison of the metal binding capacities of soluble EPS, biofilm associated EPS extracted from microbial mats and whole cell cyanobacterial supernatant EPS (WCS-EPS).

Table 4.1 Average metal concentrations in the Jurassic Navajo sandstones (HW03_04 and HW07_04) and Entrada sandstones (HW13_04 and HW12_04). Values represent ng metal / gram sandstone sample analyzed ± Standard error. Standard error values were zero when rounded off to two significant digits.

Sandstones	Mg	Zn	Cu	Mn	Fe
HW03_04 (water)	5.46 ± 0.00	0.30 ± 0.00	0.62 ± 0.00	0.88 ± 0.00	15.02 ± 0.01
HW03_04 (acid)	42.61 ± 0.00	1.01 ± 0.00	1.40 ± 0.00	9.81 ± 0.00	37.03 ± 0.01
HW03_04 (biofilm)	72.78 ± 0.00	0.61 ± 0.00	1.13 ± 0.00	43.66 ± 0.00	17.96 ± 0.00
HW07_04 (water)	6.55 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.73 ± 0.00	13.17 ± 0.00
HW07_04 (acid)	36.47 ± 0.00	0.58 ± 0.00	0.66 ± 0.00	30.03 ± 0.00	31.16 ± 0.00
HW07_04 (biofilm)	68.25 ± 0.01	0.79 ± 0.00	0.87 ± 0.00	30.58 ± 0.01	31.59 ± 0.01
HW13_04 (water)	17.18 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.32 ± 0.00	4.68 ± 0.00
HW13_04 (acid)	212.37 ± 0.03	0.13 ± 0.00	0.67 ± 0.00	14.10 ± 0.00	20.59 ± 0.00
HW13_04 (biofilm)	342.85 ± 0.01	0.00 ± 0.00	1.15 ± 0.00	35.43 ± 0.00	34.23 ± 0.00
HW12_04 (water)	321.73 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.00	0.00 ± 0.00
HW12_04 (acid)	964.13 ± 0.11	0.24 ± 0.00	0.96 ± 0.00	44.30 ± 0.01	37.84 ± 0.00
HW12_04 (biofilm)	1278.04 ± 0.06	1.16 ± 0.00	1.45 ± 0.00	70.41 ± 0.00	66.86 ± 0.01

Table 4.2 Ferrous iron binding ability of EPS in a single metal system compared to the ferrous iron binding in the presence of multiple essential cations. All the values represented indicated ng of ferrous iron per mg of EPS analyzed along with the standard errors.

	Single metal system			Multiple metal system		
	Microcosms		**Whole cell**	**Microcosms**		**Whole cell**
	Soluble	**Biofilm**		**Soluble**	**Biofilm**	
IGB	18.59 ± 0.07	87.25 ± 0.31	379.73 ± 0.27	18.22 ± 0.04	78.28 ± 0.24	224.47 ± 0.12
IGT16	15.32 ± 0.51	91.42 ± 0.33	206.34 ± 0.37	15.09 ± 0.07	90.92 ± 0.24	152.12 ± 0.40
CHK006B	15.34 ± 0.81	106.63 ± 0.30	208.83 ± 0.20	8.32 ± 0.34	68.64 ± 0.07	134.58 ± 0.10

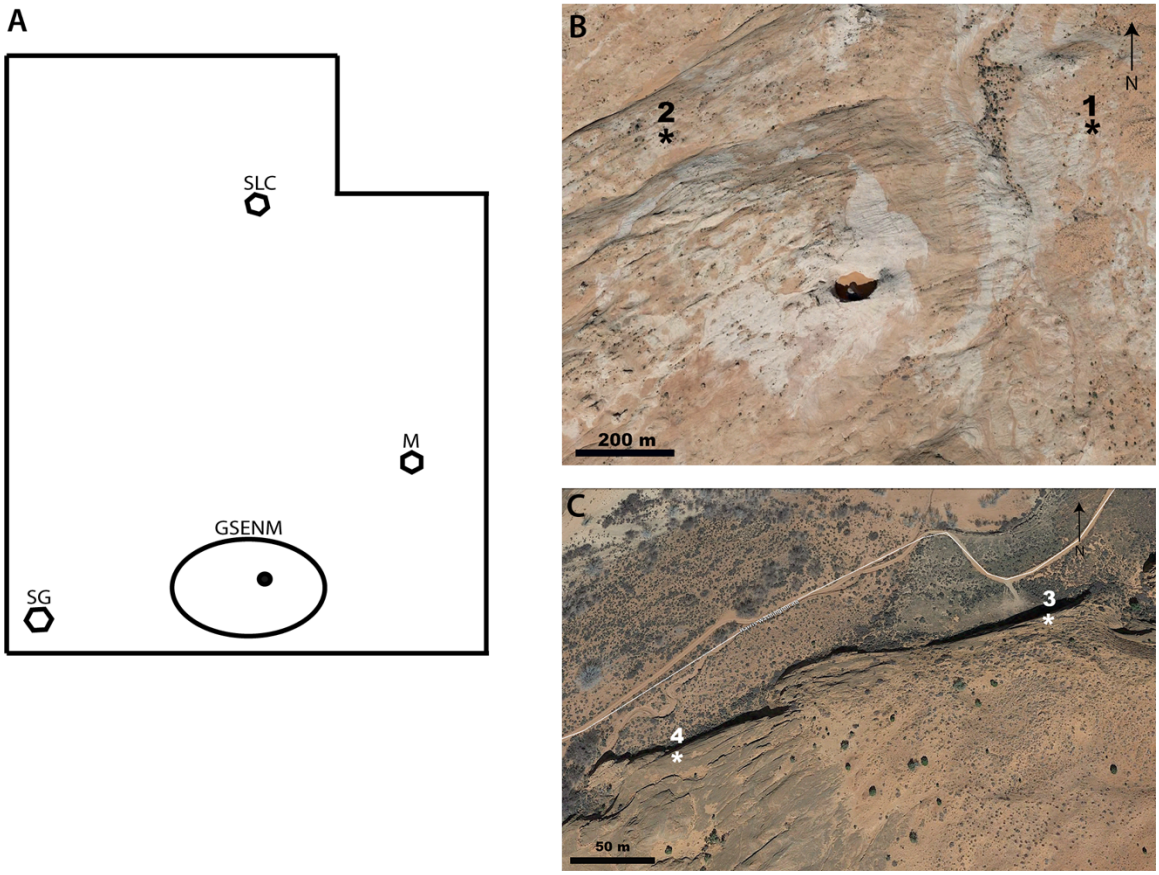
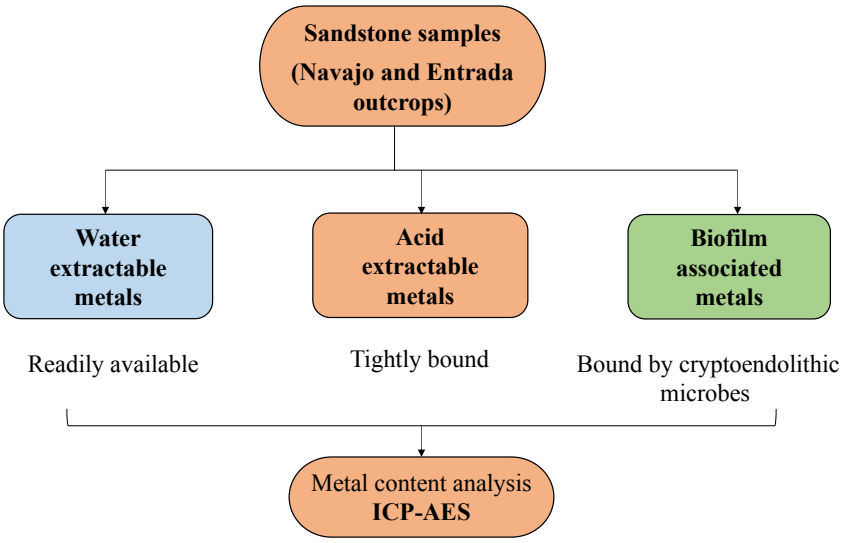


Figure 4.1 A) Location of the study area, GSENM in southern Utah (M=Moab, SLC=Salt Lake City, SG=Saint George), B) Sampling sites for the Jurassic Navajo Sandstones, 1 and 2 represent HW03_04 and HW07_04 respectively, C) Sampling sites for the Entrada Sandstones, 3 and 4 represent HW12_04 and HW13_04 respectively.

A.



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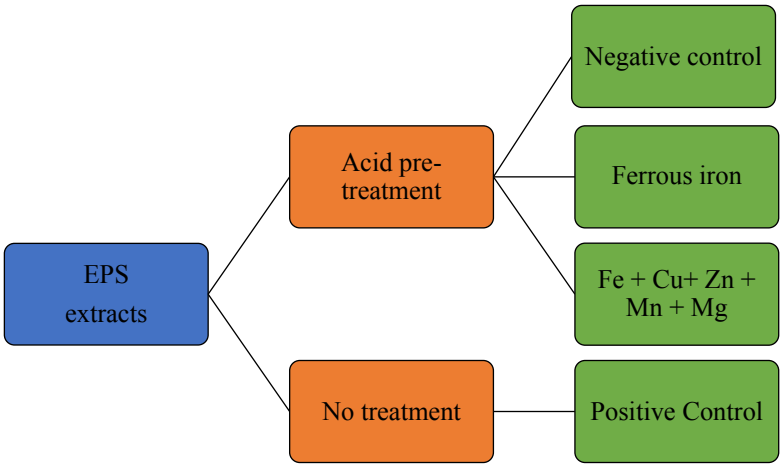

Figure 4.2 A) Experimental setup for the metal content determination in the sandstones.

B) Experimental setup for analyzing the amount of metals sequestered by EPS extracts.

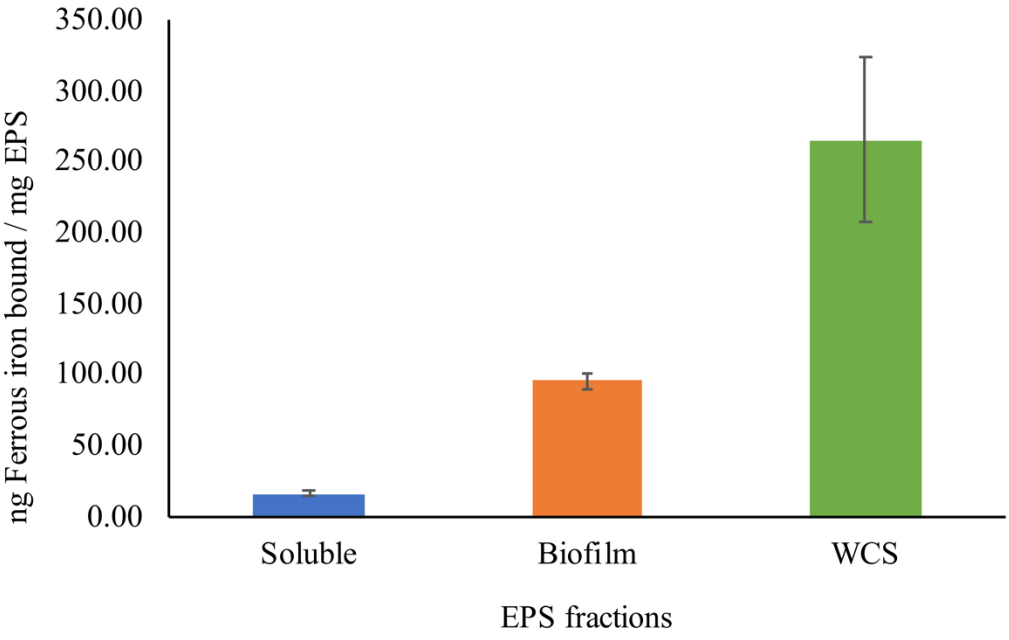

Figure 4.3 Ferrous iron binding capacities of EPS extracted from microbial mats and whole cell cyanobacterial cultures.

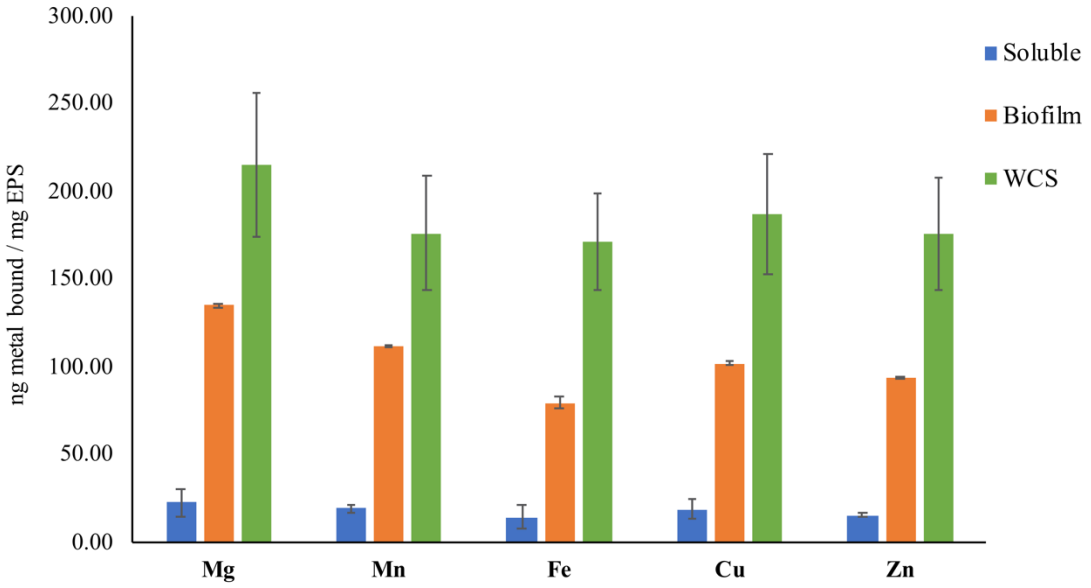

Figure 4.4 Comparison of the metal binding capacities of Soluble EPS, Biofilm associated EPS extracted from microbial mats and whole cell cyanobacterial supernatant EPS (WCS).

Chapter 5

Summary and Conclusions

One of the most resistant forms of life existing in arid landscapes are the assemblages of microorganisms in the pore spaces of rocks, known as cryptoendolithic microbial communities (Friedmann et al., 1967; Friedmann and Ocampo, 1976; Wierzchos et al., 2012). These communities serve as unique model systems for the study of microbial adaptations to survive extreme environmental conditions. This study provides a description of the composition of the bacterial cryptoendolithic diversity within the Jurassic Navajo and Entrada sandstones of the GSENM.

Cryptoendolithic bacterial communities in the Jurassic Navajo Sandstone are dominated by Cyanobacteria followed by Proteobacteria, Bacteroidetes and Actinobacteria while the other less common phyla include Chloroflexi, Deinococcus-Thermus, Verrucomicrobia, Planctomycetes and Gemmatimonadetes. Moisture availability is one of the major drivers for shaping the cryptoendolithic bacterial community structure in the Navajo Sandstone. This conclusion is based on how the communities clustered with respect to topography. A core bacterial community exists in this micro-ecosystem that is shared between the cryptoendolithic communities irrespective of the sampling locations. This core community includes members of Cyanobacteria (mainly *Leptolyngbya* along with *Chroococcidiopsis*, *Mastigocladopsis* and unclassified cyanobacteria) followed by Proteobacteria, mainly Alphaproteobacteria within which the genus *Acidiphilium* is the most prevalent (Kaur and Kurtz, in press).

Bacteroidetes is the next most abundant phylum mainly represented by the genus *Segetibacter* followed by the phylum Actinobacteria.

In the Jurassic Navajo sandstones, *Acidiphilium*, a genus with some members capable of aerobic ferric iron reduction, was present in high abundance (Kaur and Kurtz, in press). Previous studies indicated that dissimilatory reduction of ferric iron maybe constitutive or inducible depending on the strain of *Acidiphilium* spp., suggesting that ferric iron reduction by *Acidiphilium* spp. may occur in oxygenated as well as anoxic acidic environments (Johnson and Bridge, 2002). This better explains the high levels of ferrous iron in the Navajo Sandstones than the previous model where iron-reducing bacteria, such as *Anaeromyxobacter spp.,* belonging to class Deltaproteobacteria were thought to be responsible for the high levels of ferrous iron detected in these sandstones (Kurtz and Cox, 2010; Hammes et al., 2013). Over geological time, the Navajo Sandstone outcrops experienced extensive bleaching of their reddish-brown hue imparted by the hematite cladding of sand grains that occurred during early diagenesis events. Although, palaeogeochemical processes have been widely suggested to be the cause of this bleaching phenomenon, it is important to recognize the potential contribution of the cryptoendolithic microorganisms in this ongoing process. The extracellular polymeric substances (EPS) produced by the cryptoendolithic communities in this ecosystem are capable of binding ferrous iron, suggesting the existence of an active microbially mediated iron cycling in this microecosystem (Hammes et al., 2013).

Cryptoendolithic bacterial communities in the Entrada Sandstones were examined to test if the rock type affects the microbial composition. These communities were

dominated by Cyanobacteria, as expected, followed by Proteobacteria, Actinomycetes, Chloroflexi and Bacteroidetes. Other less prevalent phyla included Deinococcus-Thermus, Acidobacteria, Verrucomicrobia, Planctomycetes and Gemmatimonadetes. It was interesting to detect radiation resistant bacteria in all the Entrada samples, specifically the genus *Truepera* belonging to the phylum Deinococcus-Thermus. There is a shared bacterial community within the Entrada Sandstones that included Cyanobacteria, Proteobacteria, Actinomycetes, Deinococcus-Thermus, Chloroflexi, Gemmatimonadetes and Planctomycetes. Nearly half of the members in the shared community were assigned to unclassified genera.

Although cryptoendolithic microorganisms might appear superficially similar in the Jurassic Navajo and Entrada Sandstones, variations in the local micro-environmental conditions could influence colonization and result in distinct community assemblages. Considering the heterogeneity of geological features around this land form, it was expected that the cryptoendolithic bacterial communities in the Jurassic Navajo and Entrada Sandstones would differ from each other, with a very small bacterial community that is shared between both the habitats. When compared to the cryptoendolithic diversity in the Navajo Sandstones, Actinomycetes are relatively more abundant in the Entrada Sandstone. This can be attributed to the textural properties of the sandstones, including porosity. The Jurassic Navajo and Entrada Sandstones vary in grain size and distribution patterns; the former is fine to medium grained and well sorted, while the latter is chiefly fine grained and moderately to poorly sorted. It also reflects bacterial adaptations to available moisture regimes which can be corelated to the pore sizes of the two sandstone

substrates. The pore size in Navajo is greater than that of Entrada Sandstone which allows for more water loss through capillary rise and greater evaporation rate. As the dominant microorganisms, Cyanobacteria form the foundation of both these cryptoendolithic habitats. An initial evaluation of the sandstones indicated significant differences in cyanobacterial diversity between the two communities. Extensive analysis of cyanobacterial diversity between the Navajo and Entrada Sandstone communities, showed that the Navajo Sandstone is dominated by filamentous cyanobacteria, *Leptolyngbya* and *Mastigocladopsis*, while the Entrada Sandstone is dominated by coccoidal forms of cyanobacteria, mainly *Chroococcidiopsis*. This difference can be explained by the variations in the pore sizes of the Navajo and Entrada Sandstone, where the former has comparatively larger pores allowing filamentous cyanobacteria to grow within the sandstone. Conversely, the Entrada Sandstone has relatively constricted pore sizes that seems to encourage the growth of coccoidal forms of cyanobacteria. At the family and genus level, the cryptoendolithic community structure in the Navajo and Entrada Sandstones becomes more varied, suggesting distinctive ecological processes within these habitats. Despite the differences, a small core bacterial community is shared between both the habitats that included members of Cyanobacteria, Proteobacteria and Actinomycetes. The number of unclassified OTUs found suggests that abundant, unexplored microbial diversity exists in both the Jurassic Navajo and Entrada Sandstones, therefore indicating the need to conserve these desert communities.

Cryptoendolithic communities dominated by Cyanobacteria produce hygroscopic EPS, which supports the growth of other heterotrophic bacteria forming an intermixed

ecosystem (Omelon et al., 2006). The close physical relationship between cyanobacteria and heterotrophic bacteria likely results in nutrient cycling within cryptoendolithic communities. At the same time, reactions between organic matter and metal oxyhydroxides are important in rendering otherwise insoluble metals, such as Fe and Mn, soluble and mobile, making these nutrients accessible to the microbes growing within the rock habitats (Ferris and Lowson, 1997). EPS produced by cryptoendolithic communities in the Jurassic Navajo and Entrada sandstones can bind 15-67 ng of ferrous iron per gram of sandstone sample. The amount of selected metal ions (Fe^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, and Zn^{2+}) that were complexed by natural communities associated with Jurassic Navajo and Entrada Sandstones was measured along with the content of metals found in uncolonized sandstone samples. Comparatively, iron was less available while magnesium was higher in the Entrada Sandstone; while in the Navajo Sandstones, iron was present in greater amounts with a lower magnesium content. The literature indicates that Cyanobacteria have the ability to compensate for limiting Fe with greater Mg content (Shcolnick and Keren, 2006). The biofilm associated EPS extracted from laboratory grown microbial mats was more efficient at binding metal cations than the soluble EPS while the whole cell cyanobacterial EPS exhibited the highest metal binding capacity. The metal binding capacities of both the natural and laboratory-based communities is of the order: Mg >Mn >Fe> Cu > Zn. The amount of the essential metal cations bound by EPS is higher than the amount of these freely available metals detected in the underlying substrate. This supports the hypothesis that EPS binds and concentrates essential metal ions, making

them more accessible to the underlying cryptoendolithic microbial community for basic life processes.

Based on these findings, a generalized conceptual model is presented summarizing the basic microbial ecology in the cryptoendolithic habitats of the GSENM (Figure 5.1). The primary colonizers of cryptoendolithic communities are Cyanobacteria, that have a broad eco-physiological tolerance to cope with extreme hydrological and thermal stressors coupled with limited nutrient availability. Cyanobacteria are the main contributors to flow of carbon and energy through photosynthesis as well as potentially fixing nitrogen in these cryptoendolithic microbial communities. Cryptoendolithic communities dominated by cyanobacteria, are analogous to microbial mats or biofilms. Known survival strategies of these microbial biofilms to extreme environmental conditions include production of hygroscopic EPS, which supports the growth of other heterotrophic bacteria forming a microbial community. The most common heterotrophs in these cryptoendolithic communities include the phyla Proteobacteria, Bacteroidetes and Actinobacteria.

EPS serves many ecological functions, including protection from desiccation through the retention of water, moderating temperature fluctuations, increasing nutrient availability and hardening the surface of sandstone outcrops protecting the sandstone against wind and water erosion (Kurtz Jr. and Netoff, 2001; Omelon et al., 2006; Mager, 2010). Cyanobacteria and heterotrophic bacteria embedded in the EPS matrix are most likely involved in nutrient cycling in this micro-ecosystem. In the Jurassic Navajo Sandstones that are known for their reddish hue imparted by the hematite cladding of the

sand grains, it was interesting to find abundance of the bacterium *Acidiphilium* spp., a member of the AlphaProteobacteria. These bacteria are capable of aerobic ferric iron reduction under moderately acidic conditions, explaining the high levels of iron (II) measured in the Jurassic Navajo Sandstones.

EPSs produced by the cryptoendolithic communities in both the Jurassic Navajo and Entrada sandstones are capable of binding ferrous iron, which is then available for microbial utilization. These EPSs also exhibit the capacity to bind Mg^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+} which are all essential cofactors involved in oxygenic photosynthesis. This study indicates that EPSs produced by semi-purified cyanobacterial cultures isolated from the cryptoendolithic communities had a greater ability to bind above mentioned metal cations than the laboratory grown cryptoendolithic microbial biofilms. Consequently, EPSs produced by cryptoendolithic communities act like a biofilter to bind and concentrate essential metal ions, providing a ready source of key nutrients for cellular metabolism.

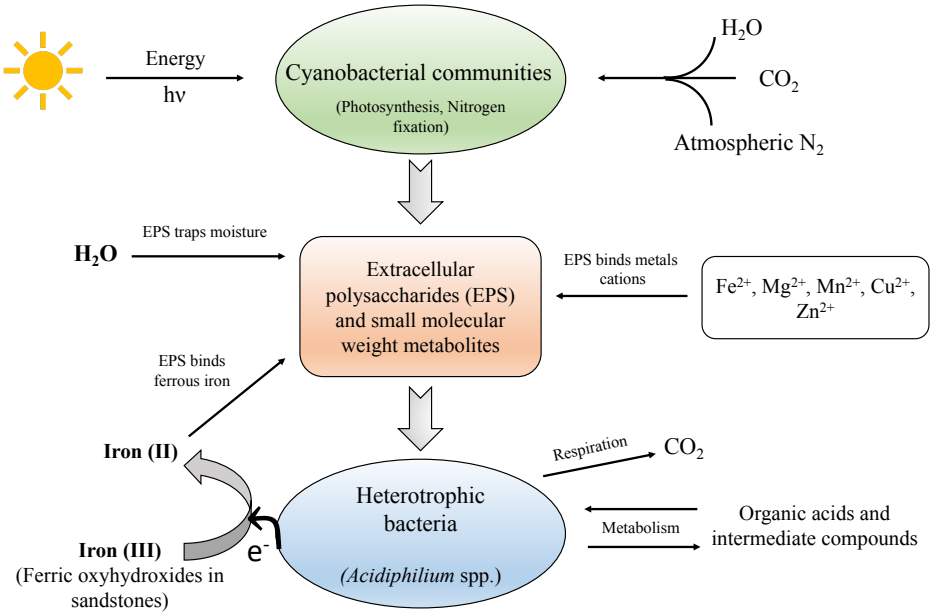

Figure 5.1 Generalized conceptual model summarizing the microbial ecology of cryptoendolithic communities in the sandstones of the GSENM.

Future directions

The results from this study are a snapshot in time, documenting the microbial diversity associated with two geological formations in 2004 and 2005. These results provide a baseline which we can use to document major changes in microbial community structure that may result from changes in precipitation patterns that are more episodic in nature and that result in additional aridification (Schwinning et al., 2008). Based upon the predicted environmental changes, we expect to see a decline in microbial diversity over time at the sites sampled in this study. Testing this hypothesis would require us to revisit the field

sites and obtain fresh samples which would then be subjected to the same set of analyses detailed in Chapters 2 and 3. The two datasets would be compared to ascertain what, if any, major changes occurred over time. With the potential for the extinction of dryland microbial communities rising (Rodriguez-Caballero et al., 2018), knowledge of which organisms are being lost allows us to potentially preserve them, protecting unexplored genetic diversity for future study.

Multiple geologic formations are found on the GSENM as shown in Figure 1.2 (Doelling et al., 2010). Our study shows that cryptoendolithic communities found on different types of sandstone are distinctly different. Based upon the preliminary data, we hypothesize that multiple distinct cryptoendolithic communities are found within the GSENM, each associated with a specific geologic unit. Additionally, while these communities may differ from a structural standpoint, we hypothesize that these communities' function in divergent and convergent ways. Divergence of function between the communities will reflect the underlying substratum, while convergent functions will reflect the need of each community's dependence upon photoautotrophs being the primary producers for carbon and reduced nitrogen. To test these hypotheses, it will be necessary to sample multiple rock types at several locations. From these samples, data regarding available cations and anions will be extracted as well as textural aspects of the substratum, i.e. porosity. Biologically, all samples would be tested for diversity to determine community structure followed by metagenomic and metatranscriptomic analyses to ascertain metabolic functions found in each community. Once these data are obtained, comparisons between the systems will show which functions are common to all

of the communities and which are unique to certain communities. Incorporating these data with the geological and geochemical data should provide insights regarding community assemblage within these ecosystems.

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