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Bacterial Communities and their Influence on the Formation and Development of Potholes in Sandstone Surfaces of the Semi-Arid Colorado Plateau

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BACTERIAL COMMUNITIES AND THEIR INFLUENCE ON THE FORMATION AND DEVELOPMENT OF POTHOLES IN SANDSTONE SURFACES OF THE SEMI-ARID COLORADO PLATEAU

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Abstract

Potholes are weathering features, of various sizes and shapes, which are found in exposed rock surfaces that lack vegetative and soil cover. Biofilms associated with the ephemeral aquatic pothole environments of the Colorado Plateau were examined as initial and intermediate stages of colonisation of arid, oligotrophic rock surfaces.

Imaging of the interface between pothole biofilms and their host rock revealed features of biological weathering; chiefly, the breakdown of silicate minerals and precipitation of clay and carbonate minerals. High pH measured in pothole water reflected deposition of secondary carbonate minerals at some locations and indicated the development of high pH microenvironments that were responsible for actively weathering the host rock. Further, the biologically-affected crust of pothole host rock contained relatively less SiO_2 and more CaO bearing minerals than the abiotic host rock, indicating that the activity of the biofilm was weathering silicate minerals while at the same time precipitating calcite.

The nutritional requirements of the biofilms were not fully met by rain water, and were supplemented by sources already present in the pothole. The aqueous geochemistry of potholes under artificial oligotrophic conditions indicated that the biofilm was active, obtained nutrients directly from sediment and the host rock, and was able to influence water chemistry without nutrients derived from rain. The sampling of potholes filled with water from rain events found that wind-blown nutrients were an important contribution from storms.

Imaging of pothole biofilms revealed novel adaptations to nutrient-limited conditions, such as microcolonies of cells and dense webs of extracellular polymeric substances, which suggested a high level of resilience in the endemic communities.

Environmental 16s rRNA analysis of three different biofilms showed that sediment accumulation is key to species diversity: deep potholes (that can retain water for longer periods, but do not have much soil) supported bacterial communities similar in diversity to biofilms on bare rock, whereas biofilms from potholes with soils hosted a much more diverse community of heterotrophic bacteria. The increase in diversity as potholes accumulate sediment underlined the importance of soil formation in the desert environment.

Keywords: Pothole, endolithic biofilm, biological soil crust, soil formation, semi-arid desert, oligotrophy, community 16s rRNA, heterotrophic bacteria, Colorado Plateau

To my parents
Marian and Perry Hughes
For their support and the many years of rock collections and pebble hunting
which have lead me here

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List of Abbreviations and Symbols

atm	Atmosphere
ATP	Adenosine triphosphate
avg	Average
BSC	Biological Soil Crust
BW	Bartlett Wash
BW-EB	Bartlett Wash Endolithic Biofilm
BW-PS	Bartlett Wash Pothole Sediments
Cal	Calcite
DDI	Distilled Deionised Water
DNA	Deoxyribonucleic acid
DGGE	Denaturing Gradient Gel Electrophoresis
EDX	Energy Dispersive X-ray Spectroscopy
EPS	Extracellular Polymeric Substances
F-spar	Feldspar
Fe-Ox	Iron Oxides
FIB	Focused Ion Beam
Fm	Formation
HCH	Hexachlorocyclohexane
IC	Ion Chromatography
ICP-AES	Inductively Coupled Plasma – Atomic Emission Spectroscopy
Ma	Mega Annum
OUT	Operational Taxonomic Unit
PAH	Polycyclic Aromatic Hydrocarbons

PET	Potential Evapotranspiration
pH	$-\log [H^+]$
Qtz	Quartz
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SMC	Seven Mile Canyon
SMC1	Seven Mile Canyon Pothole Number One
SMC2	Seven Mile Canyon Pothole Number Two
SMC-BB	Seven Mile Canyon Black Biofilm
SMC-PS	Seven Mile Canyon Pothole Sediments
SMC-CC	Seven Mile Canyon Cryptobiotic Crust
Sp.	Species
Ssp.	Species plural
UV	Ultra Violet
XRF	X-Ray Fluorescence
SEM	Scanning Electron Microscopy

CHAPTER 1

Introduction

1.1 Deserts and Dry Lands as Critical Environments

Deserts and dry lands cover approximately 47% of the Earth's land surface (Kalipeni, 2007). With the possibility of increases in global temperature of 2-3^{o}C in the next 50 years (IPCC, 2007), and increasing encroachment by human activities, this percentage will likely increase. Current intermediate environments, such as the semi-arid desert of the Colorado Plateau, are repositories of biota that live with water and nutrient-limited conditions. These organisms provide valuable insight into how the biosphere influences the terrestrial environment, producing niches in which life may survive. It is important to better understand the biological processes within these environments, particularly those that affect soil formation and stability. Semi-arid regions are especially at risk for soil erosion because desert soils may be degraded through wind erosion with excessive drying, or through water erosion by periodic torrential rains (Warren et al., 1996). Human activities exacerbate erosion through the trampling of soils in areas used as range land for livestock, and disturbances from all-terrain vehicles (Schwinning et al., 2008). Although the process of biological soil formation is well adapted to the arid and nutrient-poor natural environment, it is being detrimentally affected by these encroaching erosive pressures. Bacterial colonisation of rock speeds weathering, which is the first step in soil formation (Mottershead et al., 2003), a process through which the semi-arid environment is able to gradually support higher forms of life, reclaim arid lands from desertification and protect soils and biota that already exist.

1.2 Geological Setting: The Colorado Plateau, USA

The geology of the Colorado Plateau is exposed in four states: Colorado, Utah, Arizona and New Mexico. The project area is located near Moab Utah, where the geological formations expressed at the surface are primarily Triassic and Jurassic in age. The excellent geological exposures of the field area are due to the weathering of the Colorado Plateau, which has been substantially uplifted to elevations that currently range from approximately 1000 to 4000 m, with an average of 1500 m above sea level (Foos, 2005). The origin and timing of the uplift is still a matter of scientific debate (e.g. Flowers et al., 2008; Huntington et al., 2010; Levander et al., 2011) and is suggested to have occurred anywhere from 60 to 6 million years ago. Whatever the cause and timing of the uplift, it has caused the rivers of the area, mainly the Colorado and Green rivers and their tributaries, to deeply erode the Plateau surface, forming an extensive drainage network and many steep-sided canyons that control the hydrology of the area to this day (Freethey and Cordy, 1991). Further erosion is attributed to the semi-arid environment that limits protective soil and plant cover, exposing the rock surfaces to the effects of intense rain events, as well as the yearly freeze-thaw cycle.

The oldest rocks exposed in the study area (to the North West of Moab, Utah) are Pennsylvanian in age (320 Ma) and called the “Paradox formation”,a main component of which is a series of thick salt layers formed through evaporation in a restricted embayment of an ancient ocean known as the Paradox Basin. Periodic flooding by salt water laid down 29 identified cyclic sedimentary layers (composed of shales, siltstones, sandstones, limestone, gypsum and various salts) reaching approximately 3000 m in

thickness (Doelling, 1988). Salt beds can individually reach up to 300 m in thickness and are mined in the area for potash (Doelling, 2003). In general, the sedimentary layers of the Colorado Plateau gently dip south-west with little deformation, but locally the Paradox basin controls the structure of the geology seen at surface. The salts are deformed and forced into diapirs under pressure from the heavier overlying strata, resulting in the formation of many anticlinal structures (Cater, 1970). These anticlines trend northwest-southeast and the underlying salt walls are up to 3.2 km high and 4.8-6.4 km long (Doelling, 2003). Valleys in the study area, such as the Moab and Cache valleys, formed when the salt walls began to dissolve due to the intrusion of groundwater in the Quaternary, and the anticlines collapsed forming grabens (Graham, 2004).

Two sandstones, the Kayenta and the Moab Member of the Curtis formation, which are the focus of this study, are bench-forming units from a time of extensive desertification beginning in the late Triassic. The formations associated with this time period consist primarily of aeolian sandstones, frequently showing cross bedding and other wind influenced structures.

The Kayenta Fm. is thought to represent a period of fluvial deposition closely or conformably following the desert conditions that produced the massive, cliff forming Wingate formation (Doelling, 2003). It forms resistant ledges of sandstone and conglomerate with periodic aeolian sandstone tongues such as the one found at the field area known as Seven Mile Canyon (SMC). The Moab Member of the Curtis Formation is an easily recognised white, cliff-forming unit of aeolian origins, which is frequently cross-bedded. It is densely jointed in the study area due to the action of the adjacent Moab fault (Hurlow and Bishop, 2003).

1.3 Potholes

Depressions formed on the surfaces of many of the exposed sandstones of the Colorado plateau are able to trap rain water for various lengths of time depending on their size and location (Figure 1). This type of phenomenon has been observed in sandstones (Netoff and Shroba, 1993), granites (Dominguez-Villar et al., 2008) and limestone (Smith et al., 2000) and has been given many names (e.g. rock holes, water pockets, ganammas, rock basins). In this study they will be referred to as "potholes" based on the naming convention of previous studies in this area (Graham, 1999; Chan et al., 2005). Potholes are an important water source in an environment with limited precipitation. Individual potholes support diverse communities of microorganism and in a wider context, they provide water to macrobiotic desert inhabitants including humans. Previous studies have speculated that their formation is mainly abiotic, with little attention paid to the associated biota (e.g. Netoff and Shroba, 1993). For a recent overview of potholes and their biological communities see Chan et al., (2005).

1.4 Fundamentals of Microbiotic Life in Extreme Environments

The members of the Domain Bacteria are microorganisms that form, or contribute to the formation of, biofilms that can alter the geochemistry of their surroundings, even in some of Earth's most inhospitable environments such as the cold deserts of Antarctica (Aislabie et al., 2008), deep sea hydrothermal vents (Eberhard, 2009) and the high acid environments of abandoned mines (Johnson et al., 2002). These environments are colonised by microbiota (not only bacteria) with suites of adaptations to limiting or extreme conditions, e.g., low (Friedmann and Weed, 1978) and high (Ward et al., 1998)

Figure 1: Potholes on the Colorado Plateau near Moab, shown before and after rain. Field of view approximately 100 m.

temperature, low (Shaobin et al., 2008) and high (Kimura and Horikoshi, 1988) pH, high salinity (Post, 1977a) and the presence of heavy metals (Wolfe-Simon, 2010). Every inhabited environment possesses unique conditions to which microbiotic life has adapted; however, life in any "extreme" environment would not be possible without some access to essential nutrients and liquid water (Madigan et al., 2003). Microorganisms that colonise and live in areas with dry and nutrient poor conditions require a special set of adaptations. Despite the fundamental nature of these limitations, biofilms are still found in extremely dry (Lester et al., 2007) and nutrient-limited environments (e.g. Wanger et al., 2006; MacLean et al., 2007), actively affecting the physical and geochemical conditions. On the Colorado Plateau, these two most fundamental pressures to the persistence of life are accompanied by other restrictions such as large variations in temperature, including temperatures below freezing in the winter (NWS, 2012), as well as substantial exposure to UV radiation (Bowker et al., 2002). These restrictions result in only short periods of time each year when favourable conditions for biological activity and growth are present.

1.5 Microbial Adaptations to Limiting Environmental Conditions (Biofilms and Extracellular Polymeric Substances (EPS))

A common strategy for bacterial adaptation to almost any environment is the production of a biofilm (Costerton and Lappin-Scott, 1995). Biofilms consist of communities of bacteria and other microorganisms that provide benefits over unicellular lifestyles. Biofilms provide basic protection such as the secretion of EPS, which can make up 50-90% of the total organic carbon present in the biofilm (Flemming et al.,

2000). EPS is formed as the cell secretes layers or coatings of polymeric substances that are hygroscopic and highly viscous (Sutherland, 1999). The presence of uronic acids in EPS confers an anionic character, which allows the biofilm to attract cations such as Ca^{2+} and Mg^{2+}. Cations give the biofilm a greater binding force, stabilizing it, as they cross-link and form salt-bridges between polymer strands (Flemming et al., 2000). There is large variation in the relative quantities of EPS components; polysaccharides are usually the largest component along with proteins and more minor amounts of nucleic acids and lipids (Gralnick and Newman, 2007). Water is also an important component of EPS. In aerobic environments, such as exposed rock surfaces, EPS layers frequently contain more water than the environment they are in contact with (Hill et al., 1994) and can be used to regulate intracellular water loss in the case of moderate desiccation (Tamaru et al., 2005). The sticky character of EPS anchors bacteria to their environments, increasing their tolerance to windy conditions, as well as protecting against phagocytic predation, antibody recognition, and lysis by other bacteria and viruses (Tease and Walker, 1987). Biofilms that produce EPS structures known as nanowires are able to transfer electrons between cells (Gorby et al., 2006). Nanowires allow growth of bacteria that are not necessarily in direct contact with substrates, but may require access to a needed energy source or electron acceptor.

Differences in behaviours between species of bacteria within a biofilm have been found that are caused by large gradients in chemical and physical conditions (Baty et al., 2000). Certain bacteria such as the pathogenic *Burkholderia cenocepacia* secrete large amounts of EPS that scavenges reactive oxygen species out of the environment before they can affect the cell, creating an artificially reducing environment (Bylund et al.,

2006). This type of adaptation in a biofilm would allow anaerobic or microaerophilic bacteria to live in 'aerobic' environments. Using these types of adaptations biofilms not only protect their constituent bacteria from environmental pressures, but can also alter the environment itself to support a large variety of bacteria that would not otherwise persist individually in that location. In this way, bacteria, which may require different chemical conditions, live together in a small physical space, optimizing the efficiency of the community.

EPS associated with biofilms is especially important in nutrient limited environments. Physically, sticky EPS traps limited aeolian nutrients in sub-aerial environments (Gorbushina, 2001). Chemically, the essential character of EPS allows the biofilm to attract important nutrients more easily. Some bacteria such as *Hymenobacter aerophilus* produce an EPS high in negatively charged ligands, which increases the number of functional groups on the surface of the bacteria and causes an increase in metal adsorption (Baker et al., 2010). Any adaptation that allows organisms to obtain nutrients more efficiently is critical in oligotrophic environments.

1.6 Bacteria in Oligotrophic Environments

Bacteria show a diversity of adaptations to oligotrophic conditions, the most common of which is to simply grow and reproduce continuously, but at a very slow rate (Koch, 1997). This strategy ensures a baseline of metabolic activity, but addition of nutrients may not necessarily cause an increase in the growth rate, depending on the degree of oligotrophy (Maranon et al., 2010), and these highly adapted un-responsive types of bacteria are out-competed if conditions become more favourable for other

species. Alternatively, some slow growing bacteria are able to respond to improvements in the availability of carbon sources by releasing organic acids that help the bacteria produce energy at a higher rate (Tempest and Neijssel, 1992). Organic acid release has also been identified in bacteria under strict oligotrophic conditions, allowing the bacteria to weather nearby minerals for nutrients (Bengtsson, 1991).

Bacteria may also adapt to nutrient limitation by being metabolically versatile. Many heterotrophic bacteria found to be initial colonisers of exposed stone surfaces show exceptional metabolic diversity (Barton et al., 2004). Examples of this diversity include bacteria found in cave environments, such as species from the order *Actinomycetes* that grow on a wide variety of organic carbon compounds (Groth et al., 1999). The opportunistic genus *Sphingomonas* is commonly found in soils, and members are able to metabolize a number of aromatic carbon compounds (Blakewill et al., 1997). Other species may not have as diverse a metabolism, but are able to grow on airborne compounds; for example: The species *Hyphomicrobium* uses atmospheric methyl-halides as its carbon source (McDonald et al. 2001).

Other adaptations to oligotrophic conditions include physical strategies, such as the formation of endospores during nutrient limited periods (Torred et al., 2012). Endospores are also able to protect against desiccating conditions, which is important in the habitat of the Colorado Plateau. Other bacteria, such as the genus *Caulobacter* are dimorphic prosthecates; these bacteria produce one cell which remains at the site of growth and another motile cell that is sent out to find new, more favourable, environments (Dworking et al., 2006). The mobility of bacteria can be an important advantage in oligotrophic conditions: biofilms that form endolithically on rock surfaces

quickly work their way down into cracks (chasmoendoliths) and between mineral grains (cryptoendoliths) seeking out cooler conditions, away from intense UV radiation, where new sources of mineral nutrients may be available. It is thought that bacterial populations found isolated in the subsurface may be relics of populations that gradually worked their way deeper into the Earth (Gorbushina, 2007).

The net result of these many different adaptations is a bacterial community able to colonise inhospitable, bare rock surfaces and affect the local geochemistry, which in turn causes a variety of secondary effects, such as rock and mineral weathering and pothole formation.

1.7 Endolithic Habitats

Biofilms that colonise rock surfaces often occur as endoliths, living just beneath the rock surface, where they are best protected from harsh environmental conditions (Walker and Pace, 2007). This habitat maximises the advantages of both physical protection and the creation of microenvironments through which the microbiota can create more ideal geochemical conditions for growth. Microbiological life has been found in many rocky desert environments living just under the surface of semi-transparent rocks: the Colorado Plateau (Bell, 1993), Antarctica (Friedmann and Weed 2006; Friedmann, 1982), the Atacama desert (Wierzchos, 2006), Southern Tunisia (Sivaletta and Barbieri, 2009) and South Africa (Budel et al., 2004). Typically, these communities are located within millimetres of the host rock surface, regardless of the global location, as they are primarily photosynthetic (Omelon et al., 2006a). This mechanism of survival has several advantages, such as protecting cells from the

damaging effects of UV radiation while creating a humid microenvironment (Friedmann and Ocampo, 1976). In cold Antarctic environments these microenvironments trap heat, and although it is also the case on the Colorado Plateau, excess heat is a liability in the summer months when rock surface temperatures can reach up to 60^{o}C (Bell, 1993). A further benefit of the enclosed endolithic micro-environment 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).

Many studies have commented on the similarities between the types of bacteria found in various lithic environments, suggesting that in these types of limiting environments only a small specialised subset of bacteria are equipped to handle the multiple challenges (Budel et at. 2004; Walker and Pace, 2007a). When estimating species diversity in endolithic habitats across 15 different locations, only approximately 100 different 16S rRNA gene sequences were identified (Kemp and Aller, 2004) in contrast to other systems such as marine microbial mats and soil environments where 10^5 - 10^7 different species were identified (Dunbar et al., 2002; Ley et al., 2006). These simple biofilms are the basis for continued biological colonisation of the host rock and the development of soils. The establishment of cryptobiotic soils on the rock surface is generally attributed to the initial growth of cryptoendolithic cyanobacteria in preferentially wetted locations (Garcia-Pichel et al., 2001).

1.8 Silicate Weathering and Soil Formation

Bacterial interactions with surfaces are some of the most fundamental relationships of life. The evolution of life on earth is partially attributed to the concentrating effect of surfaces rather than water on its own, which dilutes nutrients (Reysencach and Cady, 2001). A major by-product of microbial colonisation of sandstone surfaces is the weathering of the host rock which results in the formation of soils. This process occurs both through physical and geochemical alteration of the host rock environment by the biofilm. Physical weathering is contributed to by expansion and contraction of the biofilm through wet/dry and freeze/thaw cycles (Gorbushina, 2007). This contraction causes layers of host rock held together by EPS to peel back from the surface and be removed by wind action or incorporated into desert sediments *in situ*.

Geochemical weathering is a process that can occur from cell to biofilm scale, and numerous studies have found that accumulation of bacterial products and by-products, concentrated in microenvironments adjacent to the cell, drive the dissolution process (*e.g.*, Thorseth et al., 1992; Ehrlich, 1997). Most early studies of mineral dissolution involved inorganic acids, such as carbonic acid, which were produced through microbial respiration or dissolution of $CO_{2(atm)}$ in water (e.g., Chapelle et al., 1987). Although respiration is an important factor in pH control of the environment, it is now understood that mineral surfaces are coated with secondary phases, organic polymers, and bacteria; all of which fundamentally affect the kinetics of weathering (Ullman et al., 1996). For example, low molecular weight organic ligands, produced by microorganisms, either complex with ions on the mineral surface or in solution and may decrease solution pH to accelerate weathering. Organic ligands are particularly good at increasing the solubility

of cations such as Ca^{2+}, Al^{3+}, Fe^{2+}, Mn^{2+} and Mg^{2+} by chelation from minerals (Schalscha et al., 1967; Stone, 1997). Ligands produced by bacteria include metabolic by-products, extracellular enzymes, chelates and both simple and complex organic acids (Bennett et al., 2001). Many studies point to the importance of chelating organic acids, produced by microbiota, on the weathering of minerals (e.g. Hiebert and Bennett, 1992; Vandervivere et al., 1994; Ullman et al., 1996; Maruice et al., 2001). Organic acids are much better at mineral mobilization than inorganic acids (Manley and Evans, 1986). Even at neutral pH, bacteria, which produce the organic acid gluconate, increase silicate mineral dissolution up to 1.4 fold over a control (Vandervivere et al., 1994). If the pH is not controlled and allowed to be affected by the presence of bacteria, the dissolution effects are even greater. Organic acids are produced under both reducing and oxidising conditions and the type of organic acid produced depends on the nutrient limitations of the environment (Ullman et al., 1996). Although bacteria produce many different types of organic acids, fungi have been found to produce the same acids at much higher concentrations, and so it is important to consider all organisms within a biofilm when seeking to understand mineral weathering (Palmer et al., 1991, Sterfinger, 2000).

Bacteria are also able to weather the surfaces they are in contact with indirectly, by influencing the pH of the surrounding environment. Both low and high pH conditions have been identified as a result of biological actions. The classic example of low pH is the production of surplus hydrogen ions through bacterially-enhanced oxidation of reduced sulphide minerals in acid mine situations. In acid mine environments, bacteria tolerating hyperacidity (pH 2.5 and lower) have been recorded (Johnson and Hallberg, 2003). Tolerance of low pH is useful as, in some natural systems, bacteria are able to

weather aluminosilicate minerals by producing organic and inorganic acids and lowering the pH to between 3 and 5 (Barker et al., 1998). Increasing pH is also a common occurrence, documented in several studies of mineral weathering (*e.g.*, Maurice et al., 2001, Brehm et al., 2005). Biofilms of cyanobacteria and heterotrophic bacteria were reported to increase the natural pH of 3.4 to higher than 9, causing silica dissolution (Brehm et al., 2005). Cyanobacteria are well known to produce high pH environments as a by-product of metabolism (Thompson and Ferris, 1990, Price et al., 2002; Shibata et al., 2002; Budel et al., 2004), which are ideal for the weathering of silicate minerals.

As the rock of the Colorado Plateau is weathered as a result of the above mechanisms, various soil components are produced. First, the biomass created by the presence of microbiotic biofilms adds a carbon source to what would otherwise be mainly quartz rich grains in many areas (Belnap, 2006). Second, bacterial weathering of silicate minerals (e.g., feldspars) produces clay minerals, which contribute to soil mineralogy (Barker and Banfield, 1996). These clays are found nucleated on cell surfaces and associated with biofilm EPS (Barker and Banfield, 1998). Finally, the component grains of the host rock themselves are mobilized due to physical pressure from the biofilms and incorporated into soils (Warscheid and Braams, 2000). Carbon, clay minerals and component host rock grains, are the constituent parts of a proto-soil. However, because of the exposed nature of large portions of the environment on the Colorado Plateau, another factor must be added to result in soil formation: Either the soil constituent material must be sequestered in a sheltered location or held together by biological means. These two factors are frequently combined into the formation of Biological Soil Crusts (BSCs).

1.9 Bio-Soils: Biological Soil Crusts (BSCs)

As described previously, microbial colonisation of rock surfaces usually leads to weathering and the production of constituent parts of proto soils. The semi-arid Colorado Plateau region is an environment with large areas that are commonly devoid of any significant soil cover (Schwinning et al., 2008), and so the formation of proto-soils is of great importance. These soils serve as a catalyst by supporting additional biological activity that results in the formation of BSCs. BSCs are a type of built-up biological soil found in sheltered locations on the Colorado Plateau (Garcia-Pichiel et al., 2001) and elsewhere (e.g. Zaady et al., 2000). Early work by Fletcher and Martin (1948) established that the formation of the initial surficial mat fixes the soil particles in direct contact with it, leaving the surrounding exposed soil to be weathered away, resulting in an elevated mat.

In general, BSCs are composed of a complex community of microorganisms associated with soil particles very close to the surface of the soil, within the top few millimetres (Belnap and Gardner, 1993). Their growth can directly affect hydrologic cycles, soil porosity, water retention and a variety of other soil characteristics (Belnap, 2006). The effect on the hydrology of the area depends on the type of soil crust and the climate of the area. However, it has been shown that, in general, they reduce surficial weathering, and therefore limit the production of nutrient-poor quartz-rich sediments in the southwest U.S. (Bowker et al., 2005). This maintenance of the surface environment is caused by the production of EPS, which binds soil particles to the biota of the crust, creating a cohesive and relatively stable cover. Within the crust, the microbiological diversity can range from mainly cyanobacterial communities, and cyanobacterial

associations with fungi (Friedmann and Ocampo, 1976) to a diverse community including bacteria, green algae, lichens, bryophytes and diatoms, depending on the environmental conditions (Belnap and Gardner, 1993; Johansen 1993). More recently, the genetic diversity of soil crust communities has been investigated and a better understanding of community interactions is emerging. New phylogenetic clusters have been identified based on differences in soil type, e.g., gypsum-rich crusts versus sandy- or shale-rich soil (Garcia-Pichel, 2001).

Belnap (2006) identified four separate types of BSC that each affect the hydrology of the local area differently: 1) smooth crusts in arid regions where the crusts never freeze and potential evapotranspiration (PET) is very high; 2) rugose crusts in dry-land areas with no freezing, but lower PET; 3) pinnacled crusts in cool desert regions, with a lower PET than hot deserts, and 4) rolling crusts in still colder areas with even lower PET. All of these types of crust are found at various locations on the Colorado Plateau, and may result from differences in substrate or variation in the age of the crust. Soil crusts in this area provide a more stable surface than sand. However, only crusts that have been left undisturbed for at least 20 years can adequately protect fragile soils from the strongest winds, which occur on a monthly basis (Belnap and Gillette, 1997). The importance of these crusts to the ecosystem and their inherent fragility, makes understanding these systems and their development important if we are to act to preserve them. Soil crusts are all the more important because their destruction plays an important role in desertification (Johansen, 1993; Schwinning et al., 2008).

1.10 Research Objectives/ Chapter Outlines

The objectives of this project are to understand the interaction of lithotrophic bacterial biofilms with exposed sandstone surfaces that result in the formation of potholes. The intention of this work is to improve on the current understanding of bacterial mineral weathering in conjunction with adaptations to nutrient- and water-limited conditions. Illuminating the bacterial process of pothole formation also helps to define the importance of bacteria to the overall desert environment. Programs of field- and laboratory-based investigations were conducted to these ends and three studies resulted as part of this research.

In Chapter 2, field observations and laboratory work conducted on the host sandstone and biofilms of the potholes gives insight into the formation of potholes through microbe/rock interactions. A process of host rock weathering is proposed beginning with surficial biofilms, continuing to pothole development and ending with the establishment of a biological soil crust. Examination of the host rock/biofilm interface was undertaken to examine the by-products of weathering and establish possible mechanisms that dominate the pothole environment.

Chapter 3 follows up the weathering theme of Chapter 2 with an examination of nutrient availability in the pothole environment. Field water sampling was done to establish baseline nutrient availability to the biofilms and determine if host rock weathering was contributing to the nutritional input to the biofilms. Leaching of host rock samples was conducted to investigate the possible input of micronutrients into the pothole environment from the host rock. The pothole biota was examined with scanning

electron microscopy to understand adaptations that may occur in nutrient limited conditions such as potholes.

Chapter 4 is an exploration of the bacterial communities associated with pothole development using 16s rRNA sequencing. Samples of surficial biofilm and pothole sediment biofilm were looked at in detail to gain insight into the type of strategies bacteria may employ to survive in the pothole environment. This study also provides a gauge of the diversity of the different biofilm types and locations, offering insight into the importance of potholes as biological refugia in the desert that support more life as they develop.

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CHAPTER 2

Life at the Interface: Biofilm-Rock Interactions and Pothole Formation

2.1 Introduction

Biofilms associated with rock surfaces are studied for a variety of reasons in various environments; from their detrimental effect on ancient cave art (Schabereiter-Gurtner et al., 2004) and buildings with stone facades both modern (Warscheid and Braams, 2000) and ancient (McNamara et al., 2006; Ortega-Morales et al., 1999), to their potential as analogues to life in extraterrestrial environments (Friedman and Ocampo-Friedmann, 1984) to the creation of soils through the weathering of exposed rock here on Earth (Johansen, 1993; Belnap and Gillette, 1997). Several studies have focused on the effect that nutrient limited host rock environments have on bacteria and their ability to successfully exist in those conditions (Bell, 1993; Walker and Pace, 2007). Various challenges to the microbiological colonisation of rock surfaces exist, including the limited availability of carbon or nutrient sources (Gorbushina, 2007), exposure to extremes in climactic conditions (Lester et al., 2007; Omelon, 2008) or dry desiccating conditions (Direito et al., 2011).

This study examines the various impacts of biofilms on sandstone formations in the semi-arid desert of the Colorado Plateau, at two locations, near Moab, Utah (Figure 2.1). Depressions in rock surfaces of various forms have been recognised at many locations around the world including Australia (Jutson, 1934; Twidale, 1982), Spain (Dominquez-Villar et al., 2008; Souza-Egipsy et al., 2004), Brazil (Fairbridge, 1968),

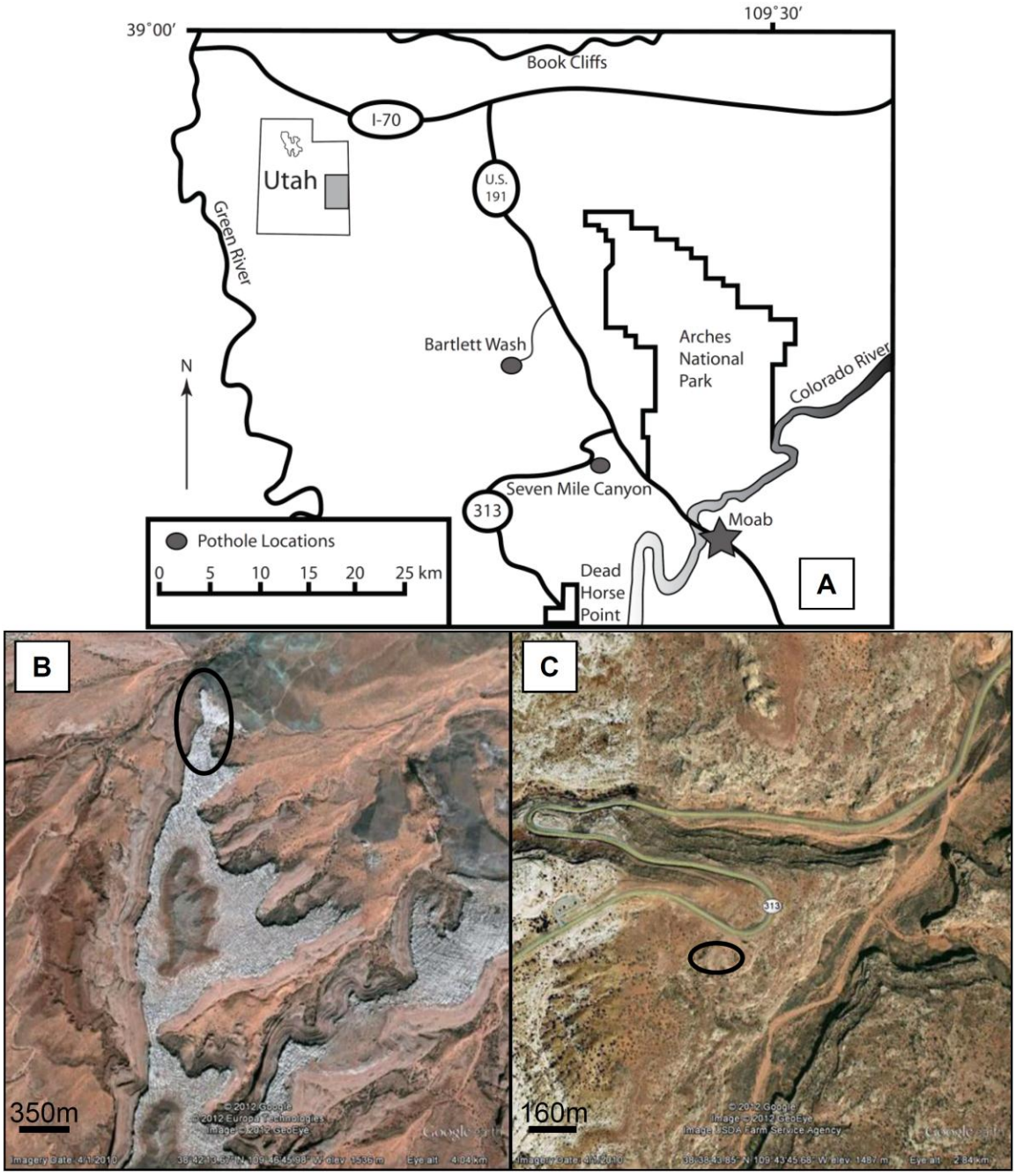


Figure 2.1: A) Field locations in Grand county Utah (after Chan et al., 2005). **B)** Bartlett Wash (BW) field site overview (N 38 degrees 42.662; W 109 degrees 47.146; 1516 m).**C)** Seven Mile Canyon (SMC) field site overview (N 38 degrees 38.648; W 109 degrees 43.862; 1482 m)

South Africa (Budel et al., 2004) and many locations on the Colorado Plateau (e.g., Netoff and Shroba, 1993; Chan et al., 2005). Many of these types of depressions have been used by humans and animals for millennia, and have been given a wide variety of names such as ephemeral pools, cisterns, tanks, caldrons, water pockets, and tinajas (Netoff et al., 1995; Bayly, 1999). In the desert surrounding Moab Utah, roughly circular depressions are found that form gradually in sandstone surfaces over time, some of which hold sediments and some that are large enough to hold water year round (Figure 2.2). It is postulated that these formations termed "potholes" and their accompanying sediment represent a source of water and soil and are therefore important components in the wellbeing of the desert ecosystem. The question is then, to determine if pothole formation is mainly a case of physical stress placed on the rock by both abiotic and biotic processes, weakening it through cyclic expansion and contraction events, or are (bio)geochemical processes at work within the biofilm, corroding the rock, forming minerals that are affecting a change in the host environment?

Early studies of potholes cited physical abrasion and wind deflation in conjunction with the expansion of salt crystals, discounting a biological component in their formation (e.g., Bradley et al., 1978; Laity and Malin, 1985; Netoff and Shroba, 1993). Over time, the effects of biofilms on environments, even bare rock surfaces, have become better understood (Warscheid and Braams, 2000). It has been found that these effects, given favourable conditions, can happen rapidly beginning with changes in chemistry in the area surrounding an individual cell as it adheres to a surface (Schwartzman, 1989; Donlan, 2002; Bludel et al., 2004); however even large bodies of water and landscapes can be influenced seasonally by biofilms. Within limiting

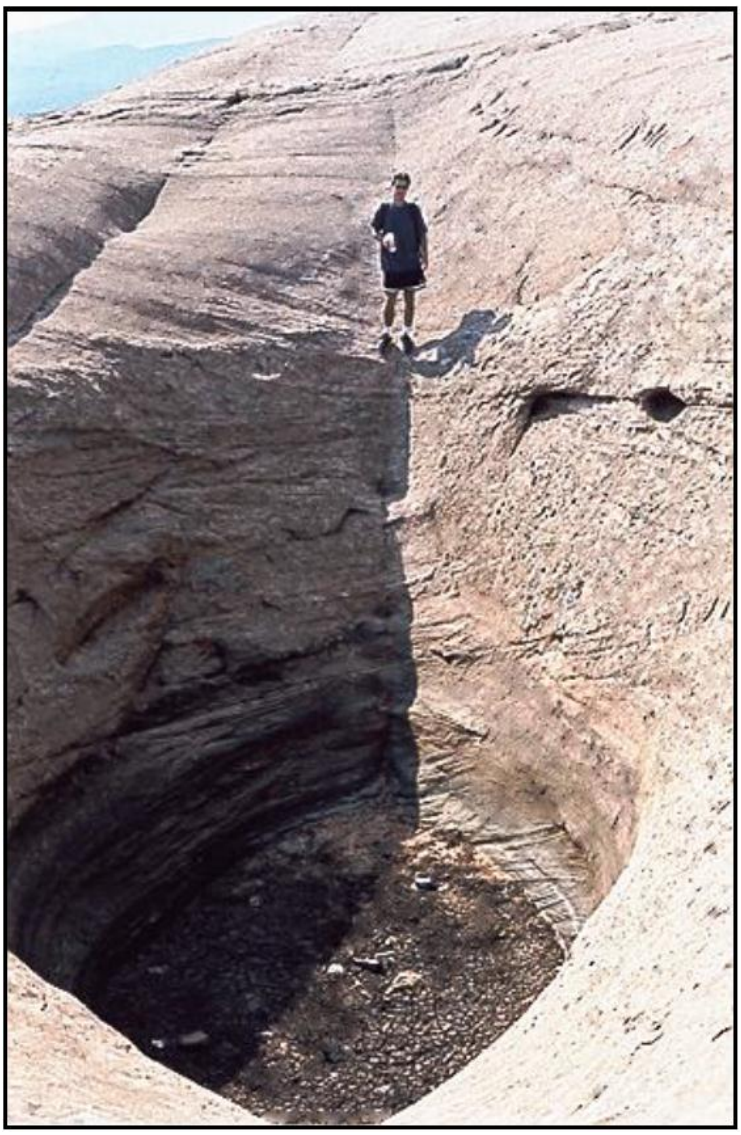


Figure 2.2: Large pothole in the Navajo Sandstone which commonly retains water most of the year, shown at the height of summer in a drought period. Person approx. 1.7 m.

oligotrophic environments nutritional constraints mean that the effects of biofilms are measured in years and decades, and make the biochemical processes more difficult to decipher (Johnson, 1993). Understanding the effects biofilms have on the rock surfaces they colonise is difficult due to their somewhat contradictory nature: Many types of bacteria and fungi produce organic acids that are corrosive, but weathered material may be re-precipitated strengthening the rock in a different location (de las Rios et al., 2003). Also, biofilms are known to produce thick layers of extracellular polymeric substances (EPS) which act to bind rock particles or even soils together (Belnap and Lange, 2001; Bronstoff et al., 2005), but at the same time apply mechanical stress to the rock due to shrinking and growth during wet/dry cycles (Dornieden et al., 2000; Warscheid and Braams, 2000).

Understanding and interpreting the net effect of these combined biological influences is a challenge in slowly evolving landscapes. There is little consensus concerning the relative importance of the processes, be they mechanical, geochemical or biological, because they cannot be observed as they occur, and often happen simultaneously, making distinction between them difficult. More recently, with the application of molecular biological techniques, more discussions have involved the relative importance of different microbiological species (Walker and Pace, 2007). Despite the rise in importance of various types of microorganisms, the general consensus is that the main component organisms in lithic habitats with exposure to solar radiation, are cyanobacteria (e.g., Friedman 1982; Bell et al., 1986; Banerjee et al. 2000; Garcia-Pichel et al., 2001; Belnap, 2002; Budel, et al., 2004; Walker and Pace, 2007). Other microorganisms have also been identified as being important, including fungi and lichens

(Sterflinger, 2000, Souza-Egipsy et al., 2004). Cyanobacteria are found in various types of lithic communities, living in (cryptoendolithic) and on (epilithic) rock surfaces as well as being the main components of proto-soils (biological soil crusts (BSCs)) (Garcia-Pichel et al., 2001; Walker and Pace, 2007). They are well adapted to the harsh conditions of these environments and are known to be able to grow in low nutrient environments, fix nitrogen (e.g., Steppe et al., 1996; Belnap, 2002), are resistant to desiccation (Tandeau de Marsac and Houmard, 1993) and have even been found to develop light attenuating pigments or "sunscreens" (Garcia-Pichel and Castenholz, 1993). Cyanobacteria have also been found to directly influence their chemical environments through a process of carbon acquisition that leads to the production of hydroxide ions and, indirectly, carbonate mineral formation (Thompson and Ferris, 1990).

The purpose of this study is to better understand how the cumulative effect of these biofilms on the host sandstone contributes to the formation of potholes and, by extension, the creation of water sources and soils in the desert environment.

2.2 Methods

Field Sampling and Observations

Initial field investigations of potholes at two locations which were typical of other pothole locations observed in the area, were conducted at Bartlett Wash (BW), and Seven Mile Canyon (SMC) both located just north of the city of Moab, Utah (Figure 2.1). The BW field location was the site of previous pothole studies and was chosen for continuity. Further, BW is a more isolated location that would have very little chance of pothole contamination either by cattle or human interference. Seven Mile Canyon was chosen to

contrast with the BW site. The potholes at SMC have a different morphology and are also more accessible. Frequently the BW location is difficult to access and was not appropriate for routine daily sampling.

At each location, samples of unaltered host rock as well as biologically-altered material were collected for petrographic analysis using an 8 lb sledge hammer (Appendix A). Samples of dry biofilm were collected using stainless steel instruments sterilised with ethanol and stored in sterile cryovials (Appendix A). Sample locations were revisited after six years to visually identify the colonisation process of biofilms.

Laboratory X-Ray Fluorescence (XRF)

Samples of surficial sandstone weighing 2 kg each were taken from representative locations at SMC (SMC-RS-6) and BW (BW-RS-1) possessing well-developed surficial (SMC) or endolithic (BW) biofilms. The samples were separated into top (biofilm-affected - 1 cm deep) and bottom (at least 5 cm from the surface) sections through abrasion (scraping) with a stainless steel spatula. Specimens were air-dried before being crushed and milled to < 75 μm by a rotary swing mill. About 1.0 g of each sample was weighed and heated at 1000°C for Loss on Ignition (LOI) determination (total carbon). An aliquot of 0.50 g heated sample was fused with 6.50 g of Lithium Borate flux (a mixture of 50% Lithium Tetra-borate and 50% Lithium Meta-borate) to form a fused disc for major oxides analysis. The major oxides were then analyzed by a PANalytical PW-2400 Wavelength Dispersive XRF Spectrometer in the Laboratory for Geochemical Analysis at the University of Western Ontario. The net peak intensities were corrected for background, spectral interferences and inter-element matrix corrections. The

concentrations were obtained by comparison against calibrations derived from 3 international standard reference materials most closely approximating the composition of the analytical sample. Based on the quality assurance samples, the analytical errors for major oxides ranged from 2% to 3%.

Cathodoluminescence

Samples of rock with black biofilm growth from pothole SMC1 and pothole SMC2 (samples SMC-RS-8 and SMC-RS-9 respectively) were selected. Thin sections were prepared showing a vertical section down into the surface to capture to the zone of biological influence. The thin sections were epoxy-embedded and ground to a thickness of 35 μm. These samples were observed under vacuum on a cold cathodoluminescence microscope, which eliminates the need for surface coating. Relion Industries Cathodoluminescence mass spectroscopy electron beam technology was employed using a Nikon Eclipse E600 petrographic microscope with Reliotron control and chamber modules, a SpectraPro 2150i low-resolution monochromator (Acton Research corporation) and Pascal 2005 SD compressor (Alcatel Vacuum Technology). CL images were taken using Nikon Act-1 software (version 2.62) in the Cathodoluminescence Spectroscopy and Imaging Facility at the University of Western Ontario.

Scanning Electron Microscopy (SEM)

Polished thin-sections of the upper layers of host sandstone were prepared showing areas known to be colonised by biofilm from both SMC-RS-8 and BW-RS-7. Both wet and dry samples of surficial biofilm from SMC-RS-9 and BW-RS-8 were

prepared for analysis. Dry samples were affixed to carbon tacks. Wet samples were fixed with 10% glutaraldehyde and dehydrated sequentially through 30 min immersions in 25%, 50%, 75% and 100% ethanol solutions. The samples were then critical point dried (Tousimis, Samdri-PVT-3B) and affixed to carbon tacks on Al-SEM studs. All samples were sputter coated with gold using a Denton Desk II to prevent surface charging. Imaging and Energy-Dispersive X-ray spectroscopy (EDX) analyses were conducted on a scanning electron microscope (SEM) with a focused ion beam (FIB) for cross-sectioning of interesting samples at 5 keV (Leo 1540 XB FIB/SEM). FIB milling was carried out using a Ga^+ ion beam in the nanofabrication facility at the University of Western Ontario.

2.3 Results

Pothole Locations

In the course of field investigations, potholes were observed in several areas in the general vicinity of Moab Utah, in different types of sandstone. Most potholes were observed forming on flat to gently sloping surfaces (approximately 2-5 degrees), usually (but not exclusively) found at the top of cliffs, which is consistent with other studies of the phenomenon of weathering pits (Netoff and Shroba, 1993) (Figure 2.3). Both sampling locations, Bartlett Wash (BW) and Seven Mile Canyon (SMC), are benches of sandstone which form gently sloping surfaces near steep drop-offs. Other pothole-like formations were observed in the area readily forming along joints in the sandstone (Figure 2.3 C D).

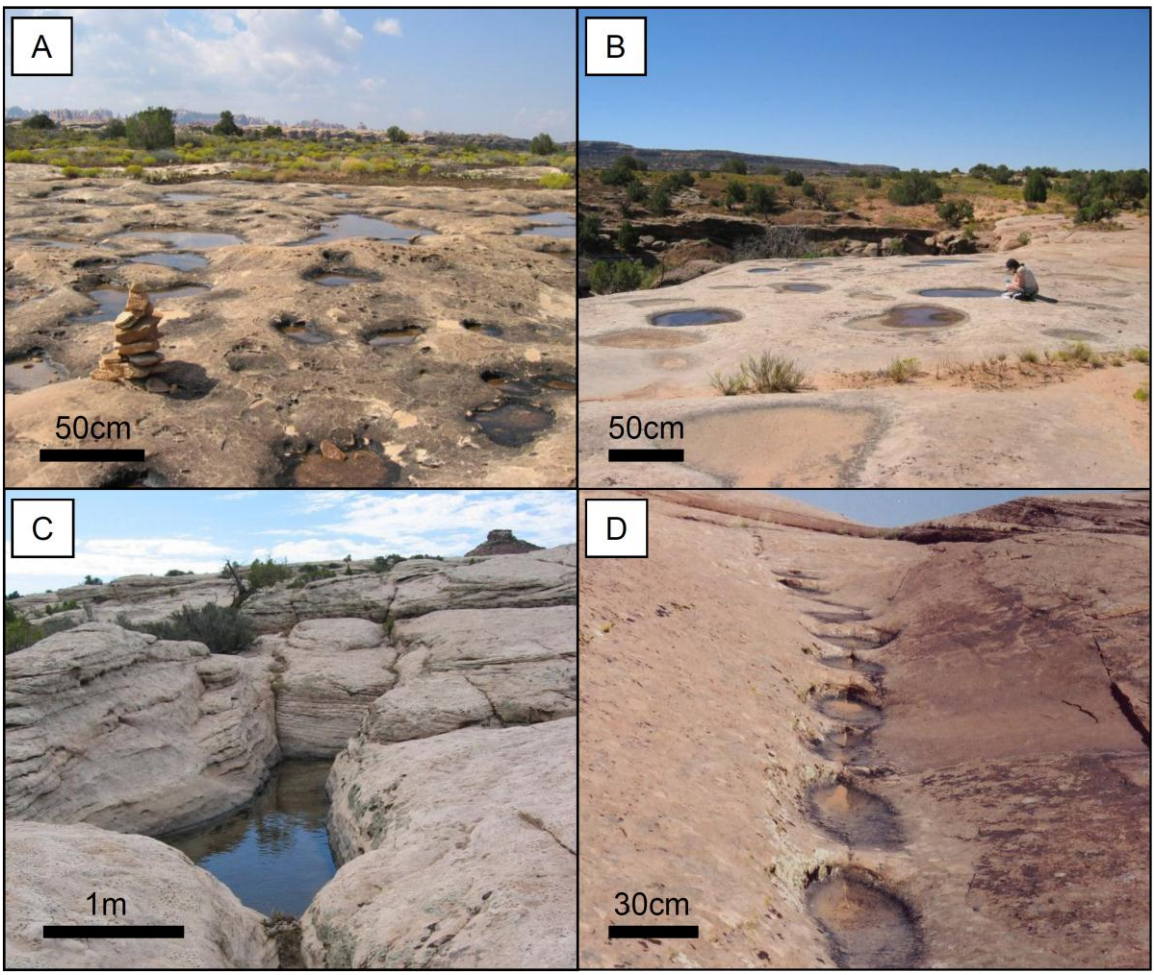


Figure 2.3: Potholes of various morphologies found at four different locations. **A)** Pothole Point, Canyonlands National Park (amorphous). **B)** Roaring Hell Canyon (flat pans). **C)** Bartlett Wash (joint-controlled). **D)** Slickrock Trail (joint-controlled).

These features were not considered in this study as their formation is presumed to be influenced by flowing water erosion, (channelling).

Pothole Morphology

Pothole morphology was observed at each field location and representative potholes were selected (BW1 and SMC1) (Figure 2.4). At BW potholes varied in depth from 10 to 50 cm with pronounced vertical sides and flat bottoms, (Figure 2.4). Potholes with no joint control were largely circular, giving them a distinctly cylindrical form, and contained only minor amounts of poor, sandy sediments at the bottom. Small lips of resistant sandstone were observed around the potholes raised 1-2 cm above the surrounding rock and slightly overhanging the pothole (approximately 1 cm). Each pothole was distinct with no overlap between adjacent potholes.

Three types of biofilms were identified either in the potholes or in the nearby host rock at BW. Black surficial biofilm was the most prominent of the three types and was observed covering the bottom and vertical sides of the potholes. Flaking of the sandstone affected by black biofilm and regrowth in exposed areas was readily observed (Figure 2.5 A B). Another biofilm was observed in the pothole sediment binding the sediment grains and causing the sediments to peel as they dried (Figure 2.5 A). The third type of biofilm was not directly associated with potholes: A layer of endolithic biofilm, usually green, was identified mainly in the BW sandstone. Endolithic biofilms were typically found approximately 1 mm below the rock surface, although penetration up to 1-2 cm depth was also observed. The endolithic biofilm was encountered whenever a surficial sample of the BW sandstone was taken.

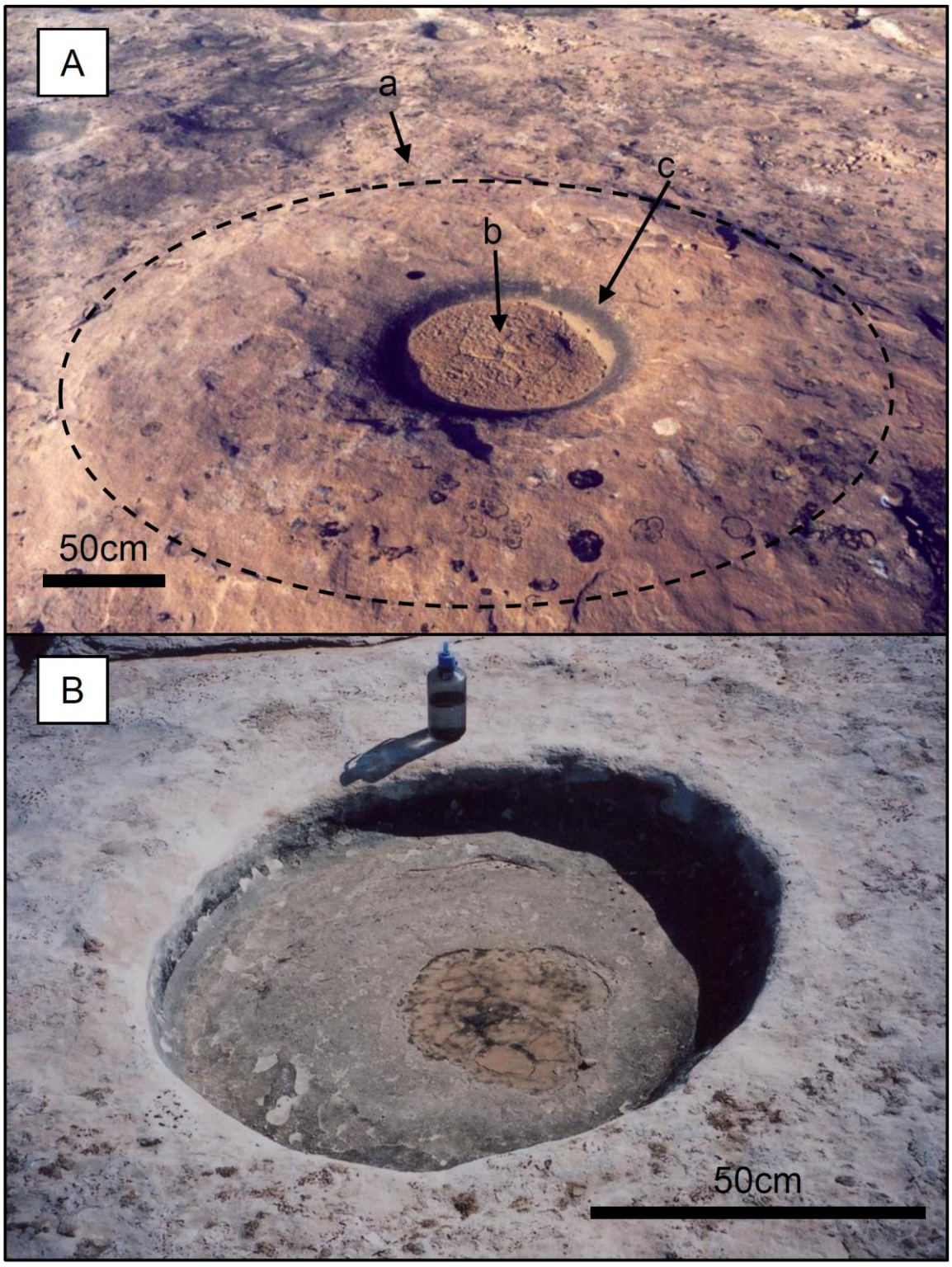


Figure 2.4: A) Typical SMC pothole with raised rounded sides (**a**) and abundant pothole sediments (**b**). Note the black surficial biofilm ring ends where it was covered by sediments and has been uncovered for the picture (**c). B)** Typical cylindrical BW pothole with sharp vertical sides and very little sediment.

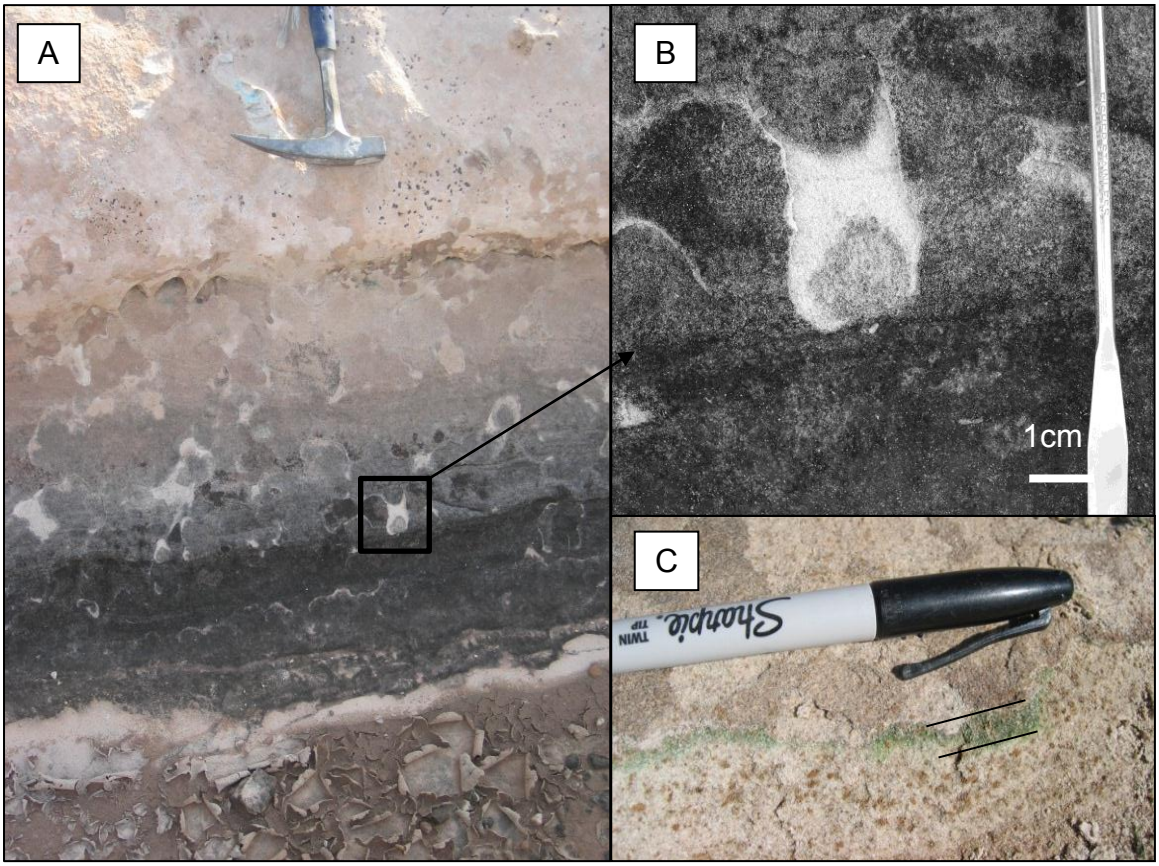


Figure 2.5: Three types of biofilm observed at BW. **A)** Black biofilm growing on the sides of a pothole became lighter in colour further away from the water source in the pothole. At the bottom pothole sediments held together by biofilm curled back from the rock surface as they dried. Hammer 31 cm **B)** An enlarged area of the pothole side where a crust of sandstone has flaked off exposing clean bleached sandstone beneath. The black biofilm is re-growing in the area both from the top down and the bottom up indicating it is not only gravity-induced movement of water staining the rock. **C)** A green layer of endolithic biofilm, which is only found in areas where the host sandstone is lighter in colour. Pen is 15 cm.

At SMC, potholes varied in depth from approximately 2-10 cm with smoothly sloping sides that were continuous with the bottom of the potholes giving them a bowl or pan morphology depending on their size (Figure 2.4). The horizontal extent of the observed potholes varied from approximately 10 cm to 1.5 m. Wider potholes appeared, in some cases, to be a result of the intergrowth of several smaller potholes (Figure 2.6). This intergrowth was also observed at other pothole locations in the Moab area not used for sampling. The potholes at SMC also show large elevated rings of resistant sandstone around the outside of each pothole that was quite extensive in some cases (+/- 50cm in horizontal thickness and +/- 10 cm vertical thickness at some locations) (Figure 2.4).

Larger potholes at SMC contained sediments that reached depths of 3-4 cm. Smaller shallow potholes were generally without sediment accumulation or hosted only minor pebbly sediments. Black biofilms in smaller, sediment poor-potholes were observed to cover the sides and bottom of the formations, and when filled with water the black biofilm was observed to define a ring of water saturation around the pothole in the sandstone. Larger potholes, which contained more sediments developed black biofilms as rings around the outside of the potholes, again defining the extent of saturated sandstone upon wetting (Figure 2.7). Black biofilm did not extend under the sediments indicating that it is largely populated by photosynthetic microbial species (Figure 2.4 A). Hand samples of the dry black biofilm layer showed a sticky carbon-rich mass full of entrained grains of host rock (Figure 2.7c). An indication of the growth rate of the black biofilm adjacent to potholes at SMC canyon was obtained through observation of a sampling scar. Samples of the black biofilm covered rock at SMC were taken from the edge of pothole SMC1 during the course of field work and the area was revisited after a

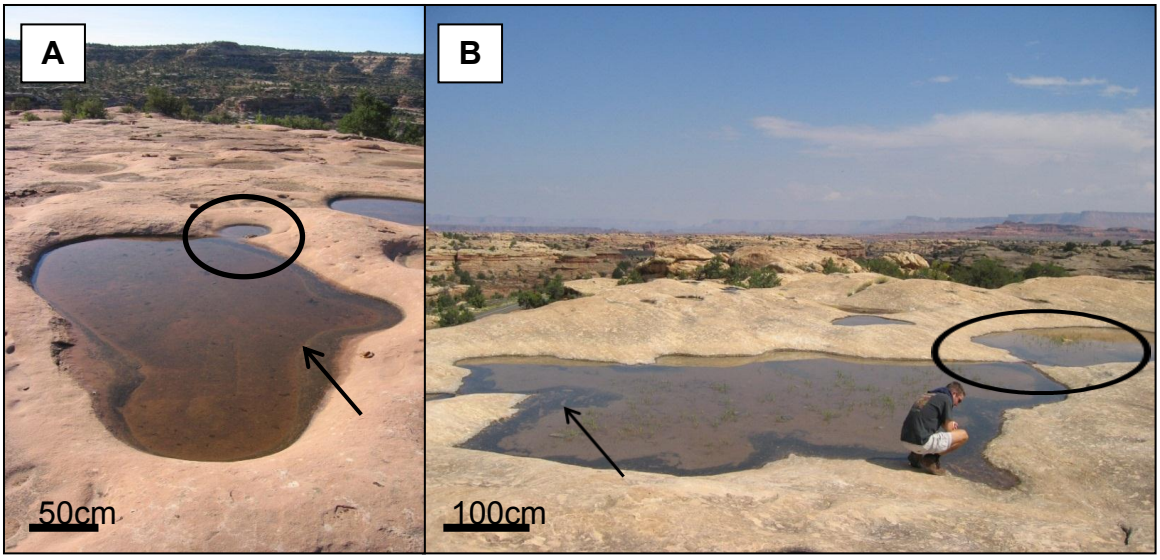


Figure 2.6: Examples of the lateral growth of large, shallow sediment-rich potholes at **A)** SMC and **B)** Canyonlands National Park. Note the growth of small plants. Black arrows indicate the interface between pothole sediments and black biofilm (the stable and growing areas of the pothole), black circles indicate where two potholes have grown into one another.

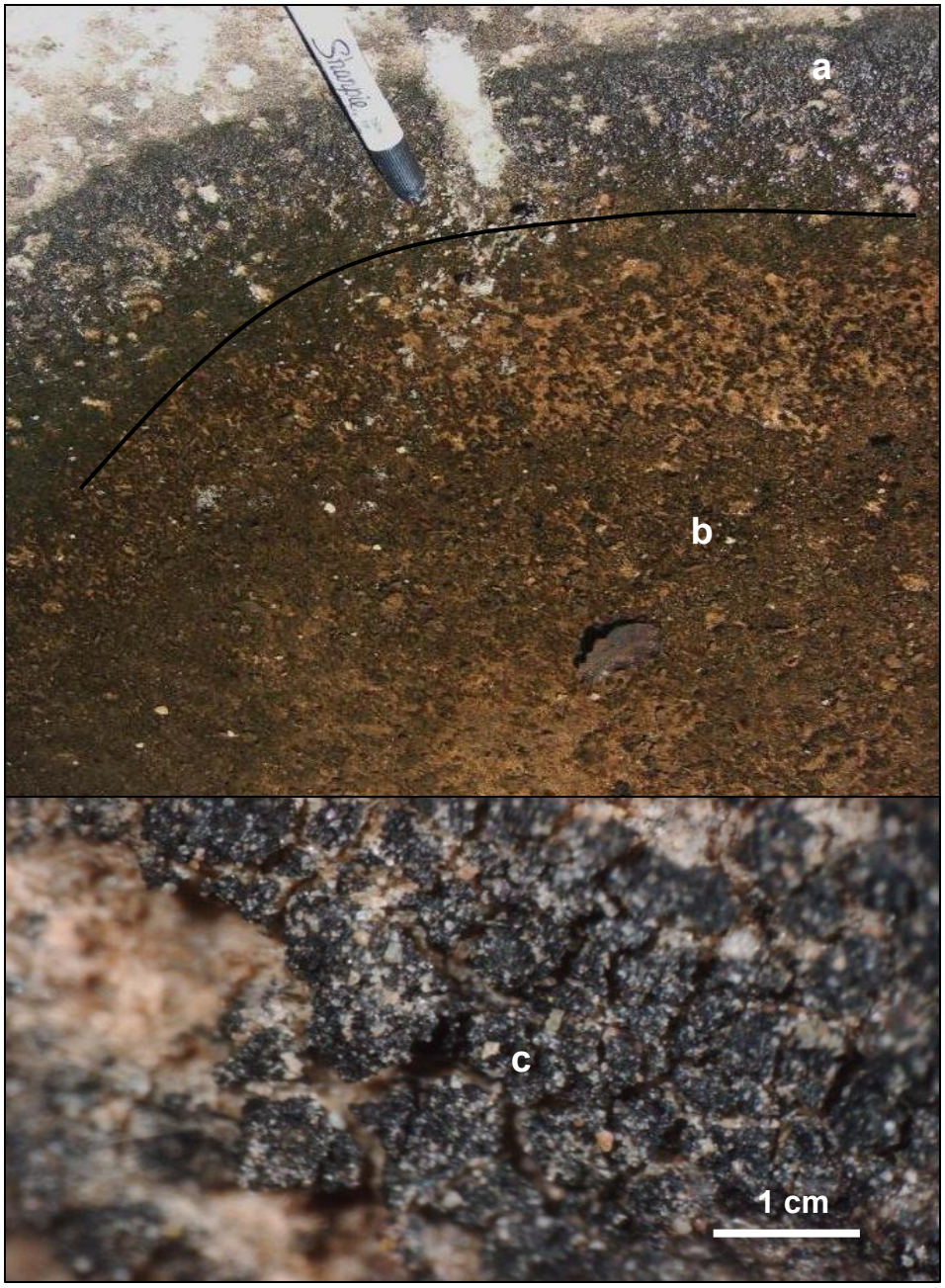


Figure 2.7: Examples of the two types of biofilm commonly encountered in the pothole environment at SMC (**a**) black epilithic biofilm and (**b**) black pothole sediment biofilm. (**c**)A close-up of dry black epilithic biofilm which has shrunk and developed desiccation cracks while entraining grains of light coloured sediment from the pothole.

period of six years. At that point it was observed that the sampling scar had been approximately 50% re-colonised by black biofilm (Figure 2.8).

Petrography

Petrographic analysis was conducted on selected samples (see Appendix A for list of samples) of pothole-forming host rock from BW and SMC to better understand how host rock composition may influence pothole morphology. Hand samples of the BW host rock were grey to white in colour and friable (easily crumbled by hand). Grain size is uniform. Petrographic analysis of generally representative host rock samples from BW field location show a very fine to fine-grained quartz-arenite sandstone. The component grains were sub-rounded to sub-angular and composed of quartz (95%), minor feldspars and lithic fragments (<5%), approximately 5% silica cement and approximately 10% porosity (Figure 2.9ABC). The silica cement appeared mainly as overgrowths on existing quartz grains which indicates secondary deposition of quartz in the system from silica-rich fluids. Further, weathered grain boundaries around quartz minerals are often observed from samples at the surface of the host rock, or in areas where biofilm was observed indicating that some dissolution of quartz grains has occurred in the rock (Figure 2.10). Isolated samples of surficial sandstone material show the accumulation of a carbonate mineral crust at the host-rock surface (Figure 2.11). As it has been noted that the BW sandstone is particularly friable this delicate crust was observed only in one sample but may be a more general phenomena that is simply too fragile to accurately sample.

The host rock at SMC adjacent to the potholes was pink-orange in hand sample

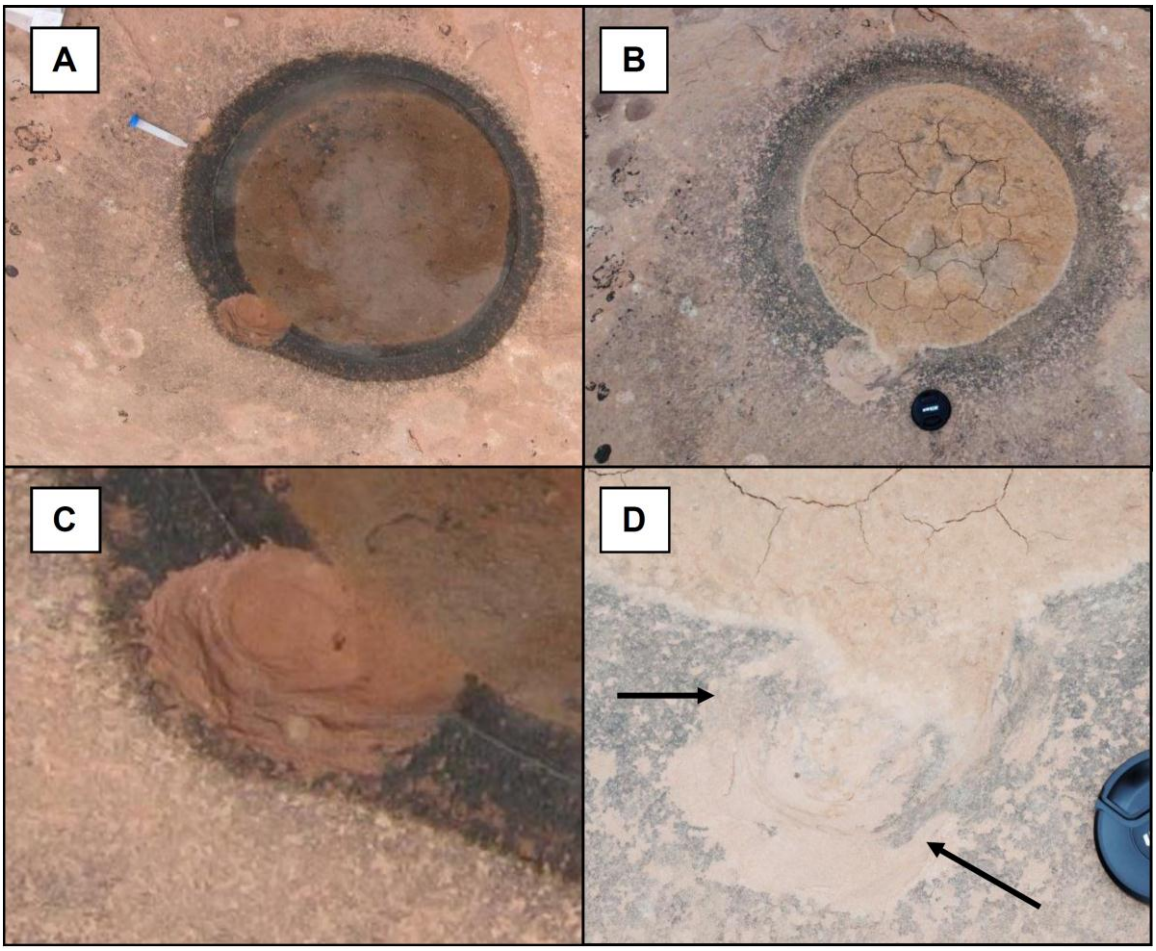


Figure 2.8: A) Pothole at SMC and **B)** the same pothole six years later**. C)** A close-up of sampling 'scar' approximately 2 cm deep (year 1). **D)** Note regrowth of black biofilm into damaged area (year 6). Lens cap 7.5 cm.

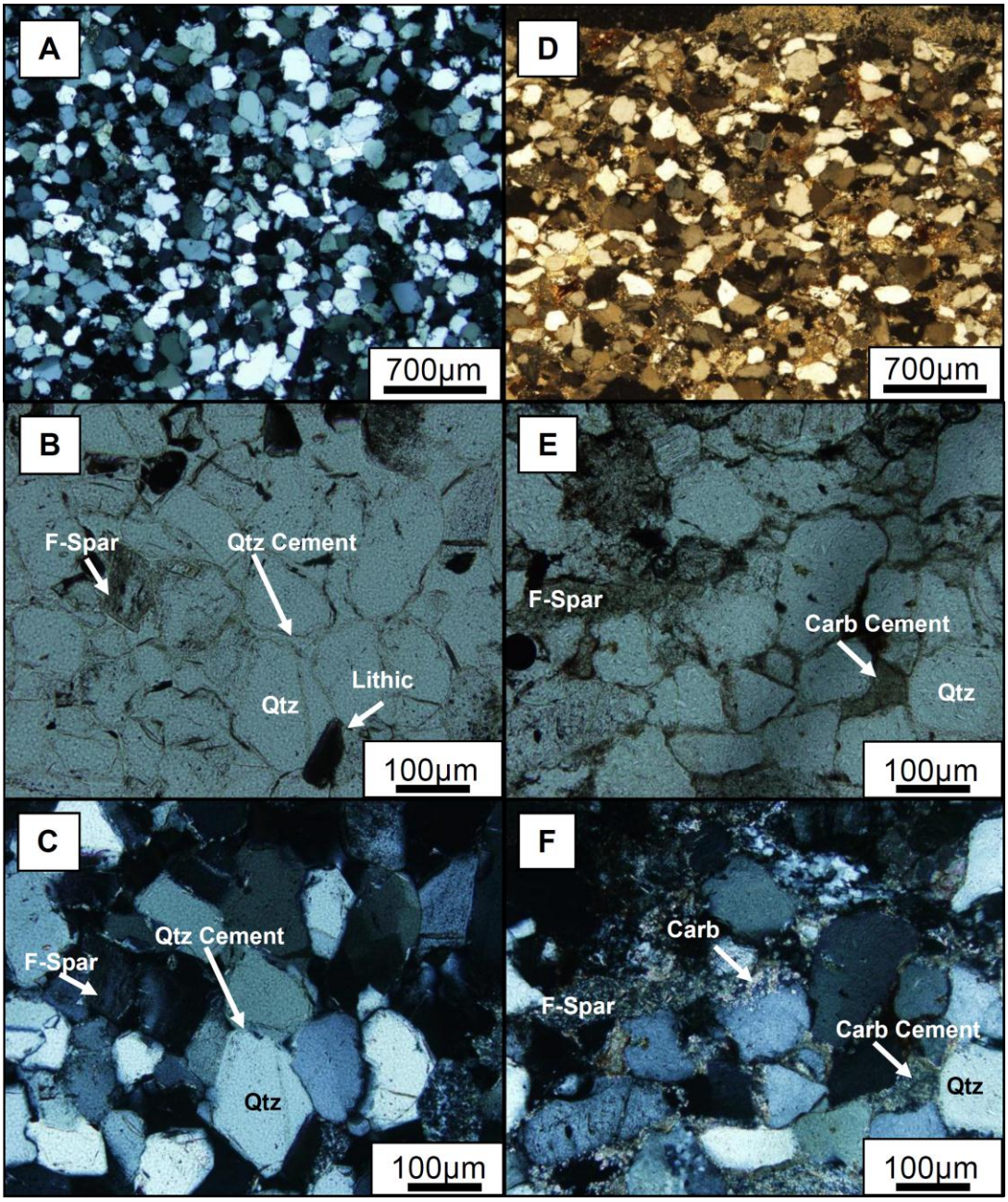


Figure 2.9: A) Cross-polarised photomicrograph of BW quartz arenite sandstone. Very little (5%) silica cement is observed. No other cementing materials, including carbonate mineral cement, were observed. **B)** Plane polarized light close-up of BW sandstone **C)** Cross-polarised light close-up of BW sandstone showing quartz (Qtz) grain overgrowth which implies the secondary mobilisation of silica in solution. Few accessory minerals are present (<5%). **D)** Cross-polarised photomicrograph of SMC sandstone from the Kayenta formation: Predominantly quartz (Qtz) with accessory feldspars (F-spar) and carbonate mineral cement (Cal). **D)** Plane polarised light close-up of SMC sandstone E) Cross polarised light close-up of SMC sandstone showing patchy carbonate mineral cement (Carb) and iron oxides (Fe-Ox).

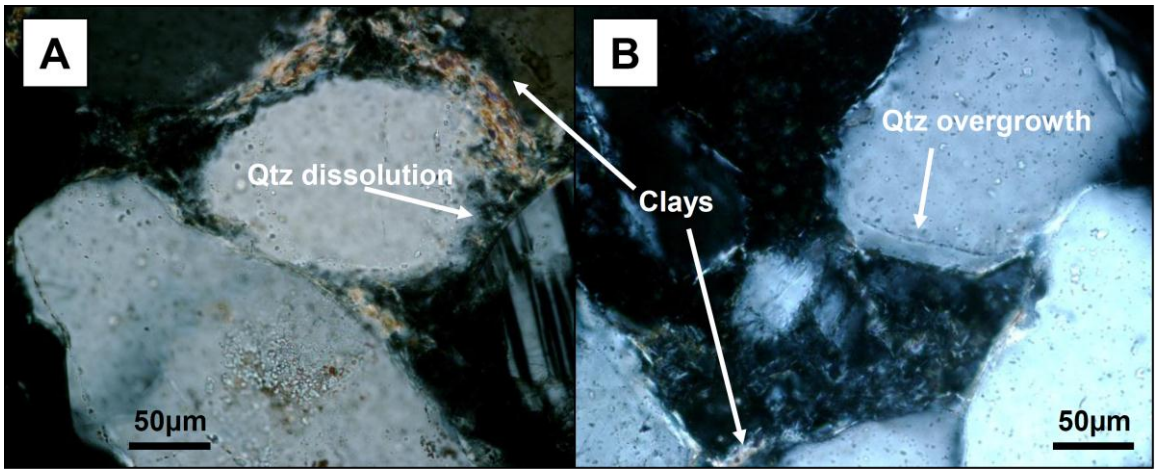


Figure 2.10: A) (BW) Silica showing ragged dissolutions along grain boundaries and clay mineral deposition. **B)** (BW) Silica showing overgrowth textures and minor clay minerals in the pore spaces.

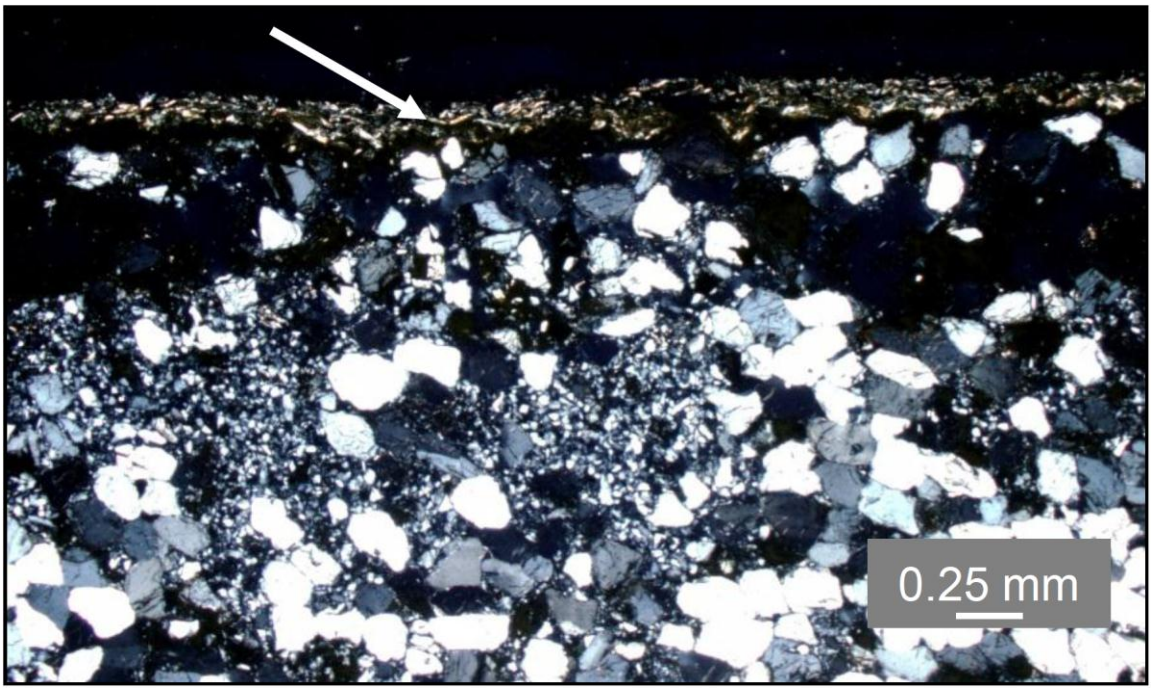


Figure 2.11: Cross-polarised photomicrograph of BW surficial sandstone with a thin, fragile carbonate mineral crust.

with occasional fine red banding from diagenetic iron-staining. The samples taken were competent and did not easily break apart. Petrographic analysis of general representative samples taken near the potholes at SMC showed very fine to fine grained arkose sandstone. The component grains were sub-rounded to sub-angular and composed of quartz (approximately 50%), feldspars and lithic fragments (approximately 35%). The sandstone was well-cemented with carbonate minerals (10%) and minor iron oxides (5%), and had a low overall porosity (1%) (Figure 2.9 DEF).

Major Oxide Analysis (XRF)

In order to further delineate the differences in host rock composition between BW and SMC XRF analysis of major oxides was conducted on representative samples from each field location (SMC-RS-6, BW-RS-1). Further, the samples were divided between biologically colonised crust and abiotic host in order to quantify changes in composition that may be caused by biological activity (Table 2.1). Major oxide analysis of the host rocks sample of BW taken from below the photic zone reflected the quartz arenite composition of the sandstone, composed of 97.5 wt % SiO_2, with Al_2O_3 (1.3 wt %) being the only other component above 1 wt % . Samples of the host rock "crust" or the portion of the rock in the photic zone (top 5 cm) showed a relative depletion in SiO_2 (-1.77 wt %), a relative enrichment in total carbon (L.O.I.) (+1.49 wt %) and no change in the relative proportion of Al_2O_2. All other major oxides are present in trace amounts with small relative changes e.g. there was a relative enrichment of CaO bearing minerals (+ 0.12 wt %).

Table 2.1: XRF major oxide analysis of rock samples from SMC and BW divided into abiotic host and biotic crust portions

Sample	SiO_2	CaO	Al_2O_3	K_2O	Fe_2O_3	MgO	TiO_2	Na_2O	P_2O_5	MnO	L.O.I.	ToTal
BW Host	97.53	0.04	1.30	0.53	0.24	0.08	0.03	0.01	0.02	0.02	0.30	100.10
BW Crust	95.76	0.16	1.30	0.50	0.39	0.06	0.02	0.01	0.03	0.01	1.79	100.02
BW Crust -Host	-1.77	0.12	0.00	-0.03	0.15	-0.02	-0.01	0.00	0.01	-0.01	1.49	
SMC Host	64.22	16.10	3.71	1.26	0.74	0.11	0.13	0.01	0.02	0.06	13.75	100.12
SMC Crust	60.41	17.64	4.17	1.55	0.88	0.16	0.17	0.01	0.03	0.08	15.02	100.12
SMC Crust-Host	-3.81	1.54	0.46	0.29	0.14	0.05	0.04	0.00	0.01	0.02	1.27	
Standard												
SY-2X	60.1	7.95	12.05	4.52	6.3	2.65	0.13	4.28	0.46	0.28	1.05	99.77
BIR-1	47.63	13.27	15.38	0.02	11.27	9.54	0.93	1.81	0.04	0.16	0.19	100.24
BHVO-1	49.9	11.49	13.7	0.49	12.21	7.17	2.73	2.23	0.28	0.16	0.25	100.61
error +/- 2-3%												

Major oxide analysis of the SMC sandstone showed it to be an arkose sandstone with 64.22 wt % SiO_2 and significant contributions (above 1 wt %) from CaO, Al_2O_3 and K_2O bearing phases as well as total carbon (LOI). Samples of the "crust" portion of the SMC rock in the photic zone (top 5 cm) showed, a relative depletion in SiO_2 (-3.81 wt %) and relative enrichments in both CaO bearing phases (+1.54 wt %) and total carbon (L.O.I) (+1.27 wt %) similar to BW. All other major oxides are present in trace amounts with only minor relative enrichment from host to crust.

Cathodoluminescence

Cathodoluminescence was used to visualise the structure of the carbonate cementing material at SMC and identify any textures that may be suggestive of biological influence. Using cathodoluminescence, two secondary carbonate mineral cement phases were identified at SMC (Figure 2.12). The majority of the carbonate mineral cement was identified as phase one (P1): coarsely filling pore spaces throughout the samples. A second smaller fraction of the carbonate mineral cement was identified as phase two (P2): finely laminated bands forming along the edges of, and penetrating into, weathered mineral grains, including quartz. The coarse nature of the P1 carbonate mineral cement did not allow for the timing of its deposition to be estimated; however, because the P2 cement has inter-grown with weathered grains it is possible to place it as a more recent addition to the rock, coeval with rock weathering.

SEM-LEO

Elemental mapping was conducted on samples of the surficial host rock from BW

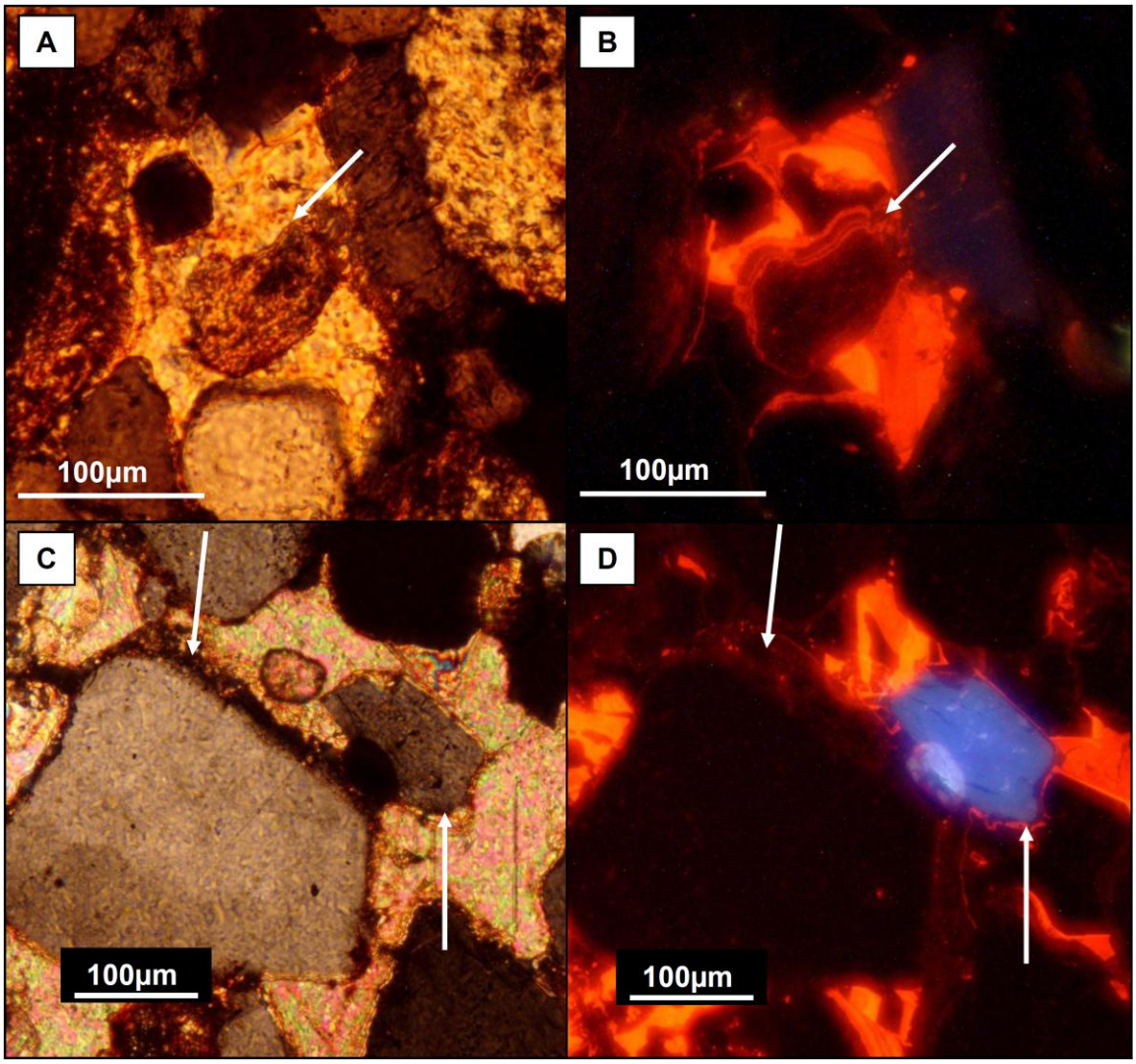


Figure 2.12: Cross-Polarised photomicrographs **(A** and **C)** and their equivalent cathodoluminescence images **(B** and **D)** of secondary carbonate minerals (orange/ yellow fluorescence) precipitation in SMC crustal sandstone. White arrows indicate fine bands of secondary carbonate minerals (P2) growing on weathered feldspar grain (brown in cross polarized light, without fluorescence under cathodoluminescence) **(B)**, or inter-grown with the edges of weathered quartz grains (grey in cross polarized light, without fluorescence under cathodoluminescence) **(C** and **D)**.These finer carbonate minerals are distinct from the pore filling carbonate mineral cement in the images.

to contrast with cathodoluminescence (CL) studies of SMC, identify any microscopic traces of possible carbonate mineral cement not visible with CL and to observe the weathering of individual silicate grains. Elemental maps of the overall sandstone showed the quartz arenite composition of the sandstone and highlighted the feldspar minerals present which were generally uniform in composition (Figure 2.13). No evidence of carbonate mineral cement was observed over background noise. Weathering of the accessory feldspar grains was mapped and showed the development of secondary clay minerals (likely illite) but only possibly very minor development of secondary carbonate minerals (Figure 2.14).

FIB-SEM

To better study the interface between the biofilm and sandstone host FIB-SEM studies were conducted on samples from BW. Black biofilm growing on BW sandstone was imaged after rehydration in the laboratory. A cross-section through the biofilm identified entrained feldspar grains surrounded by secondary clay minerals (identified with EDX) (Figure 2.15). A similar cross-section through a dried biofilm showed the interface between the biofilm and the host rock (Figure 2.16). The biofilm was growing on quartz with an enrichment of aluminum at the interface between the biofilm and the host rock indicating a weathering interface. Secondary clay minerals were observed in-filling between the quartz grains of the sandstone with possible secondary silica that was too minor to capture with EDX.

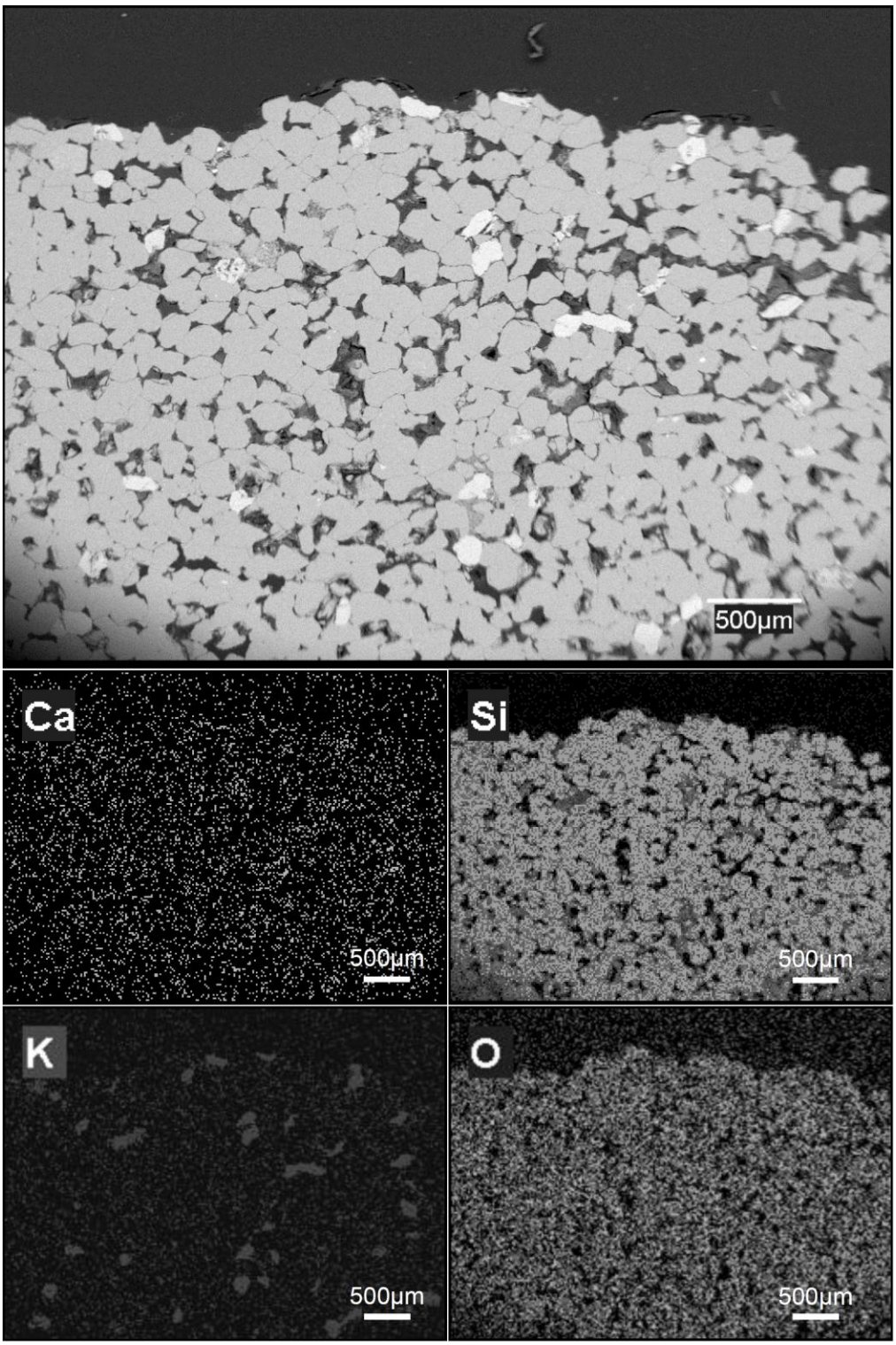


Figure 2.13: LEO EDX mapping of a polished thin section showing the bulk composition of BW sandstone crust. A backscatter electron image (top) is contrasted with EDX maps of individual elements. The bulk of the sample is composed of quartz (Si and O maps) with accessory plagioclase feldspars (K map). Calcium is a minor component of the sample and has only a very low signal above background noise (Ca map).

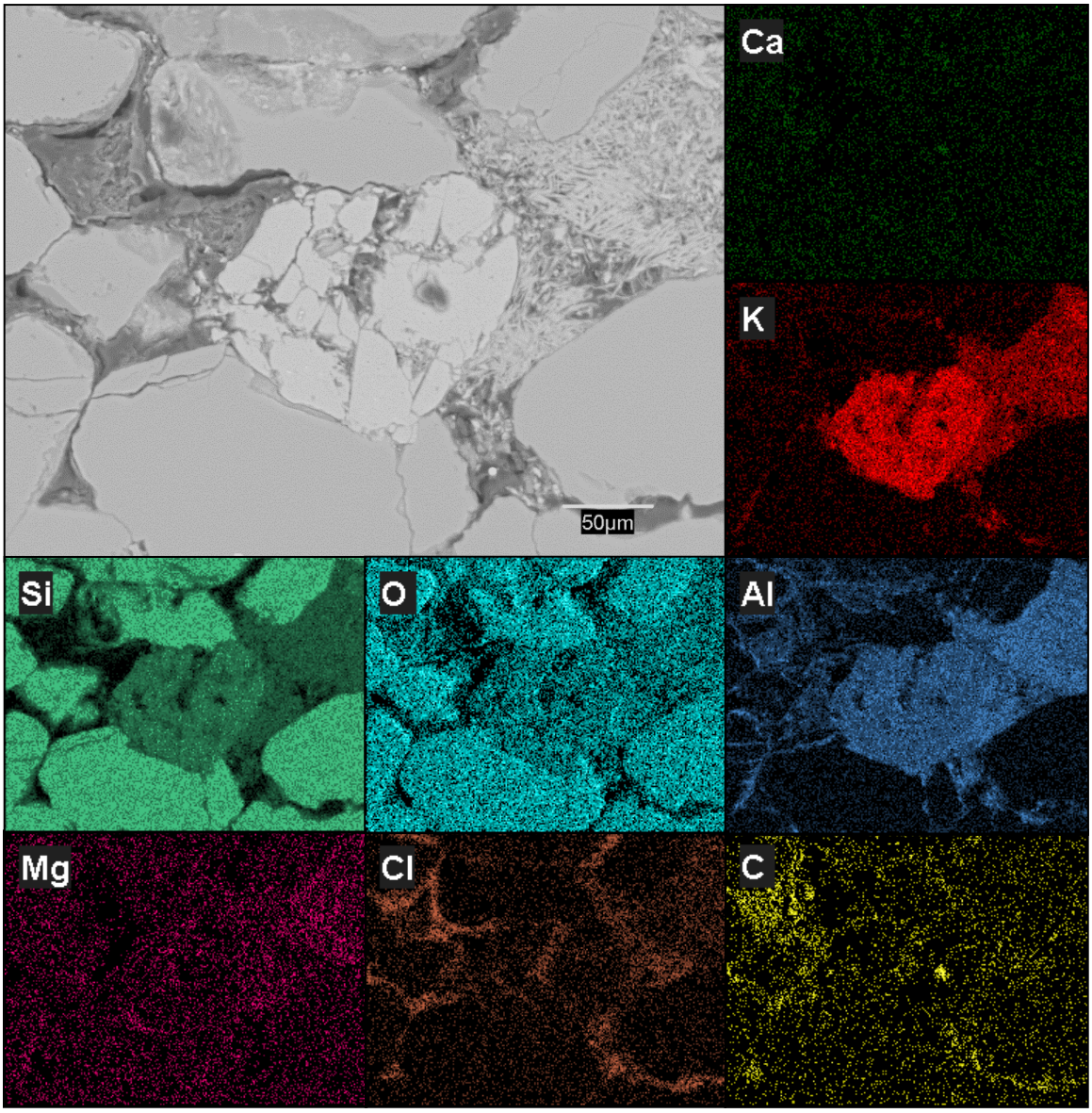
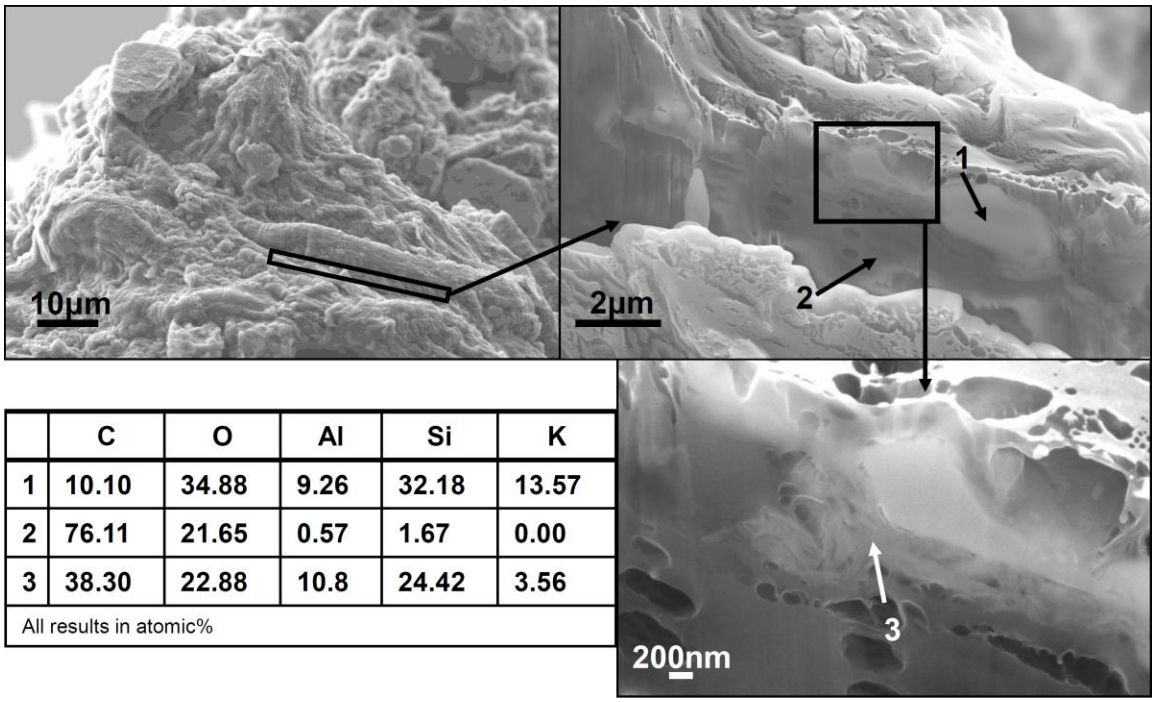
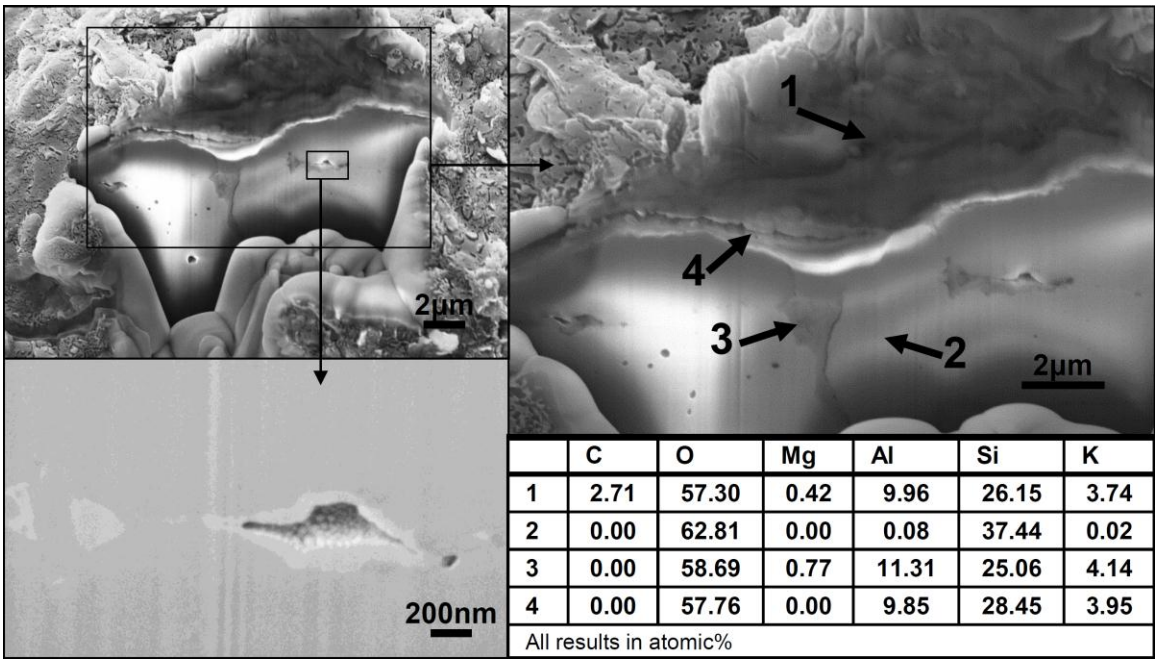


Figure 2.14: SEM EDX mapping of a polished thin section showing the area surrounding a weathered feldspar grain from the BW sandstone. A backscatter electron image (upper left) is contrasted with EDX maps of individual elements. Aluminum and potassium rich accessory clays (possibly illite) are forming (arrows) from the weathering of feldspar grains (higher contrast mineral in the centre of the image). There is only minimal evidence of possible carbonate mineral formation with some concentration of Mg above background noise. Ca does not appear in concentrations distinguishable above background. Concentrations in the carbon signal are due to the use of an epoxy containing carbon that has filled pore spaces.



	C	O	Al	Si	K
1	10.10	34.88	9.26	32.18	13.57
2	76.11	21.65	0.57	1.67	0.00
3	38.30	22.88	10.8	24.42	3.56
All results in atomic%					

Figure 2.15: FIB section with EDX analysis of a critical point dried wet biofilm **(2)** entraining silicate grains **(1)** which are surrounded by secondary clays **(3)** at BW. Note the corresponding EDX analysis for each numbered location.



	C	O	Mg	Al	Si	K
1	2.71	57.30	0.42	9.96	26.15	3.74
2	0.00	62.81	0.00	0.08	37.44	0.02
3	0.00	58.69	0.77	11.31	25.06	4.14
4	0.00	57.76	0.00	9.85	28.45	3.95
All results in atomic%						

Figure 2.16: FIB exposures and EDX analysis of the interface **(4)** of dry biofilm **(1)** and the host rock **(2)** at BW with areas of deposition of secondary minerals from weathering **(3)**. EDX results indicate that the host rock **(2)** is primarily quartz while the dry biofilm **(1)**, secondary minerals **(3)** and interface **(4)** contain the components of clay weathering products. Note the pocket of secondary minerals (enlargement in the lower left), possibly silica, which was too small to accurately analyze using EDX.

2.4 Discussion

The initiating factor, or stage zero, for pothole growth is proposed to be the presence of natural variation in a host rock surface that retains water long enough to support colonisation and biofilm growth. Potholes at the study locations (BW and SMC) were found forming on flat surfaces near cliff edges. Cliff edge locations more easily loose sediments to wind action, leaving little sand or soil to build-up on the surface that would otherwise obstruct biofilm colonisation of the host rock. After colonisation, the biofilm interacts with the specific composition of each host rock to produce potholes in unique sizes and shapes. Studies of bio-deterioration of various types of stone in numerous environments demonstrate that the structure and chemical composition of the stone defines its bio-receptivity and therefore, it is important to understand the nature of the host rock (Warscheid and Braams, 2000).

The depositional environments of the BW and SMC host sandstones were quite different, as reflected in the overall sandstone composition and pothole morphology (Figures 2.9 and 2.4). The BW sandstone is a part of the Moab Member of the Curtis Formation, a cross-bedded, aeolian, cliff-forming unit laid down between 158 and 155 Ma (Figure 2.17) (Doelling, 2003). The unit is moderately to well cemented with carbonate minerals in some locations (Doelling, 2003), although petrographic studies found cementing material to be a minor component of the exposures in our field area (Figure 2.9 A). Hydrological studies of the area indicate that the Moab member is important in the local transport of ground water causing it to be leached of its carbonate mineral cement in some areas (Hurlow and Bishop, 2003). This leaching, and its generally well-sorted composition, results in a higher overall porosity than the earlier

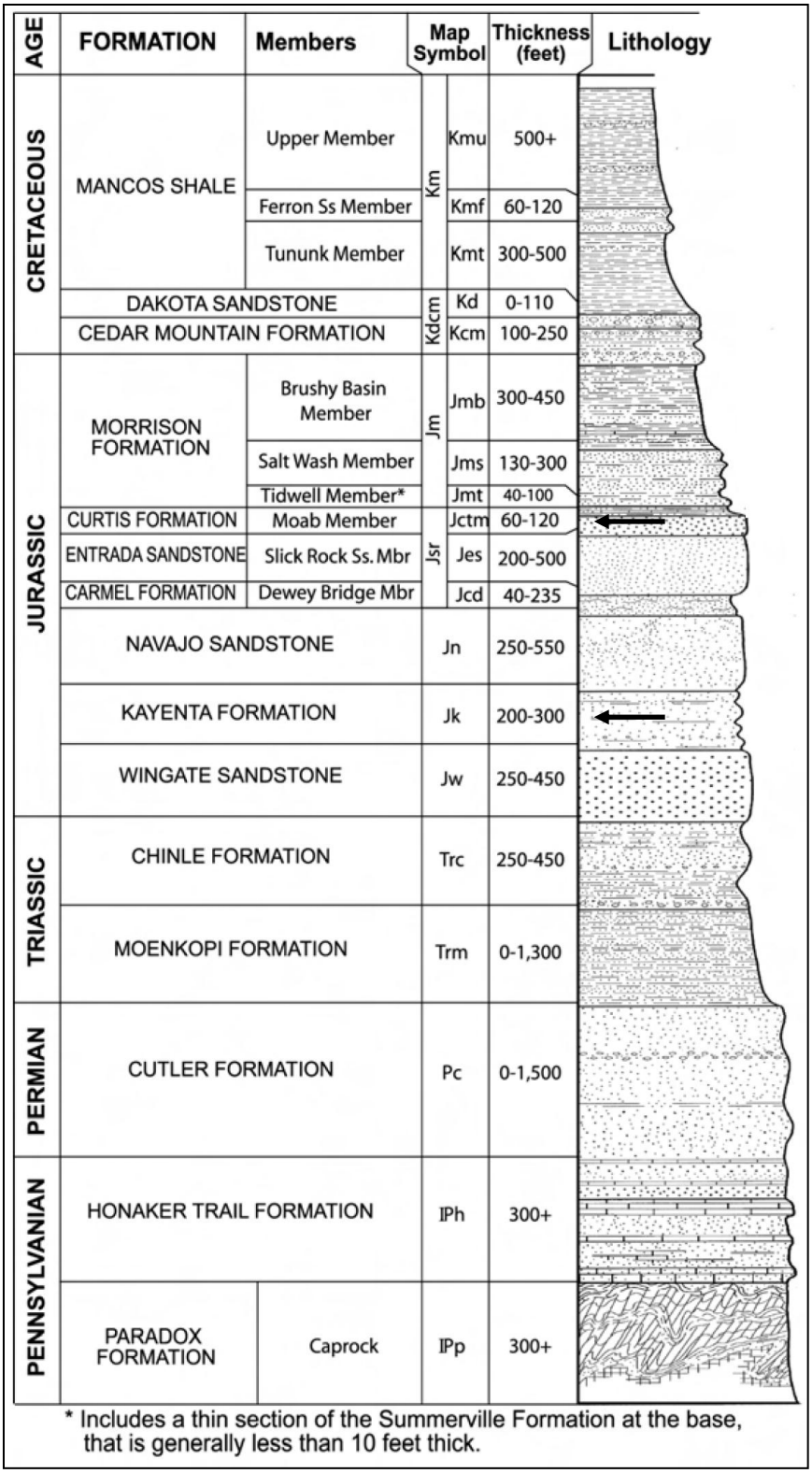


Figure 2.17: Stratigraphic column of geological formations found in the field area (after Anderson and Sprinkel, 2000).

Slick Rock sandstone member of the Entrada, which the Moab member rests on (Figure 2.17). It is moderately to densely jointed as in several areas it is found directly adjacent to the Moab Fault.

The SMC sandstone is part of the Kayenta formation, which is characterized by abundant variation, from intra-formational limestone and conglomerate to thick lacustrine material and some aeolian lenses, all from the early Jurassic (approximately190 -200 Ma) (Doelling, 2003). It was deposited between two massive aeolian cliff-forming units, the earlier Wingate and the later Navajo (Figure 2.17).

The most important differences between the BW and SMC formations are the almost complete absence of any carbonate mineral cement and the low percentage of accessory minerals in the BW sandstone. The carbonate mineral cement makes the SMC sandstone more competent than the BW sandstone, which is reflected in the pothole morphologies at each location: The potholes identified at BW are much larger and deeper and show weathering along the sides of the hole, indicating that abrasion by wind or water has a more profound effect on the weaker stone (Figure 2.4).

Physical Influence of Biofilm

Each of the three types of biofilm, surficial black biofilm, pothole sediment biofilm and endolithic biofilm were identified at both BW and SMC locations. Endolithic biofilm was more prevalent at BW likely due to the larger pore spaces in the host rock available for colonisation and the lighter colour of the sandstone which allowed more light to penetrate under the surface; a critical factor in colonisation (Omelon, 2008).

Each of these biofilms has an important role to play in pothole formation. Extracellular polymeric substance (EPS) produced by biofilms have both detrimental and beneficial effects on rock surfaces. They add mechanical stress through wet/dry cycling causing the EPS to swell and shrink, which can increase pore size and pore distribution as well as affecting the impacts of moisture and temperature fluctuations on the surfaces (Krumbien, 1988; Warscheid and Krumbien, 1996; Souza- Egipsy, 2004). The adhesive properties of EPS, however, also bind particles together, creating stabilized crusts (Belnap and Lange, 2001; Zaady et al., 2010). Without the presence of biofilms, the host rocks in this area, especially those from BW, were naturally friable. Therefore, over the short term, the EPS of the biofilms growing directly in contact with the host rock reinforce the host rock, creating the opportunity for further biotic growth (Kurtz and Netoff, 2001). This binding quality of the biofilm was especially evident in the black biofilms that line the bottoms of potholes at both SMC and BW. The continuous mat acted as a semi-permeable barrier slowing water loss by percolation or diffusion into the rock and allowing the potholes to hold water for longer than the surrounding un-colonised rock. This sealing effect was balanced by the effects of desiccation on the black biofilm. Although the surficial black biofilm was observed to bind grains together; because of the intermittent rains desiccation caused it to peel back from the host rock entraining grains of the host rock as it retreated (Figure 2.5 AB). The biofilm induced exfoliation of the rock surface which allowed the sun to penetrate the "clean" rock beneath, encouraging the activity of the cryptoendolithic bacteria and the eventual colonisation of the revealed area (Figures 2.5 and 2.8). Through these competing

processes of sealing and pealing of the sandstone surface the host rock weathered, contributing to the expansion of potholes.

Sediment accumulation was observed in many potholes particularly at SMC, beneath which the black biofilms were not observed to grow. However, as more sediment is deposited, more substantial biofilms may be supported within the sediments themselves, potentially leading to the gradual development of a permanent cryptobiotic Biological Soil Crust (BSC) (Chapter 4). The sediment load of an area could be a limiting factor to the size of the potholes there. As the bottom of a pothole is covered with sediment little to no direct exfoliation of the host rock would occur beneath it limiting expansion (Figure 2.4A). Lack of exfoliation at the bottom of the pothole would cause growth to preferentially occur on the exposed sides where the black biofilm is still active, leading to the formation of the large, flat-bottomed pools which were observed at SMC. These large potholes would eventually coalesce creating large pans of sediment. This interpretation is supported by observation of the morphology of the potholes found at SMC and large depressions that now play host to an abundance of BSCs (Figure 2.6). The lateral growth of BW potholes was not as obvious, probably due to limited soil accumulation and the friable nature of the sandstone, making it easier to weather deeply through simple mechanical abrasion. The location is also more exposed and winds which will readily remove loose material from the potholes through deflation (Netoff and Shroba, 1993).

In understanding the growth of these potholes, the biological activity may be a driving factor; however, it is limited in its extent by the nature of the substrate and the prevailing environmental conditions as well as competing forces in the pothole

environment. It is likely that the rate of formation of potholes is dominated by fluctuations in climactic conditions that lead to a disequilibrium in the many competing factors discussed above. A season of more frequent wet/dry cycles could cause the biofilms to expand more rapidly, causing greater exfoliation of the host rock. This disequilibrium condition would then be brought into check by the production of a higher than average volume of pothole sediments which would limit biofilm growth directly on the host rock.

Geochemical Influence of the Biofilm

Under purely abiotic conditions, it would be assumed that in a quartz-rich sandstone with carbonate mineral cement normal weathering would preferentially dissolve the cement over time, leaving the more resistant silica mineral grains (Keller, 1957). This relationship was not observed in our study area. Instead XRF results suggest that the sandstone crust from each of the locations had a relative abundance of calcium-bearing minerals (CaO) and carbon-bearing minerals (LOI) compared to silicon-bearing phases (SiO_2) when compared to the host rock. This occurred even at BW, where possible calcium-bearing phases are scarce (Figure 2.4 A). These results indicate that carbonate mineral cement may be precipitating in the biologically-affected crust of the sandstone, whereas silicate minerals may be depleted. Petrographic evidence from the BW sandstone showed the development of a carbonate mineral-bearing crust at the surface of the sandstone, although it was not observed in all samples (Figure 2.11).

Whole rock geochemistry of the host rock suggests that the biofilms affected the overall mineral content of the sandstone crust where they were found. Cyanobacteria,

which are found in abundance in soil crusts and cryptoendolithic communities on the Colorado Plateau (Bell, 1993 and Garcia-Pichel et al., 2001), are able to affect the precipitation of carbonate minerals as a by-product of using HCO_3^- as a secondary source of inorganic carbon (Thompson and Ferris, 1990; Price et al., 2002; Shibata et al., 2002). Because of the low solubility and slow diffusion of CO_2 in water, HCO_3^- is taken up by the cell to act as a secondary source of CO_2, producing OH^- as a by-product and raising the pH of the environment adjacent to the cell. At the cell surface, the waste OH^- ions combine with HCO_3^- in the water to form CO_3^{2-} which in turn combines with cations (e.g. Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}) attached to the bacteria by its naturally net negative surface charge (Figure 2.18) (Braithwaite and Zedef, 1996; Obst et al., 2006). The by-product is a carbonate mineral species, the composition of which depends on the availability of cations in solutions. The major carbonate mineral species identified in other studies is calcite ($CaCO_3$), as calcium is often abundant in natural waters. Also, Ca^{2+} is actively pumped out of bacterial cells to reduce the intracellular concentration (Rosen, 1987), which further increases the Ca^{2+} rich environment found at cell surfaces causing calcite to be preferentially precipitated (Thompson and Ferris,1990). In the pothole host rock crust at SMC only minor amounts of Fe_2O_3, MnO and MgO were observed, compared to the amount of CaO; Whereas at BW even CaO was minimal (Figure 2.7 and 2.8). Also, the concentration of Mg^{2+} in pothole waters was an order of magnitude less than Ca^{2+} (Chapter 3), indicating that calcite would be the major carbonate mineral phase precipitated in the pothole environment.

Geochemistry of Pothole Formation at Seven Mile Canyon

Finely banded growth of a second phase (P2) of carbonate mineral cement was

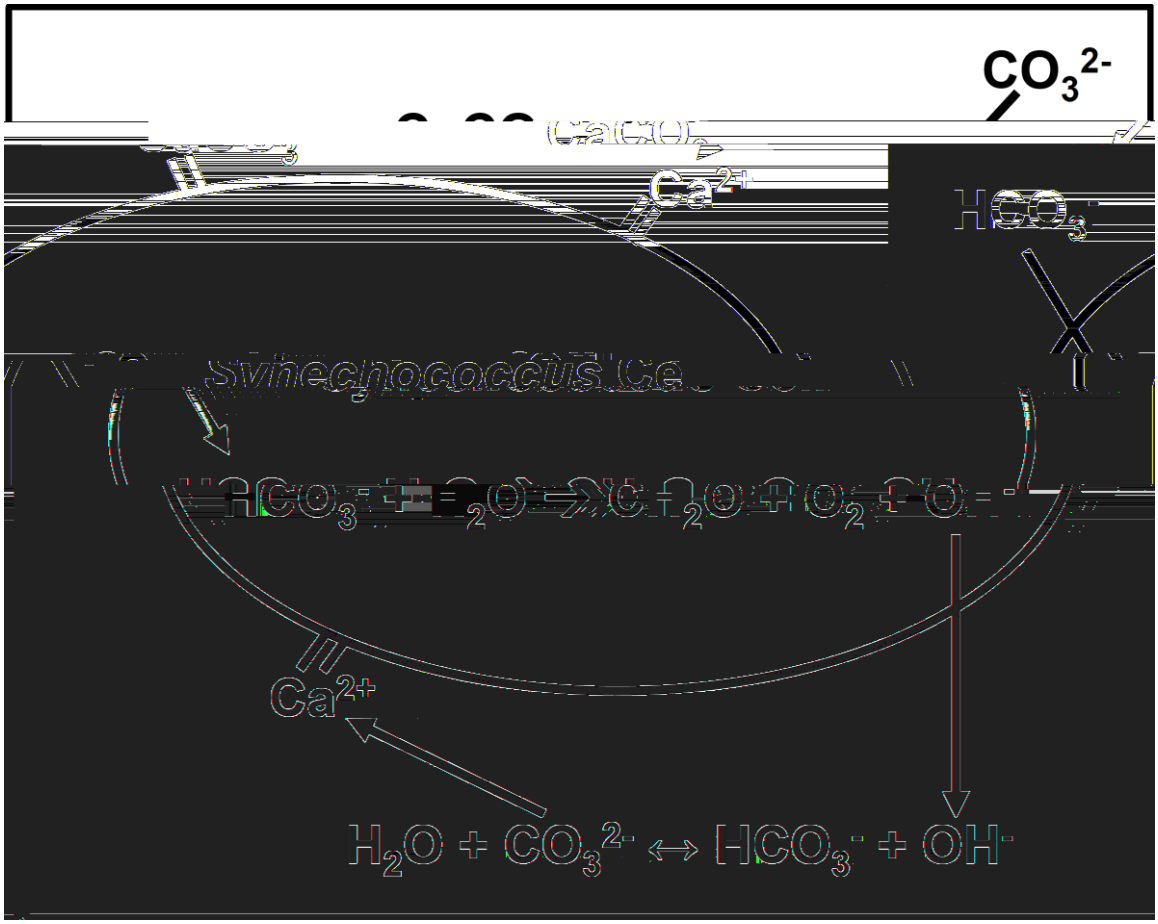


Figure 2.18: Generalised diagram of the geochemistry surrounding a cyanobacterial cell leading to an increase in pH and the precipitation of carbonate minerals (calcite). (Modified after Thompson and Ferris, 1990).

observed precipitating in samples of biologically affected host rock from SMC. In the case of carbonate minerals precipitated as a bi-product of cyanobacterial carbon acquisition, the minerals are nucleated directly on the bacterial surface. If the carbonate minerals on bacteria surfaces were visible under cathodoluminescence they would be finely banded, such as the ones observed, a morphology which would represent the periodic growth of successive cyanobacterial communities based on seasonal wetting of the pothole environment (Obst et al., 2009). Biotically catalysed carbonate minerals precipitate out of solution faster than they would abiotically and so, the fast, localized nature of this carbonate mineral precipitation represented by the P2 carbonate mineral banding may control pothole morphology at SMC. It is proposed that the sides of the SMC potholes are resistant to erosion because of this process of secondary carbonate mineral precipitation, gradually becoming higher than the surrounding area, and slightly rounded, as overlying rock mass is weathered away (Figure 2.4).

As biofilms penetrate the rock surface, weakening it through mechanical disruption, they also produce EPS and precipitate carbonate minerals, reinforcing the area. These competing processes constrain the growth of potholes, creating stable, long-term water sources in the desert. This process is not universal, but dependant mainly on the presence of free divalent cations (primarily Ca^{2+}) in solution. Because the SMC sandstone is more varied than the BW sandstone, there is a higher availability of Ca^{2+}, causing higher volumes of carbonate mineral cement to form adjacent to the potholes, thus making the cemented material more erosion resistant.

Geochemistry of Pothole Formation at Bartlett Wash

In the BW sandstone, only minor carbonate minerals were observed in petrographic investigations of the host rock. SEM-LEO elemental mapping of the biologic zone of the BW sandstone found no discernible calcium and little evidence of any carbonate mineral cementing material (Figure 2.13). The elements observed in the BW sandstone were silica, oxygen, potassium and aluminum, even when measured adjacent to weathered feldspar grains (Figure 2.14). Without carbonate mineral cement and only minor silica cement, the BW sandstone is more vulnerable to weathering and geochemical attack. Because OH^- is a by-product of the cyanobacteria-mediated reactions described above, high pH values are expected in the pothole water. Daily monitoring of pothole water following rain showed diurnal daytime pH values to a maximum of 10 (see Chapter 3). High pH values in the bulk pothole water indicate that even higher pH values may have been reached in micro-environments within the biofilms. This type of anomalously high pH has been observed in other studies of rock pools as well, showing maximum values of 9.62 in granitic ganammas in Spain (Dominguex-Villar et al., 2008) and pHs between 8.7-9.5 in other potholes from Utah (Netoff and Shroba, 1993; Graham, 1999). As the pH increases, the solubility of silica increases exponentially, and is highest at pH values above 9 (Dove, 1995). At higher pH (above pH 6) rates of silica dissolution increase with increasing pH, proportional to $a_{H^+}^{-0.5}$, and are four orders of magnitude higher at pH 11.8 (Rimstidt and Barnes, 1980; Knauss and Wolery, 1988). Proportional silica depletion was observed according to XRF data of both SMC and BW crust sandstone samples. This decrease in the silica content in the biologically-affected crust at both locations relative to the host rock, suggests that silicate minerals are dissolving, or not precipitating as readily as carbonate minerals.

Some precipitation of silica is observed in the form of quartz overgrowths, especially at BW (Figure 2.18). Without the addition of carbonate mineral cement to the potholes at BW, the already weakly-cemented sandstone is further degraded by the high pH, giving the potholes at BW their characteristic cylindrical shape. When compared with the heavily-cemented potholes at SMC, the BW potholes are much deeper (up to 0.5 m), with vertical sides that show macroscopic evidence of additional erosion (Figure 2.4).

Petrographic analysis of samples from BW found silica overgrowth textures as well as ragged dissolution at grain boundaries. Secondary clay minerals were found between grains, most likely representing dissolution products (Figure 2.14). FIB-SEM investigations of the interface between carbonate-poor host rock and biofilm at BW were conducted on both wet and dry samples to identify evidence of host rock dissolution in the presence of a biofilm producing high pH values. Possible amorphous silica deposition was observed in pore spaces in dry biofilm samples while in other areas between grains, clay minerals were observed (Figure 2.16). At the contact between the biofilm and the sand grains an increase in both aluminum and potassium contents was observed, consistent with the development of a weathering horizon. The dry biofilm itself was compositionally similar to the cementing material observed filling cracks between mineral grains, although this similarity may be the result of drying which reduces the volume of carbon rich areas in favour of the solids entrained within. FIB-SEM examination of 'wet' biofilm samples identified plucked feldspathic sandstone grains entrained in the biofilm being weathered to clay minerals along the edges (Figure 2.15). This phenomenon is observed in other studies of silicate weathering by bacteria and is thought to vary spatially within the biofilm (Barker and Banfield, 1996). In the case of

the pothole environment, it is likely that microenvironments within the biofilm concentrate the OH^- ions next to the cell envelope as they are produced, creating finite areas of high pH, increasing silicate solubility by several orders of magnitude (Bennett, 1991).

Finally, other researchers have found large diatom communities in potholes in this area (Chan et al., 2005), and diatom detritus was located by SEM investigations of the biofilms of this study (Figure 2.19). These organisms require silica in their water source to form their frustules, suggesting a ready source of silica is found in the pothole environment.

2.4 Conclusions

The growth of diatoms in pothole water is an example of how bacterial pothole communities support higher forms of life. In the end, potholes that grow deep enough have the potential to host sediments, which then, in turn, support grasses and even small trees. It is this cascade of effects, which begins with the initial colonisation of a water-filled depression that makes these seemingly inconsequential landforms important to the well-being of the desert ecosystem. The ubiquitous nature of bacteria as cryptoendoliths in the host sandstone, the slow growth of biofilms, and the slow weathering of the host rock make the importance of the process easy to discount. However, these types of microbial communities continue to weather host rock substrates presumably over geological time scales.

This work has looked at the interaction of mechanisms, both mechanical and geochemical, biotic and abiotic that combine to produce potholes. No one factor has

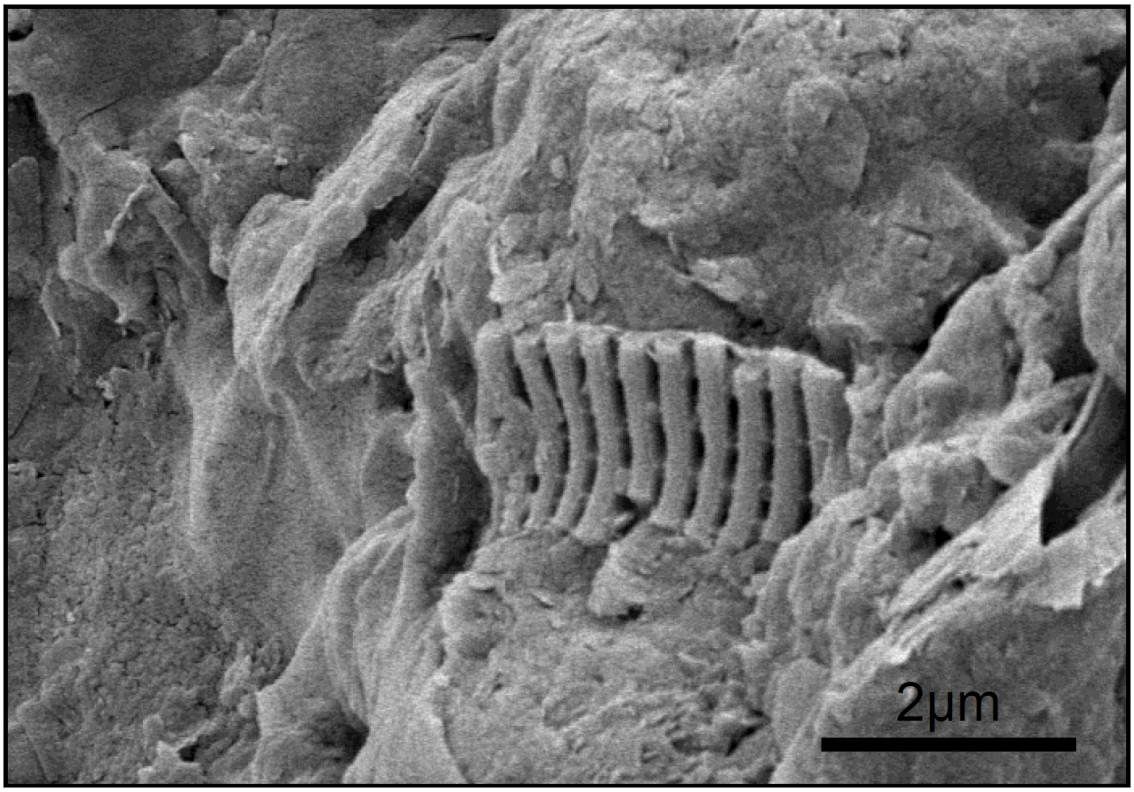


Figure 2.19: Diatom detritus in dried pothole biofilm from SMC.

been identified as all-important except for the presence of water. The many competing factors controlling these environments make them a good example of the balance between the physical and biological world, which is required for the overall well-being of this semi-arid desert environment. Changes in the environmental conditions would lead to biological, and subsequent geochemical and structural, effects: More precipitation would lead to faster biofilm growth and an increase in weathering and sediment production; less rain and the opposite is true, perhaps to the point of eliminating this mechanism for soil production entirely. Increased anthropogenic inputs from human activities would provide a different carbon source to the biofilms, perhaps shifting the species present, creating biofilms that would not produce the high pH needed to weather the host rock efficiently. Careful observation of these types of marginal ecosystems will provide insight into the potential long term effects anthropogenic impacts and climate change will have on the desert environment.

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CHAPTER 3

Nutrient Acquisition in Ephemeral Aquatic Desert Ecosystems

3.1 Introduction

Bacteria are commonly found where there was little expectation that life could survive, including some of the most hostile environments that exist today, e.g., the hot-springs of Yellowstone National Park (Roychoudury, 2004), the deep subsurface (Moser et al., 2003) and the cold, dry deserts of Antarctica (Freidmann, 1982),. In each of these extreme environments biofilms play important roles in ecosystem function. This study looks at biofilms colonising sandstone surfaces in the semi-arid desert environment of the Colorado Plateau where biofilms fill the role of primary producer, and act as an important step in soil formation. In this environment, life is limited by a lack of water and nutrients, almost no soil base, and highly variable climactic conditions. Summers are hot and dry (avg high temperature = 35.1°C, avg precipitation = 18 mm) followed by short intense fall rains (avg precipitation = 2.6 cm) and then a winter when the temperature often falls below freezing (avg low -6°C; avg precipitation (snow = 1.7 cm) (WRCC, 2012). Despite these difficulties, biofilm communities are observed forming on many rock surfaces all around the field area near Moab, Utah (see Chapter 2). In many areas general surficial weathering has evolved into unique features called “potholes”. Potholes represent refugia for desert life where biological processes go beyond simply adding to existing abiotic weathering, to create a feed-back system allowing more fragile forms of life to colonise an otherwise inhospitable environment (Chan et al., 2005). Potholes are depressions in the sandstone surface that vary greatly in size (approximately 5 m to 0.3 m

wide) and depth (approximately 2 m to a few millimeters) (Figure 3.1). At the bottom of larger potholes a granular proto-soil begins to develop. Biofilms in these deep potholes, within the soil/sediment and growing on the host rock, benefit from longer periods of hydration and exposure to external nutrient sources such as trapped, wind-blown particulate matter, which has been shown to be an important source of nutrients in oligotrophic environments (Maranon et al., 2010). It is proposed that the bacteria within the niche pothole environment enhance the weathering of the minerals present, providing nutrients for organisms up the food chain. In oligotrophic marine environments, bacteria-iron interactions increase iron availability in solution causing increased algal growth rates (Keshtacher-Liebson et al., 1995) and in forest soils, bacterial mineral weathering supports the growth of associated plant species (Marschner, 1995; Calvaruso et al., 2006). Because pothole environments are areas of both host rock weathering and proto-soil formation, they are proposed to be an intermediate stage between colonising cryptoendolithic biofilms and biological soil crusts (BSC). BSCs are a type of proto-soil base commonly found in the study area (Johansen, 1993). They are comprised of wind-blown particles held together by a cyanobacterially-dominated biofilm, and are extremely important to the desert ecosystem, being one of the only sources of soil (Garcia-Pichel and Belnap, 1996). They are often observed promoting the growth of grasses and even small trees (Figure 3.2). However, the main focus of this paper is to understand the interactions between the host rock and the initial biofilm that serves as the precursor to the eventual formation of these BSCs. Biofilms growing on or within bare rock have been recognised in many areas of the world (e.g., Antarctica (Friedman, 1980), South Africa (Budel et al., 2004), and previous studies in this field area (Bell, 1986)).

Figure 3.1: Potholes of various sizes and morphologies from the region of the Colorado Plateau near Moab, Utah. **A)** Typical pothole found at Bartlett Wash, with steeper sides due to a lack of cementing material in the host rock. Layering observed is due to primary bedding **B)** A large pothole at Bartlett Wash showing evidence of joint control. Fine layering observed inside the pothole is due to primary bedding **C)** A typical pothole (SMC2) found at Seven Mile Canyon with gently sloping sides due to carbonate cementing material, which makes the host rock more resistant. **D)** Numerous irregular potholes at Pothole Point in Canyonlands National Park.

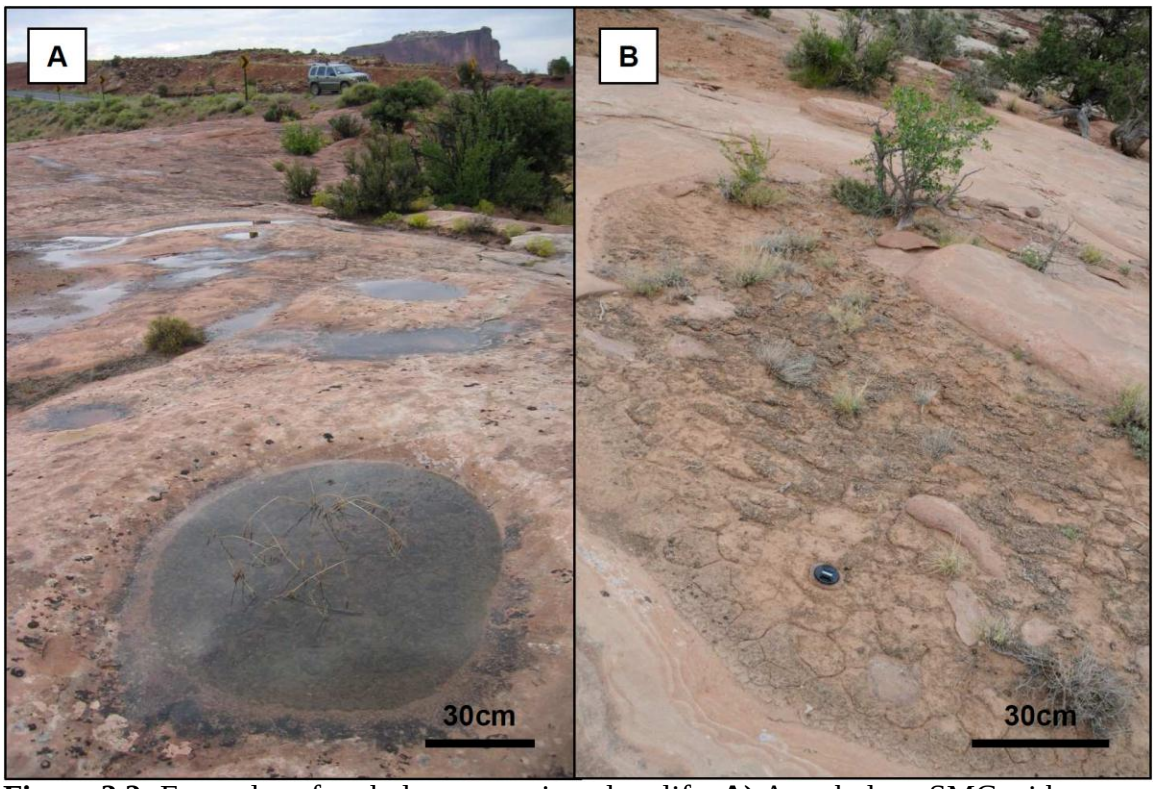


Figure 3.2: Examples of potholes supporting plant life. **A)** A pothole at SMC with a developed sediment base supporting grasses. **B)** A large pan-like pothole area at SMC developing a biological soil crust along with grasses and small shrubs.

In many cases, the biofilms are at least partly cryptoendolithic, living between the grains of the host rock (Walker and Pace, 2007). Several possible reasons exist for this extreme lifestyle: protection from the UV flux by a translucent rock barrier (Freidman, 1982); the existence of a "greenhouse" effect under the rock surface that increases humidity in dry environments (Bell, 1986); and protection from grazing predators such as gastropods (Bell, 1993). There are also limits imposed by the cryptoendolithic habitat; despite the fact that a "greenhouse" effect increases humidity, it also increases the temperature, which has been found to be 10-15°C higher just 2-5 mm under the surface (Bell, 1993). Also, within the rock, the possible sources of nutrients are even more limited than the oligotrophic surficial environment where there is some input from wind-blown material. The key environmental limitation of oligotrophy, the biological response to this condition, and the effect of that response on pothole water chemistry are the focus of this study.

It has been found that living in oligotrophic environments causes a range of biochemical changes in biofilms, such as lower cellular levels of adenosine triphosphate (ATP) and ribonucleic acid (RNA), reduced respiration potentials, and slower response to pulses of nutrients (Konopat et al., 1989). In general, oligotrophic bacterial species have slow growth rates and high substrate affinities that allow them to out-compete other bacteria that have high and specific nutritional requirements, and faster growth rates in nutrient-scarce situations (Tate, 2000). Oligotrophic conditions require bacteria to be more adaptable, and it has been found that individual bacteria grown on nutrient-poor media were able, subsequently, to utilize a wider range of substrates than bacteria grown on complete media (Upton and Nedwell, 1989). These types of adaptations enable

bacteria to colonise surfaces that seem to have little nutrient availability, such as the sandstone host considered in this study, and impact them through physical weathering. In this study area and others, in areas where cryptoendoliths are present, exfoliation of the surface rock is associated with biofilm growth (e.g., Walker and Pace, 2007a). However, opinions vary surrounding the mechanism of weathering, the manner and intensity of biological input, and if the microbiota gain any benefit from these actions (e.g., Sousa-Egipsy et al., 2004; Duane, 2006). In dry environments, just the addition of water to a dry biofilm will cause it to swell and grow within the rock, applying pressure. Biofilms with a large proportion of lichens are thought to promote the breakdown of surfaces with organic acids (Sterflinger, 2000), while in environments with certain species of cyanobacteria, OH^- is produced as a by-product of photosynthesis, causing a high pH environment that weathers the host rock (Budel et al., 2004). These possible mechanisms assume that the degradation of the substrate is simply a by-product of the presence of the biofilm, but studies have now been done showing bacteria may preferentially target minerals for the purpose of extracting nutrients (e.g., Brantley et al., 2001)

The formation of soils, such as those found at the bottom of potholes and in BSCs in the field area, is controlled mainly by mineral weathering (Anderson, et al., 2000 and Mavris et al., 2010); however, investigation of microbial enhanced weathering occurring for the acquisition of nutrients is limited (Barker et al., 1998; Uroz et al., 2009). Studies of sandstone weathering have suggested that sand grains may contain important micronutrients, such as Zn, Co, Cu, Ni and Mo (Bennett et al., 1996 and 2001), making the expenditure of the energy necessary for weathering host rock worthwhile in a nutrient-starved environment. Perhaps more telling, cultures from oligotrophic lithic

environments were found to grow better when crushed samples of the host rock were added to the media (Siebert et al., 1996) and cultures from a glacial forefield were able to grow on a minimal media with only powdered granite and glucose-NH_4Cl (Frey et al., 2010). Work identifying the efficiency of acidic mineral weathering by a specific genus of bacteria concluded that the observed traits were an adaptation to nutrient poor conditions, which allowed the bacteria to solubilize inorganic phosphorus (Uroz et al., 2009).

Basic forms of life tolerant of nutrient limitation would not survive without the presence of water. Rain is especially important in the Moab feild area as it washes particulate matter from the atmosphere and surficial debris into the potholes providing an important nutrient source. The purpose of this work was to distinguish the nutrient input of rain events from that of nutrients already present in the pothole environment. Unique weathering conditions in the potholes were examined, with particular attention to the role of pH to determine if elevated pH environments can contribute to nutrient release through mineral weathering as was previously proposed for more neutral pH environments involving the action of chelating agents or organic acids (e.g., Barker et al., 1998; Bennett et al., 2001; Uroz et al., 2009).

3.2 Methods

General Field Site Characterisation

Potholes are found in abundance throughout the area surrounding the city of Moab in South Eastern Utah, USA (Figure 3.3) and can be divided into two main groups:

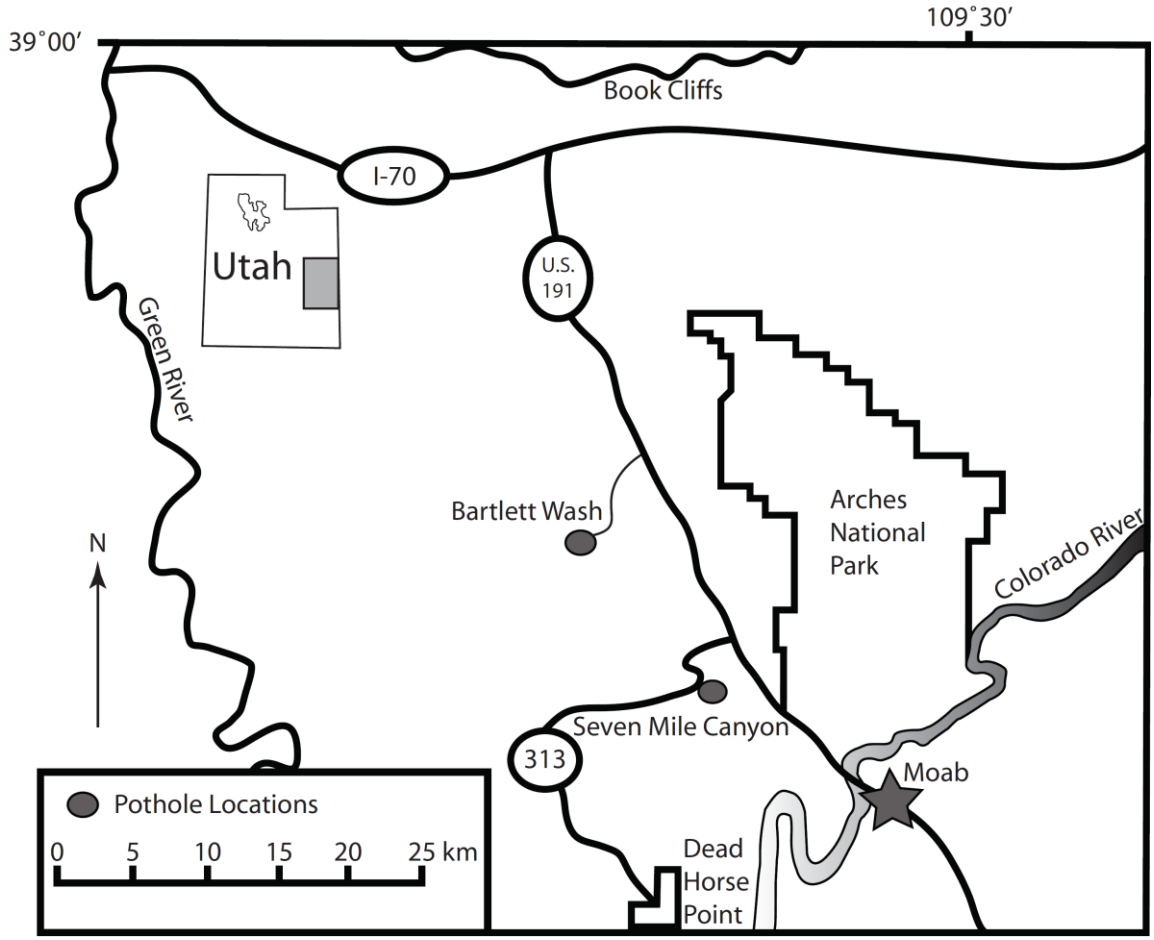


Figure 3.3: Field locations near Moab, Utah: Bartlett Wash (BW) (N 38 degrees 42.662;W 109 degrees 47.146; 1516 feet) and Seven Mile Canyon (SMC) (N 38 degrees 38.648;W 109 degrees 43.862; 1482 feet) (after Chan et al., 2005)

joint-controlled and independent potholes. Joint-controlled potholes form in areas where jointing in the sandstone host channels water during periods of rain, leading to potholes occurring in series along the joint. Independent potholes are found away from any obvious water channelling, usually on slightly sloping surfaces, forming without a pattern (Figure 3.4). Independent potholes were chosen for this study to eliminate the erosion by running water when considering their formation.

In and around the study area, the roughly circular, independent potholes observed ranged from 30 cm to 2 m in diameter and from millimetres to over a meter in depth. The depth to width ratio is largely dependent on the host rock composition (Chapter 2) and various different morphologies are observed (Netoff and Shroba, 1993). Intuitively, these different pothole morphotypes support different bacterial communities depending on the development stage of the potholes. The initial stage of pothole development is surficial colonisation by biofilms. In some locations, the biofilms are millimetre-scale thick black layers covering the bare rock surface in patches. During the rainy season, intense storms flood the sandstone surfaces with water and any irregularities, due to biofilm surface exfoliation or just natural variation, are filled. Preferential ponding of water creates a positive feedback, allowing larger biofilms to be supported and trapping wind-blown material. Eventually the enhanced biofilms cause weathering of the initial depression into a defined pothole (Figure 3.4). Potholes can retain water for days or weeks at a time depending on their depth and climactic conditions.

Description of Specific Field Sites

The field site at Seven Mile Canyon (SMC) is host to an extensive array of

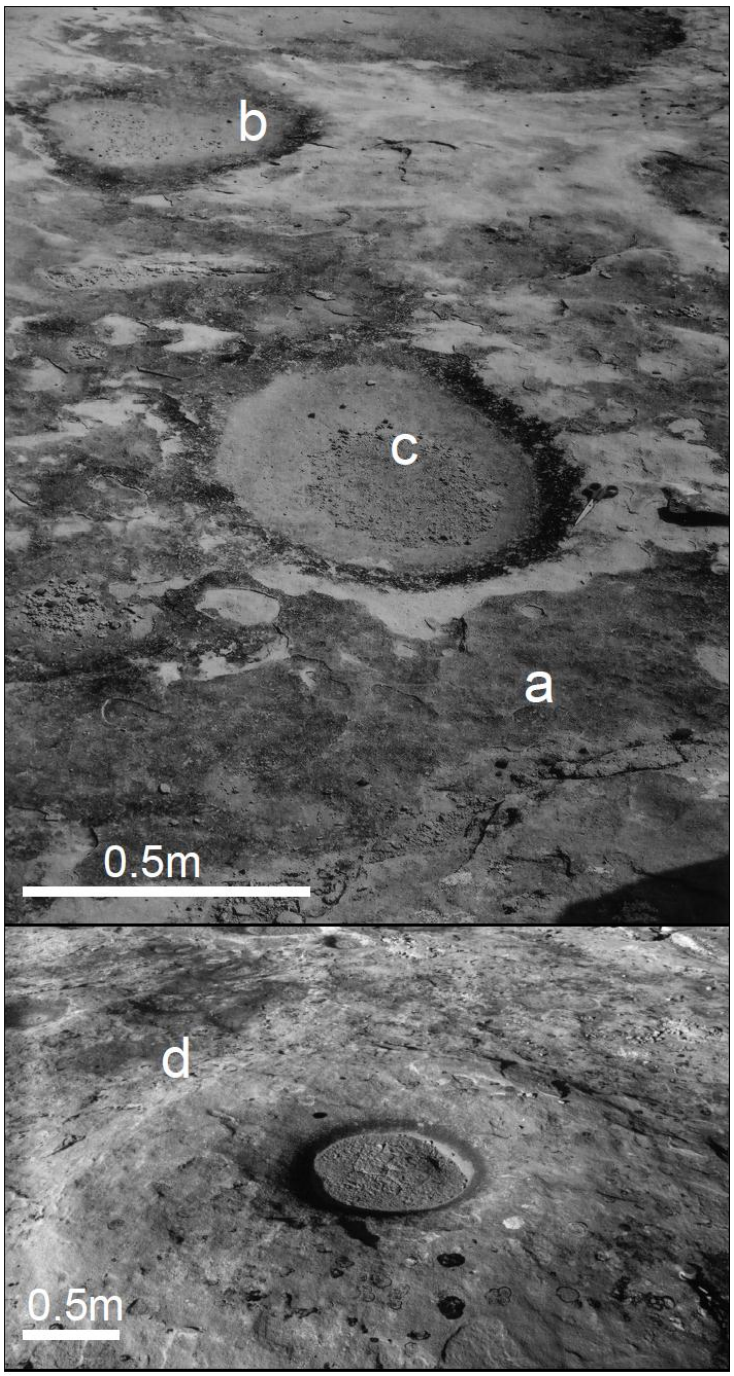


Figure 3.4: Stages of pothole development: Initial irregular surficial weathering with, black areas indicating the presence of biofilm while lighter areas have been exfoliated **(a)**. Surface irregularities develop into preferential depressions where water is retained longer **(b)**. As the depressions become deeper they become circular and rounded because of the ponding of rain-water. The edges become steeper and a sandy-soil may develop at the bottom **(c)**. A fully developed pothole with raised sides due to precipitation of secondary carbonate mineral cement. The bottom sediments are 3 cm deep **(d)**.

potholes, none of which are joint-controlled (Figure 3.5). The site is a bench of Kayenta Formation arkose-arenite sandstone which gently slopes south towards a steep drop off into Seven Mile Canyon. It was formed during the early Jurassic (approximately190 -200 Ma) (Doelling, 2003). The sandstone at SMC has a diverse composition due to its fluvial origins including minimal feldspar grains and other accessory minerals (Doelling, 2003) and a distinct pink-red-orange iron oxide stain from past periods of digenesis. Potholes cover the surface showing a wide range of sizes and stages of development, from amorphous areas of surficial black biofilm to distinct circular potholes built up from their surroundings. Potholes used for this study were generally wide, shallow pans of the type described by Netoff and Shroba (1993) with width to depth ratios of approximately 10:1, although the range of variability at SMC goes from approximately 5:1 up to 20:1 depending on the age of the potholes (Chapter 2).

The second field site at Bartlett Wash (BW) was chosen for comparison because it is slightly more isolated and at times inaccessible; therefore, it has less chance of anthropogenic influences (All Terrain Vehicles). The potholes at Bartlett Wash are also morphologically different from those at Seven Mile Canyon, which presumably reflects variation in the host sandstone. The BW sandstone is a part of the Moab member of the Curtis formation, a massive, aeolian, cliff-forming unit laid down between approximately 158 to 155 Ma (Doelling, 2003). At the field location the sandstone is a quartz-arenite, bleached and friable with very little cementing material of any kind. Potholes used for this study were generally circular shallower versions of the “cylinder” type as described by Netoff and Shroba (1993) with a large variation in width to depth ratios of approximately 2:1 to 5:1. Potholes from both field sites were chosen based on their

Figure 3.5: Overview of SMC showing a gently sloping surface with no joint control developing distinct potholes.

morphology, *i.e.*, only potholes deep enough to hold water over longer periods, which allowed for a more detailed biogeochemical study.

Field Water Sampling

Three field studies were conducted under two sets of wetting conditions: natural (*i.e.*, rain wetting conditions), and follow-up studies using distilled deionised water (DDI) (approximately pH 7) to examine the geochemical response of the biofilms to wetting under nutrient limited conditions and to try and distinguish the contribution of rock weathering from rain input in the aqueous pothole environment. The experiments were conducted primarily at the SMC field location due to accessibility, although sampling did occur at BW, as possible. In the first sampling campaign, potholes naturally wetted with rain were sampled once a day at 7 am until the potholes dried (3-5 days). Rain samples were collected separately in plastic pails cleaned with DDI. In the second sampling campaign, dry potholes were filled up to the high water mark (*i.e.*, the extent of the black biofilm) with DDI water, and allowed to dry naturally after filling. In the third campaign the potholes were again filled with DDI, however, the water level was topped-up daily to monitor water chemistry over a longer period of time. In the DDI studies, water samples were taken from each study pothole three times a day: in the early morning (between 7-8 am), at mid-day (from 12-1 pm) and in the evening (6-7 pm) (or as often as was allowed by the conditions). No rain fell during the period of DDI water sampling from the potholes. At each sampling, two 15 ml samples from each pothole were filtered with 0.45 μm filters and placed in 15 ml falcon tubes. One of the filtered samples was acidified with nitric acid to a pH of ~2 for cation analysis. All samples were kept in the dark at

4°C until analysis. Dissolved oxygen, pH and conductivity measurements were taken on site using a Hatch Sension 156 portable multiparameter meter.

Laboratory Abiotic Weathering Control Experiment

For the abiotic laboratory control study, a 250 g sample of sterilised (autoclave, 121°C, 30 min) host rock from Seven Mile Canyon rock sampled from the field location using an 8 lb sledge hammer. The sample, which did not contain biofilm, was placed into a 1.0 l beaker with 500 ml of DDI water. Field day/night conditions were simulated using a full spectrum light bulb with light exposure timed to 14 h of daylight. Water temperature was monitored hourly for the first light period, to ensure that it fell within the range of daytime water temperatures encountered in the field, and at each sampling thereafter, for 7 days. The sample was completely covered with water to simulate the high surface area: volume ratio of the potholes.

Laboratory High pH Leach Experiment

Samples of host sandstone representing a zone at least 5 cm below the rock surface were selected, which represented an area of sandstone still within the abiotic weathering zone but not colonised by biofilm. The sandstone was lightly crushed by hand and rubber mallet to liberate individual sand grains and sterilised in an autoclave at 121°C for 30 min. The samples (approximately 2.3 kg each) were then divided into 7.5 cc (approximately 9 g) portions in 15 ml falcon tubes. A beaker filled with stirred DDI water was adjusted to various pH values for the study using titration with NaOH as necessary from pH 9 to pH 12 in 0.5 increments. NaOH was chosen for adjusting pH in

the system since $NaCl_{(s)}$ occurred during natural drying of the potholes, so Na^+ is expected to be abundant in the natural system. The samples were allowed to sit for one week and then filtered at 0.45 μm and divided into two 15 ml falcon tubes for chemical analysis. Nitric acid was added to one tube until a pH of approximately 2 was reached for cation analysis. The second tube was not amended and used for anion analysis.

Water Chemistry

Concentrations of cations and anions were measured using a Perkin-Elmer Optima 3300DV inductively-coupled plasma-atomic emission spectrometry (ICP-AES) and Dionex IC-3000 ion chromatograph (IC), respectively. All blanks of DDI water were below detection limits for cations and ions.

Electron Microscopy

Samples of dry biofilm that were collected from the potholes prior to any wetting in the field were stored dry, in the dark at 4°C until use. In the laboratory, some biofilm samples were re-hydrated with rain-water under conditions of natural light, while others were left as flakes on polished pucks of the host sandstone in the dark, and re-hydrated with DDI water to simulate colonisation under nutrient-limited conditions. After a period of hydration and growth at room temperature for two weeks to simulate the longer retention times of larger potholes found at the field sites and the cooler temperatures encountered in the fall and spring when the potholes are naturally filled with water, samples were fixed with $2\%_{(aq)}$ glutaraldehyde and dehydrated sequentially through 30 min immersions in 25%, 50%, 75% and 100% ethanol solutions. The samples were then

critical point dried (Tousimis, Samdri-PVT-3B), affixed to carbon tacks and gold coated with a Denton Vacuum Desk II for analysis with a scanning electron microscope equipped with a focused ion beam (FIB) for cross-sectioning of interesting samples (Leo 1540 XB FIB/SEM) at 5 kV. FIB milling was carried out on the Leo 1540 XB FIB/SEM using a Ga^+ ion beam.

Polished geological thin sections were also analyzed at 20 keV on a Leo 440 SEM equipped with a Gresham light element detector and a Quartz Xone Energy Dispersive X-ray (EDX) analysis system.

3.3 Results

Upon sampling pH values from potholes in the field (up to three times daily) high daily pH values were quickly identified in most pothole samples. Measurements of pH in individual potholes wetted by rain ranged from circumneutral in newly filled potholes and rose, by the time of the next sampling, to pH values that averaged 8.5 to 9.1 (see Appendix B), depending on the pothole. In a separate field campaign, daily cycling of pH values was evident both in potholes that were refilled repeatedly with DDI, and those that were allowed to dry completely (Figure 3.6). The cycling was also noted in the concentration of some key ions, discussed in detail later. In the DDI water experiment, morning pH readings ranged from 8.2 to 8.8, which is consistently lower than the peak reached by the late afternoon that ranged between 9.5 and 10.1 (See Appendix B). In contrast, an abiotic laboratory control showed no cycling of pH, and reached a stable value of pH 8.35 $\pm$ 0.10 after an initial period of equilibration (48 h).

As well as studying field parameters water samples of natural rainwater and rain-

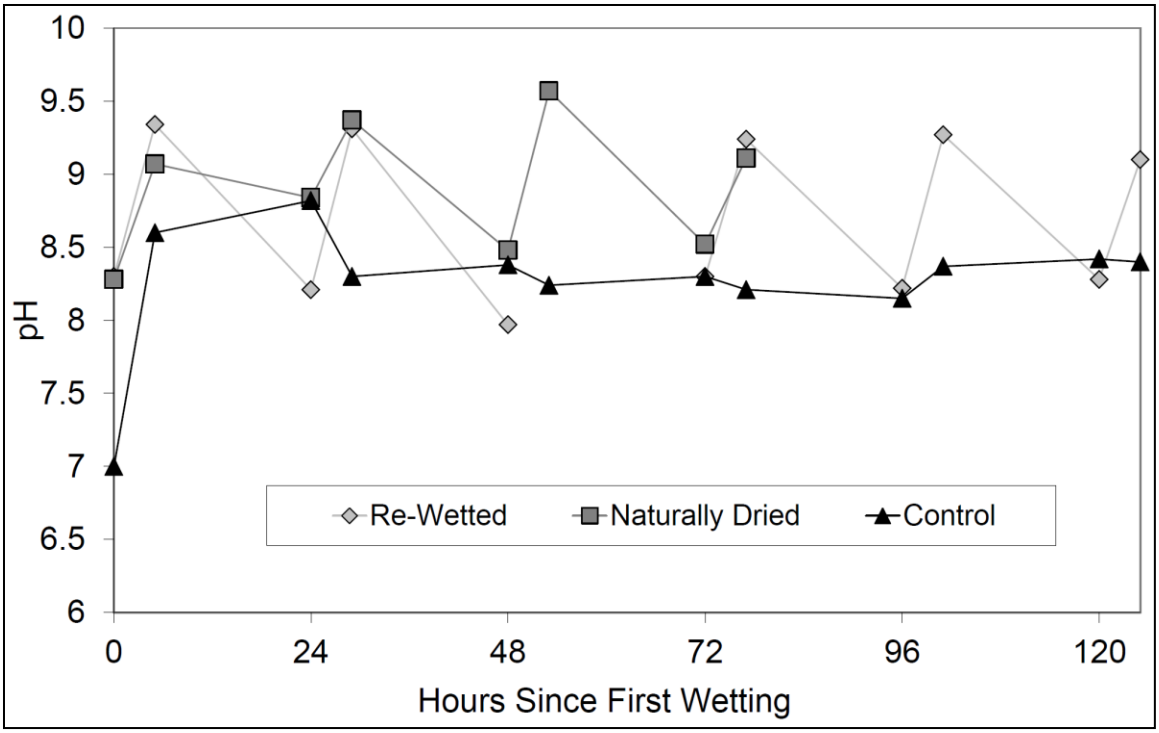


Figure 3.6: Diurnal cycling of pH in artificially wetted potholes. Lower pH values represent morning samples and the peaks represent samples taken at noon. Field samples are contrasted with an abiotic laboratory control that showed no diurnal pH cycle.

wetted pothole water were collected and compared. The rain samples were taken from two rain events that occurred approximately 24 h apart and those samples were used to identify any contribution of the rain water to the nutrient limited pothole conditions. The rain was sampled from two SMC potholes (SMC1 and SMC2) (Figure 3.7). Pothole #1 (SMC1) is a distinct circular pothole with built-up sides and a sediment depth up to 3 cm, and pothole #2 (SMC2), a distinct oblong pothole with a thin rocky sediment approximately 1 cm deep. Sediment depths varied among the potholes observed at SMC (Table 3.1) and SMC1 and SMC2 represented end members of the spectrum of sediment thickness. Rain-wetted potholes were intentionally sampled at regular intervals 24 h apart to eliminate any potential variation from diurnal cycling, which was well resolved in subsequent artificial wetting experiments. Instead, the rain wetted observations presented overall trends in ionic concentrations over the period of wetting (Figure 3.8).

The most common trend observed in rain wetted potholes was an increase in the concentration of all ions measured as the potholes dried, except for concentrations of P and PO_4^{3-} which were very low overall, and in the case of P, decreased slightly. Once the potholes were filled with rain, although general increasing trends were observed in most ions, several ions showed plateaus in the data particularly in concentrations sampled from pothole SMC2. In particular Ca^{2+} concentrations in SMC2 rose only 4.63 mg/L over the first 96 hours of wetting and then jumped in the last 24 hours an additional 7.55 mg/L. Magnesium also showed a distinct plateau in concentrations increasing only 0.24 mg/L in 96 hours and then an additional 0.5 mg/L in the final 24 hours.

When observing the aqueous ionic concentrations present in the rain itself all measured ions except for silica (Si) were present in the first rain event, but absent or

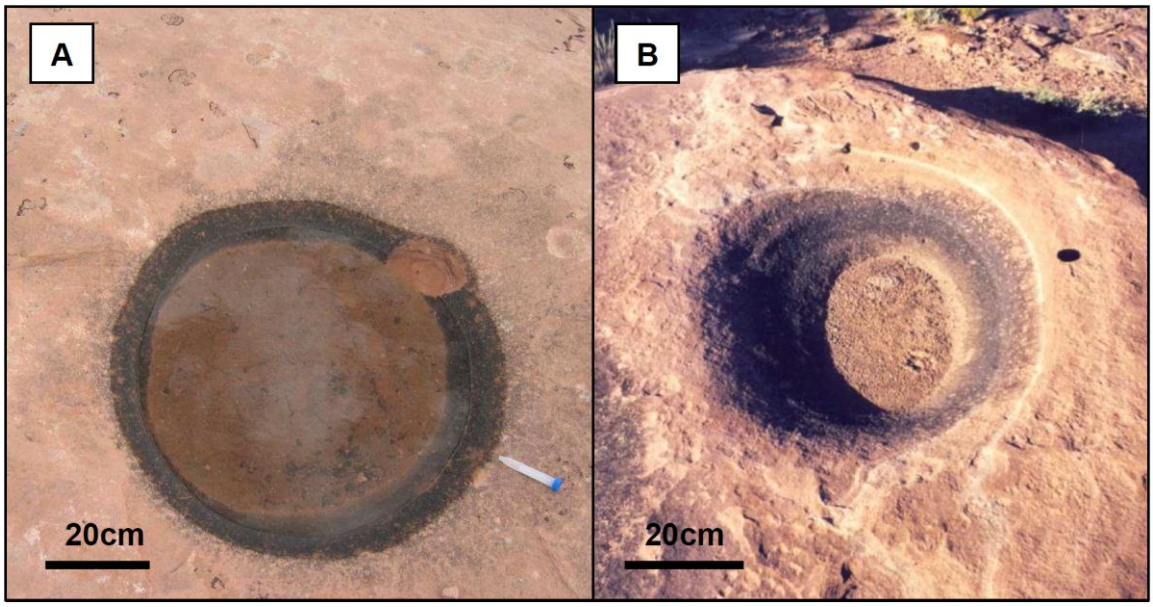
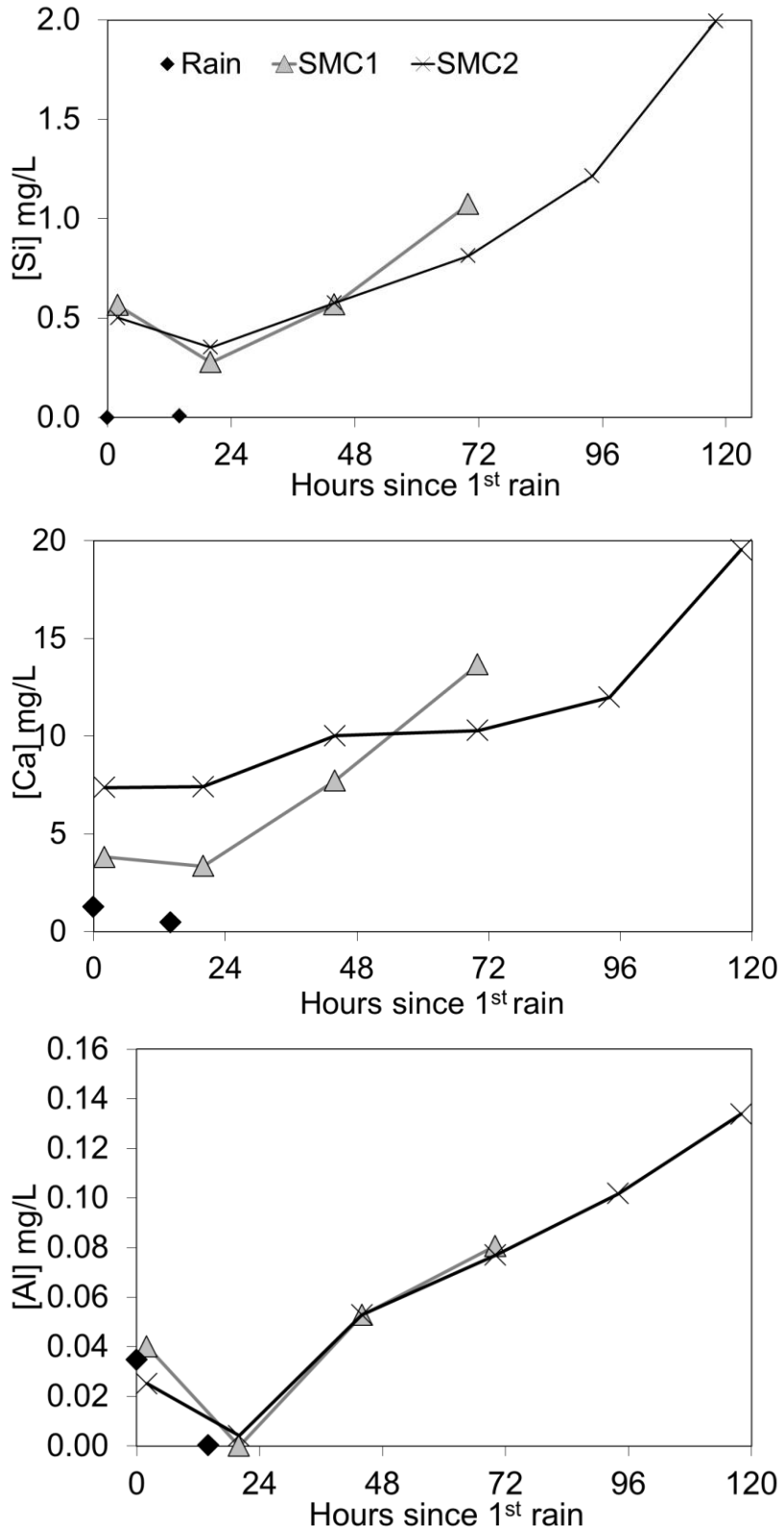
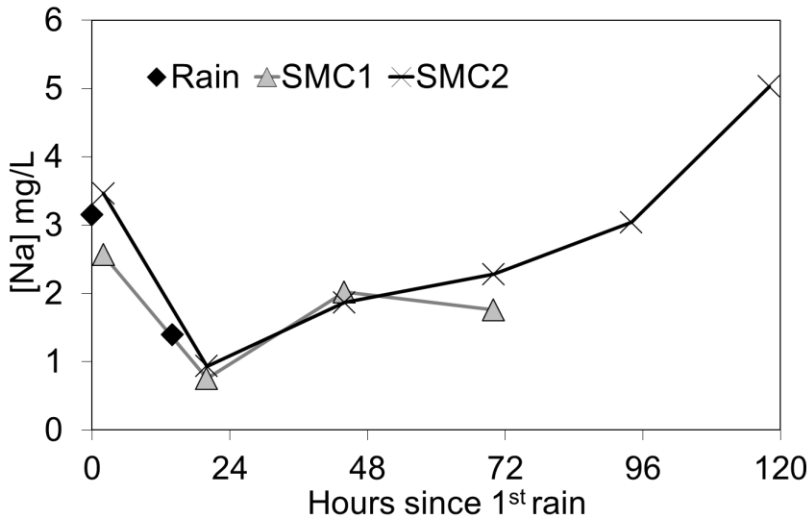


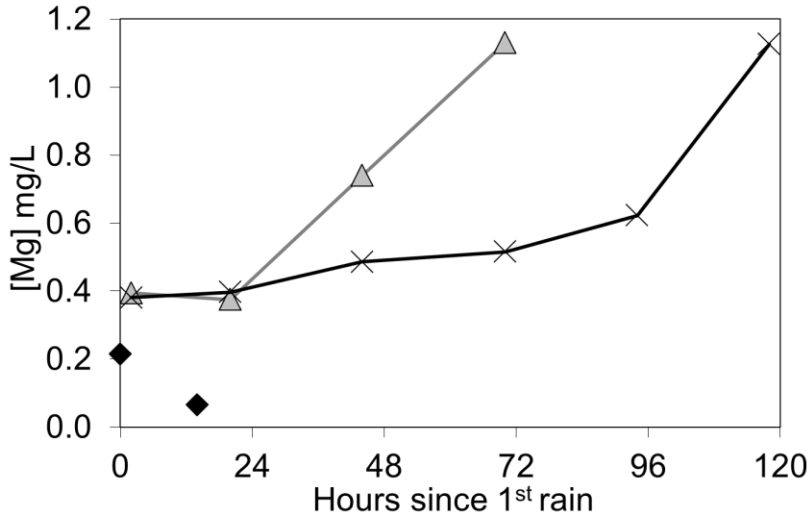
Figure 3.7: Potholes sampled in rain wetting experiments at SMC. **A)** SMC1 **B)** SMC2.

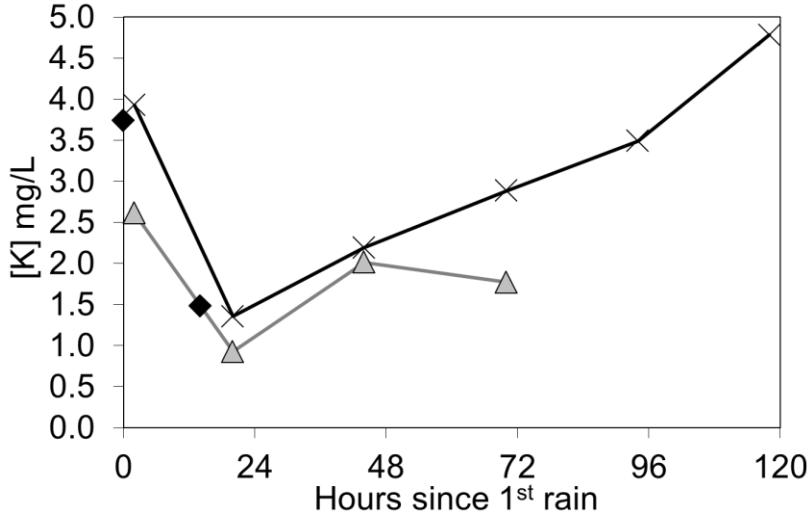
Table 3.1: Depths of potholes and their sediments at SMC

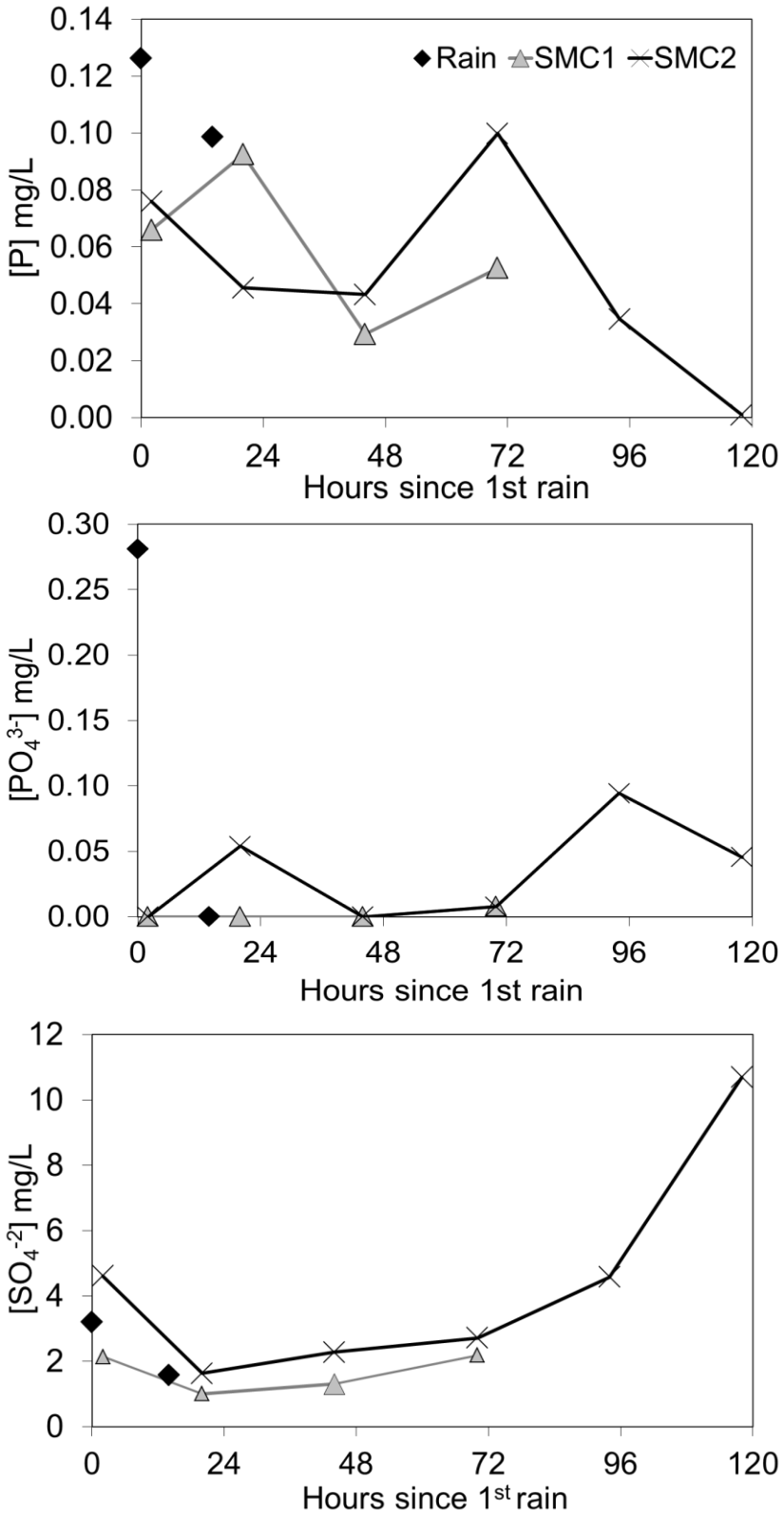
	Depth total (cm)	Depth Soil (cm)
SMC1	7.7	3.4
SMC2	9.2	0.5
SMC3	2.7	0
SMC4	2	0
SMC5	6.7	0.4
SMC6	7.8	2.2
SMC7	5.8	1.9
SMC8	9.5	3.6


Rain
SMC1
SMC2
[Si] mg/L
2.0
1.5
1.0
0.5
0.0
0
24
48
72
96
120
Hours since 1st rain
[Ca] mg/L
20
15
10
5
0
0
24
48
72
96
120
Hours since 1st rain
[Al] mg/L
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
24
48
72
96
120
Hours since 1st rain


Rain
SMC1
SMC2
[Na] mg/L
0
1
2
3
4
5
6
0
24
48
72
96
120
Hours since 1st rain


[Mg] mg/L
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
24
48
72
96
120
Hours since 1st rain


[K] mg/L
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
0
24
48
72
96
120
Hours since 1st rain


Rain
SMC1
SMC2
[P] mg/L
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
24
48
72
96
120
Hours since 1st rain
[PO4 3-] mg/L
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
24
48
72
96
120
Hours since 1st rain
[SO4 -2] mg/L
12
10
8
6
4
2
0
0
24
48
72
96
120
Hours since 1st rain

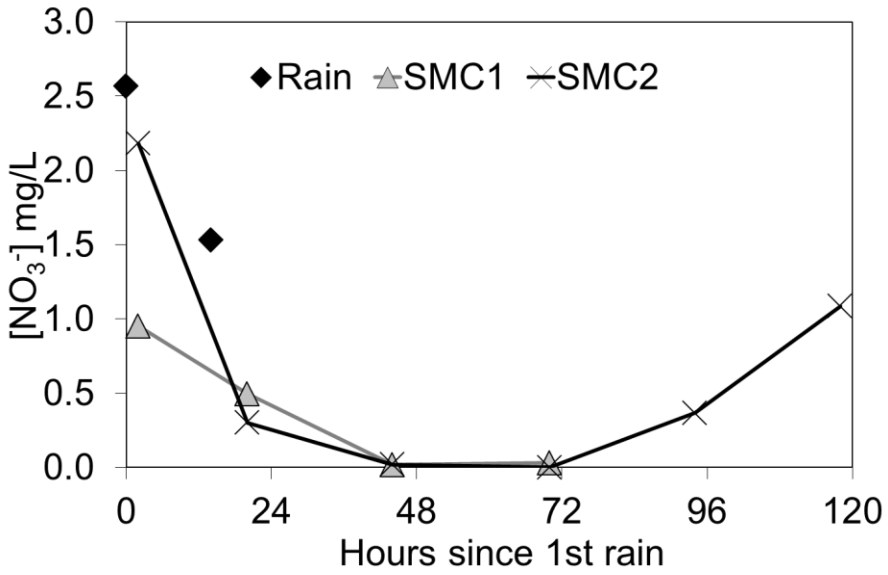


Figure 3.8: Ionic chemistry of rain events and rain filled potholes. Concentrations of ions in two rain events are shown with black diamonds. Note the decrease in aqueous concentration of most ions between the first and second rains. Also note the increasing trend in ionic concentrations in both potholes as they dry, which is not observed in P and PO_4^{3-} concentrations. The concentrations of Ca^{2+}, Si and Mg^{2+} are greater in the pothole waters than observed in the rain while the concentrations of P, PO_4^{3-} and NO_3^- are higher in the rain than observed in the potholes. PO_4^{3-}, SO_4^{2-} and NO_3^- were measured using IC as described in the methods section while all other values were measured with ICP-MS.

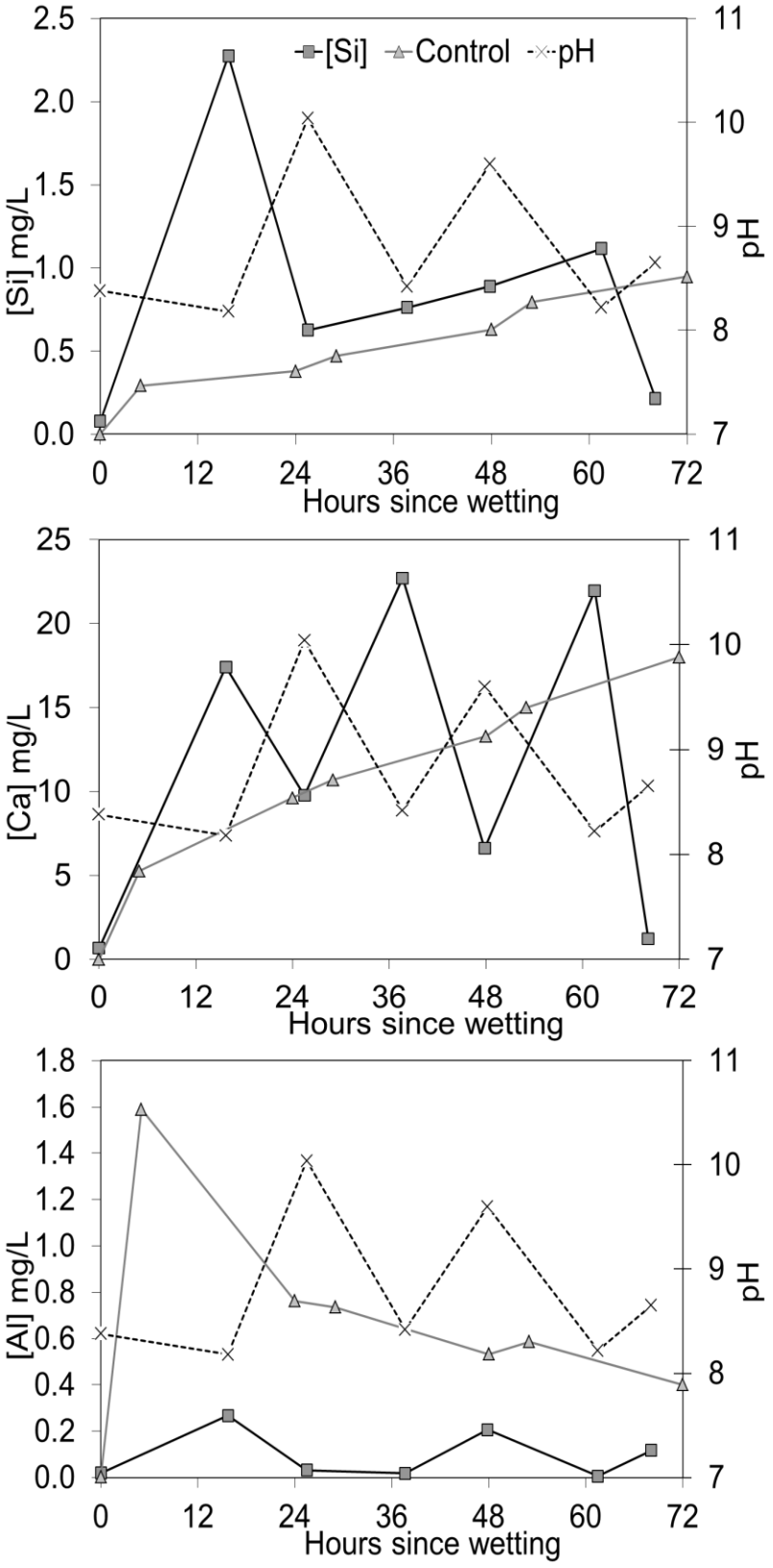
reduced in the second, including calcium (Ca^{2+},Δ_{1-2} -0.8 mg/L) magnesium (Mg^{2+}, Δ_{1-2} -0.15 mg/L), potassium (K^{+}, Δ_{1-2} -2.26 mg/L), aluminum (Al^{3+}, Δ_{1-2} -0.035 mg/L), sodium (Na^{+}, Δ_{1-2} -1.75mg/L), phosphate (PO_4^{3-}, Δ_{1-2} -0.28) (P, Δ_{1-2} -0.028 mg/L), sulphate (SO_4^{2-}, Δ_{1-2} -1.6 mg/L), and nitrate (NO_3^{-}, Δ_{1-2} 1.03 mg/L) (Figure 3.7). Results showed that the loading of nutrients from rain appeared to be variable and may be controlled by other environmental factors.

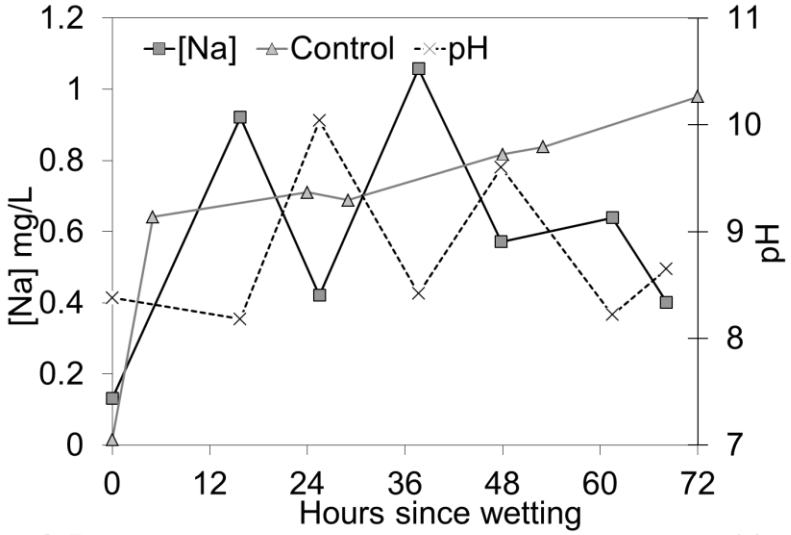
The aqueous ionic concentrations observed in the potholes beginning two hours after the first rain event indicated three types of ionic response to the addition of rain water: first ions that represent important biological nutrients were measured in the rain but the concentrations in the rain were not reflected in the pothole water. This disparity between concentrations in rain and potholes was observed for both PO_4^{3-} and NO_3^{-} where the concentrations of these ions in solution in the pothole were reduced to near the detection limit with in the first 24 hrs. Secondly, other ions had lower concentrations in the rain samples than were observed in the pothole water, including Ca^{2+} (rain 1.26 mg/L, SMC1 1st sample 3.80, SMC2 1st sample 7.35 mg/L), Mg^{2+} (rain 0.21 mg/L, SMC1 1st sample 0.39 mg/L, SMC2 1st sample 0.38 mg/L) and Si (rain 0.001 mg/L, SMC1 1st sample 0.57 mg/L, SMC2 1st sample 0.50 mg/L). This indicates a soluble source of these ions existed in the potholes themselves. Finally, the remaining ions (Al^{3+}, Na^{+}, K^{+} and SO_4^{2-}) had concentrations measured in both SMC1 and SMC2 that tracked closely with the concentrations observed in the rain-only samples. This suggests that these ions are neither limiting in the pothole environment nor quickly released into solution from sources in the potholes.

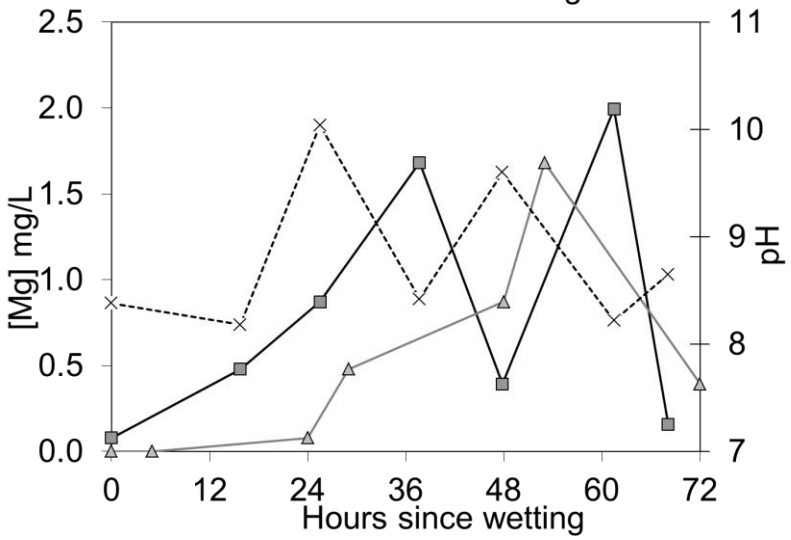
After gaining a better understanding of a naturally rain-wetted pothole system nutrient limitation experiments were conducted by filling dry potholes with DDI. Samples of pothole water were taken to track the baseline nutrients available to the biological community in the same two SMC potholes (SMC1 (Figure 3.9a) and SMC2 (Figure 3.9b)) and compared against a sterile laboratory control. Concentrations of several ions in both potholes including, Mg^{2+}, K^{+}, Na^{+}, S and Si were significantly higher in the biotic, pothole systems relative to the control while Al^{3+} was significantly lower.

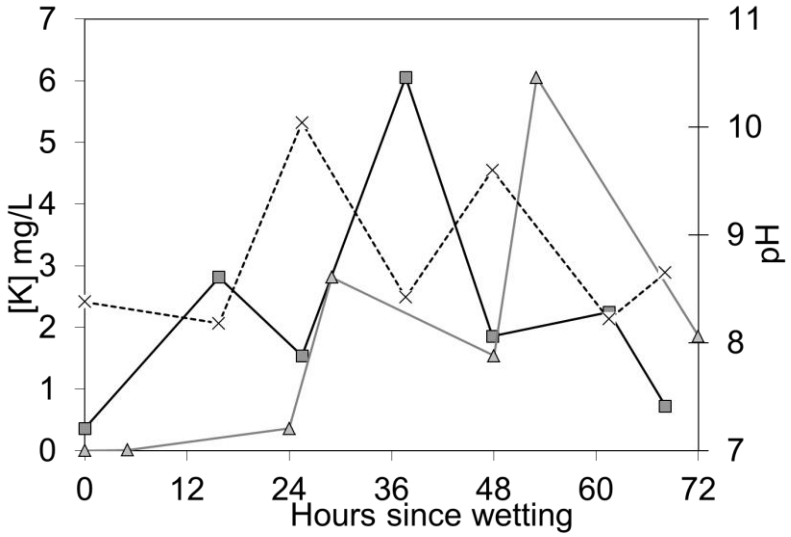
Ionic concentrations from SMC1 fall broadly into two categories: those that show diurnal cycling and those that do not. Diurnal cycling was observed in Ca^{2+}, Na^{+}, Mg^{2+}, K^{+} and P concentrations. The most pronounced cycling was observed in Ca^{2+} where concentrations were higher at night (>16 mg/L) and lower in the day (<10 mg/L). Only Al^{3+}, Si and S concentrations showed no or little evidence of diurnal influence. The control values all increased as the water volume decreased but no diurnal cycle was observed. The concentrations of several ions sampled did show variations in the control values apart from increasing concentration (e.g. Al^{3+}, Mg^{2+}, P and K^{+}) with drying, but natural variability in the more labile elements in the host rock may explain this variation.

In samples of water taken from DDI wetting of PH2 increasing concentrations of measured ions were readily observed with drying however no diurnal changes in chemistry were observed. Only concentration of P in solution did not increase with drying, and the concentration of P was lower than the observed concentrations of most other ions (+/- 0.5 mg/L). The concentrations of Si and Ca^{2+} closely tracked the abiotic control. The values of other ions such as Na^{2+}, Mg^{2+}, K^{+}, S and Al^{+} were higher than the control which may indicate the influence of salts accumulated in the pothole that were not


[Si]
Control
pH
[Si] mg/L
pH
Hours since wetting
[Ca] mg/L
pH
Hours since wetting
[Al] mg/L
pH
Hours since wetting


[Na]
Control
pH
[Na] mg/L
pH
Hours since wetting


[Mg] mg/L
pH
Hours since wetting


[K] mg/L
pH
Hours since wetting

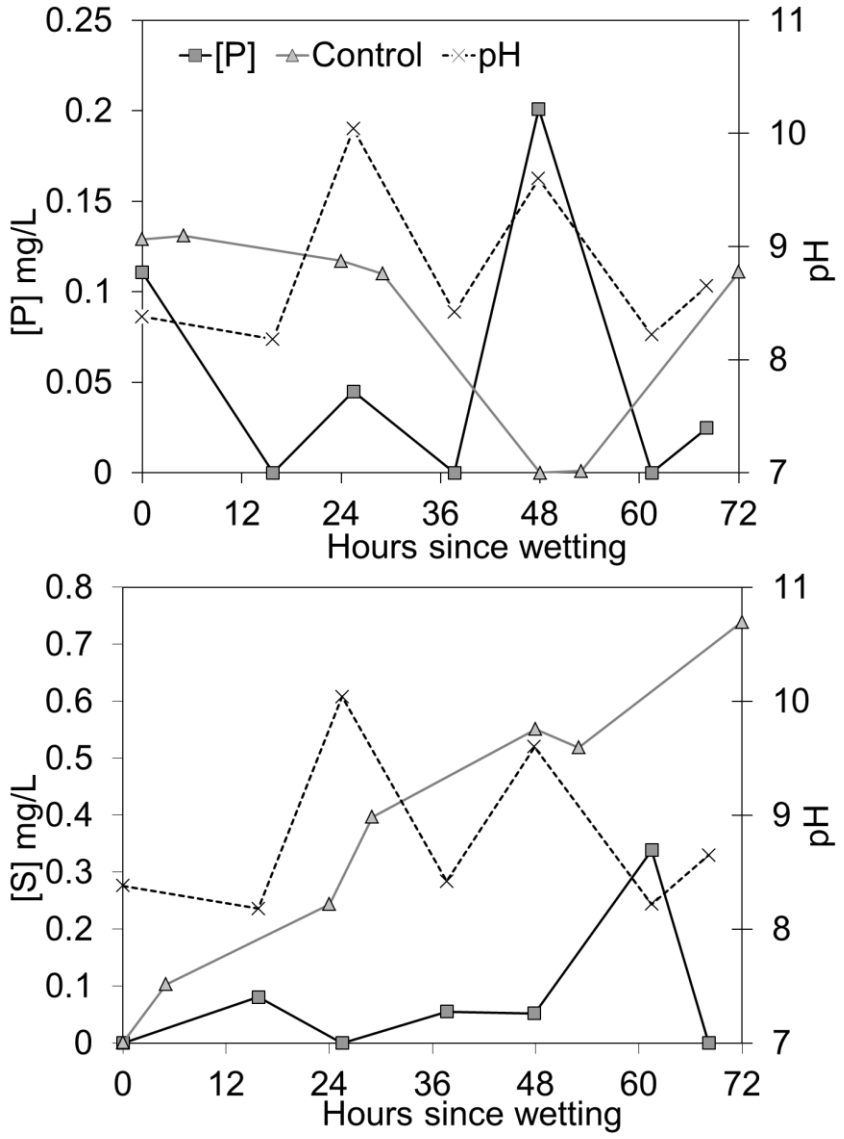
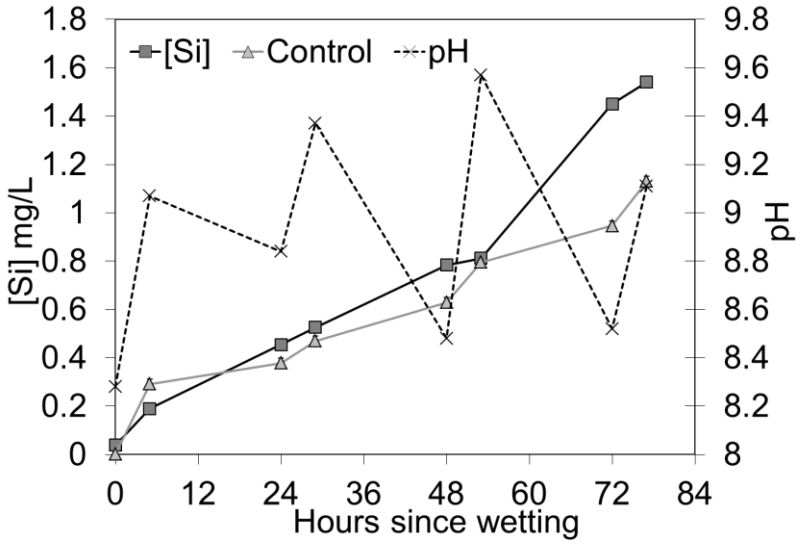
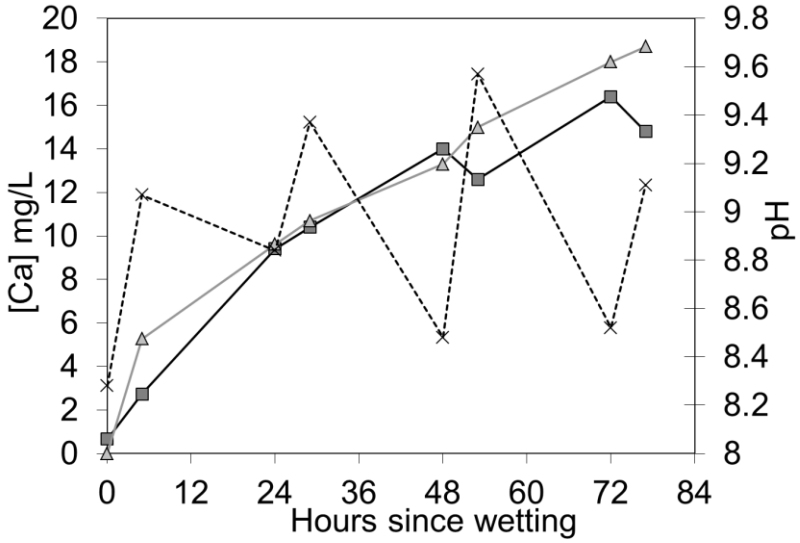
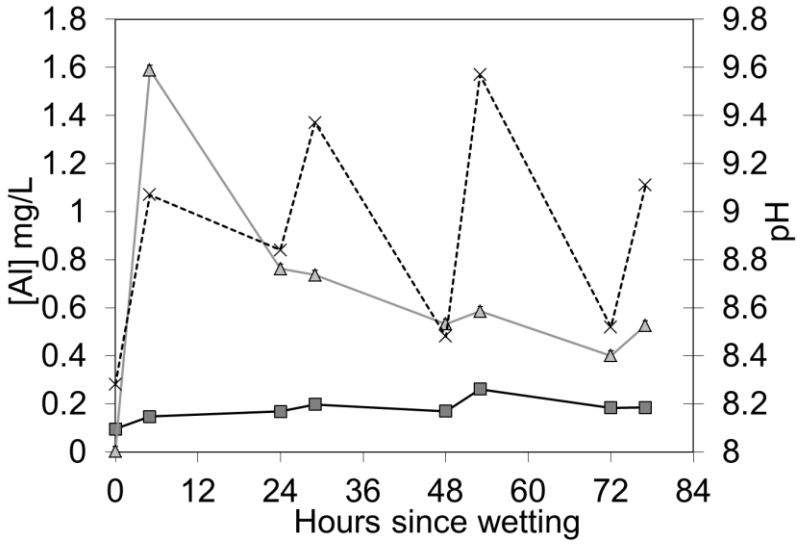
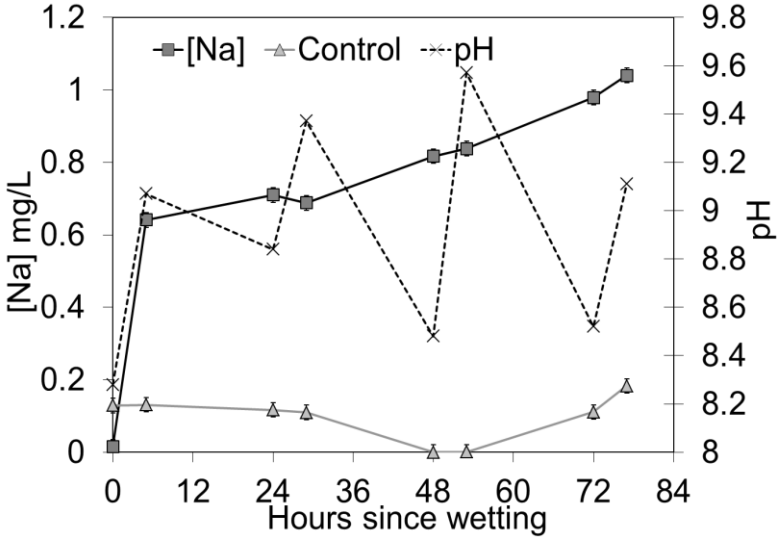


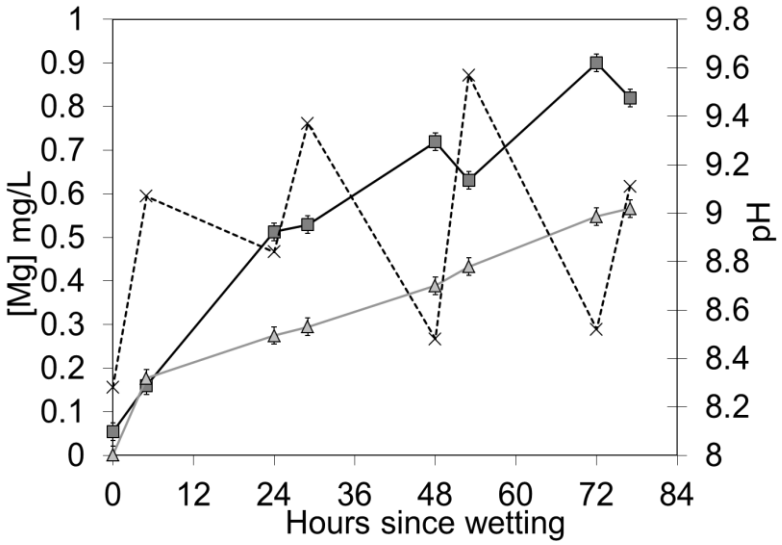
Figure 3.9a: SMC1 ionic response to wetting with DDI compared to a laboratory control. pH is shown to indicate the light/dark cycle with higher pH indicating the day. Note that P, Ca^{2+} and Mg^{2+} show evidence of diurnal cycling. SMC1 has a larger sediment load than SMC2 and therefore shows much greater diurnal cycling of ions. Time zero values of ionic concentrations represent samples taken directly after apply DDI water and so generally represent the detection limits of the ICP-MS. Variation in the control is likely due to elements liberated during the autoclave process.

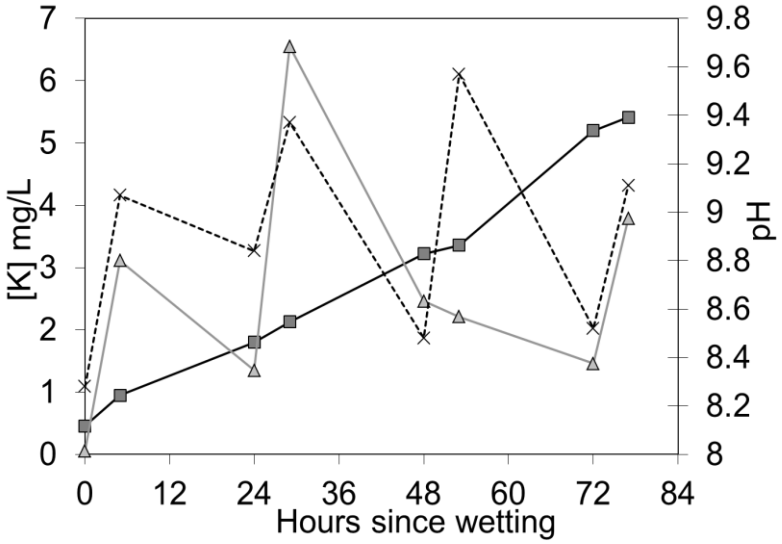

[Si]
Control
pH
[Si] mg/L
pH
Hours since wetting


[Ca] mg/L
pH
Hours since wetting


[Al] mg/L
pH
Hours since wetting


[Na]
Control
pH
[Na] mg/L
pH
Hours since wetting
0
12
24
36
48
60
72
84


[Mg] mg/L
pH
Hours since wetting
0
12
24
36
48
60
72
84


[K] mg/L
pH
Hours since wetting
0
12
24
36
48
60
72
84

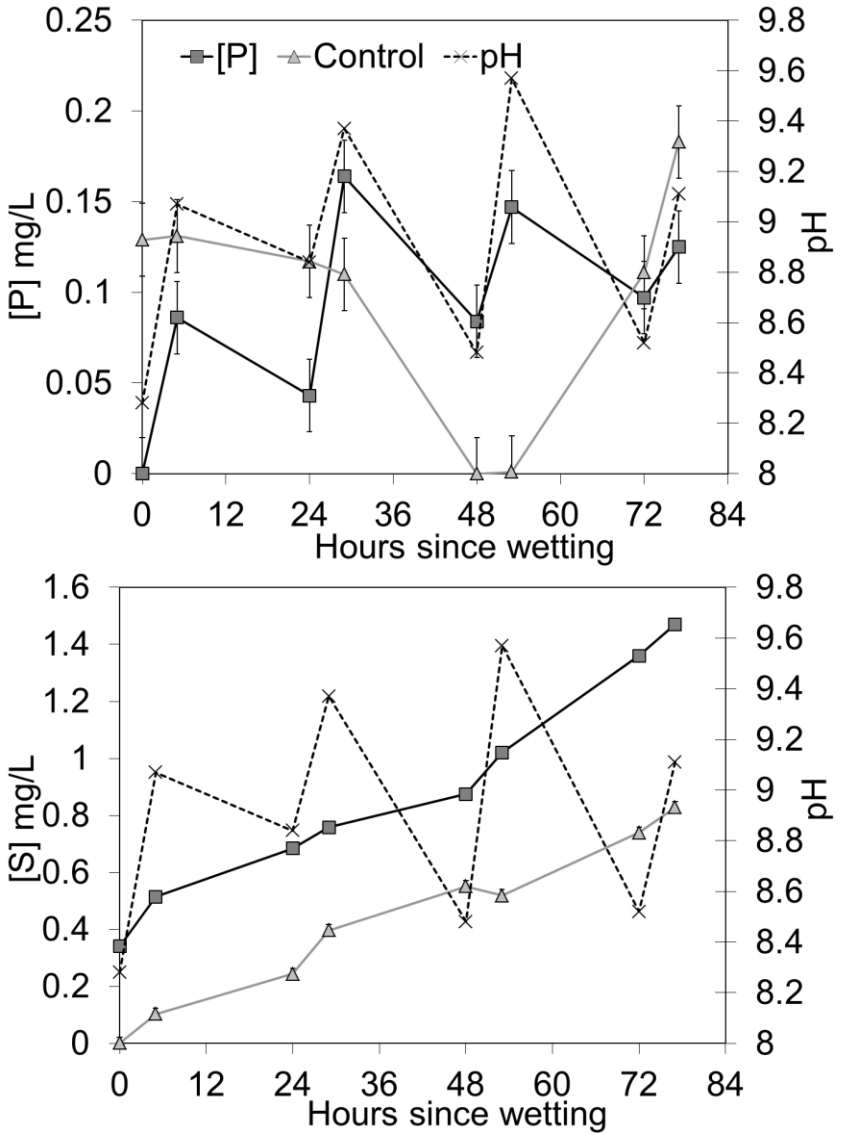


Figure 3.9b: SMC2 ionic response to wetting with DDI compared to a laboratory control. pH is shown to indicate the light/dark cycle with higher pH indicating the day. Note that P, Ca^{2+} and Mg^{2+} do not show evidence of diurnal cycling, unlike in SMC1. Time zero values of ionic concentrations represent samples taken directly after apply DDI water and so generally represent the detections limits of the ICP-MS. Variation in the control is likely due to elements liberated during the autoclave process.

represented in the laboratory control sample. A clear difference is observed between the diurnal cycling of key ions in pothole SMC1 compared to SMC2, which are mainly distinguished by the presence or absence of pothole sediments.

Pothole sediments were observed in the field for general depth (Table 3.1) and accumulation. The potholes at SMC supported the deepest sediments and after a period of six years they were revisited and assessed for changes in depth. Observations of the potholes after six years showed that no additional sediment accumulation had occurred and that the desiccation cracks in the pothole sediment occurred in the same patterns indicating very little change over the lapsed time (Figure 3.10). The most variable factor observed was the accumulation of detrital organic matter on top of the dry pothole sediments.

To further explore the possible interaction between observed high aqueous pHs in the potholes and the local host rock, representative samples of BW and SMC host rock were leached at a series of increasing pHs (9-12). The results of these leach tests showed increases in the aqueous concentrations of the majority of elements tested for (Table 3.2, Figure 3.11). Out of 62 trace elements, the concentrations of 42 elements increased significantly in solution with high pH (pH 9 and above) at SMC and 48 increased at BW. Most increases in trace elements were observed when DDI water above pH 11 was added to the host rock. Many of the major mineral forming elements, e.g., Si, Ca^{2+}, K^{+}, Mg^{2+} were released into solution; however, the Ca^{2+}, Mg^{2+} and K^{+}, showed marked decreases above pH 10.

After assessing the response of potholes to nutrient limited conditions and natural wetting, the pothole community was imaged using SEM and FIB-SEM. A dried sample

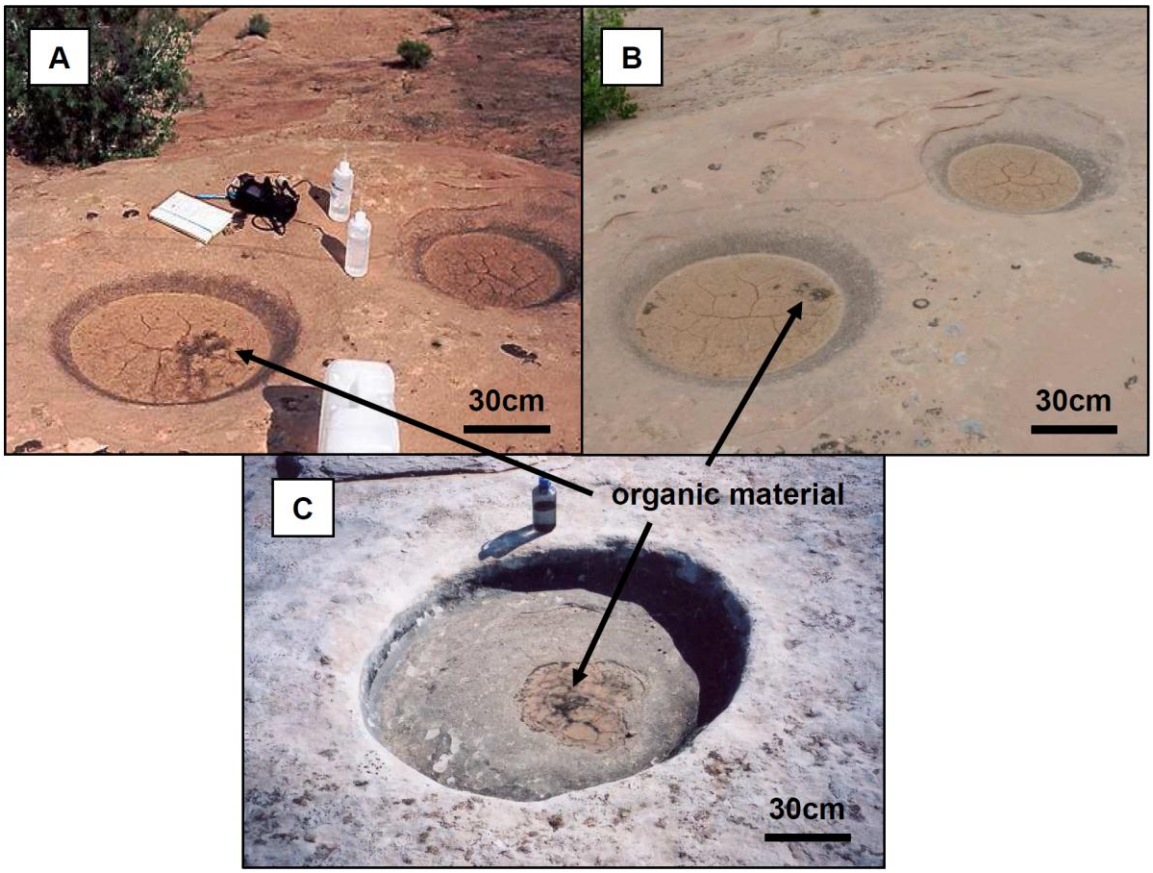


Figure 3.10: Potholes with varying accumulations of sediment. At SMC **A)** year 1 and **B)** Year 6, the potholes have a thick (+/- 3 cm) layer of sediment but the depth is static over a period of 6 years suggesting a maximum depth has been achieved relative to the overall depth of the pothole. At BW **(C)** although the pothole is deeper, there is less sediment, due to the exposed location. Note wind-blown organic matter accumulating on top of the sediments in all three images (arrows).

Table 3.2: Results of leaching of pothole host rocks at high pH (9-12) from two different field sites (SMC and BW).

Analyte	pH	Na	Li	Be	Mg	Al	Si	K	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga
Unit		ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L
D.L.		5	1	0.1	1	2	200	30	1	0.1	0.1	0.5	0.1	10	0.005	0.3	0.2	0.5	0.01
BW	9	3820	< 1	0.3	4320	317	4700	15400	1	2.4	24.5	< 0.5	9.9	< 10	0.814	0.8	> 200	19.4	0.2
BW	9.5	8030	< 1	0.2	5070	271	5100	18400	1	2.3	24.1	< 0.5	105	< 10	0.908	7.1	> 200	15.1	0.2
BW	10	10400	< 1	0.2	4800	291	4700	18800	1	2.1	24.9	< 0.5	12.9	< 10	0.789	0.8	> 200	23.8	0.2
BW	10.5	16100	< 1	0.1	6420	242	5300	17500	1	5.5	24.8	< 0.5	11.2	< 10	0.651	0.7	> 200	16.7	0.2
BW	11	33300	< 1	0.2	7840	287	5800	18000	1	11.6	27	< 0.5	8.3	< 10	0.662	0.7	> 200	16.4	0.2
BW	11.5	35000	< 1	0.2	3620	309	4500	17700	1	2.9	32.4	< 0.5	5.4	< 10	0.632	0.8	> 200	22.7	0.3
BW	12	35000	< 1	8.9	3470	2000	9800	16200	4	17.1	> 50.0	2.5	69.2	410	14.4	9.2	> 200	105	2.7
7MC	9	3810	< 1	< 0.1	635	236	3500	2890	< 1	0.5	1.5	< 0.5	34.6	< 10	0.043	0.4	10.8	14.9	0.2
7MC	9.5	6100	< 1	< 0.1	629	209	3400	2900	< 1	0.5	1.5	< 0.5	31.3	< 10	0.034	0.5	11.5	16.2	0.2
7MC	10	7960	< 1	< 0.1	585	201	3300	2880	< 1	0.4	1.5	< 0.5	27.1	< 10	0.032	0.4	10.7	13.5	0.2
7MC	10.5	13900	< 1	< 0.1	572	246	3700	2970	< 1	0.6	1.8	< 0.5	36.7	< 10	0.047	0.6	10.3	10.4	0.3
7MC	11	26900	< 1	< 0.1	405	310	3500	2370	< 1	0.8	2.4	0.8	102	10	0.081	0.5	6.5	15.4	0.3
7MC	11.5	35000	< 1	1.4	626	2000	5300	3000	1	4.4	10.3	0.8	1040	170	0.721	1.9	64.4	36.1	0.6
7MC	12	35000	8	2	738	2000	5800	3780	1	4.8	22.4	1.2	1450	350	0.955	4.4	92.3	> 250	1.2

Analyte	pH	Na	Li	Be	Mg	Al	Si	K	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga
Unit		ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L
D.L.		5	1	0.1	1	2	200	30	1	0.1	0.1	0.5	0.1	10	0.005	0.3	0.2	0.5	0.01
BW	9	3820	< 1	0.3	4320	317	4700	15400	1	2.4	25	< 0.5	9.9	< 10	0.81	0.8	> 200	19	0.2
BW	9.5	8030	< 1	0.2	5070	271	5100	18400	1	2.3	24	< 0.5	105	< 10	0.91	7.1	> 200	15	0.2
BW	10	10400	< 1	0.2	4800	291	4700	18800	1	2.1	25	< 0.5	12.9	< 10	0.79	0.8	> 200	24	0.2
BW	10.5	16100	< 1	0.1	6420	242	5300	17500	1	5.5	25	< 0.5	11.2	< 10	0.65	0.7	> 200	17	0.2
BW	11	33300	< 1	0.2	7840	287	5800	18000	1	12	27	< 0.5	8.3	< 10	0.66	0.7	> 200	16	0.2
BW	11.5	35000	< 1	0.2	3620	309	4500	17700	1	2.9	32	< 0.5	5.4	< 10	0.63	0.8	> 200	23	0.3
BW	12	35000	< 1	8.9	3470	2000	9800	16200	4	17	50.0	2.5	69.2	410	14.4	9.2	> 200	105	2.7
7MC	9	3810	< 1	< 0.1	635	236	3500	2890	< 1	0.5	1.5	< 0.5	34.6	< 10	0.04	0.4	11	15	0.2
7MC	9.5	6100	< 1	< 0.1	629	209	3400	2900	< 1	0.5	1.5	< 0.5	31.3	< 10	0.03	0.5	12	16	0.2
7MC	10	7960	< 1	< 0.1	585	201	3300	2880	< 1	0.4	1.5	< 0.5	27.1	< 10	0.03	0.4	11	14	0.2
7MC	10.5	13900	< 1	< 0.1	572	246	3700	2970	< 1	0.6	1.8	< 0.5	36.7	< 10	0.05	0.6	10	10	0.3
7MC	11	26900	< 1	< 0.1	405	310	3500	2370	< 1	0.8	2.4	0.8	102	10	0.08	0.5	6.5	15	0.3
7MC	11.5	35000	< 1	1.4	626	2000	5300	3000	1	4.4	10	0.8	1040	170	0.72	1.9	64	36	0.6
7MC	12	35000	8	2	738	2000	5800	3780	1	4.8	22	1.2	1450	350	0.96	4.4	92	> 250	1.2

Analyte	pH	Ba	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	Hf	Ta	W
Unit		ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L
D.L.		0.1	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.02
BW	9	> 400	0.95	0.97	0.38	1.81	0.53	0.15	0.47	0.07	0.32	0.05	0.13	0.01	0.07	0.01	0.01	0.001	0.2
BW	9.5	> 400	0.66	0.71	0.27	1.27	0.37	0.11	0.34	0.05	0.23	0.04	0.1	0.01	0.06	0.01	0.01	0.001	0.2
BW	10	> 400	0.88	0.95	0.37	1.74	0.5	0.15	0.47	0.07	0.32	0.06	0.13	0.01	0.07	0.01	0.01	0.001	0.2
BW	10.5	359	0.37	0.4	0.14	0.667	0.2	0.06	0.18	0.03	0.12	0.02	0.05	0.01	0.03	0	0.01	0.001	0.2
BW	11	233	0.4	0.43	0.16	0.723	0.21	0.06	0.2	0.03	0.13	0.02	0.06	0.01	0.04	0.01	0.01	0.001	0.3
BW	11.5	> 400	0.51	0.58	0.21	0.975	0.29	0.08	0.27	0.04	0.17	0.03	0.08	0.01	0.05	0.01	0.02	0.001	0.5
BW	12	> 400	4.78	5.3	2.03	9.66	2.96	0.74	2.82	0.42	1.99	0.34	0.81	0.08	0.5	0.07	0.14	0	0.7
7MC	9	6	0.14	0.6	0.05	0.231	0.08	0.02	0.07	0.01	0.06	0.01	0.02	0	0.02	0	0.01	0.001	0
7MC	9.5	5.5	0.11	0.53	0.04	0.195	0.07	0.02	0.06	0.01	0.04	0.01	0.02	0	0.02	0	0	0.001	0
7MC	10	5.2	0.1	0.42	0.04	0.165	0.05	0.02	0.05	0.01	0.03	0.01	0.02	0	0.01	0	0	0.001	0.1
7MC	10.5	5.7	0.14	0.6	0.05	0.219	0.08	0.02	0.08	0.01	0.06	0.01	0.02	0	0.02	0	0	0.001	0
7MC	11	11.4	0.61	1.73	0.16	0.684	0.19	0.05	0.2	0.03	0.13	0.02	0.06	0.01	0.05	0.01	0.01	0.001	0
7MC	11.5	114	3.27	12.8	1.28	5.86	2.07	0.58	2.06	0.32	1.47	0.24	0.59	0.07	0.44	0.06	0.06	0	0
7MC	12	154	3.24	14.1	1.21	5.51	1.88	0.54	1.95	0.31	1.45	0.23	0.56	0.06	0.43	0.06	0.11	0	0.2

Analyte	pH	Re	Os	Pt	Au	Hg	Tl	Pb	Bi	Th	U	Ba	Al	Mg	Ca	Cu	Na	V	Zn
Unit		ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	mg/L	mg/L	mg/L	ug/L	mg/L	ug/L	ug/L
D.L.		0.001	0.002	0.3	0.002	0.2	0.001	0.01	0.3	0.001	0.001	20	0.1	0.1	0.1	2	0.1	10	5
BW	9	0	0.01 < 0.3		0.01 < 0.2	0.02		0.6 < 0.3	0.21		34.7	630	--	--	52	1480	--	--	--
BW	9.5	0	0.01 < 0.3		0.01 < 0.2	0.02		0.6 < 0.3	0.2		40.3	630	--	--	61	1250	--	--	--
BW	10	0	0.01 < 0.3		0 < 0.2	0.03		0.5 < 0.3	0.24		41.6	630	--	--	66	1340	--	--	--
BW	10.5	0.001	0.01 < 0.3		0 < 0.2	0.02		0.3 < 0.3	0.11		38.9	--	--	--	52	997	--	--	--
BW	11	0	0.01 < 0.3		0.01 < 0.2	0.02		0.4 < 0.3	0.12		33.8	--	--	--	51	1170	--	--	--
BW	11.5	0	0.01 < 0.3		0.03 < 0.2	0.02		0.4 < 0.3	0.24		59.5	420	--	--	47	1340	67	--	--
BW	12	0	0.01 < 0.3		0.02 < 0.2	0.03	9.4	3.7	0.45		69.3	860	5.1	--	22	38000	219	70	--
7MC	9	0.001	0.002 < 0.3		0.09 < 0.2	0.01		0.4 < 0.3	0.01		0.17	--	--	--	29	--	--	--	--
7MC	9.5	0.001	0.002 < 0.3		0 < 0.2	0.01		0.4 < 0.3	0.01		0.17	--	--	--	29	--	--	--	--
7MC	10	0.001	0.002 < 0.3		0 < 0.2	0.01		0.4 < 0.3	0.01		0.16	--	--	--	28	--	--	--	--
7MC	10.5	0.001	0.002 < 0.3		0.002 < 0.2	0.01		0.4 < 0.3	0.01		0.17	--	--	--	24	--	--	--	--
7MC	11	0.001	0.002 < 0.3		0.01 < 0.2	0.01		0.7 < 0.3	0		0.42	--	--	--	--	--	--	--	--
7MC	11.5	0.001	0.002 < 0.3		0.63 < 0.2	0.01		3.3 < 0.3	0.04		0.27	--	1.7	--	40	--	55	--	--
7MC	12	0.001	0 < 0.3		0.02 < 0.2	0.01		3.1 < 0.3	0.03		0.45	--	2.7	--	36	--	147	--	882

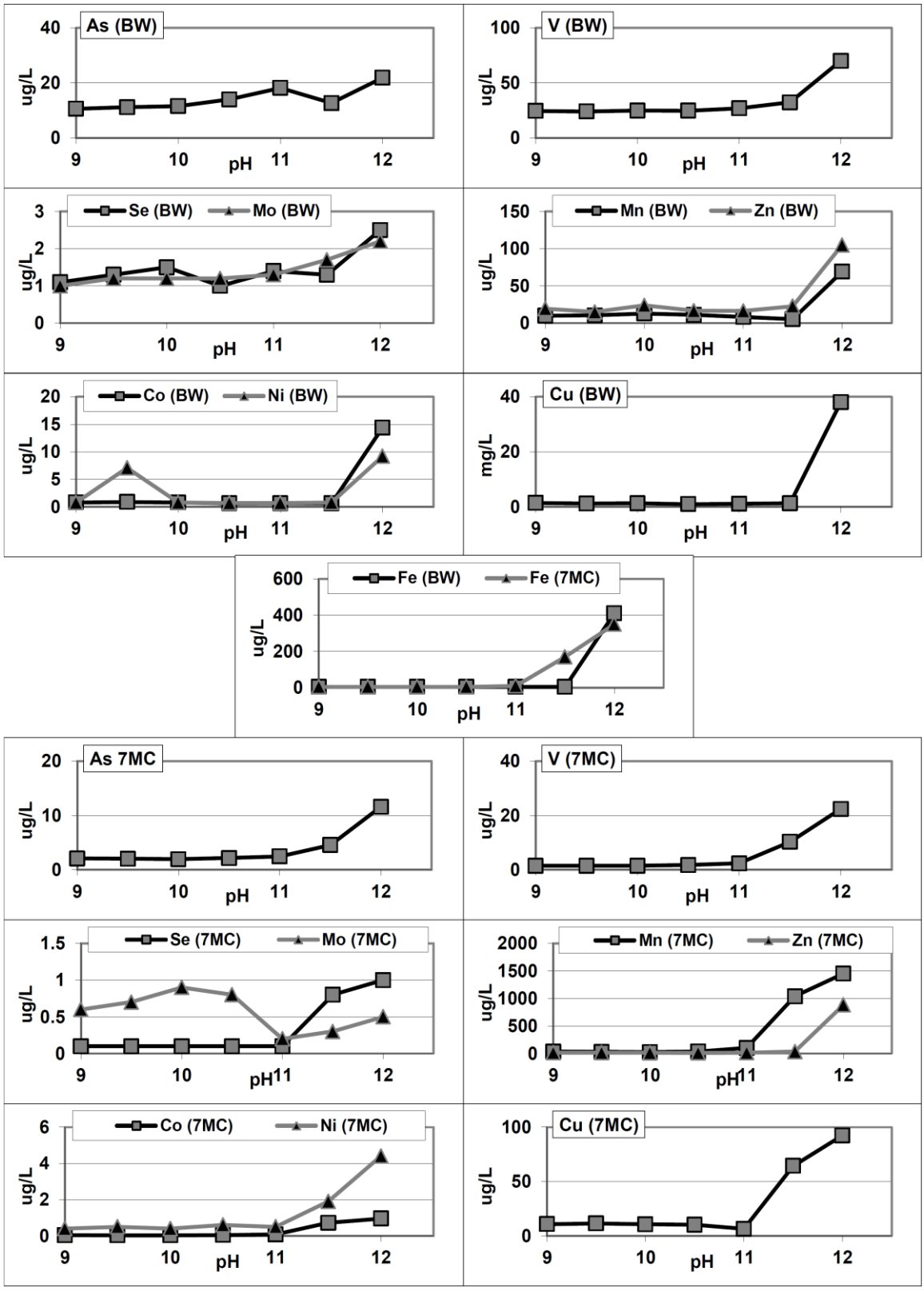


Figure 3.11: Selected trace metals leached at from the host rocks of SMC and BW increasing in concentration with pH.

black biofilm was re-wetted with DDI and kept in the dark to limit the growth of phototrophic organisms. Under sun-lit DDI wetted conditions the black biofilm quickly developed a distinct cyanobacterially dominated biofilm (Figure 3.12A). However, when presented with the nutrient limitations of DDI and a lack of sunlight, the structure of the biofilm was altered and occurred mainly along the edges of the mineral grains (Figure 3.12B). Compared with samples exposed to sunlight, the nutrient-starved biofilm kept in the dark did not support the growth of cyanobacteria. Within the oligotrophic heterotrophic community two types of adaptation to the desiccating and nutrient limiting conditions were identified. First, throughout the biofilm webs of extracellular polymeric substances (EPS) were observed associated with rod-shaped cells (Figure 3.12 C and F). The webs of EPS formed distinct clusters which were several times larger than the accompanying cells. The second distinct feature observed is large spheres (approximately 10 μm) which EDX determined were composed of carbon (data not shown) (Figure 3.12C). Upon desiccation of the spheres, or cutting with the focused ion beam, the forms of smaller cells were identified within, indicating that each large sphere represented a distinct microcolony (Figure 3.12 D and E). In FIB cross-sections small amounts of refractory material (too small to identify with EDX) was observed accumulating around the cells indicating metabolic activity.

3.4 Discussion

The first step in determining the importance of the host rock in supplying biologically relevant compounds and elements to the biofilms growing on them is to understand the contribution of other sources of chemical components to the system. In

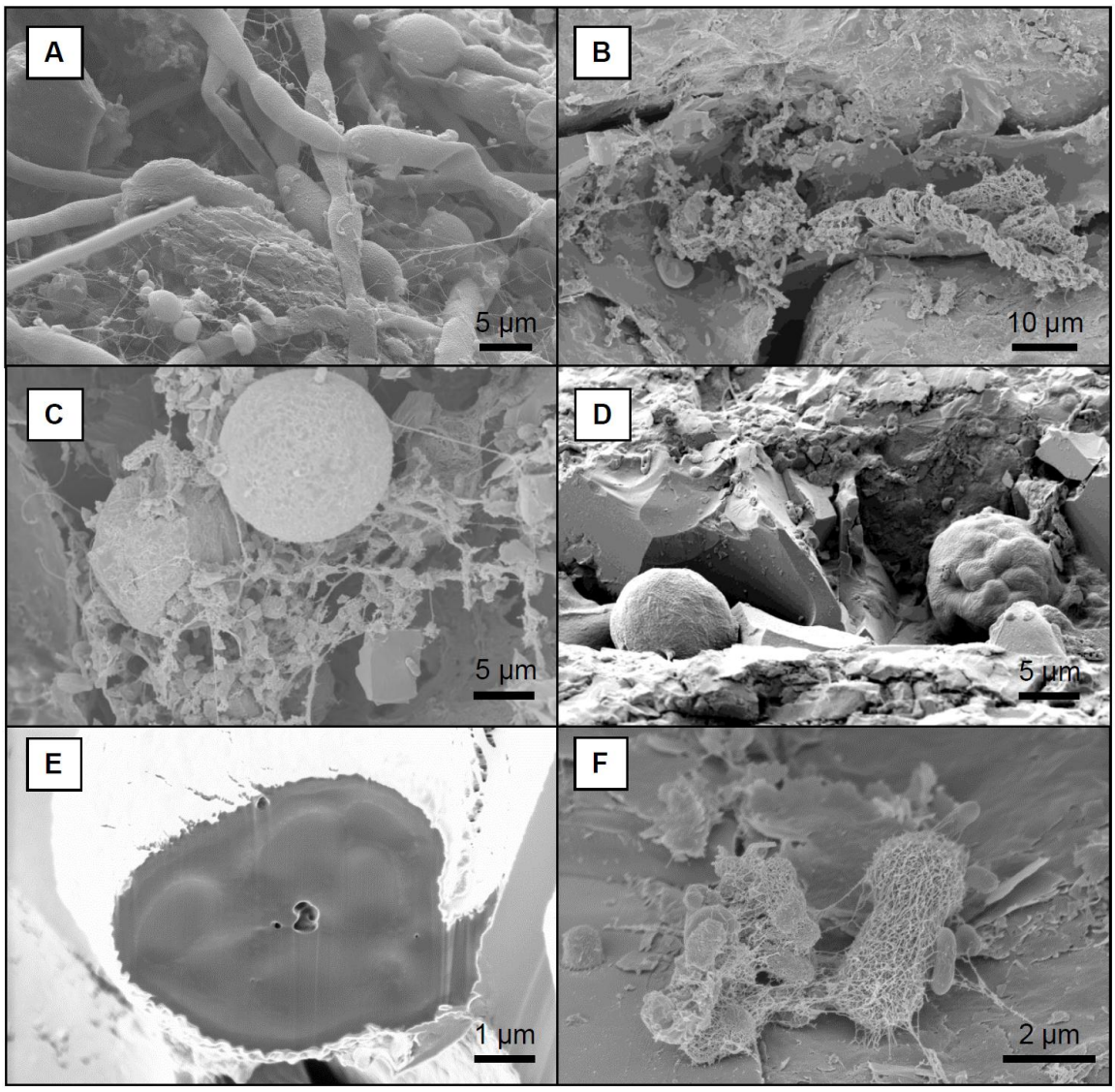


Figure 3.12: Scanning Electron Microscope (SEM) images of SMC biofilms: **A)** Rain wetted pothole biofilm grown under natural light showing filamentous cyanobacteria. **B)** A "dark "biofilm sample wetted with DDI water preferentially colonizing pore space and cement between sand grains. **C)** Large carbon spheres common in the nutrient limited biofilm. **D)** Slightly desiccated carbon sphere showing internal cell-like shapes. **E)** Focussed Ion Beam cross-section of a carbon sphere showing cell-like internal components. **F)** Dense bundles of EPS associated with bacteria.

particular, a better-defined understanding of the nutritional input of rain water to the pothole environment versus the input of nutrients from the host rock. The primary importance of rain events to the pothole environment is in providing critical hydration to the biofilm. In the semi-arid desert environment this input, above any contribution of nutrients, is of primary importance, as other possible sources of water, such as fogs and periods of increased humidity in the air, are rare. Previous work has suggested that the cryptoendolithic environment could provide somewhat of a greenhouse effect, concentrating humidity within the rock (Bell, 1993), however this would not support the large biofilms observed in the pothole sediments. With this in mind, the potholes were sampled after being filled with both rainwater and DDI water.

Characterization of Naturally High Pothole pH

Previous studies have highlighted the development of alkaline pH values in pothole waters on the Colorado Plateau (Netoff and Shroba, 1993; Graham, 1999 and Chan et al., 2005) and in other locations (Dominguez-Villar et al., 2008). Rain-filled potholes from this study also developed high pH values both at SMC (maximum observed value of 9.96) and BW (maximum observed value of 10.10). The high pH values measured during field monitoring agree with other studies that show biofilms rich in cyanobacteria produce a high pH, which in turn weathers the host rock (Budel et al., 2004). This diurnal pH increase results from certain species of cyanobacteria that employ carbonic anhydrase to fix CO_2 from bicarbonate (HCO_3^-) as their carbon source, and in the process produce hydroxide ions (OH^-) as a by-product of metabolism (Thompson and Ferris, 1990). The high pH observed in the sampled potholes can be understood in the

context of this process, in that, if the pH values are controlled by the metabolic action of photosynthetic organisms, the pH will cycle diurnally. The observed diurnal cycle of pH values that could not be duplicated in the abiotic control indicated that the pH of the potholes is biologically controlled. It also indicated that even when the environment was intentionally stressed by using DDI water the biofilm survived and continued to influence pothole chemistry, possibly through obtaining nutrients from the host rock and sediments, *i.e.,* water was the limiting growth factor.

Geochemistry of DDI Wetted Potholes

As suggested by the diurnal cycling of pH even in DDI wetted potholes, the microbiota of the potholes continue to function even under extremely nutrient limited conditions. Differences between the SMC1 and SMC2 systems are found in the diurnal cycling of aqueous concentrations of specific ions in SMC1 that were not observed in SMC2. For example in SMC2 concentrations of Ca^{2+} were slightly less than the control and showed no diurnal cycling while in SMC1, which has a much greater sediment load, Ca^{2+} concentrations showed pronounced cycling. Similar results were observed for other major cations which are important in biological processes. These disparities indicate a larger biological influence on SMC1 over SMC2 as the deeper sediments allow for more abundant and diverse biological activity. It is also likely that the deeper sediments support the nitrogen economy of the pothole through increased nitrogen fixation (Billings et al., 2003). Studies of atmospheric deposition of nitrogen from dust and precipitation have found that the contribution of nitrogen from dust far outweighs the biological demand (McNamara, 2006) and so residual materials would likely be partially conserved

in the pothole sediments, which would otherwise be lost through wind action upon drying. The pothole sediments appear to aid in pothole formation by holding water and nutrients which encourage growth of cyanobacteria that are responsible for increasing the pH and weathering the host rock.

Geochemistry of Rain-Wetted Potholes

If biological activity in the pothole environment continues without nutrient input from rain, it raises the question of how important the input of nutrition content from rain is to the system. Two rain events were observed, the first rain event came after a dry period of at least two weeks and so it is possible that that dust from the dry desert was thrown up onto the pothole plateaus by the preceding winds and then washed by the rain into the potholes. The second rain came only a day after the first and, as the area was still damp, less dust was coating the surfaces and less dust was transported off the damp surfaced into the atmosphere and therefore less material was deposited into the potholes. This difference in the moisture conditions preceding the rains may account for the drop in concentration of many aqueous ions between the two rains although longer term studies of the rains in the field area are required to further support this suggestion.

Despite the input of atmospheric dust, some major ions such as calcium (Ca^{2+}) and silicon (Si) are thought to be derived primarily from the sediments or host rock. The first pothole water samples following the first rain-event possessed higher Si and Ca^{2+} concentrations than present in rain water samples after being wet for only two hours, suggesting soluble sources of Ca^{2+} and Si in the potholes, coupled with bacterial communities that are very responsive to wetting. The main repository of Si in the host

rock is quartz. Because the solubility of silicate minerals decreases as a function of how ordered and dense the silicate tetrahedral are (Drees et al., 1989), and quartz is well ordered, it is unlikely to be the source of the initial concentration of soluble silica in solution. The initial Si values probably result from the weathering of other Si bearing minerals such as feldspars which are more susceptible to alteration in high pH conditions (Welch and Ullman, 1993). It is also possible that a secondary silicate phase, such as biogenic opal (from diatoms) or amorphous silica may be a source of more readily available Si, both of which are much less ordered and therefore more soluble than quartz.

A steady increase in Ca^{2+} and Si values was observed after the initial wetting in SMC1 presumably due, in part, to evaporative concentration because it dried out much more quickly than SMC2. In SMC2, however, Ca^{2+} values plateaued before increasing dramatically due to evaporative concentration as the pothole dried. The plateau in the data may indicate that in the intermediate period a carbonate mineral, possibly calcite, was being precipitated as a by-product of cyanobacterial carbon fixation described above. In this process, soluble Ca^{2+} is attracted to the negatively charged cell wall of the bacteria and complexed with CO_3^{2-}, which is more abundant in a high pH solution (Thompson and Ferris, 1990). The Ca^{2+} results from rain wetting correlated well with the results from artificial wetting. As in the rain wetted samples, although no Ca^{2+} was present in the DDI water, high values of Ca^{2+} (over 20 mg/l) were still observed in the pothole along with a pronounced diurnal cycling indicating that the biological component of the pothole controls Ca^{2+} concentrations aside from natural concentration with drying. The diurnal cycle of Ca^{2+} values was opposite to that of pH, indicating that the carbonate phase was precipitated while the photosynthetic organisms were most active. A similar plateau

response found in the Mg^{2+} values was interpreted to be derived primarily from the host rock or sediments. SMC2 Mg^{2+} values plateaued after initial wetting with rain only to increase as the final water evaporated and, in DDI wetted potholes, a diurnal cycle was observed in SMC1. This suggests that a smaller portion of the precipitated carbonate phase may be a Mg-calcite, Mg-carbonate or perhaps dolomite. The abundance of Mg in the pothole is of importance because its significance to bacterial metabolism is often overlooked in favour of other nutrients such as PO_4^{3-} and NO_3^- (Wackett et al., 2004). In fact, Mg^{2+} plays a vital role in ATP formation and is a cofactor in enzymes that maintain cellular pH balance and control iron transport and metabolism (Chamnongpol and Groisman, 2002).

The most important contribution of the rain events is shown in the ionic nutrient values, *i.e.*, N and P, which are limited in the potholes. Both PO_4^{3-} and NO_3^- were found in higher concentrations in the rain than in the potholes. In both cases these important nutrients were used so quickly that the concentration of these ions in pothole water never matched the concentration in rain water (Figure 3.8). Consumption of important nutrients immediately after their addition shows that rain water provides some significant nutritional input to the potholes. Other ions did not change in concentration upon reactivation of the biosphere. In the case of K^+, Al^{3+}, Na^+, and SO_4^{2-} no immediate draw down of concentrations was observed, nor were the concentrations initially supplemented to a large degree by contributions from the host rock and sediments. As the potholes dried SO_4^{2-}, Al^{3+}, K^+ and Na^+, values increased. These increases were probably supplemented by additions from dissolution in the sediments and host rock.

Alternate Nutrient Sources

The results of wetting the potholes with DDI suggest that there is some limited source of nutrients already present in the potholes from wind-blown dust and accumulated sediments so that bacterial communities can function without nutrients from rain. The likely limiting nutrient is bio-available phosphate as cyanobacterial communities are able to fix nitrogen and produce nitrate (Billings et al., 2003). It is possible that the added protection created by the pothole sides as depth increases, supplements the potholes ability to collect wind-blown sediments, which contribute useful, although inherently unreliable, particulate nutrients to the environment. This possibility is supported by observing the presence of sediments collected in many potholes, some with detrital organic material visible on the surface. However, these sediments are not abundant in all locations; for example, the potholes at BW had only minimal soils in all but the largest potholes. Also, in comparing the sediment depth from potholes at SMC revisited after a period of six years, no appreciable increase was observed. This indicates that either sediment accumulation is a slow process or that once a base soil has accumulated, additional input brings the sediment level up too high to be adequately protected from wind action and a static level is reached until the pothole increases in size. Wind-blown contributions of sediment to oligotrophic water bodies are an area of particular interest. Many studies of oligotrophic marine environments have evaluated the contributions of wind-blown materials, finding that they frequently contribute to an increase in biomass (Guieu et al., 2010; Maranon et al., 2010).

A second possible nutrient source is the natural cycling of the biological community itself. After a biological community has been established during wet periods, the initial, more favourable conditions would support a less oligotrophic community of bacteria. As labile carbon sources are consumed and competition for resources increases, the initial community may die back, being out-competed by the oligotrophic community, but providing some residual nutrient sources (Fiere et al., 2007). Further, as the pothole dries, there will be an abrupt decline in the active and viable pothole biota. Some bacterial species in the potholes are spore forming and will survive dry periods, however the majority of the biomass does not have the capability to produce resting stages and will presumably die-back upon desiccation (see Chapter 4). This remnant biotic material will form an important initial nutrient base for the community forming during the next wetting cycle.

A final possible source of required nutrients is from dissolution of the host-rock. Although the host rock in the study areas is generally considered to be a nutrient poor substrate, minerals such as apatite and feldspars were occasionally present and would be readily weathered at higher pH (Brady and Walther, 1989; Welch and Ullman, 1993). Also, the quartz grains themselves, which make up the bulk of this sandstone, are frequently observed to host fluid inclusions, which may represent formation fluids or subsequent reworking through diagenesis (Figure 3.13) (Schiano, 2003). In either case fluid inclusions, although volumetrically small (usually observed to be <1 μm each in the host rock), may provide a possible, if irregular, source of trace elements. Other work on mineral weathering frequently suggests the actions of organic acids as chelators (Vandervivere et al., 1994), which function over a range of pHs from acidic to near

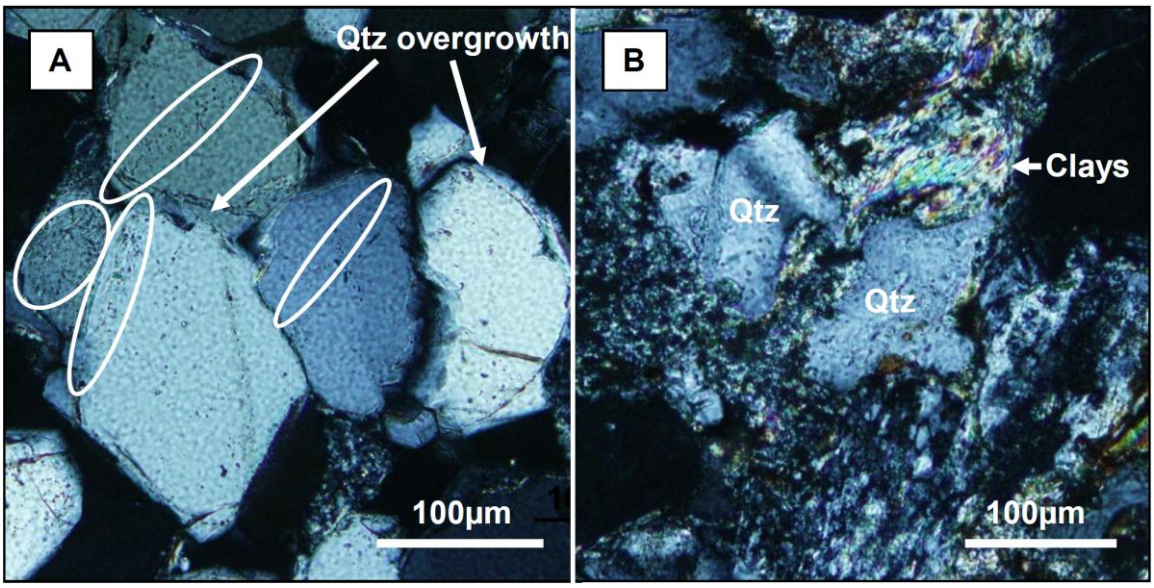


Figure 3.13: A) fluid inclusions (circled) in quartz grains and quartz overgrowths (arrows) in the host rock from BW **B)** weathered quartz grains with secondary clays at SMC.

neutral. The action of organic acids is not precluded from the more alkaline pothole environment, it is instead, more prevalent during the shift in pH to more acidic conditions at night or in the early morning. These hydronium-based mechanisms of mineral weathering could well be in effect at night, while the uniquely high daytime pH of the potholes provides a different complimentary weathering mechanism.

Leaching of Pothole Sandstone at High pH

To better understand the possible nutrient contribution of the sandstone to the biofilm community at two different locations, the elevated pH values observed in the field (of up to 10.1) were used as the basis for a laboratory study. Biofilms are able to create microenvironments, often forming isolated pockets of unique geochemical conditions (Wolfaardt et al., 1999). It is clear from the diurnal nature of the pH values and the values of other ions, such as calcium Ca^{2+}, that their concentration in solution is linked to photosynthetic activity mainly controlled in the pothole environment by cyanobacteria, as previously discussed. Within the biofilms of the pothole, it is possible that the pH values are locally much higher thereby having a more concentrated, profound effect on the host rock (Power et al., 2009) and so values of up to pH 12 were evaluated. Samples of host rock from the potholes frequently showed rugged weathering horizons in quartz and other silicate grains in thin section, as well as evidence of re-precipitated material in quartz grain overgrowths which indicate both weathering of the host rock and re-precipitation of secondary silica out of solution (Figure 3.13AB). Alkaline leach testing of host rock samples, found that many of the major mineral forming elements, e.g., Si, Ca^{2+}, K^{+}, Mg^{2+} were released into solution; however the Ca^{2+}, Mg^{2+} and K^{+},

showed marked decreases above pH 10. Quartz and feldspar, which are known to occur at BW and SMC (see Chapter 2) are more soluble at alkaline pH (above pH 9) (Dove, 1995; Vandervevre, 1994). Therefore, this decrease suggests that the precipitation of secondary mineral phases such as illite or smectite (layered alumino-silicate clays) occurred (Meunier and Velde, 2004) or perhaps, that mineral carbonation was occurring. Aqueous geochemistry samples from the field correspond well with this data, as they showed decreases in Ca^{2+} and Mg^{2+} during the day when the bacteria are most active, and the pH is highest. Si values continued to increase at high pH suggesting dissolution of silicate minerals is occurring. Of more interest was the increased concentration of trace elements in solution at high pH. Most trace elements analysed were released into solution. Not all trace elements are beneficial to the pothole biota, for example, concentrations of biologically toxic arsenic (As) in solution increased at higher pH; However, the effect of As on the microbial community is likely negligible. Microbial life has co-existed with As, and many other toxic elements, for billions of years leading to adaptations to their presence. Genomic studies have identified the ability to produce the arsenate reductase enzyme in most prokaryotes DNA, indicating that they have developed mechanism to deal with As in low concentrations (Mukhopadhyay et al., 2002). Other elements may be of use to some bacteria but toxic to others, such as tungsten (W) (Sugio, 2001) or be able to fulfill the functions of more important elements if they are absent e.g., Cs^{+} replacing K^{+} (Jasper, 1978), which may be of significance in oligotrophic environments such as this one. Specific trace elements, which increased with leaching and are of importance to bacterial life include selenium (Se), vanadium (V), molybdenum (Mo), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), and zinc (Zn).

All showed distinct increases in concentration at high pH (Figure 3.12). The presence of a wide range of transition metals is of particular importance: transition metals have important roles in all facets of bacterial metabolism and are associated with many enzymes. For example, V has been found in important enzymes, particularly nitrogenases (Eady, 1995) and nitrate reductases (Antipov et al., 2003). Further, a greater diversity of trace elements and micronutrients supports a greater bacterial metabolic diversity. As seen with V, its presence allows for both reduction and oxidation of nitrogen giving important flexibility to an ecosystem stressed by many outside factors.

Electron Microscopy

Since it has been established that the bacterial community of the potholes can influence the geochemistry of their environment even under nutrient limited conditions, it is of interest to examine the biofilm itself for physical adaptations. Because cyanobacteria are so dominant in these biofilms, if their growth is not discouraged very few other bacterial types were observed; although they are known to be present from molecular studies at this location and other similar sites (see Chapter 4). Light limited conditions allowed for a closer examination of the adaptations of heterotrophic microbiota to nutrient limited conditions. The large carbon spheres observed with SEM may represent microcolonies of bacteria protected from losing moisture and nutrients to the surrounding environment by being encapsulated in EPS. Comparable to this growth strategy, other groupings of cells were found entrained within, or associated with a dense web of EPS material, likely polysaccharides. This adaptation would increase the reactive surface area of the microcolony, thereby allowing access to a larger share of the limited

available resources through ion-exchange processes and through water absorption. These unusual structures imply that although the water alone lacks nutrients, there is still some support to be gained from the substrate itself and the biofilm is a fluidly adaptable community that can survive even starvation conditions for some time. Numerous studies have examined the importance of EPS to biofilms and shown their importance to the accumulation of nutrients (Barker et al., 1996; Wolfaardt et al., 1999; Hassler et al., 2011), heavy metals (De Philippis et al., 2011) and abiotic precipitates (Pentecost, 1988). Both morphologies will be affected differently by the other limiting factor of pothole life: access to water. The production of hygroscopic EPS was proposed as a way for bacteria to retain water in desiccating terrestrial environments (Ortega-Calvo et al., 1995) and both of these morphologies show different usages of EPS. The large, relatively dense spheres and their contained environments probably provide additional tolerance of desiccation. Although the potholes are frequently dry for periods of time this type of adaptation could help to bridge periods of desiccation between rain events in the wetter periods of the year. Field observations revealed that potholes can dry-out within 4-5 days of a rain event leaving only damp sediments. If the next rain comes before the sediments have completely dried the type of microenvironment provided by the spheres would provide a large competitive advantage over other desiccated bacteria. The second EPS web morphology is unlikely to provide as much desiccation tolerance; the exopolymer web surrounding the bacteria may retain moisture slightly longer than a lone bacteria, however the large surface area would be prone to drying.

3.5 Conclusions

The addition of DDI water produced a baseline of biological activity that was observed through diurnal cycling of pH and certain ions (e.g. Ca^{2+}). Furthermore, unique biological assemblages were observed in the biofilm, seemingly adapted to nutrient-starved conditions. This indicates that the life within the potholes can not only survive extreme temperatures and extended periods of drought, but also that it can grow on limited nutrient input from the sandstone substrate. The possible contribution of nutrients from the host substrate is an important source for the biofilm in deriving what nutrients it can from a limited environment. Geologic formations are large repository of elements essential to life, e.g., H, O, C, P, N and S, which bacteria can access, as shown in this study, and perhaps repackage for use by higher forms of life (Douglas and Beveridge, 1998). The higher biomass and diversity of microorganisms in the biofilm observed by SEM images shows that the pothole, with the inputs provided by rain-water, is a step up from the dryer environment of cryptoendoliths colonizing a flat surface where limited water resources also mean a limited availability of nutrients. Potholes are a second stage of development in the process of colonizing the desert. From this stage, potholes trap wind-blown particulates, building a sandy soil that, with sufficient thickness, can be colonised by biological soil crusts, completing the first step of colonizing bare rock by producing a stable soil base accessible to higher life. Despite the limitations of the environment, the simple chemistry of obtaining sufficient carbon for photosynthesis in cyanobacteria creates high pH weathering conditions, which appears to be the basis of an entire desert ecosystem.

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CHAPTER 4

An Assessment of the Heterotrophic Bacterial Community at Various Stages of Pothole Development Using 16S rRNA

4.1 Introduction

Bacteria have evolved to exploit most environments on Earth, where they play key roles in ecosystem function, especially in environments that are inhospitable to most other forms of life. In such environments, the role of bacteria as pioneer species and primary producers is particularly important in terraforming these ecosystems (Friedman and Weed, 1978; Ohad et al., 2005; Lester et al., 2007). Initial colonisation of dry, nutrient poor environments, in many cases, begins to alter the area to allow it, through proto-soil creation, to be used by less tolerant biota, marking the boundary in between a landscape with productive soils and a desert (Belnap et al., 2001; Omelon, 2008; Mager and Thomas, 2011). The high desert of the Colorado Plateau near Moab, Utah, is one of these types of environments. Many areas within this region consist of expanses of exposed sandstone that are subjected to large fluctuations in temperature and intense solar radiation, with only limited precipitation. The bacteria that are the focus of this study have colonised these surfaces through the ability to exploit any available advantage (see Chapters 2, 3). The result of this colonisation is the development of ephemeral pond environments, or "potholes", through preferential weathering of the sandstone surfaces. These pothole environments provide insight into a transitional phase between large-scale biological soil crusts (BSCs) and the initial colonisation of bare rock surfaces. BSCs accumulate particulates, gradually producing a thin base of soil upon which plant species

may develop a foothold, reducing desertification (Bamforth, 2008; Pendleton, 2003; Johansen, 1993).

Indentations in the rock surfaces, or potholes, exacerbated by the work of the microbiota, wind and the freeze thaw seasonal cycle, expand over time to accumulate sediments and begin to hold water for longer periods. These larger potholes become semi-permanent sources of water that support a diversity of life and develop thick layers of sediments at the bottom (Appendix A; Duane, 2006). Marginal microbial communities exist throughout the world, where they contribute to lithic degradation despite having the lowest community diversity of most ecosystems on Earth (Walker and Pace, 2007; Omelon, 2008).

Within the pothole environment, distinct subsets of living conditions exist which, although physically close, vary greatly in important variables such as the duration of exposure to liquid water and the ability to retain windblown material that may contain vital nutrients. Potholes begin as shallow surface depressions lined with a black biofilm while later stages develop enough depth to begin to hold sediments in the bottom year round. These stages of development are also partially-controlled by the host sandstone environment: More porous sandstones may provide places for endolithic bacteria to grow but are also less able to retain water. Samples were taken to evaluate the differences in bacterial species diversity between these adjacent environments in order to suggest how the bacterial community changes through the development of a pothole, and also evaluate the importance of slight differences in substrate.

An environment such as this one, which is mainly composed of an inhospitable semi-arid desert, is especially vulnerable to any shifts in the climate. Any water source is

valuable in a desert environment, and potholes are particularly valuable because of their potential role in the production of BSCs. BSCs are less prevalent in areas such as the Great Basin Desert due to lower rainfall and poor soil conditions (Johansen, 1993) and it is a struggle to find any bacteria at all in the hyper-arid Atacama Desert (McKay et al., 2003; Lester et al., 2007). Even increases in rainfall can be problematic to BSC development, as too much concentrated precipitation causes erosion and leaching of nutrients from soils, soil crusts (Johnson et al., 2007) and even vegetated areas (Schlesinger et al., 1999). Potholes provide ideal environments to retain both moisture and nutrients, promoting 'internal' recycling, so it is important to understand if the diversity of the bacterial population found therein gives a picture of the resilience of this community to possible changes in its larger environment.

Extensive work to date has examined the photosynthetic communities present in BSCs (Garcia-Pichel et al., 2001; Belnap et al., 2004; Yeager et al., 2004) and endolithic communities in arid environments (Friedman, 1982; Walker and Pace, 2007; Omelon, 2008). It has been well established that cyanobacteria are dominant in these environments, providing a primary source of nitrogen fixation (Belnap et al., 2006) as well as symbiotic associations with lichens in the area. However, although photosynthetic organisms are, undoubtedly, the most important primary producers, other types of bacteria are also known to play important roles in these communities. It has been found that chemoorganotrophic bacteria may frequently colonise stone surfaces, as they are able to obtain mineral nutrients and organics from the air (Viles and Gorbushina, 2003). Heterotrophic bacteria are also commonly identified in lithic environments, filling important niches and living in places photosynthetic organisms cannot (Reddy and

Garcia-Pichel, 2007). For example, at some locations sediments accumulate within the potholes excluding direct light which encourages forms of metabolism other than photosynthesis.

4.2 Methods

Pothole Type Selection

Potholes are found in abundance throughout the Colorado Plateau region (Figure 4.1) and can be divided into two main groups: joint-controlled and independent potholes. Joint controlled potholes form in areas where jointing in the host sandstone channels water during periods of rain, leading to potholes occurring in series along the joint. Independent potholes are found away from any obvious method of water channelling, usually on slightly sloping surfaces forming without a pattern. Independent potholes were chosen for this study because joint controlled potholes rarely contain sediments.

Field Site Descriptions

Two field sites were selected, one at Seven Mile Canyon (SMC) and a second at Bartlet Wash (BW), both in close proximity to the city of Moab, Utah (Figure 4.2). The field site at Seven Mile Canyon (SMC) is host to a higher than average concentration of potholes, none of which are joint-controlled. The SMC sandstone is part of the Kayenta formation, which is characterized by abundant variation; from intra-formational limestone and conglomerate to thick lacustrine material all from the early Jurassic (approximately190 -200 Ma) (Doelling, 2003). The site is a bench of mainly feldspar-arenite sandstone gently sloping south towards a steep drop off into SMC. Potholes

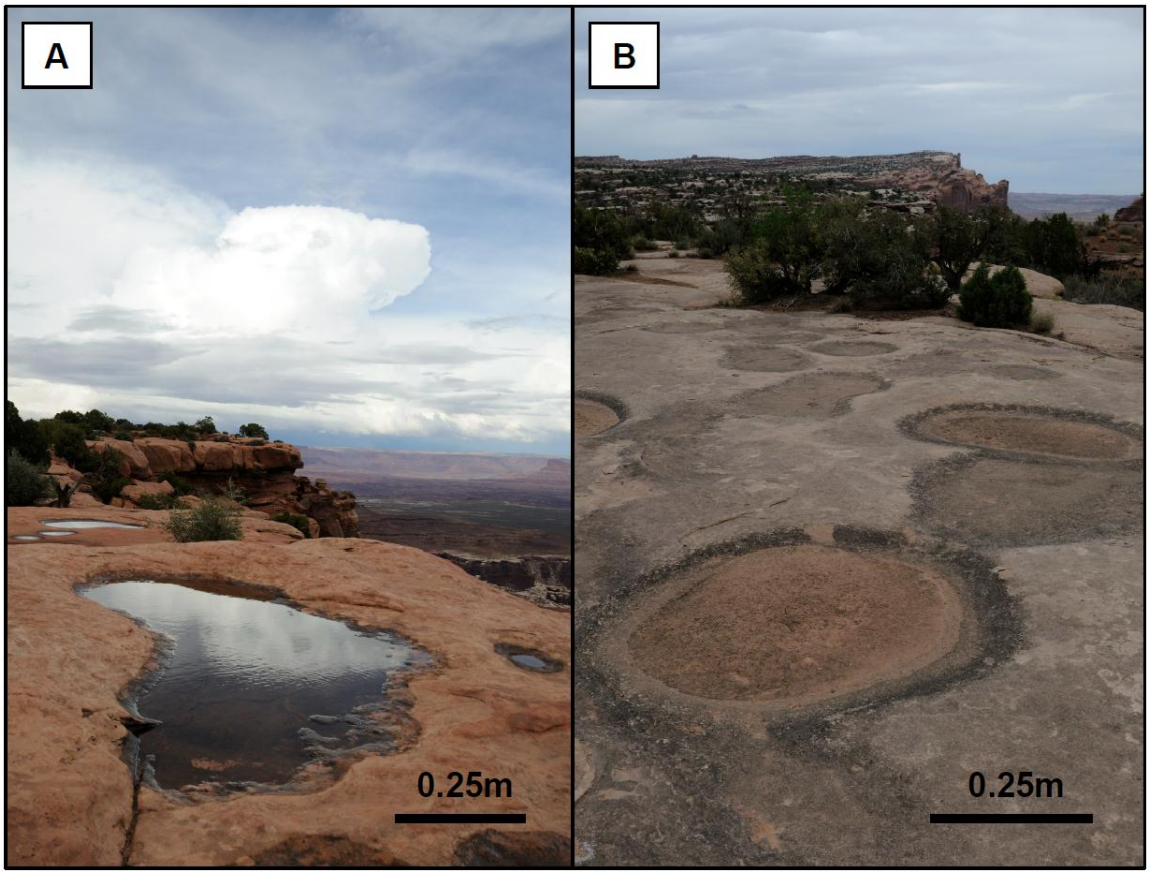


Figure 4.1: Potholes forming near Moab, Utah. **A)** Potholes at Dead Horse Point State Park. **B)** Potholes at Seven Mile Canyon.

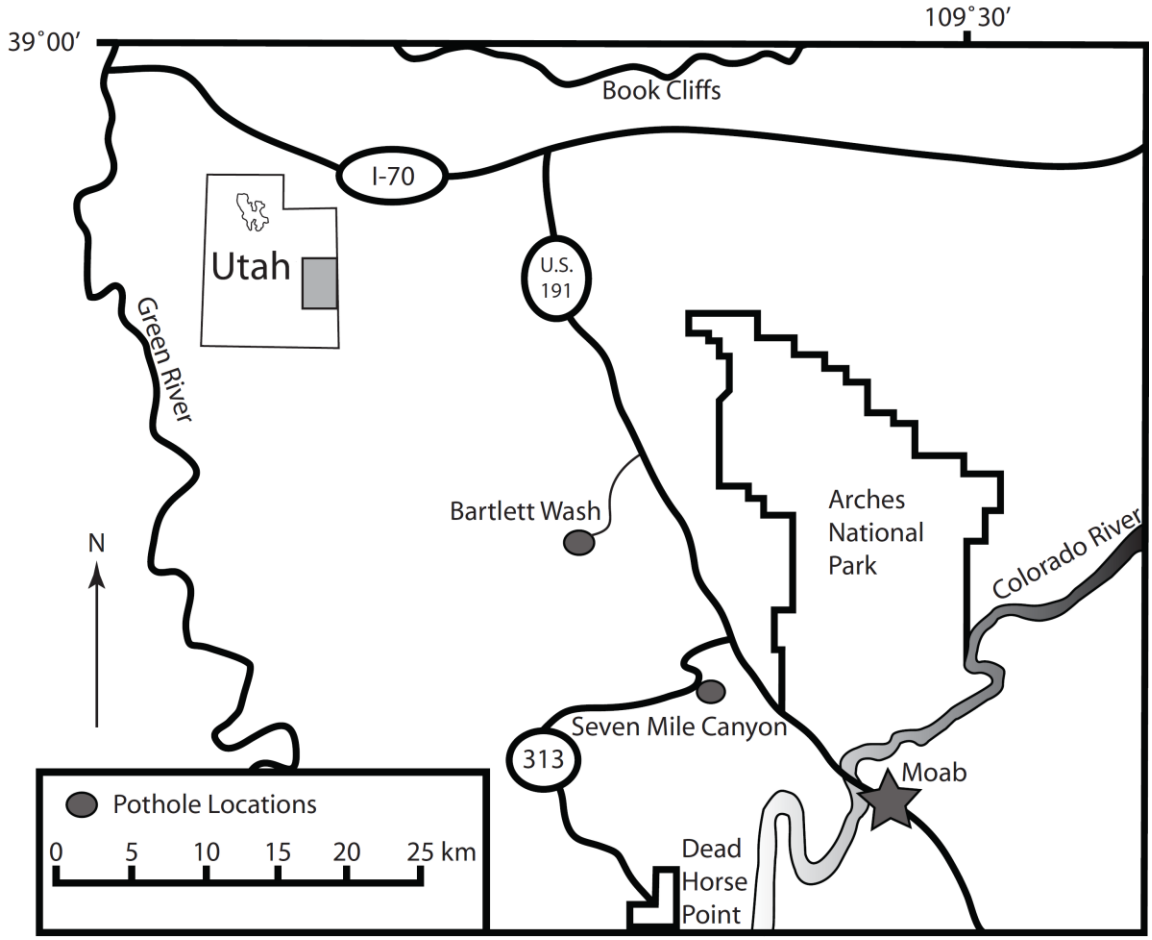


Figure 4.2: Sampling locations from this study (after Chan et al., 2005). Bartlett Wash (BW) (N 38 degrees 42.662;W 109 degrees 47.146; 1516 m); Seven Mile Canyon (SMC) (N 38 degrees 38.648;W 109 degrees 43.862; 1482 m).

cover the surface showing a wide range of sizes and stages of development, from amorphous areas of surficial black biofilm to distinct circular potholes 'dug in' within their surroundings. Evidence of animal life (foot-prints and scat) was noticed in and around the potholes, which is to be expected, as they are the only water source to be found in that area. A second field site at BW was chosen for comparison because it is more isolated and at times inaccessible. It has less chance of animal and human contact with the potholes. The potholes at BW are morphologically different than those from SMC, which presumably reflects the slight variation in the host sandstone. The BW sandstone is a part of the Moab Member of the Curtis Formation, a massive, aeolian, cliff-forming unit laid down between approximately 158 and 155 Ma (Doelling, 2003). The sandstone at BW has very little cement and is therefore more friable than at SMC; it also has fewer accessory minerals and is a quartz-arenite (See Chapter 2).

Sample Selection

Five separate samples were selected for rRNA analysis, which represented the different niches present in the pothole environment (Figure 4.3). One sample of the black surficial biofilm was selected from SMC (SMC-BB). Surficial black biofilms did not readily develop at BW due to the porosity of the sandstone. Instead, endolithic layers of green biofilm were more ubiquitous, observed 1 mm – 1 cm under the rock surface. A sample of this endolithic biofilm was taken (BW-EB). One sample of pothole sediment from SMC was selected (SMC-PS), and one sample of sediment from BW (BW-PS). The pothole sediments from SMC varied in thickness, up to a maximum of 3 cm while the sediments of BW were much thinner, measuring only a few millimeters deep. Finally

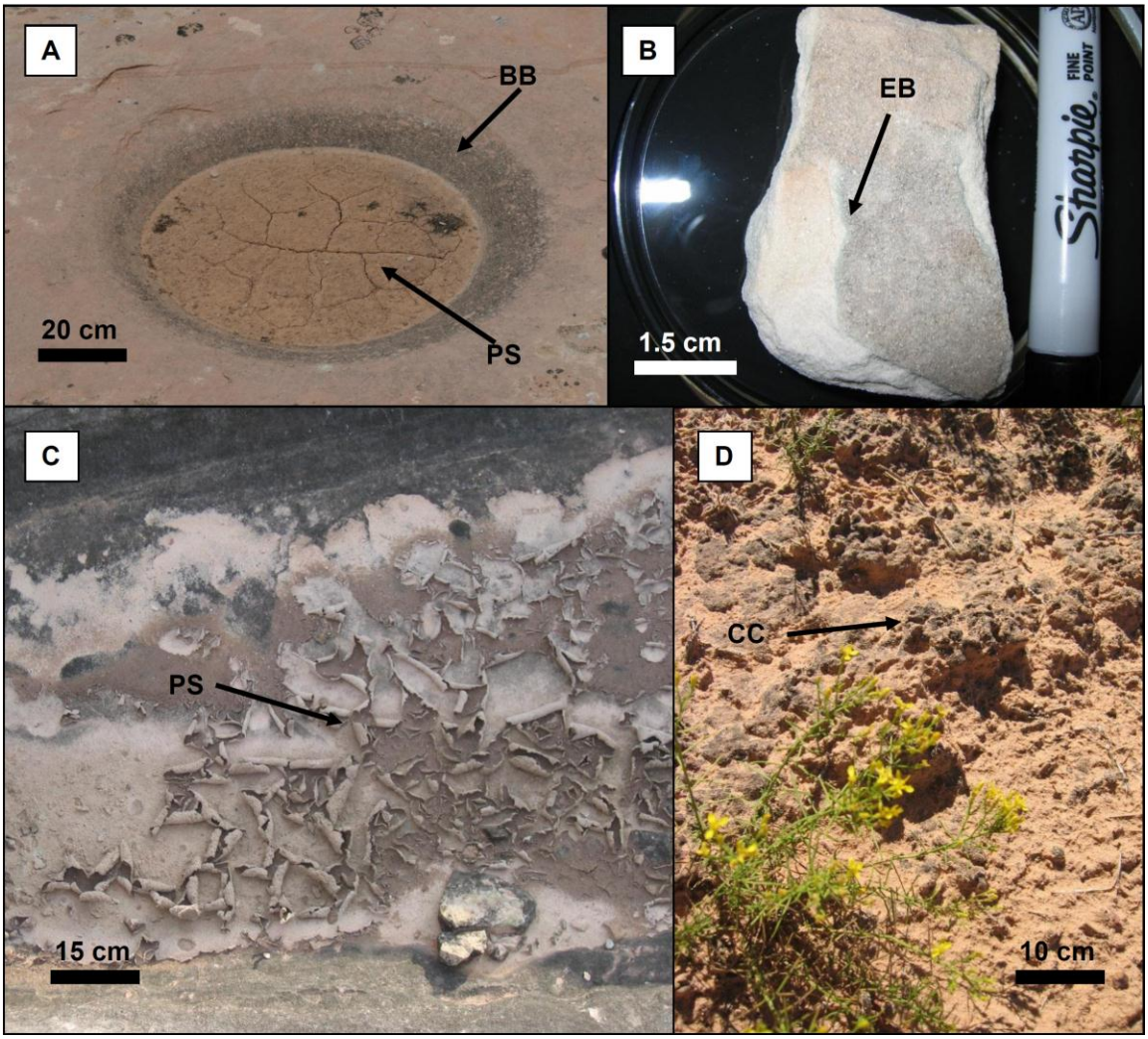


Figure 4.3: A) Sampling location of SMC-PS and SMC-BB. **B)** Larger sister sample to sample of BW-EB. **C)** Sampling location of BW-PS. **D)** Sampling location of SMC-BSC.

a sample of biological soil crust, the end stage of pothole development was included from SMC (SMC-BSC).

DNA Extraction and Amplification

DNA was extracted from each sample with a Soil DNA Isolation kit (Mo Bio Laboratories, Inc., Solana Beach, CA.) used according to the manufacturer's suggested protocol. rRNA was amplified from DNA extracts by polymerase chain reaction (PCR) by using JumpStart REDTaq ReadyMix (Sigma, Saint Louis, USA) and universal eubacterial primers pA and pH, product ca. 1500 bp (Massol-Deya et al., 1995). PCR reactions were performed in a Biometra T1 Thermocycler (Montreal Biotech, QC) under the following conditions: initial denaturation at 94°C for 120 sec; 35 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 30 sec, extension at 72°C for 2 min; and a final extension at 72°C for 7 min. PCR products were held at 4°C prior to further analysis. Agarose gel electrophoresis was performed and the PCR product was purified with QIAquick PCR Purification Kit (Qiagen, Mississauga, ON).

Cloning and Sequencing of 16S rRNA

Purified rRNA was cloned into vector pCR2.1 by using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Clones containing inserts from picked colonies were identified by direct PCR analysis by using M13 primers after being purified using a PureLink HQ Plasmid Purification Kit (Invitrogen, Carlsbad, CA). The purified rRNA was digested by restriction fragment length polymorphism (RFLP) using restriction enzymes with 4-bp recognition sites (Hin6I, RsaI and BsuRI). Fifty (50) clones from

each site, representing the main pothole condition or environment, were selected for sequencing. The sample of black surficial biofilm from SMC (SMC-BB) is used to represent the earliest stage of pothole development and pothole sediments, one from each field location, (SMC-PS1) and (BW-PS) were chosen to represent more mature potholes. Clones with a distinct RFLP patterns were selected for sequencing. The nucleic acid concentration of selected clones was measured using a NanoDrop ND-1000 spectrophotometer to assure sufficient DNA was present for sequencing. DNA sequencing was performed by the Robarts Research Institute DNA sequencing facility at the University of Western Ontario, London, ON.

The sequences were visually evaluated for noise and meaningful length using Chromas Technelysium Pty. Ltd., (Tewantin, Queensland, Australia). Problematic sequences and possible chimeras were identified with Greengenes (DeSantis et al. 2006) using Belleophon v.3 (Huber et al. 2004) and Mallard v1.02 (Ashelford et al., 2006). Sequences were subjected to a sequence similarity analysis using National Center for Biotechnology Information (NCBI) Blast search tool. The sequences were aligned using CLUSTALW (Thompson et al., 1994) and alignments were corrected by hand. Neighbour-joining analysis was performed using the Seaview package (Gouy et al., 2010). Bootstrap analysis (1000 replicates) was used to obtain confidence estimates for phylogenetic tree topologies. The sequences determined in this study have been deposited in the GenBank database under accession numbers JQ11698 through JQ711760.

4.3 Results

The samples of black pothole biofilm SMC-BB showed a low diversity with only 14% of the species being unique. The pothole sediment sample SMC-PS was found to have the greatest variety of clones (88%); almost all species identified were unique, although many were closely related. The BW sediments BW-PS contained 30% unique species. The species phylogenies are represented in Figures 4.4, 4.5 and 4.6, and individual operational taxonomic units (OTUs) identified using the BLAST tool and NCBI database are summarised in Tables 4.1, 4.2 and 4.3. For a brief general description of the phlyla/classes identified in this work see Appendix B. Each location hosts a different dominant group of bacteria (Figure 4.7). Sample SMC-BB was composed of mainly α and γproteobacteria making up 89% of all clones. The diverse community from SMC-PS is dominated by the αproteobacteria and clostridia, making up 43% of the clones. BW-PS sample was composed mainly of α and βproteobacteria, making up 87% of the clones. Within these larger groupings are clusters of species that are closely related or, in the cases of SMC-BB and BW-PS, the same species. Within sample SMC-BB, the dominant species is a grouping of 13 clones related to the genera *Brevundimonas* comprising three different species. Secondary groups include 5 clones related to three species of the genera *Sphingomonadaceae* and six clones of three species of the genera *Enterobacteriaceae*. Although SMC-PS shows the greatest variety of clones, small clusters of certain species are observed. The largest grouping contains four clones related to different species of *Sphingomonas* as well as smaller groups related to the genera *Geobacter* (2) and *Clostridium* (2). The diversity of the sediment sample from BW shows the least diversity with 22 clones closely related to two species of *Acidovorax*,

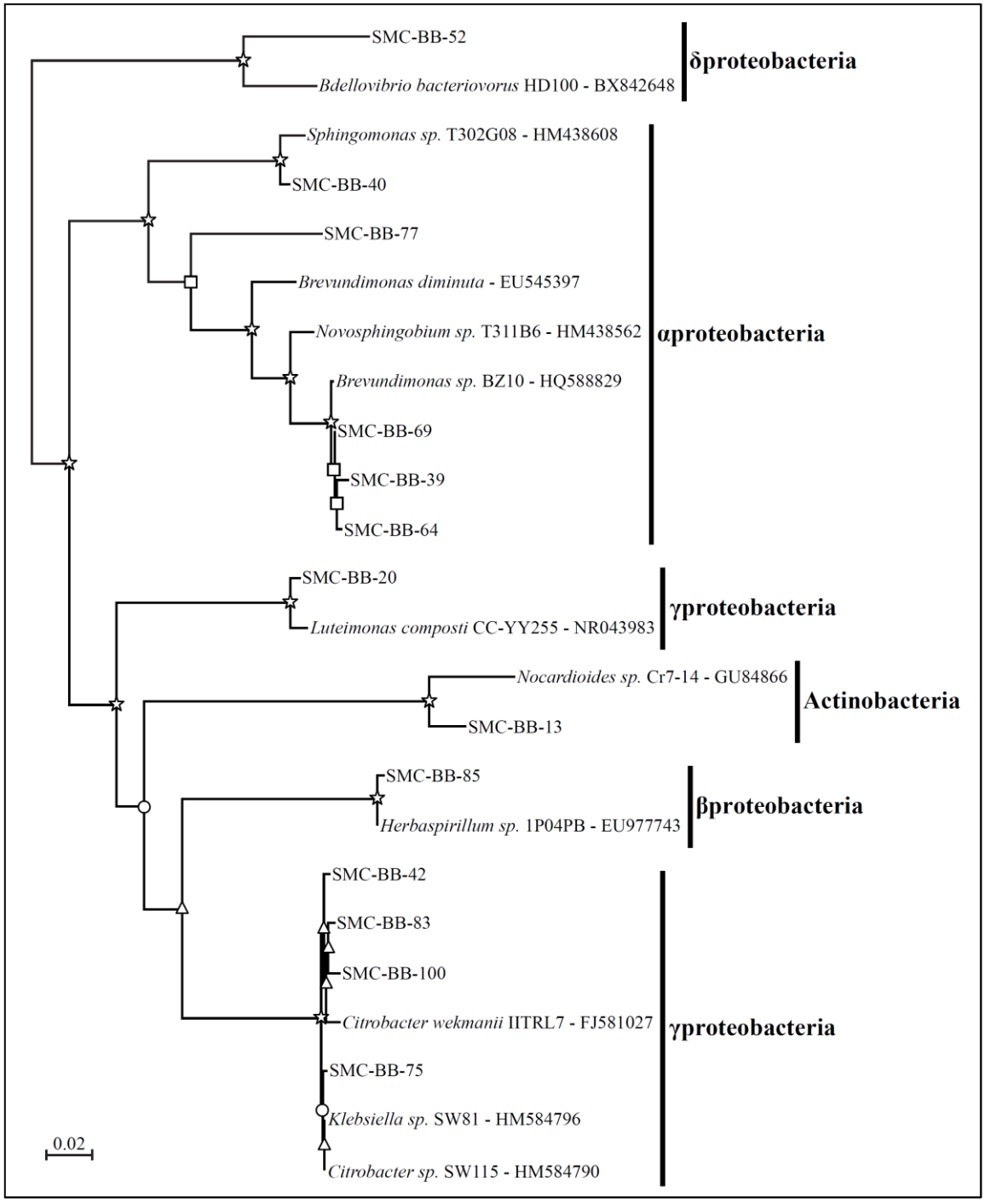


Figure 4.4: Phylogenetic tree based on 16s rRNA sequences from (SMC-BB). Symbols on the branches indicate bootstrap confidence values, as follows:☆≥80%; □60-79%; Δ40-59%; ○≤ 39%. The branch lengths indicate the expected number of changes per sequence position as per the scale with each tree. A selection of closest comparable sequences from BLAST results are included for clarity.

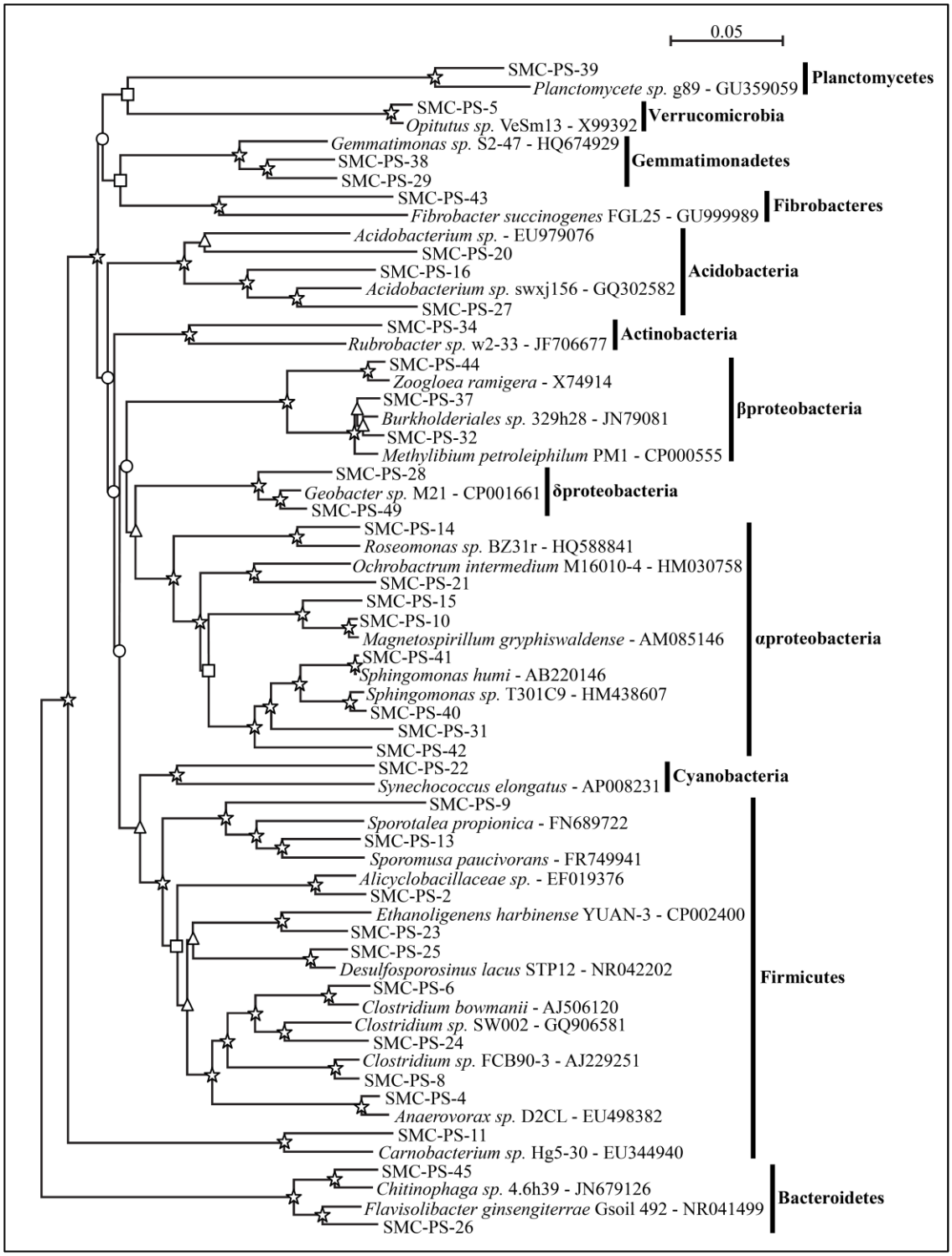


Figure 4.5: Phylogenetic tree based on 16s rRNA sequences from SMC-PS. Symbols on the branches indicate bootstrap confidence values, as follows:☆≥80%; □60-79%; Δ40-59%; ○≤ 39%. The branch lengths indicate the expected number of changes per sequence position as per the scale with each tree. A selection of closest comparable sequences from BLAST results are included for clarity.

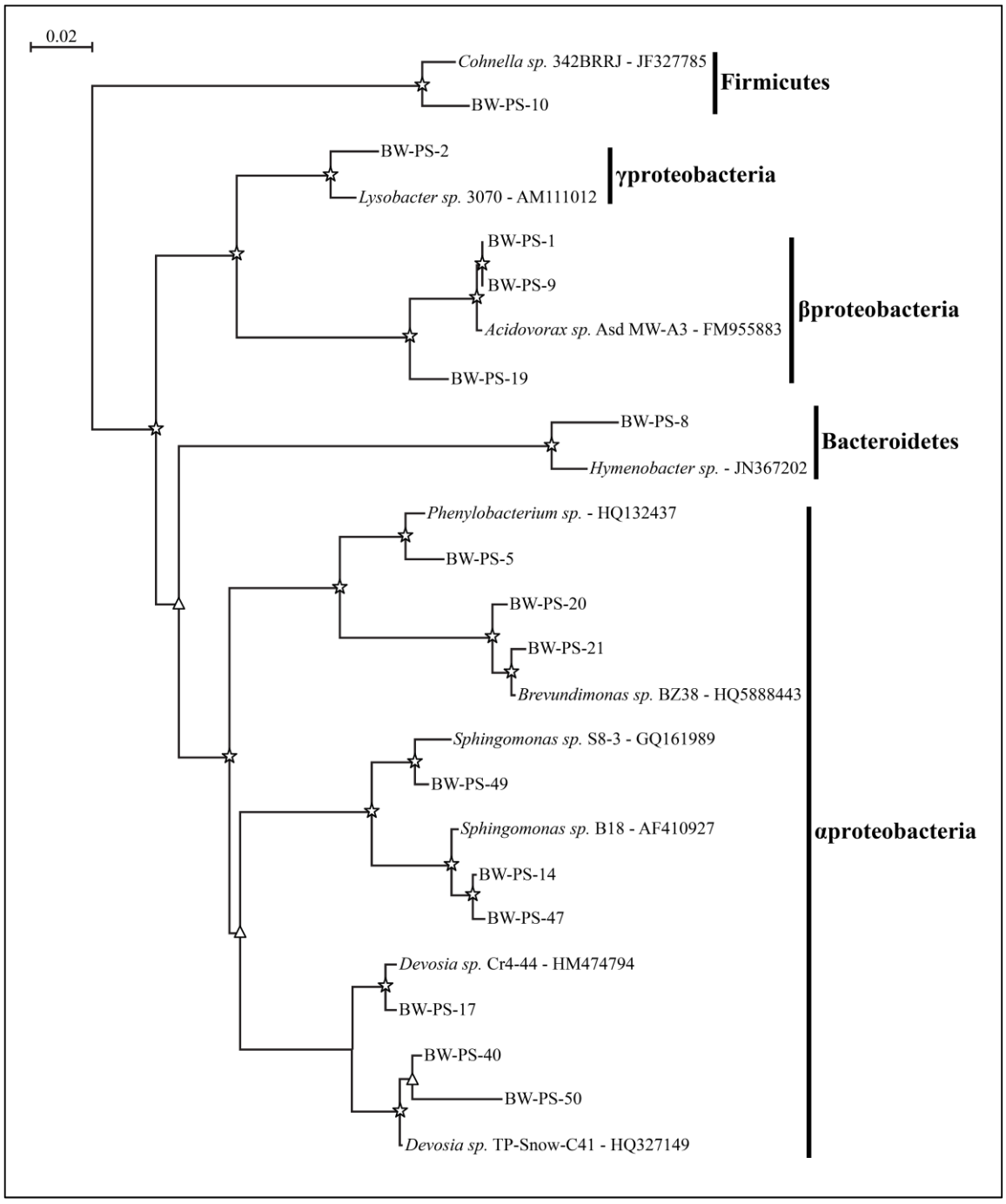


Figure 4.6: Phylogenetic tree based on 16s rRNA sequences from BW-PS. Symbols on the branches indicate bootstrap confidence values, as follows: ☆≥80%; □60-79%; Δ40-59%; ○≤ 39%. The branch lengths indicate the expected number of changes per sequence position as per the scale with each tree. A selection of closest comparable sequences from BLAST results are included for clarity.

Table 4.1: Bacterial BLAST Matches (SMC-BB)

OTU	# of clones	From rflp	Group/phylum	% coverage	% similarity	Name	Environment	Assention #
39	10	40	αproteobacteria	100	98	*Brevundimonas* sp.BZ10	Hydrocarbon-contaminated soil	HQ588829
77	1	-	αproteobacteria	100	94	*Brevundimonas diminuta*	River polluted with poultry waste	EU545397
64	1	-		100	99	Uncultured bacterium Clone EDW07B003_2	Transition from fresh to saline water	HM066429
			αproteobacteria	100	99	*Brevundimonas sp*. BZ10	Hydrocarbon-contaminated soil	HQ588829
69	1	-	αproteobacteria	100	97	*Novosphingobium sp*.T311B6	Anthracene contaminated soil	HM438562
40	5	15	αproteobacteria	97	97	*Sphingomonas sp*. clone T302G08	Anthracene contaminated soils	HM438608
85	1	1	βproteobacteria	96	98	*Beta proteobacterium* R-37018	Antarctica:Transantarctic Mountains, Lundstrom	FR691425
			βproteobacteria	92	98	*Herbaspirillum sp*. 1P04PB	Clean-room floor	EU977743
75	2	5	γproteobacteria	100	99	*Citrobacter sp*. SW115	Silkworm intestine	HM584790
83	2	-	γproteobacteria	99	98	*Citrobacter werkmanii* strain IITRL7	Pulp paper mill waste contaminated site	FJ581027
100	1	-		100	98	Uncultured bacterium clone T60_G12	Bacterial community in 'taberna' a beverage	GU808383
			γproteobacteria	99	98	*Citrobacter murliniae* strain CDC 2970-59	Type strain	NR028688
42	2	1	γproteobacteria	100	99	*Klebsiella* sp. SW81	Silkworm intestine	HM584796
20	1	1	γproteobacteria	97	98	*Luteimonas composti* Strain CC-YY255	food waste	NR043983
52	1	-	δproteobacteria	100	96	Uncultured bacterium Clone Hot Creek 2	Arsenite oxidizing biofilm	AY168736
				100	94	*Bdellovibrio bacteriovorus* HD100	n/a	BX842648
13	1	-	Actinobacteria	95	99	*Nocardioides sp*. Cr7-14	Glacier cryoconite in Austria	GU84866

Table 4.2: Bacterial BLAST Matches (SMC-PS)

OTU	# of clones	Group/phylum	% Coverage	% Similarity	Name	Environment	Assention #
31	1	αproteobacteria	99	97	*Sphingomonas sp.* Clone SIA181-1A1	Fresh water	AF395032
41	1		97	99	Uncultured bacterium Clone 41D	Heavy metals-contaminated soils	EU676399
		αproteobacteria	96	99	*Sphingomonas humi*	Terrestrial and freshwater environments	AB220146
40	1		100	99	Uncultured bacterium Clone bar-b70	Soil under lichen and moss crusts	JN023996
		αproteobacteria	100	98	*Sphingomonas sp.* Clone T301C9	Anthracene contaminated soil	HM438607
42	2	αproteobacteria	95	99	*Sphingomonadaceae* Clone T313H5	Anthracene contaminated soil	HM438359
10	1	αproteobacteria	98	98	*Magnetospirillum sp.* Clone B03-03D	Eisenia fetida (earthworm) intestine	FJ542973
15	1	αproteobacteria	100	97	*Magnetospirillum gryphiswaldense*	n/a	AM085146
21	1		98	91	Uncultured bacterium Clone Bms_CK228	Petroleum-Contaminated Saline-Alkali Soils	
		αproteobacteria	100	90	*Ochrobactrum intermedium* strain M16-10-4	Oil reservoir production water	HM030758
14	1		98	97	Uncultured bacterium Clone JSC9-C	Clean room	DQ532254
		αproteobacteria	99	93	*Roseomonas sp.* Clone BZ31r	Soil with Hydrocarbons	HQ588841
32	1		98	98	Uncultured bacterium Clone BR98	Oil field	HQ190449
		βproteobacteria	98	97	*Burkholderiales* Clone 329h28	Bioreactor	JN79081
37	1		97	98	Uncultured bacterium Clone bar-c11	Soil under lichen and moss crusts	JN024002
		βproteobacteria	100	97	*Methylibium petroleiphilum* PM1	Methyl tert-butyl ether-degrading bacteria	CP000555

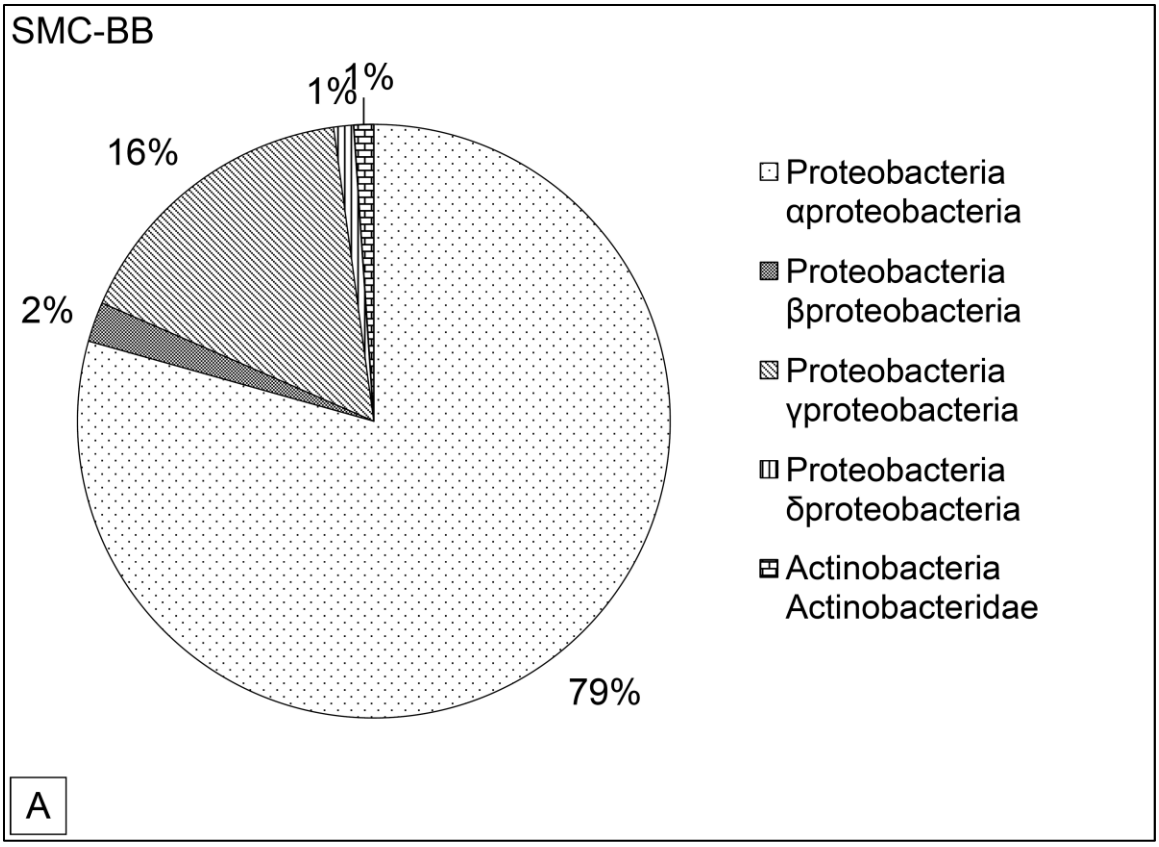
44	1	βproteobacteria	100	97	Uncultured beta proteobacterium Clone 16L13	Painted and unpainted concrete structures	EU409854
		βproteobacteria	100	97	*Zoogloea ramigera*	n/a	X74914
28	2		100	98	Uncultured bacterium Clone MA-49-V94B	Pristine confined aquifer	HM141865
		δproteobacteria	100	96	Geobacter sp. M21	n/a	CP001661
16	1		98	96	Uncultured bacterium Clone BN67	Oil field soil	HQ190319
		Acidobacteria	100	95	Uncultured Acidobacteria	n/a	Z95709
20	1	Acidobacteria	98	85	Uncultured Acidobacteria bacterium	Rhizosphere of faba bean	EU979076
27	1		100	91	Uncultured Acidobacteria bacterium	n/a	Z95711
		Acidobacteria	97	91	*Acidobacterium sp.* Clone sw-xj156	Non-sulfide, low-salt cold spring sediment	GQ302582
34	1		99	97	Uncultured bacterium G01-2	Gold mine biofilm	FM253670
		Actinobacteria	81	88	*Rubrobacter sp.* Clone w2-33	Atacama Desert, Chile	JF706677
45	1		98	97	Uncultured bacterium Clone AK1AB2_04B	Unvegetated, recently-deglaciated soil	GQ396857
		Bacteroidetes	98	95	*Chitinophaga sp.* Clone 4.6h39	n/a	JN679126
26	1		92	99	Uncultured Sphingobacteria bacterium Clone SeqSEEZ202	Corn rhizosphere	JN367222
		Bacteroidetes	99	94	*Flavisolibacter ginsengiterrae* Clone Gsoil 492	Ginseng cultivating soil	NR041499
22	1	Cyanobacteria	100	91	*Synechococcus elongatus*	Freshwater	AP008231
43	1		99	90	Uncultured bacterium Clone 258ds10	Water downstream of manure	AY212707
		Fibrobacteres	97	84	*Fibrobacter succinogenes* Strain FGL25	Goat rumen	GU999989
2	1		100	99	Uncultured bacterium Clone 500-37	Pyrene in soil	FR687441
		Firmicutes	99	98	*Alicyclobacillaceae* bacterium	Trembling aspen soil with elevated CO_2	EF019376
4	1		95	99	Uncultured bacterium Clone MUP3H01	Sediment frozen for preservation	HQ178883
		Firmicutes	97	95	*Anaerovorax sp.* D2CL_Bac_16S_clone16	Chloro-ethene degrading culture	EU498382

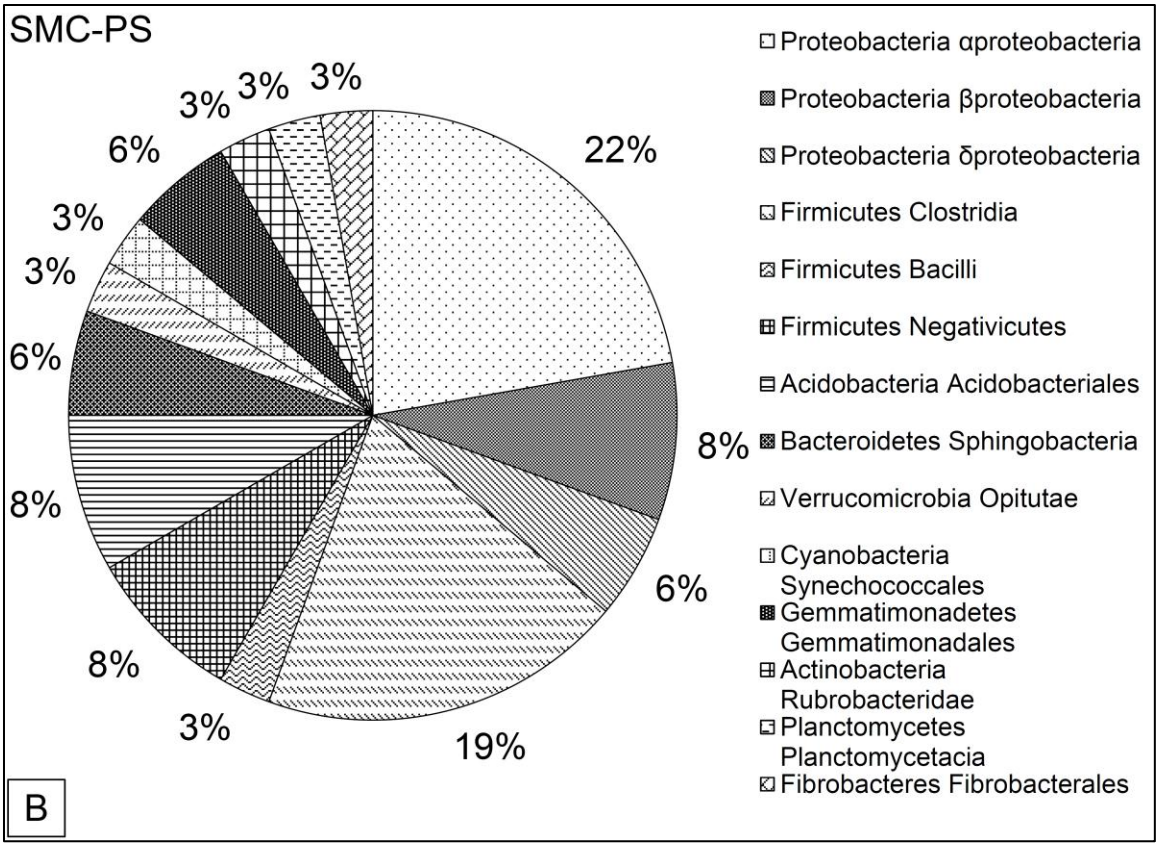
11	1		97	92	Uncultured bacterium clone HKT_RR46	Electronic waste dumping site	JN030437
		Firmicutes	97	89	*Carnobacterium sp.* Clone Hg5-30	Larval guts	EU344940
24	1		97	97	Uncultured bacterium Clone kab90	Volcano mud	
		Firmicutes	100	92	*Clostridium sp.* SW002	Yak rumen (anaerobic)	GQ906581
6	2		100	98	Uncultured bacterium Clone S36	Pond sediment	JF775630
		Firmicutes	99	96	*Clostridium bowmanii*	Antarctic microbial mat	AJ506120
8	1		96	98	Uncultured bacterium Clone AKAU4083	Uranium contaminated soil	DQ125852
		Firmicutes	92	96	*Clostridium sp.* FCB90-3	Anoxic soil of rice paddy	AJ229251
25	1		99	98	Uncultured bacterium Clone ZZ14C3	Benzene-contaminated groundwater	AY214191
		Firmicutes	99	96	*Desulfosporosinus lacus* Strain STP12	Freshwater lake sediments	NR042202
23	1	Firmicutes	100	90	*Ethanoligenens harbinense* ClonevYUAN-3	n/a	CP002400
13	1	Firmicutes	98	96	*Sporomusa paucivorans*	n/a	FR749941
9	2		98	96	Uncultured bacterium Clone G07	57 deg. C green filament	AF407698
		Firmicutes	99	84	*Sporotalea propionica*	Tetrachloromethane contaminated aquifer	FN689722
29	1		98	91	Uncultured soil bacterium Clone bac2nit44	Nitrogen amended dry meadow surface soil	EU861947
		Gemmatimonadetes	100	90	*Gemmatimonas aurantiaca* T-27	Aerobic/anaerobic batch reactor	AP009153
38	1		100	96	Uncultured bacterium Clone TX2_5E10	Extreme saline-alkaline soil	JN78339
		Gemmatimonadetes	98	92	*Gemmatimonas sp*. Clone S2-47	Weathered feldspar mineral	HQ674929
39	1		95	94	Uncultured bacterium Clone 1S5	Rhizosphere soil of peanut	GU359059
		Planctomycetes	98	91	*Planctomycete* Clone g89	Rhizosphere of faba beans	EU979098
5	1	Verrucomicrobia	97	97	*Opitutus sp*.VeSm13	Anoxic rice paddy soil	X99392

Table 4.3: Bacterial BLAST Matches (BW-PS)

OTU	# of clones	From RFLP	Group/phylum	% coverage	% similarity	Name	Environment	Assention #
21	2		αproteobacteria	100	98	*Brevundimonas sp*. BZ38	Hydrocarbon-contaminated soil	HQ588843
20	1			98	95	Uncultured bacterium Clone ZBAF1-9	Coking wastewater	HQ681994
	-		αproteobacteria	95	94	*Brevundimonas sp.* EMB 358	Anareobic/Aerobic SBR Reactor	DQ413173
17	1		αproteobacteria	100	98	*Devosia sp.* Cr4-44	Glacier cryoconite in Austria	HM474794
40	2		αproteobacteria	100	99	*Devosia sp.* Cr7-05	Glacier cryoconite in Austria	GU441678
50	1		αproteobacteria	97	99	*Devosia sp.* TP-Snow-C41	Snow pit, Tibetan Plateau	HQ327149
5	1	1		100	99	Uncultured bacterium Clone 319MICcrown	Concrete sewer biofilm	JF41876
	-		αproteobacteria	100	98	*Phenylobacterium sp.*	Heavy metal contaminated estuarine sediment	HQ132473
14	1	3	αproteobacteria	99	98	*Sphingomonas sp.* B18	Bacteriophages in freshwater	AF410927
49	1			97	97	Uncultured bacterium Clone kab217	Volcano mud	FJ936934
			αproteobacteria	100	96	*Sphingomonas sp.* S8-3	Alpine soil	GQ161989
47	1			100	99	Uncultured bacterium Clone 1C226554	Aquatic biofilm	EU799003
	-		αproteobacteria	100	99	*Sphingopyxix sp.* Clone XZXXH49	High-altitude lakes of the eastern Tibetan plateau	EU703439
9	1		βproteobacteria	100	98	*Acidovorax sp.* Clone Van 23	Anaerobic phenolic compound biodegradation	HQ222268
1	19	7	βproteobacteria	99	98	*Acidovorax sp.* Clone Asd MW-A3	Sediment from Glacial melt water stream	FM955883
2	1	2	γproteobacteria	97	97	*Lysobacter sp.* 3070	Deep sea pacific sediment	AM111012
19	1		γproteobacteria	97	92	Uncultured bacterium Clone B12-814	Sludge	EF174240
			γproteobacteria	98	91	*Lysobacter spongiicola*	Deep-sea sponge	NR041587

8	2	1		97	95	Uncultured bacterium Clone ZQMB08	Coal bed	JF417834
	-		Bacteroidetes	95	96	Hymenobacter group bacterium	Soil at the corn rhizosphere and surround	JN367202
10	1			97	96	Uncultured bacterium Clone ADB-39	Urban aerosols	HM366499
	-		Firmicutes	99	96	*Cohnella sp.* 342BRRJ	n/a	JF327785


SMC-BB
1%
1%
16%
2%
79%
Proteobacteria αproteobacteria
Proteobacteria βproteobacteria
Proteobacteria γproteobacteria
Proteobacteria δproteobacteria
Actinobacteria Actinobacteridae
A


SMC-PS
3%
3%
3%
22%
6%
3%
3%
6%
8%
6%
8%
3%
19%
Proteobacteria αproteobacteria
Proteobacteria βproteobacteria
Proteobacteria δproteobacteria
Firmicutes Clostridia
Firmicutes Bacilli
Firmicutes Negativicutes
Acidobacteria Acidobacteriales
Bacteroidetes Sphingobacteria
Verrucomicrobia Opitutae
Cyanobacteria Synechococcales
Gemmatimonadetes Gemmatimonadales
Actinobacteria Rubrobacteridae
Planctomycetes Planctomycetacia
Fibrobacteres Fibrobacterales
B

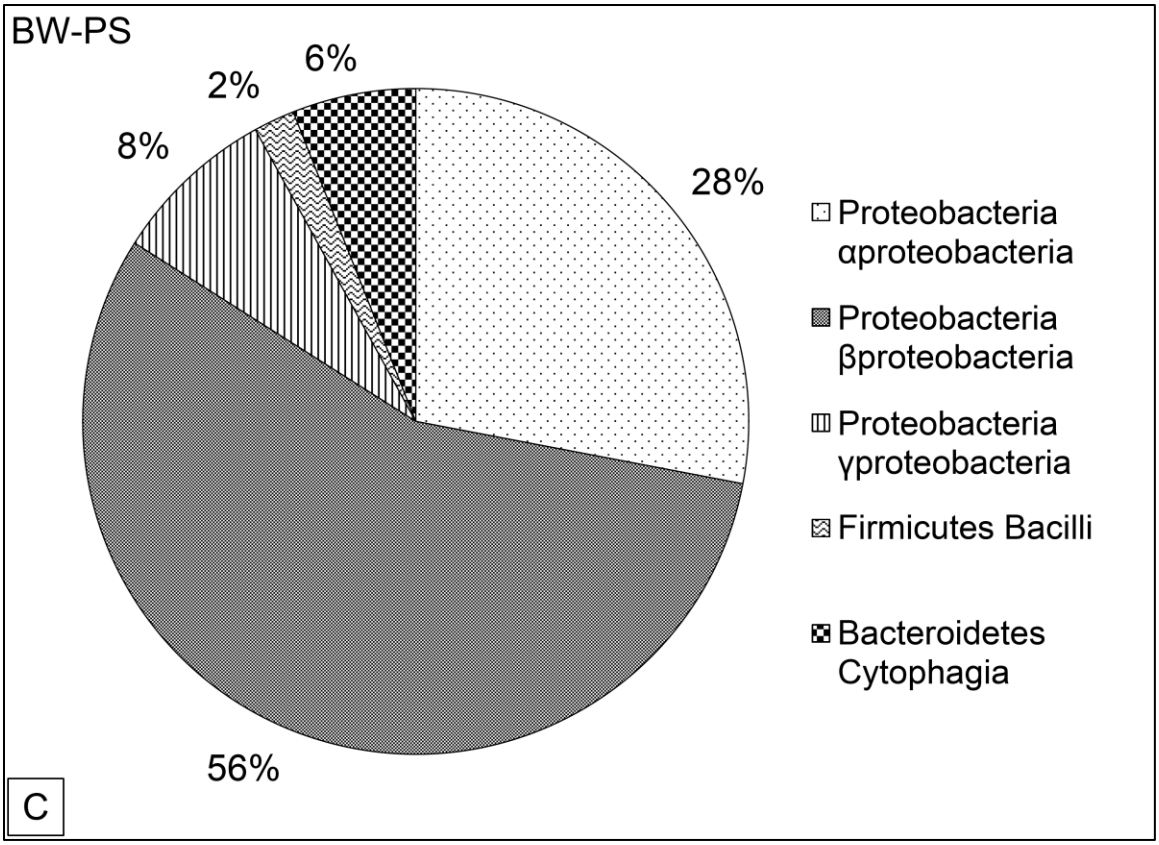


Figure 4.7: Phyla/Classes represented at each sampling location and the percentage of clones assigned to each group. **A)** SMC-BB; **B)** SMC-PS; **C)** BW-PS

and smaller groupings of two clones of two species of *Brevundimonas*, three clones of two species of *Devosia*, three clones of two bacteria of the family *Xanthomonadaceae* and two clones of one species of *Hymenobacter*.

4.4 Discussion

The diversity of colonies found in each location suggests that the initial host sandstone is important to the pothole sediment environment and the overall success of the pothole in supporting higher forms of life. The sediments sampled from the bottom of the pothole BW had a low diversity that is more closely related to the species diversity in the sample of surficial black biofilm from SMC. The low diversity of the BW sediments suggests that, although large and deep potholes form there, other key factors in the development of a supportive sedimentary environment are missing. The sandstone at BW is poorly cemented, porous and arenitic; factors which allow easy access to the protective rock surface, with good light penetration, for initial endolithic colonisation (Bell 1993, Kurtz and Netoff, 2001). However, the porosity limits the ability of the BW sandstone to pool water, restricting the biofilm development beyond the stage of initial colonisation despite the ability of cyanobacteria to produce large amounts of EPS that can act to reduce water loss (Mager and Thomas, 2011). Additionally, the BW location is much more exposed (high on a sandstone mesa) than the SMC location, which is located near the top of its namesake canyon. The canyon location may give more shelter from drying winds, and erosion of the canyon itself may provide more sedimentary material for proto-soils. It is also possible that the greater abundance in secondary minerals at SMC may provide limited metals for biogeochemical cycling (Northup et al., 2000). In fact,

any increase in the mineral diversity of the host rock may be important as studies of lithotrophic oligotrophic organisms in culture have shown that their growth improves with the addition of host rock extracts (Siebert et al., 1996). Finally, evidence of animal traffic was regularly noted at the SMC field site probably because of its' somewhat sheltered location and easy accessibility. It is obvious that the wild inhabitants of the area contaminate the potholes (Enterobactereaceae), with scat and urine while using them as a valuable nutrient and water source. Any addition of animal by-products, transferred soils or plant materials would serve as a tremendous resource in such an oligotrophic environment. Although nitrogen compounds have been suggested to be the main additions from animal by-products (Hawkes, 2003), secondary nutrients may well be more important, as studies have found that the biological demand for nitrogen is more than met by abundant deposition from precipitation and dust (Nienow and Friedman, 1988).

With these factors taken into account, the quartz-dominated BW sediments seem to be the best example of a subsistence biofilm to compare more directly with the black surficial biofilm from SMC. The stage of biofilm development does not indicate the relative ages of host rock colonisation as it is quite possible that, although it is less diverse and sediment poor relative to SMC-PS, the BW biofilm may be older. Biofilm communities tend to colonise rock relatively quickly until the carrying capacity is reached (Walker and Pace, 2007; Hoppert et al., 2004). These two biofilms, therefore, represent early stage colonisation, and the bacteria found in these environments are more likely to be highly opportunistic and adaptable species.

Sample SMC-BB was dominated by two genera: *Brevundimonas* and *Sphingomonas*. The αproteobacteria genus *Sphingomonas* was represented in each field location and related to several clones in the SMC pothole sediments. The αproteobacteria are the most heavily represented class in both samples from SMC and the second most important after the βproteobacteria at BW. *Sphingomonas* ssp. are studied mainly as degraders of toxic compounds in the environment such as polycyclic aromatic hydrocarbons (PAHs) (Leys et al., 2005) and hexachlorocyclohexane (HCH) (Kumari et al., 2002). PAHs can be used as carbon and energy sources for bacteria growing on stone building facades in urban areas (Saiz-Jimenez, 1999). The source of most of these pollutants is vehicle emissions, which is a possible source at SMC, located, as it is, close to a popular stretch of road leading to a state park. The ability of the *Sphingomonas* spp. to degrade a wide range of toxic compounds is due to a general metabolic diversity, enabling them to exist, optimistically exploiting a variety of carbon sources and making these obligate aerobes ideal candidates to colonise the oligotrophic desert pothole environments.

To get to the comparatively rich pothole sediment environment of SMC, the bare rock must first be colonised and, as discussed, the BW pothole sample represents the first step to building a pothole biofilm. The most abundantly represented class in BW-PS was the βproteobacteria, more specifically the genus *Acidovorax*, a group of chemo-organotrophic bacteria. Some species of *Acidovorax* are known to be lithotrophic (inorganic energy), using hydrogen as an energy source (Schneider and Schlegel, 1977). Clones related to these bacteria were not found in the other two samples indicating that wind-blown inputs may be more important in the BW environment. Although wind-

blown material is no doubt important at SMC, some surrounding potholes in the area already support plants and so may contribute organic matter or nutrients more readily into the overall ecosystem (Ruiz et al., 2008). The *Acidovorax* were previously classified as *Pseudomonas*, a genus known for their large genomes and metabolic diversity giving them an advantage to colonise the oligotrophic conditions by having the capacity to 'eat' almost anything (found at BW), and exist opportunistically on whatever windblown nutrient additions appear (Willems et al., 1990).

The second most abundant class in BW-PS was the αproteobacteria, which has a greater diversity of species with eight distinct clones, which are broken down into three families the *Caulobacteraceae*, the *Sphingomonadaceae* and the *Hyphomicrobiaceae*. The family *Sphingomonadaceae* was found in all three locations in this study, while the *Caulobacteraceae* were only represented in the black biofilm from SMC and not found in the sediments. The genus *Breundimonas* of the family *Caulobacteraceae* hosts a large group of extremely oligotrophic species. This group of organisms tends to grow best at slightly higher temperatures (25-30°C) with maximum tolerable temperature for most species reaching 37°C (although specific species may grow at temperatures as high as 42°C) (Garity et al., 2003). This range of temperatures suggests that the black biofilm would be able to grow at ambient pothole water temperatures even during the peak of summer in July which were measured at 33-35^{o}C (midday). Morning and evening temperatures were more moderate 24-27^{o}C. Growth limitations of these types of organisms would occur during the spring when pothole water is derived from snow melts and in the late fall when temperatures fall below freezing (NWS, 2012). These indications of restricted growth periods for the biofilm emphasise the vulnerability of the

pothole environment to changes in temperature and particularly to any variation in precipitation including the time of year when most rainfall occurs. The genus *Breundimonas* of the family *Caulobacteraceae* is highly similar to clones in this study in BW-PS and SMC-BB and has been found in other oligotrophic environments such as Antarctic soils and aerosols (Gonzales-Troil, 2009). Temperature variation is a major obstacle to life in this environment: The summer months are hot and dry with temperatures often reaching 40°C while winter months can see temperatures below 0°C. Sequence data shows microbes related to those previously cultured from glacial environments (e.g., BW-PS #1; see Reddy et al. 2009) and those found in higher temperatures environments such volcanic mud (BW-PS #49), thus underlining the variability of the desert environment (Table 4.3). Mesophillic species are represented in samples of the SMC pothole sediments including a clone related to the family *Alicyclobacillacae* with a possible growth range of between 4 and 70°C (Garity et al., 2003).

The *Hyphomicrobiaceae* were only found at BW in this study and the sequences are all generally related to the genus *Devosia*, which are all aerobic heterotrophs frequently isolated from soils (Yoo et al., 2006), some associated with symbiotic nitrogen fixation (Rivas et al., 2003). They are not known for tolerance to desert-like conditions, indeed all three clones are related to *Devosia* isolated from cold, aqueous environments. This may indicate that the species found at the study location are more adapted to colder times of year and presumably take advantage of spring snow melt or late fall rains.

The sediments of SMC-PS possess a much higher diversity of clones than the other samples. The sediment environment at SMC is more varied, receiving inputs of

wind-blown material and retaining moisture for much longer than the much more severe environment found centimetres away in the sandstone at the pothole rim. Further, the deeper sediments provide shelter from UV radiation and many more micro-niches, which can be colonised by slightly less resistant microbiotic life, much like the BSCs of the area (Belnap et al., 2008; Viles, 2008). Through this simple accumulation of a few centimeters of sediment a positive feedback occurs, with a greater richness of species creating more opportunities for nutrient extraction and a more active/diverse biosphere. A 'larger biofilm' will produce more EPS being able to retain water for longer and protect less desiccation tolerant microbes, which in turn supports more growth and so on (Knowles and Castenholz, 2008).

Although the main groupings of clones in BW-PS and SMC-BB indicate a focus on obtaining the basics of life in an oligotrophic environment, the sediments at SMC were found to host other species that suggest the next possible steps once a baseline community has been established. Some of the SMC sediment clones are related to genera that specialise in living at the oxic-anoxic interface. For example, one clone is most closely related to the species *Citrobacter freundii*, which has been found to transition between anaerobic and aerobic environments although its optimal growth conditions are aerobic (Qui et al., 2009). Also, genera such as the *Magentospirillum* were found, which are microaerophilic but live at the oxic-anoxic interface and can survive an oxygen atmosphere (Bazylinski et al., 2000). The presence of microaerophilic species indicates that another entire anaerobic lifestyle may be available within those sediments. The second most dominant class of bacteria related to the clones from SMC-PS are the clostridia which in general are spore forming obligate anaerobes. The importance of

anaerobic bacteria in the sediments indicates that areas in even the thin (up to 3 cm deep) sediment layers develop relatively stable anaerobic conditions. None of the clostridia were found in the other two samples. However other obligate anaerobes were represented in the black biofilm at SMC, albeit in much smaller percentages. The family *Enterobacteriaceae* is represented by four different clones in SMC-BB, suggesting a possible influence from animal scat. Although anaerobic bacteria were not found in BW-PS, it is possible that they were simply not sampled, i.e., they only represent a minor fraction (< 2%) of the bacterial population. It is also possible that the more porous nature of the sandstone makes the establishment of even micro-anaerobic environments unlikely or very tenuous.

Some anaerobic bacteria have been found to tolerate oxic environments using a system of diurnal migration vertically through bacterial mats (Teske et al., 1998). This allows anaerobes to avoid daytime oxygen produced in upper layers by cyanobacteria while still having access to nutrients at night (Krekeler et al., 1998). Although the clostridia-related clones found in the pothole sediment are not known to migrate, they are still a marker to suggest that a milestone of sediment formation has been reached. Support of an anaerobic community indicates stability in both sediment depth and retention of water. The pothole from which the sample was taken held water for a total of five days after a rain even longer than most other formations at that location. It is likely that with further study of the bacterial communities in pothole sediments this type of interface lifestyle, requiring a high level of flexibility, would be common, especially in groups that have the ability to form spores as a resting stage, such as the clostridia. Despite the environment within the pothole sediments being more hospitable to

microbiotic life, many of the same challenges are present in all locations: Desiccation and the ability to survive long periods without water is one of the greatest challenges, with spore formation being the best answer to this environmental reality.

4.5 Conclusions

Although it has been well established in various studies that biological soil crusts in desert environments are dominated by cyanobacteria (e.g., Garcia-Pichel et al., 2001, Belnap et al., 2001), they alone do not provide a complete picture of the bacterial diversity that is integral to colonising these inhospitable environments. A larger picture of pothole bacterial diversity has been observed that must be more fully studied to develop a clear picture of how communities of microorganisms support the formation of essential soils in desert environments. The community structure of these potholes is still unclear, perhaps because of the opportunistic nature of additions to the potholes, with the potential for new species of bacteria and nutrients to be added with wind-blown sediments. Further comparison of the DNA of pothole communities through approaches such as denaturing gradient gel electrophoresis (DGGE) would provide a more detailed picture of the importance of various species and the overall diversity in these unique ecosystems. This study has found that, not only are the various micro-niches in the pothole environment variable with respect to the diversity of bacteria present, but the types of bacteria varied by location (within a few centimetres within the same pothole) and based on the host rock in which the pothole developed (host rocks that support the development of pothole sediments and those that don't). This progression in the diversity of bacteria from surficial biofilms to pothole sediments underlines the importance of the

BSCs found in the semi-arid desert environment: They represent an end stage of pothole development, supporting (intuitively) the most diverse community of bacteria. This diversity enables them to further develop the cryptobiotic soil to the point where eukaryotic organisms are able to colonise it. These soils may also act as reservoirs of biological diversity in the desert environment providing a ready source of wind-blown particulates to nucleate the next biofilm in a developing pothole. These communities are finely balanced: Any change in the climate that reduces rain fall or increases daily temperatures would result in a potential reduction in these soils and the potential they represent. More study is warranted to understand the full importance of bacteria to the succession of soil development in the semi-arid desert so that it may be better managed and preserved.

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CHAPTER 5

Conclusions

This research has focussed on the cumulative effects desert bacterial populations can have, on its ecosystem, specifically, populations of bacteria growing as biofilms. On this small scale, the effects of the microbial inhabitants of lithic desert surfaces are easily visible in the form of potholes and cryptobiotic or biological soil crusts (BSCs). These concentrated effects allow for an appreciation of the profound affect that these organisms can elicit in their environment. The impacts of human interaction in marginal areas, such as the semi-arid regions of the Colorado Plateau are increasing, and understanding them is important to monitoring the natural habitats of the Plateau for their preservation and as indicators of desertification. Today, on the Colorado Plateau, much of its more than 340 000 km^2 is used as rangelands while 15 438 km^2 are preserved in national parks (Schwinning et al., 2008). The use of the area as rangeland makes the region less drought-tolerant as the surface disturbances from cattle and their human minders destroys BSCs and increases erosion, which in turn reduces soil carbon and nitrogen stocks (Schwinning et al., 2008). Because the area is semi-arid, it receives only 250 mm/yr of precipitation on average, and its unique geographical location makes what rains it receives somewhat unpredictable, leaving the area prone to extended periods of drought (Hereford et al., 2002). Global climate change will only exacerbate this problem causing more erratic precipitation and a predicted increase in temperatures (IPCC, 2007). A temperature increase of more than 3^oC in a century would increase the area of the United States affected by severe desertification; for example, such a temperature increase in the

Great Basin region of the USA would increase the area of the basin experiencing severe desertification by 20% (Nettleton and Mays, 2007). Aside from the ecological damage this sort of desertification causes, it has a direct human effect as over 1 billion people rely on semi-arid or marginal environments throughout the world (Reynolds and Stafford-Smith, 2002). Due to its sensitivity to climate change, this environment serves as a sentinel ecosystem.

From a scaling perspective, it seems unlikely that a global scale problem such as desertification could be in any way affected by the formation of potholes; however, through this project, I have demonstrated that on a local scale, these potholes are sources of water and more importantly, that they have a direct impact on the local formation of desert proto-soils, both of which are essential in mitigating desertification (Chapters 2 and 3). Although the rate of soil production is difficult to assess and may be quite slow, any addition or development of soil is important in marginal environments.

Water sources are limited in semi-arid environments and potholes can retain water for several days after a rain-fall. Pothole biofilms from this study swelled upon wetting and produced EPS sealing the porous host rock and enabling water to be retained for longer periods of time. These pools of water then supported small communities of eukaryotic organisms (Chapter 2, and Chan et al., 2005) providing interesting refugia in the arid environment.

Potholes are the result of a combination of physical weathering and the colonisation of bare rock surfaces by surficial and endolithic microbial biofilms, which are ubiquitous in most arid environments that lack soils and an abundance of vascular plants (e.g., Friedman, 1982; Budel et al., 2004; Walker and Pace, 2007). These biofilms

locally weather the host rock, sometimes producing catchments for sediment that develop into BSCs due to the potholes' Advantageous bowl-like shape (Chapter 2). The extent of this process is beyond the scope of this thesis and would require years of careful work to catalogue the prevalence and distribution of potholes in marginal and semi-arid environments. Further, because these processes cannot be observed as they occur, areas of past pothole development, now covered by BSCs, would be difficult to identify. Still, it is important to gain an understanding of the development of potholes because BSCs have been found to slow or even gradually reverse desertification of the areas in which they occur, depending on favourable environmental conditions (Bowker et al., 2006; Belnap, 2006).

Potholes have important survival advantages, even though they lack BSCs, which enable them to endure periods of uncertain precipitation and oligotrophic conditions. These advantages allow potholes to add to local soil production, aiding in the establishment of local BSCs. From an ecosystem perspective, BSCs are disadvantaged by producing an immediate burst of CO_2 from respiration as they are first wetted, which occurs for 30-60 minutes before the cyanobacteria can begin to replace the organic carbon by photosynthesis (Lange, 2003). Therefore, BSCs must remain wet for longer than an hour during day light to replace the lost carbon or there will be an overall net carbon loss to the soil system. Even if this explanation of BSCs undervalues the role of heterotrophic bacteria, cyanobacteria are the dominant microorganisms in most of these crusts, and the longer they are wet, the better the system will fare. Further, studies have shown that the deposition of aeolian organic matter varies with precipitation (Zaady et al., 2001), so any decrease in precipitation would lead to a corresponding decrease in

available organic carbon and nutrient sources. Potholes are better able to trap both precipitation and wind-blown material due to their shape, giving the organisms that inhabit them a distinct advantage. Where BSCs, in general, are slightly raised and only retain small amounts of water, biofilms forming in pothole soils are kept wet much longer due to the raised potholes sides. Although BSCs occur in many locations, those formed in potholes would have the advantage of additional periods of hydration leading to a greater reservoir of carbon.

Aside from working against desertification, BSCs are also important indicators of desertification in the environments in which they occur (Bowker et al., 2005 and 2006); potholes could also be used as indicators of the impacts of desertification, through such variables as the quality of the soil they develop, the length of time they are filled with water during the year, and the diversity of the pothole microbiological community.

Although the physical enlargement of potholes is too slow to be readily observed, smaller scale effects such as peeling of surficial layers of sandstone and soil accumulation are easy to measure, and would provide good indicators that the biological community is viable and active, as well as an initial indication of the rate of soil formation. The geochemical mechanisms of pothole formation are found to vary based on the composition of the host rock, showing that these versatile communities are able to exploit the local environment, precipitating minerals in the surface layers of the host rock that temporarily strengthens it and makes it less permeable to water (Chapter 2). The enlargement of the potholes is carried out through a combination of physical and geochemical weathering: Natural freeze/thaw and wet/dry cycles are exacerbated by the properties of extracellular polymeric substances (EPS) produced by the biofilms

observed growing on and within the host rock with SEM studies in Chapter 3, which expand and contract with wetting; and the host rock is geochemically attacked by high daytime pH values found in all potholes sampled, which can reach 10 (Chapter 3) and are assumed to be higher in the biofilm microenvironments surrounding individual cells. The production of high pH conditions is well documented in cyanobacteria, which also precipitate carbonate minerals as an accessory process, is well documented by other researchers (e.g., Thompson and Ferris 1990, Budel et al., 2004). Carbonate minerals that were found cementing the host rock Seven Mile Canyon (SMC), which accounts for the unique raised edges of the potholes found there. High pHs will also contribute to silicate weathering, breaking down pothole minerals (Dove, 1995), a process which was suggested by petrographic, SEM-EDX and XRF data collected at the biofilm/host rock interface (Chapter 2). Because the development of high pH values is important to weathering of the host rock, tracking them throughout the year would give an idea of the rate of rock weathering occurring in the environment from year to year. The failure to develop high pH values with a distinct daily cycle would be an indication of stress to the main cyanobacterial component of the ecosystem, which over the long term could limit desert soil production.

The significance of this weathering regime in the aqueous geochemistry of the potholes was highlighted in Chapter 3. Nutrient availability in the entire semi-arid desert ecosystem is low, and so, although potholes are oligotrophic environments compared with other types of soil producing environments, in the desert their unique form makes them relatively nutrient rich. When potholes wetted with distilled deionised (DDI) water were compared with potholes filled naturally by rain to determine which elements were

being weathered from the host rock, and if rain water contributed any nutrients to the potholes, it was determined that rain was important for wetting the potholes and for depositing and transporting atmospheric dust into the potholes but did not, itself, contribute much in the way of nutrients. Potholes wetted with DDI were still found to support biologically viable microbial communities indicating that the nutrients required for life were already present in the pothole environment, either due to aeolian deposits or from micronutrients in the constituent mineral of the rocks. Sampling of rain water in the field (Chapter 3) suggested that dust in the atmosphere was deposited by rain into the potholes providing a source of nutrients such as nitrate and phosphate. Further the ability of the pothole communities to react to hydration with distilled deionised water (Chapter 3) and produce a recognisable diurnal cycle and grow an observable biofilm (SEM Chapter 3) indicates that windblown inputs into the potholes are sufficient to support biofilm growth upon wetting.

Leaching of the host rock is a secondary source of important trace nutrients as shown by the abundance of trace elements being released from host rock samples at high pH (Chapter 3), which are important to both biological growth and chemical diversity in soils. Studies of soil micronutrient limitations have found that BSC development is determined by pre-existing soil fertility and that diversity of the BSC species is dependent on the presence of certain critical micronutrients (Bowker et al., 2005). Because pothole biofilms can obtain these micronutrients through host rock weathering, they increase the rate of BSC formation. Once established, BSCs in desert environments are important in the global budgets of both trace gases and trace elements (Zaddy et al., 2000) and when the biofilms associated with potholes are also considered, the effect is

even greater, making these ecosystems important in the global soil cycle. The geochemistry of the pothole water shows the influence of the active microbiological population on the presence of various elements such as Ca^{2+}, Mg^{2+} and Al^{3+}, as well as the presence of important nutrients such as PO_4^{3-} and NO_3^-. Monitoring the cycling of nutrients in pothole waters would give a good indication of the activities of the bacteria and indicate the robustness of the communities and their ability to support the development of BSCs.

As potholes develop, deepen, and are able to hold more sediment and water, a greater diversity of bacteria is observed (Chapter 4). Such monitoring carried out on a temporal scale in the same location would be a useful indicator of the health of the pothole environment. Intuitively, a shift back to the initial colonising species would indicate stress on the environment. Because potholes and BSCs are important ecosystem components on the Colorado Plateau and elsewhere, a greater understanding of their microbiological communities is needed. Many studies focused on the main biological components of the ecosystem, i.e., cyanobacteria (e.g., Garcia-Pichel et al., 1996; Belnap et al., 2008), without integrating other important biota such as lichens on the open rock surfaces (Johansen, 1993) fungi, which were not observed in the potholes (Sterflinger, 2000), and heterotrophic bacteria supported by the cyanobacteria (Chapter 4). Even when considering just bacterial populations, much work remains to be done. It is clear that, although much is known about certain species of bacteria, little is known about bacterial communities in specific environments and the numerous types of adaptations that enable survival in difficult conditions. Several studies have pointed to biases in the general practice of using universal bacterial primers to evaluate the diversity of bacteria present

in environmental samples of soils (e.g., Gaddy et al 2011; Blaire et al., 2011). These studies suggest that entire phyla may be underrepresented by current data, severely limiting the potential to understand soil systems based on microbial diversity. Only a fraction of the bacteria on Earth have been identified and only a fraction of those have been isolated in pure culture. The task set before those interested in studying the influences of bacteria on soils and their formation is extensive but increasingly important.

It is easy to look out over the arid-landscapes of the Colorado Plateau and imagine it to be so inhospitable and devoid of life that one could be standing on another planet; but in reality, it is a habitat more closely attuned to the fundamentals of life on this planet than those that are lush and nutrient rich. Here is an ecosystem developing unique features, such as potholes, that create an oasis of water retention and soil formation out of benches of arenitic sandstone. Despite desiccation by the intense summer sun and freezing in the winter, these environments provide benefits up the food chain fundamental to the survival of complex multicellular life. The work presented in this thesis discussed physical, geochemical and structural community elements of the function of the lithic pothole-forming biofilms. Work remains to be conducted, specifically in the understanding and control of bacterial contributions responsible for pothole and desert soil formation; as well as more generally to fully understand the complexities of biofilms and their function. Through this continued work towards a deeper understanding of the fundamental processes of soil formation and biofilm function we can better appreciate the importance of the biosphere to arid environments, and use mechanisms already at work to preserve them.

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APPENDIX A

Field Samples Used for Testing

A.1: Potholes Used for Water Sampling

SMC				BW			
	DDI Continuous	DDI Drying	Rain		DDI Continuous	DDI Drying	Rain
PH1	X	X	X	PH1	-	X	
PH2	X	X	X	PH2	-	X	
PH3	X	X		PH3	-	X	
PH4	X	X		PH4	-	X	
PH5	X			PH5	-		X
PH6	X			PH6	-		X
PH7			X		-		

A.2: Rock Samples

Number Seven Mile Canyon	Description	Petrography	XRF	CL	Leach	Biofilm regrowth	SEM-LEO
SMC-RS-1	Weathered host sandstone coming off in layers	x					
SMC-RS-2	Darker fissile unit	x					
SMC-RS-3	With black biofilm at edge of a pothole						
SMC-RS-4	Black biofilm coated pebble in pothole (Dry)	x					
SMC-RS-5	Host sandstone with lichen	x			x	x	
SMC-RS-6	Host sandstone with black biofilm	x	x			x	
SMC-RS-7	Host sandstone				x	x	
SMC-RS-8	Black biofilm covered rock from side of SMC1	x		x			x
SMC-RS-9	Black biofilm covered rock from side of SMC2			x			x
SMC-RS-10	Black biofilm covered rock from side of SMC3						

SMC-RS-11	Black biofilm covered rock from side of SMC4						
SMC-RS-12	Black biofilm covered rock from side of SMC5						
SMC-RS-13	Black biofilm covered rock from side of SMC6						
SMC-RS-14	Black biofilm covered rock from side of SMC7						
Number Bartlett Wash	**Description**	**Petrography**	**XRF**	**CL**	**Leach**	**Biofilm regrowth**	**SEM-LEO**
BW-RS-1	Host Rock with endolithic biofilm	x	x	x			
BW-RS-2	Host Rock				x		x
BW-RS-3	Black covered Pebbles from the bottom of BW3	x					
BW-RS-4	Host Rock with lichens	x					
BW-RS-5	Black material at side of pothole	x					
BW-RS-6	Weathered host rock	x					
BW-RS-7	Black biofilm covered rock from side of BW1	x				x	x
BW-RS-8	Black biofilm covered rock from side of BW2	x					x
BW-RS-9	Black biofilm covered rock from side of BW3	x					
BW-RS-10	Black biofilm covered rock from side of BW4	x					
BW-RS-11	Black biofilm covered rock from side of BW5						
BW-RS-12	Black biofilm covered rock from side of BW6						

A.3: Biofilm Samples

Number	Description	Biofilm regrowth	SEM/ FIB	16S rRNA
SMC-BF-1	Black Biofilm SMC1 (Dry)	x	x	
SMC-BF-2	Black Biofilm SMC1 (Wet)			x
SMC-BF-3	Black Biofilm SMC2 (Wet)	x	x	

SMC-BF-4	Black Biofilm SMC2 (Dry)			
SMC-BF-5	Black Biofilm SMC3 (Wet)	x		
SMC-BF-6	Black Biofilm SMC3 (Dry)			
SMC-BF-7	Black Biofilm SMC7 (Wet)			
SMC-BF-8	Black Biofilm SMC7 (Dry)			
SMC-BF-9	Sediment Biofilm SMC1 (Wet)			x
SMC-BF-10	Sediment Biofilm SMC2 (Wet)			
SMC-BF-11	Sediment Biofilm SMC7 (Wet)			
SMC-BF-12	Biological Soil Crust			x
SMC-BF-13	Biological Soil Crust			
Number	**Description**	**Biofilm regrowth**	**SEM/ FIB**	**16S rRNA**
BW-BF-1	Black Biofilm BW1 (Dry)			
BW-BF-2	Black Biofilm BW1 (Wet)			
BW-BF-3	Black Biofilm BW2 (Dry)			x
BW-BF-4	Black Biofilm BW2 (Wet)			
BW-BF-5	Black Biofilm BW3 (Dry)			
BW-BF-6	Black Biofilm BW3 (Wet)			
BW-BF-7	Black Biofilm BW4 (Dry)			
BW-BF-8	Black Biofilm BW4 (Wet)			
BW-BF-9	Sediment Biofilm BW1 (Wet)			x
BW-BF-10	Sediment Biofilm BW2 (Wet)			
BW-BF-11	Sediment Biofilm BW3 (Wet)			
BW-BF-12	Sediment Biofilm BW4 (Wet)			
BW-BF-13	Endoliths BW Host			x
BW-BF-14	Endoliths BW Host			

APPENDIX B

Geochemical Data

SMC1: Field Measurements, continuous wetting with DDI

Date	Time	pH	Dissolved Oxygen	Conductivity	Temperature
	24hrs		mg/L	µS/cm	^{o}C
30-Jun	16:00	8.38	4.60	19.64	32.33
01-Jul	7:47	8.18	8.01	66.10	20.17
	19:00	10.04	12.40	107.50	26.80
02-Jul	7:10	8.42	6.87	185.10	21.63
	12:00	7.09	6.84	6.30	30.13
	17:20	9.60	7.62	56.20	31.43
03-Jul	7:00	8.22	6.40	186.70	22.83
	13:37	8.65	5.21	10.94	27.90
	17:55	8.73	7.42	39.10	26.60
04-Jul	7:12	8.06	6.27	97.00	21.37
	12:50	9.05	8.67	105.60	24.17
06-Jul	7:35	8.47	4.12	9.83	23.53
	10:10	9.10	4.81	23.70	28.00
	13:00	9.36	5.38	39.50	35.40
	16:05	9.55	5.75	51.40	33.83
	19:15	9.73	6.58	59.20	26.57
	22:10	9.09	4.85	71.10	24.20
07-Jul	1:00	7.22	4.46	92.70	21.87
	4:00	8.32	4.65	112.90	20.70
	12:15	9.30	5.07	24.80	33.87
	19:15	9.70	9.42	53.90	29.37
08-Jul	7:17	8.30	9.54	109.00	22.00
	11:52	9.34	9.64	107.70	33.87
	19:50	9.53	9.44	58.30	26.93
09-Jul	6:55	8.21	8.01	108.80	22.03
	13:25	9.31	9.20	108.10	35.83
	20:10	9.13	8.48	112.70	25.30
10-Jul	7:50	7.97	5.74	7.46	25.43
11-Jul	7:10	8.30	7.64	164.70	22.40
	11:55	9.24	8.34	35.40	33.93
	19:17	9.40	7.97	59.00	28.70

12-Jul	7:25	8.22	7.19	121.20	21.93
	12:45	9.27	8.68	60.20	34.80
	19:25	9.51	8.44	83.30	25.63
13-Jul	7:30	8.28	6.84	164.60	21.50
	12:35	9.10	7.23	49.10	34.97
	19:40	9.24	6.40	79.70	24.77
14-Jul	7:25	8.30	6.30	180.00	22.07
	12:25	9.27	8.02	61.50	34.17
	19:35	9.14	8.12	89.50	26.03
15-Jul	7:25	8.22	7.15	212.00	21.37
	12:25	9.21	7.52	37.10	33.50

SMC2: Field Measurements, continuous wetting with DDI

Date	Time	pH	Dissolved Oxygen	Conductivity	Temperature
	24hrs		mg/L	µS/cm	^{o}C
01-Jul	8:00	8.35	7.95	67.2	21.2
	19:10	10.08	14.82	101.8	26.8
02-Jul	7:20	8.28	6.41	103.6	20.9
	12:10	9.56	11.78	127	31.8
	17:25	10.09	10.42	133.7	31.0
03-Jul	7:10	8.18	6.01	152	20.9
	12:25	9.74	13.89	151.2	27.9
	18:05	9.79	9.11	85.8	26.5
04-Jul	7:45	8.14	6.19	36	22.4
	12:55	9.72	9.73	57.3	24.1
	18:50	10.14	10.4	81.4	23.6
05-Jul	7:25	8.28	6.92	104.5	19.3
06-Jul	7:40	8.49	4.08	7.88	23.6
	10:15	9.33	7.63	24.1	29.2
	13:15	9.6	5.45	41.2	34.7
	16:10	9.7	5.79	53.4	34.4
	19:20	9.85	6.07	56.9	28.3
	22:15	9.47	4.6	52.5	25.2
07-Jul	1:10	8.87	4.27	57.8	23.8
	4:05	8.9	4.03	67.5	21.7
	12:20	9.45	7.1	84.5	34.2
	19:20	9.9	10.07	92.7	29.7
08-Jul	7:20	8.29	8.78	113.5	22.3

	11:55	9.36	11.75	105.9	32.8
	20:00	9.82	10.31	94.2	27.5
09-Jul	7:02	8.16	8.2	151	21.7
	13:30	9.55	8.08	65.8	35.7
	20:20	9.68	9.51	54.9	26.7
10-Jul	7:54	8.16	6.31		22.7
11-Jul	7:20	8.14	7.11	113.3	21.4
	12:00	9.39	11.55	105.8	33.4
	19:20	9.8	8.9	95.8	28.9
12-Jul	7:30	8.16	7.47	153.6	22.0
	12:50	9.67	9.78	66	34.3
	19:28	10.05	9.19	87.3	26.7
13-Jul	7:38	8.19	6.88	113.5	20.8
	12:40	9.61	9.55	107.2	34.5
	19:45	9.39	6.39	95.2	25.7
14-Jul	7:30	8.29	5.57	73.7	21.8
	12:32	9.55	9.24	70.4	33.5
	19:42	9.89	9.39	83.5	27.6
15-Jul	7:29	8.07	6.33	110.4	19.9
	12:30	9.59	11.16	105	32.5

SMC5: Field Measurements, continuous wetting with DDI

Date	**Time**	**pH**	**Dissolved Oxygen**	**Conductivity**	**Temperature**
	24hrs		**mg/L**	**µS/cm**	**^{o}C**
01-Jul	7:30	7.42	9.18	13.14	34.9
02-Jul	7:25	8.03	6.16	66.4	22.7
	12:15	9.4	9.4	92.1	32.4
	17:30	9.88	9.29	115.4	31.5
03-Jul	7:15	8.04	6.1	148.2	21.9
	12:30	9.72	15.24	135.5	26.3
	18:10	9.9	9.05	162.2	28.2
04-Jul	7:55				
	13:00	9.75	8.37	40.4	24.3
	18:50	10.03	9.04	60.2	23.8
05-Jul	7:30	8.06	6.84	87.5	19.8
06-Jul	7:50	8.53	4.7	20.8	22.8
	10:20	9.54	5.98	34.7	28.2
	13:16	9.68	6.2	54.8	34.2

	16:12	9.83	6.14	70.09	33.4
	19:22	9.96	6.37	76.6	27.8
	22:20	9.51	4.64	69.1	24.9
07-Jul	1:15	8.72	4.22	74.9	23.5
	4:10	8.12	4.31	87	21.8
	12:30	9.63	7.54	106.8	33.8
	19:25	10.11	9.92	115.1	29.3
08-Jul	7:25	8.12	8.6	93	23.1
	12:00	9.52	11.82	48.5	32.6
	20:01	9.97	9.78	108.6	27.7
09-Jul	7:06	8.07	7.63	185.6	23.4
	13:34	9.6	8.84	55.5	35.5
	20:22	9.6	9.52	50.2	25.9
10-Jul	8:00	7.98	6.23	78.4	23.4
11-Jul	7:25	8.03	6.89	136	22.4
	12:08	9.53	11	121.1	32.8
	19:25	9.68	8.39	55.1	29.7
12-Jul	7:35	8	6.85	78.4	22.2
	12:55	9.62	10.93	9.24	33.5
	19:30	10.14	9.22	111.8	26.7
13-Jul	7:45	8.15	5.56	136	21.5
	12:40	9.61	9.36	119.9	34.7
	19:50	9.79	6.82	70.2	26.1
14-Jul	7:35	8.05	6.13	120.4	22.1
	12:40	9.59	8.23	49.4	33.3
	19:45	9.79	8.53	69.9	27.7
15-Jul	7:30	7.98	6.37	102.8	20.5
	12:32	9.64	10.5	107.5	32.6

SMC6: Field Measurements continuous wetting with DDI

Date	Time	pH	Dissolved Oxygen	Conductivity	Temperature
	24hrs		mg/L	µS/cm	^{o}C
03-Jul	17:45	7.82	7.51	8.80	26.9
04-Jul	7:15	7.54	5.60	55.20	21.7
	12:45	9.63	10.82	61.30	24.1
	18:45	9.78	9.80	43.10	24.3
05-Jul	7:20	8.08	7.34	66.70	20.3
06-Jul	7:25	6.72	5.98	9.41	22.0
	10:00	9.38	6.36	26.30	27.4

	13:00	9.52	5.84	44.00	34.0
	15:55	9.59	6.15	60.90	34.2
	19:00	9.72	7.45	73.40	26.6
	21:50	8.71	3.92	99.60	24.7
07-Jul	1:00	8.34	4.72	131.50	23.6
	3:50	8.29	4.60	172.70	21.7
	12:10	8.21	4.68	7.43	33.9
	19:10	9.54	8.75	37.30	30.0
08-Jul	7:08	8.16	8.98	67.90	21.7
	11:45	9.42	9.85	47.00	32.0
	19:40	9.77	11.43	65.30	27.3
09-Jul	6:45	8.10	7.93	94.10	22.1
	13:10	9.60	10.53	77.40	34.6
	20:05	9.68	10.94	57.50	25.1
10-Jul	7:36	8.23	6.75	92.10	22.4
11-Jul	7:10				
	11:45	7.22	7.00	6.87	30.7
	19:05	9.49	8.45	33.90	29.4
12-Jul	7:15	7.78	7.13	74.20	21.4
	12:40	9.51	10.00	51.90	33.1
	19:15	9.90	10.48	70.20	26.6
13-Jul	7:20	7.84	6.85	92.20	20.2
	12:25	9.71	9.07	99.60	33.9
	19:30	9.64	7.56	110.70	24.7
14-Jul	7:40	8.17	6.22	6.52	23.1
	12:15	9.35	7.40	31.80	33.0
	19:25	9.61	9.24	50.20	27.2
15-Jul	7:20	7.75	6.51	95.00	20.1
	12:11	9.54	9.51	96.60	33.0

SMC7: Field Measurements, continuous wetting with DDI

Date	Time	pH	Dissolved Oxygen	Conductivity	Temperature
	24hrs		mg/L	µS/cm	^{o}C
03-Jul	17:45	7.70	5.76	700.00	28.7
04-Jul	7:15	8.18	6.18	745.00	21.3
	12:45	8.93	12.57	584.00	23.1
	18:45	8.70	11.31	643.00	23.8
05-Jul	7:20	8.36	7.76	693.00	19.7
06-Jul	7:25	7.78	5.07	733.00	21.5

	10:00	8.27	6.99	719.00	27.7
	13:00	8.60	7.83	644.00	33.7
	15:55	8.83	7.97	616.00	33.1
	19:00	9.05	8.56	652.00	26.4
	21:50	8.67	4.36	709.00	24.0
07-Jul	1:00	8.48	4.48	763.00	22.4
	3:50	8.47	4.41	835.00	20.2
	12:10	7.88	5.39	700.00	33.6
	19:10	9.01	12.29	615.00	29.0
08-Jul	7:08	8.65	9.76	870.00	21.8
	11:45	8.52	13.43	612.00	32.1
	19:40	9.05	12.52	655.00	26.4
09-Jul	6:45	8.44	7.42	996.00	21.5
	13:10	8.67	12.98	578.00	34.9
	20:05	8.96	11.63	673.00	23.8
10-Jul	7:36	8.50	7.87	1002.00	23.6
11-Jul	7:10	8.46	7.10	917.00	21.0
	11:45	8.49	13.31	620.00	32.8
	19:05	8.99	11.49	640.00	28.0
12-Jul	7:15	8.48	8.52	884.00	21.0
	12:40	8.63	12.97	548.00	33.1
	19:15	9.12	12.23	633.00	25.2
13-Jul	7:20	8.36	8.36	992.00	23.7
	12:25	8.66	11.13	607.00	33.5
	19:30	9.01	8.12	711.00	23.8
14-Jul	7:40	7.79	6.21	705.00	22.5
	12:15	8.60	11.38	650.00	32.9
	19:25	9.07	11.95	680.00	26.3
15-Jul	7:20	7.73		770.00	22.3
	12:11	8.65	12.02	697.00	32.3

DDI Continuous Filling: Pothole Water Depths (cm)

Date	Time	SMC1	SMC2	SMC5	SMC6	SMC7
30-Jun	16:00	8.2	8			
01-Jul	7:47	5.5	6.67			
	19:00	4	5	6.7		
02-Jul	7:10	2.8	4.7	5.7		
	12:00	0	4.4	5.5		
	17:20	5.1	3.6	4.5		
03-Jul	7:00	3.1	2	2.9		
	13:37	6	1.3	1.9		
	17:55	5.4	2.4	0.9	7.8	5.8
04-Jul	7:12	4.4	1.1	5.7	6	3.9
	12:50	3.1	4.4	5.1	5.6	3
	18:45	0	4.8	4.7	7.2	5.1
05-Jul	7:20	0	3.1	4	5.4	4.5
06-Jul	7:35	6.6	8.8	6.5	5.5	5.9
	10:10	6	8.1	6.2	5.6	5.8
	13:00	5.7	7.9	5.9	4.7	5
	16:05	5.2	7.4	5.5	4.4	4.7
	19:15	4.8	7.2	5.1	3.9	3.9
	22:10	4.8	7.2	4.8	3.6	3.9
07-Jul	1:00	4.4	6.9	4.8	3.4	3.4
	4:00	4	6.8	4.5	3.2	3.6
	12:15	3.5	6.2	3.8	7.8	5.5
	19:15	5.5	3.5	2.9	6.8	4.3
08-Jul	7:17	4.5	4.6	4.3	5.8	3
	11:52	3.8	4.2	3.7	7.1	5.8
	19:50	5.7	2.9	2.4	6	4
09-Jul	6:55	4.6	2.1	1.3	4.9	2.8
	13:25	3.9	5.6	5.7	4.5	5
	20:10	3.6	6.9	6.1	5.8	3.9
10-Jul	7:50	5.5	6.3	5.5	4.7	2.7
11-Jul	7:10	2.8	4.2	3.2	0	2.7
	11:55	6.3	3.9	2.3	7.6	5.6
	19:17	5	2.9	6.4	6.4	4.4
12-Jul	7:25	4.3	2.3	3.6	5.7	3.1
	12:45	4.4	4.8	4.8	7	5.4
	19:25		3.7	3.6	5.9	3.9
13-Jul	7:30	3.4	2.8	2.8	4.9	2.2
	12:35	5.7	2.2	1.9	4.4	4.6
	19:40	4.5	1.4	3.9	2.9	3.1

14-Jul	7:25	3.6	5.6	3.2	0	0
	12:25	5.1	6.1	6.5	7	5.5
	19:35	4	5.3	5.7	6	4
15-Jul	7:25	0	4	4.2	4.8	0.5
	12:25	5.1	4	3.8	4	4.7

Shading indicates filling of the pothole with DDI

SMC1: ICP-MS Data, continuous wetting with DDI

Date	**Time**				
Analyte		**Al**	**Ca**	**Fe**	**K**
Units	**24hrs**	mg/L	mg/L	mg/L	mg/L
Detection Limit		0.0005	0.001	0.0005	0.001
30-Jun	16:00	0.0194	0.675	<0.0005	0.361
01-Jul	7:47	0.2678	17.414	<0.0005	2.811
	19:00	0.0318	9.767	<0.0005	1.540
02-Jul	7:10	0.0185	22.716	<0.0005	6.056
	17:20	0.2061	6.623	<0.0005	1.854
03-Jul	7:00	0.0057	21.974	<0.0005	2.248
	13:37	0.1164	1.230	0.0277	0.724
06-Jul	7:35	0.0462	1.679	0.0057	0.506
	10:10	0.0971	3.014	0.0110	0.881
	13:00	0.0968	2.478	0.0021	0.595
	22:10	0.1318	9.159	0.0013	1.463
07-Jul	1:00	0.1310	15.921	0.0012	2.282
	12:15	0.1941	21.307	0.0002	3.081
08-Jul	11:52	0.1510	19.821	<0.0005	2.533
	19:50	0.1781	10.063	<0.0005	1.587
09-Jul	13:25	0.1472	19.772	<0.0005	2.502
	20:10	0.0918	21.325	<0.0005	2.542
11-Jul	11:55	0.0376	2.545	<0.0005	0.440
12-Jul	7:25	0.0370	23.503	<0.0005	2.187
	12:45	0.0761	7.677	<0.0005	0.976
13-Jul	12:35	0.0634	7.760	<0.0005	1.053
	19:40	0.1265	15.435	<0.0005	1.745
14-Jul	12:25	0.0784	8.016	<0.0005	1.127
	19:35	0.0968	13.016	<0.0005	1.642
15-Jul	7:25	0.0347	36.945	<0.0005	5.648
	12:25	0.0697	4.785	<0.0005	1.048

Date	Time					
Analyte		**Mg**	**Na**	**P**	**S**	**Si**
Units	**24hrs**	mg/L	mg/L	mg/L	mg/L	mg/L
Detection Limit		0.001	0.001	0.001	0.001	0.001
30-Jun	16:00	0.079	0.131	0.111	<0.001	0.077
01-Jul	7:47	0.478	0.921	<0.001	0.081	2.276
	19:00	0.872	0.421	0.045	<0.001	0.625
02-Jul	7:10	1.681	1.058	<0.001	0.056	0.762
	17:20	0.393	0.571	0.201	0.052	0.889
03-Jul	7:00	1.994	0.639	<0.001	0.339	1.117
	13:37	0.157	0.401	0.025	<0.001	0.215
06-Jul	7:35	0.211	0.149	<0.001	0.053	0.193
	10:10	0.316	0.180	<0.001	<0.001	0.521
	13:00	0.216	0.139	<0.001	<0.001	0.486
	22:10	0.536	0.308	0.057	<0.001	1.019
07-Jul	1:00	1.089	0.472	0.037	0.073	1.156
	12:15	1.486	0.718	0.078	0.199	1.215
08-Jul	11:52	1.326	0.572	0.156	0.063	1.260
	19:50	0.533	0.378	0.008	0.030	1.142
09-Jul	13:25	1.290	0.716	<0.001	0.061	1.739
	20:10	1.377	0.772	<0.001	0.162	2.009
11-Jul	11:55	0.200	0.134	0.007	<0.001	0.275
12-Jul	7:25	2.044	0.472	<0.001	0.117	1.926
	12:45	0.565	0.241	0.222	<0.001	0.843
13-Jul	12:35	0.615	0.262	0.137	0.178	0.913
	19:40	0.977	0.402	<0.001	0.145	1.673
14-Jul	12:25	0.635	0.221	<0.001	0.186	0.936
	19:35	0.814	0.306	<0.001	0.078	1.569
15-Jul	7:25	3.155	1.102	<0.001	1.163	3.097
	12:25	0.363	0.275	<0.001	0.188	0.677

SMC2: ICP-MS Data, continuous wetting with DDI

Date	Time				
Analyte		**Al**	**Ca**	**Fe**	**K**
Units	**24hrs**	mg/L	mg/L	mg/L	mg/L
Detection Limit		0.0005	0.001	0.0005	0.001
01-Jul	8:00	0.0326	12.988	<0.0005	1.905
	19:10	0.0637	22.168	<0.0005	3.485
02-Jul	7:20	0.1457	16.418	<0.0005	3.387
	12:10	0.1364	13.524	<0.0005	4.017
	17:25	0.0745	12.329	<0.0005	5.058
	18:05	0.0546	4.264	<0.0005	1.659
04-Jul	7:45	0.0346	30.035	<0.0005	6.250
	12:55	0.1444	9.908	<0.0005	2.794
06-Jul	7:40	0.1550	11.298	<0.0005	2.924
	10:15	0.0372	18.412	<0.0005	3.779
	19:20	0.0216	2.803	<0.0005	0.866
07-Jul	4:05	0.0700	4.360	<0.0005	1.006
	12:20	0.0513	3.443	<0.0005	0.716
	19:20	0.1187	6.595	<0.0005	1.417
08-Jul	11:55	0.1268	8.992	<0.0005	1.910
	20:00	0.0809	7.025	<0.0005	1.434
09-Jul	7:02	0.0741	12.183	<0.0005	2.077
	13:30	0.1328	14.289	<0.0005	2.487
	20:20	0.2074	15.198	<0.0005	3.054
11-Jul	7:20	0.1454	19.068	<0.0005	3.815
	19:20	0.1985	14.321	<0.0005	4.856
12-Jul	7:30	0.1339	9.524	<0.0005	2.369
	19:28	0.1314	8.667	<0.0005	2.033
13-Jul	12:40	0.1170	17.133	<0.0005	3.555
	19:45	0.1507	11.480	<0.0005	3.932
14-Jul	12:32	0.1742	12.817	<0.0005	2.968
	19:42	0.1485	18.855	<0.0005	4.712
15-Jul	12:30	0.1255	12.982	<0.0005	5.156

SMC2: ICP-MS Data, continuous wetting with DDI

Date	Time					
Analyte		**Mg**	**Na**	**P**	**S**	**Si**
Units	**24hrs**	mg/L	mg/L	mg/L	mg/L	mg/L
Detection Limit		0.001	0.001	0.001	0.001	0.001
01-Jul	8:00	0.693	0.996	<0.001	0.203	0.486
	19:10	1.125	1.729	<0.001	0.506	0.904
02-Jul	7:20	0.898	1.821	0.108	0.739	0.871
	12:10	0.483	1.948	0.012	0.838	1.218
	17:25	0.829	3.083	0.077	1.167	1.268
	18:05	0.158	0.913	<0.001	0.227	0.373
04-Jul	7:45	1.894	3.280	<0.001	1.011	1.399
	12:55	0.586	1.495	0.099	0.212	0.670
06-Jul	7:40	0.520	1.447	0.204	0.346	0.765
	10:15	1.048	1.591	<0.001	0.243	0.827
	19:20	0.120	0.334	0.041	<0.001	0.156
07-Jul	4:05	0.157	0.390	0.044	<0.001	0.295
	12:20	0.105	0.266	<0.001	<0.001	0.203
	19:20	0.194	0.488	0.025	0.117	0.502
08-Jul	11:55	0.293	0.638	<0.001	0.011	0.651
	20:00	0.261	0.525	<0.001	0.001	0.442
09-Jul	7:02	0.486	0.660	0.137	<0.001	0.598
	13:30	0.552	0.786	<0.001	<0.001	0.715
	20:20	0.456	0.977	0.039	0.056	1.032
11-Jul	7:20	0.831	1.273	<0.001	0.051	1.245
	19:20	0.499	1.776	<0.001	0.128	1.937
12-Jul	7:30	0.397	0.798	<0.001	<0.001	0.850
	19:28	0.348	0.738	<0.001	0.055	0.765
13-Jul	12:40	0.776	1.242	0.008	0.101	1.539
	19:45	0.428	1.390	<0.001	0.089	1.906
14-Jul	12:32	0.357	0.958	0.017	0.123	1.542
	19:42	0.885	1.616	0.020	0.193	2.395
15-Jul	12:30	0.493	1.963	0.032	0.199	3.031

SMC1: DDI Wetting and drying field measurements

Date	Time 24hrs	Depth mm	Temp °C	pH	Cond µS/cm
11-Jul	7:30	77	21.4	8.4	2.59
	12:00	71	32.5	9.01	26.5
	14:00	64	33.3	9.73	52.8
12-Jul	7:00	45	23.3	8.89	127.3
	11:00	44	34.9	9.62	106
	15:00		31.9	9.42	119

SMC2: DDI Wetting and drying field measurements

Date	Time 24hrs	Depth mm	Temp °C	pH	Cond. µS/cm
11-Jul	7:30	92	24.8	8.28	8.45
	12:00	89	32.1	9.07	30.2
	14:00	82	33.9	9.69	47.1
12-Jul	7:00	72	23.5	8.84	91.1
	11:00	67	32.9	9.37	94.1
	15:00	59	33.9	9.95	115.9
13-Jul	8:00	50	23.8	8.48	121.6
	12:00	43	31.6	9.57	119.3
				10.1	
	15:00	42	33.2	8	137.5
14-Jul	8:00	28	25.2	8.52	154.8
	11:00	23	21.9	9.11	144.9
	16:00	15	33	9.71	158.7

SMC3: DDI Wetting and drying field measurements

Date	Time 24hrs	Depth mm	Temp °C	pH	Cond. µS/cm
11-Jul	7:30				
	12:00				
	14:00				
12-Jul	7:00	72	25	7.01	13.45
	11:00	65	31.5	6.89	32.5
	15:00	58	31.9	9.02	45.2
13-Jul	8:00	42	18.8	7.55	89.2
	12:00	36	29.4	9.31	84.5
	15:00	31	30.8	10.17	109.3
14-Jul	8:00	3	22.4	8.15	244
	11:00				
	16:00				

SMC4: DDI Wetting and drying field measurements

Date	Time 24hrs	Depth mm	Temp °C	pH	Cond. µS/cm
11-Jul	7:30				
	12:00				
	14:00				
				6.7	
12-Jul	7:00	15	23.3	6	56.4
	11:00	8	32	7.2	95.2

SMC1: ICP-MS DDI wetting and drying

Analyte	Units	Time Wet (h) Detection Limit	0	5	24
Al	mg/L	0.001	0.168	0.143	0.232
Ca	mg/L	0.001	0.409	2.53	4.65
K	mg/L	0.001	0.414	0.894	1.21
Mg	mg/L	0.001	0.069	0.27	0.401
Na	mg/L	0.001	0.374	0.374	0.451
P	mg/L	0.001	0.016	0.045	0.047
S	mg/L	0.001	0.425	0.349	0.37
Si	mg/L	0.001	0.058	0.331	0.699

SMC2: ICP-MS DDI wetting and drying

Analyte	Units	Time Wet (h) Detection Limit	0	5	24
Al	mg/L	0.001	0.095	0.147	0.201
Ca	mg/L	0.001	0.666	2.73	4.28
K	mg/L	0.001	0.458	0.948	1.4
Mg	mg/L	0.001	0.054	0.16	0.229
Na	mg/L	0.001	0.344	0.683	0.978
P	mg/L	0.001	0.001	0.086	0.2
S	mg/L	0.001	0.341	0.515	0.599
Si	mg/L	0.001	0.039	0.19	0.373
Analyte	**Units**	**Time Wet (h) Detection Limit**	**72**	**77**	**96**
Al	mg/L	0.001	0.17	0.261	0.259
Ca	mg/L	0.001	14	12.6	13.2
K	mg/L	0.001	3.22	3.36	4.3
Mg	mg/L	0.001	0.719	0.631	0.437
Na	mg/L	0.001	2.12	2.39	2.88
P	mg/L	0.001	0.084	0.147	0.1
S	mg/L	0.001	0.875	1.02	1.1
Si	mg/L	0.001	0.784	0.812	1.13

SMC3: ICP-MS DDI wetting and drying

Analyte	Units	Time Wet (h) Detection Limit	0	5	24
Al	mg/L	0.001	0.141	0.14	0.216
Ca	mg/L	0.001	1.12	3.03	5.49
K	mg/L	0.001	0.563	0.787	1.04
Mg	mg/L	0.001	0.11	0.261	0.774
Na	mg/L	0.001	0.409	0.455	1.3
P	mg/L	0.001	0.004	0.266	0.152
S	mg/L	0.001	0.612	0.697	1.47
Si	mg/L	0.001	0.062	0.187	0.425

SMC4: ICP-MS DDI wetting and drying

Analyte	Units	Time Wet (h) Detection Limit	0	5	24
Al	mg/L	0.001	0.152	0.189	0.14
Ca	mg/L	0.001	4.53	10.6	23.6
K	mg/L	0.001	1.63	1.64	3.47
Mg	mg/L	0.001	0.289	0.636	1.56
Na	mg/L	0.001	0.65	0.847	1.82
P	mg/L	0.001	0.087	0.249	0.33
S	mg/L	0.001	1.22	1.85	3.02
Si	mg/L	0.001	0.18	0.873	1.85

Control Data: ICP-MS for comparison with pothole field data

Time since wetting (h)			0	5	24
Analyte	Units	Detection Limit			
Al	mg/L	0.001	0.002	1.59	0.762
Ca	mg/L	0.001	<0.001	5.27	9.62
Fe	mg/L	0.0005	0.004	0.013	<0.0005
K	mg/L	0.001	0.057	3.11	1.35
Mg	mg/L	0.001	<0.001	0.177	0.275
Na	mg/L	0.001	0.015	0.641	0.71
P	mg/L	0.001	0.129	0.131	0.117
S	mg/L	0.001	<0.001	0.103	0.244
Si	mg/L	0.001	<0.001	0.292	0.378
Time since wetting (h)			**29**	**48**	**53**
Analyte	**Units**	**Detection Limit**			
Al	mg/L	0.001	0.736	0.533	0.586
Ca	mg/L	0.001	10.7	13.3	15
Fe	mg/L	0.0005	<0.0005	<0.0005	<0.0005
K	mg/L	0.001	6.55	2.46	2.21
Mg	mg/L	0.001	0.295	0.389	0.433
Na	mg/L	0.001	0.688	0.817	0.838
P	mg/L	0.001	0.11	<0.001	<0.001
S	mg/L	0.001	0.397	0.551	0.519
Si	mg/L	0.001	0.47	0.629	0.795

Time since wetting (h)			72	77	96
Analyte	**Units**	**Detection Limit**			
Al	mg/L	0.001	0.401	0.526	0.403
Ca	mg/L	0.001	18	18.7	23.2
Fe	mg/L	0.0005	<0.0005	<0.0005	<0.0005
K	mg/L	0.001	1.46	3.79	7.92
Mg	mg/L	0.001	0.548	0.566	0.0751
Na	mg/L	0.001	0.979	1.04	1.31
P	mg/L	0.001	0.111	0.183	0.217
S	mg/L	0.001	0.739	0.828	1.2
Si	mg/L	0.001	0.947	1.13	1.36
Time since wetting (h)			**101**	**120**	**125**
Analyte	**Units**	**Detection Limit**			
Al	mg/L	0.001	0.494	0.328	0.298
Ca	mg/L	0.001	25.5	34.8	45.2
Fe	mg/L	0.0005	0.001	<0.0005	<0.0005
K	mg/L	0.001	14.5	6.07	8.88
Mg	mg/L	0.001	0.822	1.23	1.98
Na	mg/L	0.001	1.57	2.08	4.09
P	mg/L	0.001	0.12	0.267	0.103
S	mg/L	0.001	1.3	2.12	4.58
Si	mg/L	0.001	1.67	2.07	3.25

Rain Wetted Potholes: Field Data

Pothole #	Date	Time	pH	Temp. ^{o}C	Cond. µS/cm
BW1	Sep-07	8:00	7.93	20.2	106.4
BW1		18:00	8.19	25.3	106.9
BW1	Sep-08	8:00	7.93	19.3	135
BW2		9:00	7.98	19.4	230
BW1		10:00	7.91	19.2	136.1
BW1		18:30	7.64	23.5	81.8
BW2			9.33	21.6	55.6
BW3			8.92	22.1	88.9
BW1	Sep-10	18:00	8.03	19.1	81.9
BW1			9.57	18	71.5
BW3			9.14	19.2	89.4
BW1	Sep-11	18:20	8.33	19.7	87

BW2			10.1	18.2	99.2
BW3			9.06	21	99
Pothole #	**Date**	**Time**	**pH**	**Temp. ^{o}C**	**Cond. µS/cm**
SMC1	Sep-08	13:45	8.57	30.5	75.3
SMC2			8.62	29.5	91.2
SMC6			9.27	28.2	56.7
SMC1	Sep-09	8:30	7.72	18.6	431.1
SMC2			7.56	18.2	59.7
SMC6			7.81	18.6	31.1
SMC1	Sep-10	9:30	9.32	18.7	72.3
SMC2			8.69	17.2	88.4
SMC6			8.8	17.6	59.9
SMC1	Sep-11	10:00	9.96	21.2	109.2
SMC2			9.32	19.5	92.5
SMC6			9.32	20.4	92.4
SMC2	Sep-12	8:30	8.09	13.8	117

Chemistry of Rain Water: ICP-MS and CL

Analyte	**Units**	**Detection Limit**	**Hours Since Wetting**	
			0	14
Al	mg/L	0.0005	0.035	<0.0005
Ca	mg/L	0.1000	1.26	0.47
Fe	mg/L	0.0005	0.004	0.003
K	mg/L	0.001	3.74	1.48
Mg	mg/L	0.001	0.21	0.06
Na	mg/L	0.001	3.15	1.39
P	mg/L	0.001	0.126	0.099
S	mg/L	0.001	0.798	0.186
Si	mg/L	0.001	0.001	0.009
PO_4^{3-}	mg/L	0.001	0.281	<0.001
SO_4^{2-}	mg/L	0.001	3.196	1.576
NO_3^{-}	mg/L	0.001	2.564	1.531

SMC1 Rain-Filled: ICP-MS and CL

Analyte	Units	Detection Limit	Hours Since Wetting			
			2	20	44	70
Al	mg/L	0.0005	0.040	0.001	0.053	0.081
Ca	mg/L	0.1000	3.81	3.33	7.71	13.66
Fe	mg/L	0.0005	<0.0005	<0.0005	<0.0005	<0.0005
K	mg/L	0.001	2.61	0.93	2.01	1.77
Mg	mg/L	0.001	0.39	0.38	0.74	1.13
Na	mg/L	0.001	2.56	0.75	2.02	1.76
P	mg/L	0.001	0.066	0.093	0.029	0.053
S	mg/L	0.001	0.372	0.126	0.423	0.750
Si	mg/L	0.001	0.565	0.278	0.569	1.073
PO_4^{3-}	mg/L	0.001	<0.001	<0.001	<0.001	0.008
SO_4^{2-}	mg/L	0.001	2.141	1.005	1.303	2.185
NO_3^-	mg/L	0.001	0.952	0.496	0.017	0.030

SMC1 Rain-Filled: ICP-MS and CL

Analyte	Units	Detection Limit	Hours Since Wetting		
			2	20	44
Al	mg/L	0.0005	0.025	0.004	0.053
Ca	mg/L	0.1000	7.36	7.41	10.00
Fe	mg/L	0.0005	<0.0005	<0.0005	<0.0005
K	mg/L	0.001	3.93	1.36	2.19
Mg	mg/L	0.001	0.38	0.40	0.49
Na	mg/L	0.001	3.46	0.93	1.87
P	mg/L	0.001	0.076	0.046	0.043
S	mg/L	0.001	0.999	0.109	0.265
Si	mg/L	0.001	0.503	0.353	0.576
PO_4^{3-}	mg/L	0.001	<0.001	0.054	<0.001
SO_4^{2-}	mg/L	0.001	4.613	1.629	2.280
NO_3^-	mg/L	0.001	2.181	0.301	0.019

Analyte	Units	Detection Limit	Hours Since Wetting		
			70	94	118
Al	mg/L	0.0005	0.077	0.102	0.134
Ca	mg/L	0.1000	10.29	11.98	19.53

Fe	mg/L	0.0005	<0.0005	<0.0005	<0.0005
K	mg/L	0.001	2.89	3.49	4.781
Mg	mg/L	0.001	0.52	0.62	1.126
Na	mg/L	0.001	2.28	3.04	5.028
P	mg/L	0.001	0.100	0.035	0.001
S	mg/L	0.001	0.882	1.254	2.722
Si	mg/L	0.001	0.814	1.216	1.993
PO_4^{3-}	mg/L	0.001	0.008	0.094	0.046
SO_4^{2-}	mg/L	0.001	2.714	4.576	10.696
NO_3^-	mg/L	0.001	0.000	0.368	1.085

Leach Testing: Anion Data

Sample	pH	Analyte	Amount mg/L	Detection Limit mg/L
SMC	9	Cl	17.95	0.1
SMC	10	Cl	8.25	0.1
SMC	10.5	Cl	8.70	0.1
SMC	11	Cl	10.20	0.1
SMC	11.5	Cl	10.31	0.1
SMC	12	Cl	10.61	0.1
SMC	12.5	Cl	13.67	0.1
SMC	13	Cl	12.21	0.1
BW	9	Cl	8.53	0.1
BW	10	Cl	8.80	0.1
BW	10.5	Cl	12.52	0.1
BW	11	Cl	5.91	0.1
BW	11.5	Cl	5.81	0.1
BW	12	Cl	7.77	0.1
BW	12.5	Cl	5.95	0.1
BW	13	Cl	11.53	0.1
BW	DDI	Cl	1.97	0.1
SMC	9	SO4-2	17.27	0.001
SMC	10	SO4-2	11.69	0.001
SMC	10.5	SO4-2	11.87	0.001
SMC	11	SO4-2	12.23	0.001
SMC	11.5	SO4-2	12.36	0.001
SMC	12	SO4-2	13.30	0.001

SMC	12.5	SO4-2	23.47	0.001
SMC	13	SO4-2	23.50	0.001
BW	9	SO4-2	5.51	0.001
BW	10	SO4-2	5.69	0.001
BW	10.5	SO4-2	8.58	0.001
BW	11	SO4-2	6.61	0.001
BW	11.5	SO4-2	7.95	0.001
BW	12	SO4-2	12.77	0.001
BW	12.5	SO4-2	7.00	0.001
BW	13	SO4-2	8.81	0.001
std	DDI	SO4-2	5.64	0.001
SMC	9	NO-3	14.53	0.001
SMC	10	NO-3	12.37	0.001
SMC	10.5	NO-3	13.36	0.001
SMC	11	NO-3	3.24	0.001
SMC	11.5	NO-3	12.97	0.001
SMC	12	NO-3	8.40	0.001
SMC	12.5	NO-3	23.52	0.001
SMC	13	NO-3	15.13	0.001
BW	9	NO-3	3.58	0.001
BW	10	NO-3	3.62	0.001
BW	10.5	NO-3	5.09	0.001
BW	11	NO-3	3.69	0.001
BW	11.5	NO-3	3.52	0.001
BW	12	NO-3	16.17	0.001
BW	12.5	NO-3	3.41	0.001
BW	13	NO-3	4.93	0.001
std	DDI	NO-3	5.22	0.001
SMC	9	PO4-3	0.06	0.001
SMC	10	PO4-3	0.05	0.001
SMC	10.5	PO4-3	0.10	0.001
SMC	11	PO4-3	0.23	0.001
SMC	11.5	PO4-3	0.36	0.001
SMC	12	PO4-3	0.11	0.001
SMC	12.5	PO4-3	1.14	0.001
SMC	13	PO4-3	0.13	0.001
BW	9	PO4-3	0.40	0.001
BW	10	PO4-3	0.26	0.001
BW	10.5	PO4-3	0.94	0.001
BW	11	PO4-3	1.46	0.001
BW	11.5	PO4-3	0.57	0.001

BW	12	PO4-3	0.70	0.001
BW	12.5	PO4-3	0.24	0.001
BW	13	PO4-3	2.49	0.001
std	DDI	PO4-3	0.46	0.001

APPENDIX C

Overview of Major Bacterial Phylogenetic Groupings Found in the 16s rRNA Environmental Sequencing of Pothole Biofilms (Chapter 4)

Unless otherwise identified, information for this appendix was drawn from Bergey's Manual of Systematic Bacteriology (Garrity et al., 2003)

Phylum Proteobacteria

The most abundant group represented in this work is the phylum Proteobacteria. The phylum was first defined as a class by Stackebrandt et al. (1988), using 16S rRNA sequences from a group of, at the time, unrelated eubacteria. Within the Proteobacteria five separate classes are defined using the Greek letters: α, β, γ, δ, ε. The Alpha, Beta, Gamma and Delta Proteobacteria are represented in this thesis. The Proteobacteria were formally adopted by the second edition of Bergey's Manual of Systematic Bacteriology (Garrity et al., 2003) and raised to the phylum level, with each of the five groups becoming classes. The Proteobacteria is the largest and most phenotypically diverse phylum encompassing a large proportion of the gram-negative bacteria (Kersters et al., 2006). In general, the Proteobacteria are mainly mesophilic, free-living and are motile through the action of polar or peritrichous flagella. To each of these generalisations there are numerous exceptions given the large diversity of the phylum.

Class Alphaproteobacteria

Within the Proteobacteria, the Alphaproteobacteria is the largest class, and most clones from this thesis fall within it. Alphaproteobacteria are found in abundance in both

terrestrial and marine environments (Giovannoni et al., 2005) and the class contains a large number of photorophs, chemolithotrophs, chemoorganotrophs and aerobic photoheterotrophs; dominated by the purple non-sulphur bacteria (Kersters et al., 2006). Some photoheterotrophs are also able to grow photoautotrophically. A few species can grow methylotrophically. One order is composed exclusively of parasitic and mutualistic bacteria. Recently studies have identified proteins that are unique to the Alphaproteobacteria; helping to distinguish them based on more than the branching of phylogenetic trees (Gupta and Mok, 2007).

Class Betaproteobacteria

Members of the Betaproteobacteria are phenotypically, metabolically, and ecologically diverse. Members include aerobic and facultative anaerobic chemoorganotrophs, such as those found to dominate in soils contaminated with polycyclic aromatic hydrocarbons (PAHs) (Martin et al., 2012). Members also include: obligate and facultative chemolithotrophs, lithoautotrophs that oxidize ammonia, rare photoheterotrophs and nitrogen-fixing organisms. Tolerated temperature ranges are generally mesophilic but some thermophilic species have been identified. They are found in many environments including marine and fresh water, soils and as plant, animal, and human pathogens. Obligate and facultative aerobes and facultative anaerobes are identified. Unique pockets of Betaproteobacteria, found in Brazil and South Africa symbiotically fix nitrogen in plant roots, a function that is usually performed by species of Alphaproteobacteria (Gyaneshwar et al., 2011).

Class Gammaproteobacteria

The Gammaproteobacteria class is not currently defined by any unique molecular or biochemical characteristic and is based solely on the branching of phylogenetic trees (Gao et al., 2009). It contains many well-known pathogenic bacteria such as *Escherichia coli* and *Vibrio cholerae*. It is also home to the anoxygenic phototrophic bacteria known as phototrophic purple sulphur bacteria. The metabolic diversity of the Gammaproteobacteria is comprehensive, including the already mentioned photosynthetic bacteria as well as: chemotrophs, autotrophs, chemoorganotrophs (respiratory and fermentative), methylotrophs, and chemolithotrophs (e.g., sulphur oxidizing organisms). They are found in both aerobic and anaerobic environments and represent both obligate and facultative responses to those conditions. Some microaerobic strains have also been identified. Halotolerant and halophilic strains have been found as well as, obligate acidophiles. Temperature ranges tolerated encompass psychrophilic and psychrotolerant lifestyles through the spectrum to mesophilic and moderately thermophilic ranges. The Gammaproteobacteria include a wide variety of obligate and facultative parasites of protozoa, fish, insects, arachnids, mammals and humans. In the environment the Gammaproteobacteria are encountered in fresh water, and marine environments, as well as soils.

Class Deltaproteobacteria

Members of the Deltaproteobacteria are divided into one group of dominantly obligate anaerobic genera and another of obligate aerobic genera. Most species are chemoorganotrophic or chemolithoautotrophic. The anaerobic genera include many metal

and sulphur reducing genera (e.g., *Geobacter* and *Desulfovibrio*) some of which are thermophilic although most are mesophilic. Aerobes include parasitic genera that prey on other bacteria (e.g., *Bdellovibrio*; Madigan et al., 2003). Members are found in marine and freshwater settings, soils and sewage sludge, as well as being important contributors to acid rock drainage in environments affected by mining. New species of thermoacidophilic Deltaproteobacteria that reduce elemental sulphur have been isolated from deep sea hydrothermal vents (Flores et al., 2011)

Phylum Cyanobacteria (Waterbury, 2006)

The Cyanobacteria are a phylum of oxygenic photosynthetic bacteria with some species that can carry out anoxygenic photosynthesis when hydrogen sulphide is present at high concentrations. A limited number of species have been found to grow chemoheterotrophically in the laboratory (Rippka et al., 1979). Cyanobacteria are among the most morphologically and developmentally diverse groups of prokaryotes ranging from simple, single cells to filamentous forms which have many different cell types. These filamentous forms represent a truly multicellular lifestyle where photosynthesis and nitrogen fixation are handled in separate cells. The Cyanobacteria tolerate a wide range of environmental conditions as long as there is some light exposure. They are primary colonisers of many environments, and are found in soils, marine and fresh waters, as well as hypersaline environments. Most cyanobacteria are mesothermic, but psychrophiles and thermophiles have also been identified. They are also well known symbionts, growing with other species such as lichens and other eukaryotes.

Phylum Planctomycetes

The Planctomycetes are gram-negative bacteria observed in, or isolated from seawater, freshwater, groundwater, soil, peat bogs, compost, manure, sewage sludge, and animal tissues (e.g., prawn, sponge, coral, lice, termite, and human colon). The role of this phylum in the environment is poorly understood, but it has been found to dominate biofilms in certain locations (Bengtsson and Øvreås 2008) and is not rare in general. Like the phylum Verrucomicrobia it is thought that a bias when using universal bacterial primers limits the recovery of Planctomycetes sequences in environmental samples (Blaire et al., 2011). Generally growth temperatures are mesophilic but the occurrence of Planctomycetes in some extreme environments has been recorded (eg., high temperature, hypersaline, acidic and alkaline). So far most isolated species are aerobes or anaerobes (facultative or obligate) and have chemoheterotrophic, or chemolithoautotrophic metabolism. Many use carbohydrates as their primary source of carbon. A unique feature of this phylum is its possession of intracellular membrane compartmentation in the form of a nuclear membrane, which makes its study important for understanding the origins of complex organisation in eukaryotic cells (Fuerst, 2005).

Phylum Bacteroidetes

The phylum Bacteroidetes is a phenotypically diverse group of gram-negative rods that occur in many environments. The phylum is composed of three classes, but the class Bacteroides is the most studied because it contains many human pathogens. When found in the gut and mouth these pathogens are beneficial, but can be dangerous outside

of these environments. Of all known anaerobic pathogens certain species of the Bacteroides have the most antibiotic resistance mechanisms (Wexler, 2007). The Bacteroidetes are found in the environment in sea water and soils where some psychrophilic members have been identified although most are mesophilic. They are found in aerobic, anaerobic and microaerobic conditions and species have been identified that adopt obligate and facultative lifestyles in each condition. Many are chemoorganotrophs (both respiratory and fermentative).

Phylum Acidobacteria

The phylum Acidobacteria is relatively poorly understood when compared to other, large phylums such as the Proteobacteria. Many species have been identified, particularly in soils and sediments, but few have been well isolated in pure culture (Rappe and Giovannoni, 2003). They have also been found in a variety of other environments (e.g., hot springs, marine snow, feces, caves, and sites contaminated with toxic metals) indicating a ubiquitous nature. Species that have been characterised are in general found to be either fermentative or respiratory chemoorganotrophs. They have been found in both aerobic and anaerobic conditions, and although the environments they are found in are generally mesophilic, both thermophilic and psychrophilic growth conditions are tolerated in some species. A limited number of species were found to be acidophilic.

Phylum Fibrobacter

The phylum Fibrobacter is small with only one genus divided from the Bacteroidetes in 1988 (Montgomery et al., 1988). The gram-negative cells are rod-shaped, vary in length and grow primarily on cellulose. They are predominantly found in the stomachs of ruminants (Suen et al., 2011); however, new work has found them in more diverse environments (McDonald, 2008). They are in general, obligate anaerobes.

Phylum Gemmatimonadetes

The first member of this small, gram-negative phylum was discovered in 2003. The grouping was first considered a family (Zhang et al., 2003), and only promoted to phylum in 2008. Only one strain has been isolated however, uncultured strains have been found in many terrestrial and aquatic habitats. They are non-spore forming heterotrophic bacteria that are generally found in mesophilic aerobic environments.

Phylum Verrucomicrobia

The gram-negative phylum Verrucomicrobia is represented in a wide variety of environments including salt and freshwater, vertebrate digestive tracts, as nematode endosymbionts and in soils (Wagner and Horn, 2006). Recent work by Bergmann et al. (2011) speculates that the phylums' diversity in soils is drastically underestimated due to primer bias. Most isolated species are chemoheterotrophs, growing preferentially on carbohydrates including complex natural polysaccharides. Some species are chemoorganotrophs (fermentative or respiratory) and able to oxidize a wide variety of organic molecules making them possibly important in oligotrophic environments. They are found in mesophilic environments and species with both strictly aerobic and

anaerobic oxygen requirements have been indentified. The recent isolation of the complete genome of a methylotroph which is both hyperacidophilic (pH 2-2.5) and a thermophilic (60^{o}C) has shown that the Verrucomicrobia are also possibly important extremophiles (Hou et al., 2008).

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