ENVIRONMENTAL INFLUENCES ON THE GENETIC DIVERSITY OF BACTERIAL COMMUNITIES IN ARCTIC STREAMS

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Julia Rose Larouche

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Thesis Examination Committee:

_____ **Advisor**
William Breck Bowden, Ph.D.

_____
Gregory K. Druschel, Ph.D.

_____
Rosanna Giordano, Ph.D.

_____ **Chairperson**
Jane Hill, Ph.D.

_____ **Vice President for Research**
Frances E. Carr, Ph.D. **and Dean of Graduate Studies**

Date: September 30, 2008

ABSTRACT

The National Park Service (NPS) Inventory and Monitoring (I&M) Program is designed to collect baseline data on "vital sign" indicators across the entire NPS system. The project presented in this thesis was designed to supplement to efforts of the Artic Network (ARCN) to catalogue the physical, chemical and biological metrics associated with the Stream Communities and Ecosystems vital sign and to foster a better understanding of the basic structure and function of these remote systems. This data is essential to assess the impacts of current and future environmental change in the ARCN parks.

The primary objective of this project was to quantify the genetic diversity of microbial communities of selected arctic stream ecosystems. Microbes are a fundamentally important but poorly understood component of arctic stream ecosystems. They are responsible for recycling organic matter and regenerating nutrients that are essential to the food webs of aquatic ecosystems. Recent research (Jorgenson et al. 2002) in the ARCN parks has shown that two fundamentally different lithologies – ultramafic and non-carbonate – influence terrestrial productivity and impart different geochemical characteristics to stream water. Microbes are found in different stream habitats – sediment (epipssamon) and rock (epilithon) biofilms. In this work we test the hypothesis that these differences in lithology and stream habitat influence the genetic diversity of bacterial biofilm communities in arctic streams and whether these patterns can be correlated to stream biogeochemistry. A microbial community fingerprinting method, T-RFLP, as well as 16S rRNA gene sequencing were used to explore the genetic diversity of microbial communities in sediment and epilithic biofilms in stream reaches that drain watersheds with contrasting lithologies in the Noatak National Preserve, Alaska.

Differing patterns in bacterial community composition at both the large-scale (lithology) and small-scale (stream habitat) were observed. Non-metric multidimensional scaling (NMDS) ordination of T-RFLP peaks and Analysis of Similarity (ANOSIM) showed a high degree of separation (ANOSIM $P < 0.001$) between the non-carbonate and ultramafic lithologies, as well as the two habitats, sediment and epilithon. Significant ($P < 0.005$, Bonferroni corrected) positive correlations were detected between particular nutrients, base cations, and dissolved organic carbon and bacterial community structure unique to each lithology. Although clone libraries indicated high bacterial OTU diversity within and across stream sites, biogeographical patterns were observed depending on locality type. Rarefaction analyses indicated that streams arising from the non-carbonate lithology may be more diverse than streams arising from the ultramafic lithology. Analysis of MOlecular VAriance (AMOVA) indicated that sediment and epilithon samples had genetically different microbial communities ($P = 0.01$) and taxonomic identifications revealed markedly different bacterial residents between sediment and epilithon habitats. Our results show relationships at large- and small-scales at the landscape level and in ecological niches within a single stream.

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At the heart of all beauty lies something inhuman, and these hills, the softness of the sky, the outline of this [arctic tundra] at this very minute lose the illusory meaning with which we had clothed them henceforth more remote than a lost paradise…that denseness and that strangeness of the world is absurd. -Albert Camus

TABLE OF CONTENTS

Page

LIST OF TABLES v

LIST OF FIGURES……vi

CHAPTER 1: COMPREHENSIVE LITERATURE REVIEW 1

1.1 Introduction 1

1.2 The National Park Service Inventory and Monitoring Program..... 2

1.3 The Noatak National Preserve, Alaska 4

1.4 The Importance of Microbial Communities 6
1.4.1 Typical Freshwater Bacteria: Function, Biofilm Habitat, and Composition 8

1.5 The Concept of Microbial Biogeography 11
1.5.1 Microbial Community Composition and Function: The Environment Selects 11
1.5.2 Microbial Community Composition and Function: Geographical Patterns 12

1.6 Methods of Microbial Community Characterization 13
1.6.1 Microbial Community Fingerprinting: Terminal-Restriction Fragment Length Polymorphism (T-RFLP) 16
1.6.2 16S Ribosomal RNA (rRNA) Gene Cloning and Sequencing 18

1.7 Linkages between Biogeochemistry and Microbial Community Composition.... 20

1.8 Microbial Communities of Arctic Aquatic Ecosystems 22

1.9 Research Goals 23

LITERATURE CITED 24

CHAPTER 2: MICROBIAL BIOGEOGRAPHY OF ARCTIC STREAMS: EXPLORING INFLUENCES OF LITHOLOGY AND HABITAT 43

2.1 Introduction 44

2.2 Material and Methods 48
2.2.1 Study Area 48
2.2.2 Sampling Design 48

2.2.3 Sample Collection of Benthic Microbial Communities……48
2.2.4 DNA Extraction 49
2.2.5 T-RFLP Profiles..... 49
2.2.6 Analysis of T-RFLP Profiles 51
2.2.7 T-RFLP Statistical Analyses..... 52
2.2.8 Building Clone Libraries for 16S rRNA Gene 53
2.2.9 Processing of Sequence Data 55
2.2.10 Estimations of richness and diversity. 56
2.2.11 Clone Library Statistical Analyses..... 57
2.2.12 Taxonomic Associations of 16S rRNA Bacterial Clones 58
2.2.13 Nucleotide sequence accession numbers 58

2.3 Results 59
2.3.1 Biogeochemistry of stream study sites 59
2.3.2 Lithology and Habitat Comparisons using T-RFLP 59
2.3.3 Lithology and Habitat Comparisons using Clone Libraries 60

2.4 Discussion..... 63
2.4.1 Comparisons using T-RFLP 64
2.4.2 Relationships between Bacterial Communities and Biogeochemistry 64
2.4.3 Comparisons using 16S rRNA Gene Sequencing..... 65
2.4.4 Conclusions and Relevance to Microbial Biogeography..... 68

LITERATURE CITED..... 72

COMPREHENSIVE LITERATURE CITED 90

APPENDIX..... 102

LIST OF TABLES

Page

Table 2.1. Summary of individual study streams with GPS……..78

Table 2.2. Values of biological, chemical and physical parameters for study streams by lithology 79

Table 2.3. Phylogenetic affiliation of clones amplified from sediment and epilithon.. …80

Table A.1. Phylogenetic affiliation of clones amplified from sediment and epilithon with closest GenBank identification……103

Table A.2. Diversity indices (Chao1, ACE, Shannon, Simpson) for 16S rRNA clone libraries……104

Table A.3. AMOVA results at each level of partitioning……105

LIST OF FIGURES

Page

Figure 1.1. The 32 National Park Service (NPS) Inventory and Monitoring (I&M) Networks (taken from nps.gov) 33

Figure 1.2. The four NPS Inventory and Monitoring Networks in Alaska: Southeast Alaska, Southwest Alaska, Central Alaska and the Arctic (taken from nps.gov) 34

Figure 1.3. The Noatak National Preserve resides in the NPS Arctic Network, Alaska (credit: A. Balser)..... 35

Figure 1.4. Conceptual diagram of the NPS three-phase approach to establish individual network I&M Programs (taken from nps.gov) 36

Figure 1.5. A cross-sectional diagram of a microbial biofilm (taken from nps.gov) 37

Figure 1.6. Phylogenetic tree on bacterial small subunit (SSU) rRNA sequences. Large drops indicate typical and dominant groups of freshwater bacteria and small drops represent other groups observed in, but not unique to freshwater (taken from *Logue et al.* [2008])..... 38

Figure 1.7. Schematic of terminal restriction fragment polymorphism (T-RFLP) methodology (credit: B. Crump)..... 39

Figure 1.8. Schematic of the 16S rRNA gene cloning from environmental samples (credit: B. Crump)..... 40

Figure 1.9. Relationships between methodologies (dotted lines) and phylogenetic diversity coverage. Rarefaction curves show theoretical depths of coverage (phylotypes of OTUs observed) for two microbial communities, indicating that the coverage achieved differs by methodology depending on the overall phylogenetic diversity (taken from *Stackebrandt* [2006]). The two methods employed in this study are community fingerprints and clone libraries 41

Figure 1.10. The redox ladder showing environmental niches for microbial metabolism (taken by Drever [2002]) 42

Figure 2.1. Study area of Feniak Lake region with stream site locations across contrasting lithologies in the Noatak National Preserve..... 81

Figure 2.2. Non-metric multidimensional scaling (NMDS) ordination (1st & 2nd of the 3-dimensional solution) of stream sediment bacterial communities based on pairwise similarity estimates (Bray-Curtis). Points that are close together represent sediment biofilm communities with similar bacterial community composition based on the T-RFLP (terminal restriction fragment length polymorphism) method. The associated normal stress value of the ordination is 0.11, indicating a good approximation of the overall structure of the data in multivariable space. Significant ($p<0.005$), Bonferonni adjusted biogeochemical variables were overlaid (arrows) showing the degree of correlation with microbial data. Abbreviations: Nutrients = TDN-NO_3^-, total dissolved nitrogen – nitrate and TDN/TDP, total dissolved nitrogen/total dissolved phosphorus; DOC, dissolved organic carbon; Cations = Ca^{2+}, Mg^{2+}, K^+, and Na^+; NO_3^-/TDN, nitrate/total dissolved nitrogen……..82

Figure 2.3. Non-metric multidimensional scaling (NMDS) ordination (2-dimensional solution) of stream sediment and epilithon bacterial communities based on pairwise similarity estimates (Bray-Curtis). Points that are close together on the right side of the ordination represent epilithon samples (n = 8) and those points close together on the left side of the ordination represent sediment samples (n = 42)…….....……..83

Figure 2.4. (a) Rarefaction curves of observed OTU richness in sediment and epilithon samples within each lithology (non-carbonate and ultramafic). (b) Rarefaction curves of observed OTU richness in sediment and epilithon samples regardless of lithology. The variance of the number of OTUs drawn in 100 randomizations at each sample size was calculated as 95% confidence intervals (not shown). 84

Figure 2.5. Rarefaction curves of observed OTU richness in individual stream and stream habitat comparisons (a) Sediment non-carbonate streams; (b) Epilithon non-carbonate streams; (c) Sediment ultramafic streams; (d) Epilithon ultramafic streams…….85

Figure 2.6. AMOVA results showing percent variation at each level of partitioning…...86

Figure 2.7. Non-metric multidimensional scaling (NMDS) plot of genetic and community structure for sediment and epilithon samples. Distances between points are based upon the F_{ST} statistic for all samples pairs 87

Figure 2.8. Distribution of represented bacterial divisions and families detected in the sediment clone libraries in each lithology…….88

Figure 2.9. Distribution of represented bacterial divisions and families detected in the epilithon clone libraries in each lithology……..89

Figure A.1. Distribution of T-RFs by habitat within each lithology…….....105

Figure A.2. Rarefaction curves for the five sediment clone libraries (n = number of OTUs observed)……..106

Figure A.3. Rarefaction curves for the four epilithon clone libraries (n = number of OTUs observed)……..107

Figure A.4. Rarefaction curves for sediment and epilithon clone libraries at individual stream sites (n = number of OTUs observed)……..108

Figure A.5. Diversity richness estimators, Chao1 and ACE, with 95% confidence intervals……109

CHAPTER 1: COMPREHENSIVE LITERATURE REVIEW

1.1 Introduction

The purpose of this chapter is to provide background for this study. The following topics will be reviewed: (1) the National Park Service (NPS) Inventory and Monitoring (I & M) Program, (2) Noatak National Preserve, Alaska, (3) the importance of microbial communities to ecosystem function and a description of typical freshwater bacteria including their habitat and role in aquatic ecosystems, (4) the concept of microbial biogeography, (5) the patterns in distribution and function of bacterial communities in aquatic environments, (6) the molecular methods and approaches available to characterize microbial communities in their natural environment, focusing on the methods employed in this study, (7) the linkages between biogeochemical constituents in the environment and microbial community composition, and (8) the microbial community observations by previous studies in arctic aquatic ecosystems. This study investigates a microbial component of stream ecosystems in the Noatak National Preserve and explores the feasibility of using a microbial fingerprint as a metric in the Stream Communities and Ecosystems vital sign as part of the NPS Inventory and Monitoring Program to provide information about landscape and ecosystem-level functioning.

1.2 The National Park Service Inventory and Monitoring Program

In 1992, the NPS established an Inventory and Monitoring Program as a strategy to improve park management through greater reliance on scientific information, in particular, "to develop scientifically sound information on the current status and long-term trends in the composition, structure, and function of park ecosystems and to determine how well current management practices are sustaining those ecosystems" (NPS 2005). Nationwide, 270 national parks have been grouped into 32 ecosystem-based networks, Vital Sign Networks, linked by geographic similarities, common natural resources, and resource protection challenges (Figure 1.1) (NPS 2005a). The network approach allows for collaboration, information sharing, and economies of scale in monitoring of natural resources that park managers are directed to preserve "unimpaired for future generations".

The Arctic Network (ARCN) is one of the largest of these Vital Sign Networks, consisting of five contiguous NPS units encompassing 19.3 million acres (7.8 million hectares), or roughly 25 percent of all NPS acreage in the United States (Figure 1.2). These five NPS units are Gates of the Arctic National Park and Preserve, Noatak National Preserve, Kobuk Valley National Park, Cape Krusenstern National Monument, and Bering Land Bridge National Preserve. The Noatak National Preserve is outlined in Figure 1.3. These Arctic Parks harbor some of the most unique and relatively undisturbed freshwater ecosystems in North America.

"Vital signs" are measurable indicators of ecosystem health. As used by the National Park Service, vital signs are "a subset of physical, chemical and biological

elements and processes of park ecosystems that are selected to represent the overall health or condition of park resources, known or hypothesized effects of stressors, or elements that have important human values. Vital signs may occur at any level of organization including landscape, community, populations, or genetic level, and may be compositional (referring to the variety of elements in the system), structural (referring to the organization or pattern of the system), or functional (referring to ecological processes)" (Sanzone et al. 2006). ARCN has chosen 28 candidate vital signs to monitor within their parklands (e.g. Lake/Stream Communities and Ecosystems, Permafrost and Thermokarsting, Climate and Weather, and Air Contaminants, etc.).

The monitoring objectives for the freshwater ecosystems set forth by ARCN are: 1) to collect baseline data on the physical, chemical, and biological parameters of streams, lakes, and surrounding watersheds within ARCN, 2) to determine long-term trends in the physical, chemical, and biological characteristics of streams, lakes, and surrounding watersheds within ARCN, and 3) to understand how the landscape components interact at various spatial and temporal scales to affect freshwater ecosystems.

Networks in the NPS Inventory and Monitoring Program develop their monitoring strategies through a three-phase approach (Figure 1.4). Phase I includes gathering background information, developing conceptual ecological models, and formulating initial objectives, Phase II involves the selection of vital signs, and Phase III involves developing overall sample design, sampling protocols, and a data management plan. The challenge remains in choosing appropriate metrics, or specific features, to quantify an

indicator (e.g. nutrients, chlorophyll *a* and algae, metals, benthic invertebrates, etc.) that will provide the most valuable information towards understanding the dynamics of stream ecosystems.

The primary objective of this project was to test the value of using measures of microbial community diversity as metrics within the Stream Communities and Ecosystems vital sign for the ARCN parks. In addition to its potential value in measuring a vital sign indicator, microbial diversity provides fundamentally important information about the structure of arctic stream ecosystems that may ultimately be useful in understanding associated biogeochemical processes that drive stream ecosystem function in this environment.

1.3 The Noatak National Preserve, Alaska

The Noatak River and its watershed occupy 6.6 million acres and extend from the Kotzebue Sound through the Arctic foothills of the Brooks Range located in northern Alaska. The headwaters of the Noatak River arise in the Gates of the Arctic Park within the central Brooks Range, a granitic northern extension of the Rocky Mountains, and are fed primarily by snowmelt, with some groundwater and glacial contribution (Elias et al. 1999). The Noatak River is the longest continuous river segment in the U.S. National Wild and Scenic system and the largest mountain-ringed river basin that is virtually unaffected by humans in the United States (Milner et al. 2005).

Due to its complex geology and variety of climate and landscape conditions, the Noatak River basin harbors a wider array of ecosystems than does any other watershed of

comparable size in the Arctic region (Jorgenson et al. 2002). The vast and remote nature of the Noatak River basin has left the area and its ecosystems poorly documented from a scientific perspective. Aside from a few isolated studies in soil and parent material (Binkley et al. 1994, Binkley et al. 1995, Stottlemyer et al. 2003), vegetation (Young 1974, Oswald et al. 1999, Suarez et al. 1999), and lakes and rivers (Smith 1913, O'Brien et al. 1975), knowledge of the ecosystems of the Noatak River basin is limited, particularly for the freshwater environments. A 1973 expedition led by Steve Young was the first "coordinated, interdisciplinary scientific inquiry into the natural environment of a piece of Arctic terrain considerably larger than a number of states in the northeastern United States" (Young 1974). Following the work by Young et al. (1974) the Noatak watershed was established as a Biosphere Reserve in 1976, a National Monument in 1978, and a National Preserve in 1980 (Jorgenson et al. 2002).

The highest elevation in the Noatak River basin is Mount Igikpak (elevation = 2594 m) in the Schwatka Mountains. There are three distinct elevation gradients based on slope from the steeper headwater branches of the Noatak River to the medium-gradient main stem to the estuarine lowland segments near the coast (Milner et al. 2005). The basin is characterized by six major regions. These six regions were designated by P.S. Smith in 1913 as Headwater Mountains, Aniuk Lowlands, Cutler River Upland, Mission Lowland, Zigichuck Hills, and the Coastal Lowland (Young 1974).

The Noatak River basin has an arctic climate, with long cold winters and short cool summers. Mean temperatures for July and February are approximately 11ºC and -25ºC, respectively (Anderson et al. 1994). The floor of the basin and the surrounding

uplands are essentially underlain by continuous permafrost. Arctic streams vary according to the permafrost characteristics and duration of seasonal thaw periods. The streams of the Noatak region begin to freeze in October, with no discharge from the upper basin later in winter. River ice breakup occurs in early May and then rapid streamflow is observed in June due to spring snowmelt (Milner et al. 2005). Ice extension to the substrate of the freshwaters during the Arctic winter creates a limited environment for the benthic macroinvertebrates in which adaptations and physiological tolerances to freezing are critical for survival (Milner et al. 2005).

Air temperatures over the last decade (1990-2000) were the warmest in the last 400 years (Overpeck et al. 1997). This warming event has triggered substantial tree growth in the Noatak Valley allowing for spruce forest to pervade the tundra landscape (Suarez et al. 1999). Microbial growth and processing of organic matter should be strongly linked to temperature; however, the effect of increased temperature on microbial activity is unclear (Rouse et al. 1997).

1.4 The Importance of Microbial Communities

Over the past four decades of molecular-phylogenetic studies, researchers have gradually built an impressive map of evolutionary diversification revealing that the primary diversity of life is microbial. Microbial diversity was shown by Woese (1990) to be distributed across a three-domain classification system based on the differences in the sequences of the nucleotides in the cell's ribosomal RNA (rRNA): Archaea, Bacteria, and Eucarya. Characteristics across these three domains provide evidence that inorganic

material sustained earlier life and that photosynthesis and use of organic compounds for carbon and energy metabolism evolved later (Pace 1997).

Microbial life encompasses the vast majority of all metabolic and genetic diversity on Earth; microbes can survive and thrive in any environment where it is thermodynamically favorable (Gleeson 2007). Bacteria act as geochemical agents in various environments by influencing primary mechanisms of mineral mobilization, redox activity, and elemental (C, N, S, and P) cycling (Ehrlich 1998). The activity of bacteria and other microorganisms play numerous roles in regulating atmospheric composition, recycling inorganic and organic matter, and keeping the planet habitable for all forms of life (Ehrlich 1998).

Comparing the rRNA structure of bacteria is informative because rRNA molecules throughout nature perform the same functions with little structural change over time. For this reason, similarities and differences in rRNA nucleotide sequences is indicative of the degree of relatedness between organisms. The development of molecular-phylogenetic methods to study natural microbial ecosystems without the traditional culture-based techniques has resulted in the discovery of many microbial lineages (Pace 1997).

Our current understanding regarding the composition of the natural microbial world is rudimentary given the limited number of environments studied so far with modern molecular methods, however the progress observed in this field each day is substantial. The rRNA genes isolated from the environment of organisms that represent different types of genomes can be targets for further characterization if they seem

interesting or useful towards systems processes (Pace 1997). An inventory of the microbial diversity across ecosystems may provide further understanding of the mechanisms of our biosphere.

1.4.1 Typical Freshwater Bacteria: Function, Biofilm Habitat, and Composition

Numerous studies confirm the importance of bacteria and their consumers to aquatic ecosystem function in terms of energy flow and biogeochemical cycling. Cole et al. (1988) estimated that approximately 40% of the total primary productivity was consumed by bacteria in aquatic ecosystems in environments ranging from highly oligotrophic to highly eutrophic. Peterson et al. (2001) showed the importance of bacteria to nitrogen cycling in headwater streams via bacterial assimilation of ammonium.

Bacteria also play an important role in the cycling and transformation of metals within the aquatic environment. Bacteria can cause localized accumulation or association of metals around their cells as well as affect the solubility and availability of metals by promoting either the oxidation or reduction of certain elements (Bremer and Geesey 1994). Bacteria or reactions mediated by enzymes or metabolic products from bacteria influence the availability of metals to other aquatic organisms and the surrounding environment.

Bacteria in aquatic systems can adopt two different lifestyles: sessile (attached) or planktonic (free-floating). A biofilm (Figure 1.5) provides a refuge for sessile bacteria and in this state they are defined as “polysaccharide-encased, surface-adherent microbial communities” (McLean 2002). A sessile mode of life for bacteria within a biofilm offers

numerous advantages over the planktonic mode, including the ability to acquire and store nutrients from the water column in the biofilm matrix (Paul et al. 1991), symbiotic relationships with neighboring organisms (bacteria, algae, fungi) (Sigee 2005), resistance to toxic chemicals and environment stressors via enzymatic activity and structural protection (Costerton et al. 1987), and genetic resilience due to mechanisms of lateral gene transfer (Christensen et al. 1998, Hausner and Wuertz 1999).

The importance of sessile bacteria in aquatic environments has been recognized and explored for many years starting with early biofilm research largely focused on alpine streams (Geesey et al. 1978, McFeters et al. 1978). Biofilm-associated microbes drive biogeochemical cycling due to their ubiquity, diverse metabolic capabilities, and high enzymatic activity (Moss et al. 2006). Furthermore, biofilm organisms (photo- and heterotrophic) play a major role in ecosystem processes because of their high abundance and metabolic contribution.

Sessile bacteria adhere to rock and sediment surfaces through the excretion of polymeric fibers, anchoring the cell and allowing bacteria to take hold within the biofilm matrix. Bacteria act as initial colonizers to submerged surfaces and prepare the substrate for subsequent colonization for other organisms (Geesey et al. 1978). These heterotrophic bacteria are major players in biogeochemical cycles utilizing sediment and rocks as substrate for both biological and chemical reactions. Organic matter and minerals (iron and manganese oxides) coat sediments at the bottom of streams or rock surfaces (Thurman 1985). This coating is referred to as a 'conditioning film' and provides the first opportunity for bacterial adhesion and attachment for biofilm

development (Sigee 2005). Suspended sediment particles also have organic matter and mineral coatings to form macro aggregates, sometimes referred to as “snow” or “floc” in aquatic systems (Wotton 2007). These macroaggregates also act as substrate for bacterial adhesion.

Depending on available resources, only certain types of bacteria communities will thrive within the biofilm. Competition, succession, and mutualism within the bacterial community enhances weathering processes (mineral dissolution and deposition), regulates uptake of both organic matter and inorganic nutrients, and even catalyzes the exchange of genetic material resulting in new evolutionary lineages (McLean 2002).

Freshwater bacterial communities have been shown to be characterized by the following classes: Proteobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Verrucomicrobia, and Planctomycetes (Glockner et al. 2000, Zwart et al. 2002). Figure 1.6 depicts a phylogenetic tree based on bacterial small subunit (SSU) rRNA sequences from freshwater environments.

Microbial ecologists have only recently begun exploring linkages between microbial community structure and microbial community functioning in a variety of aquatic and aquatic-terrestrial interface environments, such as in soils (Dunbar et al. 2002, Nemergut et al. 2005, Oline 2006), marine (Cottrell and Kirchman 2000, Fuhrman 2002, Kirchman et al. 2007), glaciers (Skidmore et al. 2005), lakes (Zwart et al. 2002, Langenheder et al. 2005, Lindstrom et al. 2006), wetlands (Gutnecht et al. 2006) and rivers and streams (Crump and Hobbie 2005, Hullar et al. 2006, Fierer et al. 2007, Anderson-Glenna 2008). Although the importance of bacterioplankton and sediment

bacteria in biogeochemical cycles of freshwater ecosystems is well known, our current knowledge on the functioning and phylogeny of stream microbial communities remains unclear, especially for streams at high altitudes and latitudes in which the ecological importance of cold ecosystems under a changing climate is evident.

1.5 The Concept of Microbial Biogeography

Several studies support the conclusion that microorganisms are distributed ubiquitously in the biosphere, because they are abundant and easily transported by wind or water, suggesting that community composition within a habitat may be determined by local environmental conditions (Finlay 2002). This statement refers to one of the oldest concepts in microbiology that "everything is everywhere, but the environment selects" (Baas-Becking 1934). The concept reflects that microbes are so small that their ecological niche is defined below a millimeter scale and as a result suitable habitats are widespread.

However, recent studies dispute the idea that 'everything is everywhere' and a growing body of evidence shows that microorganisms vary in abundance, distribution and diversity, over taxonomic and spatial scales (Whitfield 2005, Hughes-Martiny et al. 2006, Anderson-Glenna et al. 2008).

1.5.1 Microbial Community Composition and Function: The Environment Selects

Surveys of bacterial diversity in lakes and streams have identified many populations common to freshwater systems worldwide, but the processes by which these populations interact with their environments are poorly understood (Crump et al. 2003).

Many variables that directly influence stream microbiota have not been identified and the mechanisms of microbial community response to environmental controls are not clear.

Findlay (2003) showed changes in ecosystem functioning resulting from changes in the genetic structure in microbial communities. Similarly, Franklin (2001) used batch culture experiments to assess microbial community structure and functional potential and found that structural differences between communities maintained in the same environment can exhibit differences in community organization and function.

Findlay (2003) showed that biofilm communities responded to nitrogen additions by changes in function with no apparent change in community composition. Thus functional similarity under similar environmental conditions may not be a result of similar bacterial community composition. Likewise, Langenheder (2005) found that communities of varying composition existed under similar conditions and noted that bacterial communities are comprised of "populations of generalists that can grow under most conditions as well as populations with the life strategy of specialists". Furthermore, Langenheder (2006) observed that differently composed communities were different with regard to specific enzyme activities, but maintained similar broad-scale functions, such as biomass production and respiration.

1.5.2 Microbial Community Composition and Function: Geographical Patterns

Recent research suggests that at least some bacterial taxa can exhibit geographical patterns. The strongest evidence to date for biogeographical patterns in prokaryotic organisms comes from a study by Whitaker (2003) in which a survey of the genetic diversity of hotspring archaen *Sulfolobus* isolates from five geographically distinct

regions was found to have significant correlation between genetic distance and geographical distance.

At the stream reach scale along a single riffle, Franken (2001) noted that there is a typical flow pattern in which surface water enters the hyporheic zone in a downwelling zone at the head of the riffle and hyporheic water returns to the stream surface in an upwelling zone at the tail of the riffle. Boulton (1998) describes the system as upwelling subsurface water supplying stream organisms with nutrients while downwelling stream water provides dissolved oxygen and organic matter to microbes and invertebrates in the hyporheic zone. Findlay and Sobczak (2000) noted that bacterial abundance in the hyporheic zone was greater in shallow hyporheic sediments than in deeper sediments and greater in sediments of downwelling zones than upwelling zones. These observed microbial community dynamics in shallow hyporheic sediments may influence the composition of microbial communities in the stream channel at the habitat scale.

The studies reviewed above have attempted to differentiate the effects of geographic distance versus environment on microbial community composition. There is a clear indication that microbial assemblages can exhibit both environmental isolation and biogeographical provincialism at a range of scales, which in some cases 'the environment selects', but it is not always the case that 'everything is everywhere'.

1.6 Methods of Microbial Community Characterization

Despite the importance of bacteria and their role in biogeochemical cycling, their taxonomy is poorly understood. Conventional methods for taxonomic classification of

bacteria are contingent upon culture-based methods of bacterial strains. However, less than 10% (and frequently less than 1%) of environmental bacteria are culturable (Handelsman 2004). Due to the fact that traditional methods for the identification of bacteria rely on pure cultures, the majority of environmental bacteria are unidentifiable using conventional methods (Pusch et al. 1998).

The development of DNA based techniques has provided new methods for the identification and quantification of environmental microorganisms (Saylor and Layton 1990). There are numerous methodologies currently available to characterize microbial consortia, which are broadly classified as nucleic acid-based, biochemical-based, and microbiological-based methods. Nucleic acid-based techniques are the optimal approach for providing definitive information on naturally occurring microbial communities (Spiegelman et al. 2005). Nucleic acids-based studies have differentiated novel types of rRNA sequences in the environment that diverge more deeply in phylogenetic trees than those of cultivated organisms, suggesting that the divergent organisms recognized by rRNA sequence are potentially more different from known organisms in the lineage than the known organisms are from one another (Pace 1997).

Method development in the field of microbial ecology has made leaps and bounds since the 1960s when the most advanced studies focused on pure cultures but could not provide insight into interactions among microorganisms and between microorganisms and their natural environment. It was not until the 1980s when researchers first considered factors such as density, diversity and function of overall microbial populations in their natural environment, rather than just a single cultured organism. Thomas D.

Brock theorized and clarified that the characteristics of an organism cultivated in a laboratory setting may not reflect its true physiology or activity in their natural environment where resource competition, predation and other dynamic environmental variables persist (Brock et al. 1994). Meanwhile, Pace (1985) found that microbial diversity could be studied using molecular applications without traditional cultivation, but by retrieving macromolecules (DNA, RNA, proteins) of different organisms in an environmental sample.

Many of these culture-independent techniques are possible because of the advent of the polymerase chain reaction (PCR), a method that detects microorganisms in a sample based on a target sequence in their rRNA genes, by amplifying the rRNA signal relative to the noise of other genes present in each organism's DNA. Amplification of the rRNA genes is valuable because these genes are ubiquitous and highly conserved in all cell-based organisms.

Ideally, a comprehensive study on the microbial communities of a stream ecosystem requires the following three approaches as suggested by (Pusch et al. 1998):

1) overall analysis of the community giving a general overview of the diversity and identification of dominant species,
2) identification of strains and physiological work on relevant species
3) the single-cell approach (fluorescent antibodies or molecular probes) to study the functional niches of a specific species or a functional group situated in the habitat.

This study employed the first approach mentioned above to establish "who is there?" in the environment with the caveat that the next logical study would attempt to

answer "what are they [the microbes] doing?" in their particular environment. In this study two methods were used – terminal restriction length polymorphism and 16S rRNA gene cloning and sequencing – to assess the genetic diversity and to infer the identity of dominant species of bacteria across the arctic streams studied.

1.6.1 Microbial Community Fingerprinting: Terminal-Restriction Fragment Length Polymorphism (T-RFLP)

T-RFLP is a semi-quantitative, culture-independent approach developed for rapid analysis of microbial diversity from various environments (Liu et al. 1997). T-RFLP is one of many molecular fingerprinting techniques that separate PCR products of 16S rRNA genes by nucleotide base pair size. Bacterial DNA is amplified from the collective, extracted DNA using "universal" primers based on the conserved sequences in 16S rRNA. The PCR pool is digested with restriction enzymes that make cuts in the DNA at specific nucleotide sites. Because each digestion could potentially yield multiple fragments per amplicon, generating complex patterns that are too difficult to resolve, the refined T-RFLP approach was developed. T-RFLP uses fluorescently-labeled primers (one of the two primers used in the PCR amplification has a fluorophore) so that only one terminal-restriction fragment (T-RF) is visualized, resulting in a simplified restriction pattern for analysis (Avaniss-Aghajani et al. 1994). The T-RFs are then separated according to length by agarose gel electrophoresis. The gel banding patterns that result based on the positions of individual restriction sites varies between the different "ribotypes" detected. The T-RFLP method assesses microbial communities and gives a qualitative estimation based on the number of unique PCR fragments and relative

frequencies of the various polymorphisms, or ribotypes, present in a given sample (Liu et al. 1997). See Figure 1.7 for a schematic of T-RFLP analysis.

Advantages to T-RFLP include high-resolution, accurate sizing and fluorescence detection of the individual fragments by the use of internal size standards, and high sample throughput due to the use of automated fragment analyzer instrumentation. Many studies have successfully applied T-RFLP in comparing the similarity of microbial communities from different ecosystem localities such as soils (Dunbar et al. 1999, Dunbar et al. 2000, Dunbar et al. 2002, Blackwood et al. 2003), glaciers (Bhatia et al. 2006), and lakes (Langenheder and Ragnarsson 2007), as well as detecting seasonal shifts in stream microbial community composition in response to environmental factors (Hullar et al. 2006) and landscape-scale biogeography (Fierer et al. 2007).

Limitations of this method are that sequencing of the T-RFs is not possible and phylogenetic information can only be inferred indirectly by comparison of T-RF lengths of 16S rRNA databases of theoretical restriction enzymatic sites, resulting in a probable T-RF length (Kent et al. 2003). However, T-RFs of the same size can be generated from microbial taxa that are distantly related, resulting in an underestimation of diversity (Blackwood et al. 2007). There is considerable debate in the literature as to whether T-RFLP analysis qualitatively reflects minimum differences in community composition or can be used to quantitatively describe community diversity (Blackwood et al. 2007, Fierer et al. 2007). The current consensus is that T-RFLP is most appropriately considered as an assessment of diversity on a very coarse scale, not at the species scale of taxonomic resolution.

1.6.2 16S Ribosomal RNA (rRNA) Gene Cloning and Sequencing

Clone libraries are developed from sequences of phylogenetic genes from environmental nucleic acids. The most common is the small subunit (SSU) ribosomal 16S rRNA, an approach proposed by Carl Woese in the 1970s. The power of this approach is due to particular characteristics of the 16S rRNA gene: its ubiquity in prokaryotes, its three-dimensional structure is highly conserved among members with close phylogenetic differences, it contains both conserved and variable regions, and is resistant to lateral gene transfer.

The basics of building a 16S rRNA clone library from an environmental sample are as follows (see Figure 1.8): (1) extract DNA contained in the sample, (2) amplify the bacterial DNA from the collective DNA using "universal" primers based on the conserved sequences in 16S rRNA, (3) insert or "ligate" the PCR products (16S rRNA genes from various bacterial organisms present in the sample) into plasmids (small circular double-stranded DNA from natural bacteria) that act as vectors to form new recombinants with the foreign DNA that are then (4) introduced or "transformed" into bacterial cells that can produce many copies of the inserted DNA, which are (5) grown on plates for the isolation of individual environmental clones that can be prepared for sequence analysis.

Databases such as GenBank "BLAST" (Altschul et al. 1990) can be used to compare the obtained 16S rRNA sequences with other known sequences to identify microorganisms with the closest match. This information can also be used to estimate

genetic distances between sequences and used to reconstruct phylogenetic trees that suggest evolutionary relationships.

The advantage of sequence-based techniques over fingerprinting methods is that clone libraries provide information for both the phylogenetic identity and to some extent, the relative abundance of community OTUs (operational taxonomic units) – a term used to describe the diversity, or species richness, of a sample (Stackebrandt 2006). This information can be stored and compared to sequences obtained from other studies. As long as sequences from multiple samples from one locality are retrieved, there is a higher probability of discovering novel phylogenetic groups. Clone libraries also allow for a greater sampling coverage of phylotype diversity over T-RFLP (see Figure 1.9).

Fingerprinting and sequence-based methods are both subject to all of the biases of PCR in that the primers preferentially bind to the dominant template in a sample, thus masking minor populations and rare organisms. Moreover, another major limitation of clone library characterization is that the high bacterial diversity in most environments, especially soils and sediments, is not a true reflection of the true microbial community present in the environment. Unlike fingerprinting methods (T-RFLP), sequence-based analyses are laborious and expensive which makes it difficult to analyze multiple samples with replication. However, recent technologies and private enterprises are facilitating the gathering of DNA data.

1.7 Linkages between Biogeochemistry and Microbial Community Composition

There is little understanding of microbial communities and their relationships with other organisms or their fluctuations with respect to environmental conditions and seasons. Algae and bacteria are known to form symbiotic communities within biofilms, which provide an ecologically important source or organic nutrients for the growth of heterotrophic bacteria within aquatic environments (McFeters et al. 1978).

Various biotic and abiotic factors likely influence microbial community composition in aquatic ecosystems, some of which are physical variables (i.e. temperature variations, climate, topography, and light availability) and biogeochemical variables (i.e. underlying and surrounding lithology, terrestrial vegetation and solute composition including carbon sources, inorganic nutrients, and electron acceptors).

Microbes are capable of metabolizing via various metabolic pathways, each consisting of a different combination of redox reactions, and each producing a different net gain in energy (Figure 1.10). Microbes fill niches covering all possible scenarios of the availability of electron acceptors. Depending on the redox potential of a particular environment there are specific microbes that carry out redox reactions specific for differing geochemical environments (e.g oxic, anaerobic, sulfidic). As the environmental conditions of an ecosystem become anoxic, microorganisms adapt and perform the appropriate redox reactions as their metabolic pathway. The microbial and the geochemical environments in aquatic ecosystems exert feedback on each other through microbial metabolism and numerous naturogenic and anthropogenic environmental processes (e.g. mineral dissolution and nutrient cycling). All the constituents, reducers

and oxidizers, are not only critical to microbial viability, activity and growth, but they are also critical components to geochemical cycling in aquatic ecosystems.

The availability of inorganic nutrients and the redox speciation in freshwater ecosystems affects bacterial abundance and activity. For example, dissolved inorganic phosphorous is a common limiting factor of bacterial growth. Furthermore, the presence of energetically favorable terminal electron acceptors can exert strong control on the ability of bacteria to metabolize organic matter at redox surfaces in sediments, rocks and soils (Hedin et al. 1998, Kainanen et al. 2002).

Few researchers have investigated the effect of lithogical and biogeochemical variations on microbial community structure. Takai (2003) found that archaeal organisms present in subsurface Cretaceous rock shifted over small scales to changes in the lithology and geochemical gradients. Skidmore (2005) found that microbial community composition was correlated with chemical weathering products that were in turn controlled by bedrock mineralogy of two geographically distant glaciers.

Resolving the interactions between microbial communities and their geochemical environment remains a challenge for microbial ecologists and biogeochemists. A circular cause and consequence question persists: Does microbial community composition drive biogeochemical processes or does biogeochemistry control the composition of particular microbial assemblages?

1.8 Microbial Communities of Arctic Aquatic Ecosystems

The literature on microbial communities in arctic freshwater ecosystems is limited. Many studies of microbial community structure and functioning have been conducted in the sub-arctic region of Sweden primarily restricted to lakes (Lindstrom 2001, Lindstrom and Bergstrom 2004, Langenheder et al. 2006) or arctic Norway in wetlands (Hoj et al. 2005). Microbial research in arctic Alaska includes bacterial studies in Toolik Lake on the North Slope of Alaska, examined by Bahr (1996) and Crump (2003), surrounding lakes and inlet and outlet streams near Toolik Lake (Crump et al. 2007) as well as archaea and bacteria studies in a river-influenced coastal arctic ecosystem in the Beaufort Sea (Galand et al. 2006, Garneau et al. 2006) and the Chukchi Sea and the Canada Basin (Kirchman et al. 2007) of the western Arctic Ocean.

Bahr (1996) cultured a variety of phyla potentially capable of metabolizing a wide range of compounds, suggesting that extreme arctic conditions do not prevent adaptation by species more commonly found in temperate freshwater environments. Crump (2003) hypothesized that shifts in planktonic bacterial community composition would occur due to major seasonal changes in the source and quality of dissolved organic matter. They observed that community composition shifts resulted from both changes in the relative abundance of autochthonous bacteria (native to the system) as well as advection of allochthonous bacteria (from outside the system) via the Toolik Lake inlet stream during the spring thaw. This study poses a localized microbial biogeographical question regarding how inlet streams affect lake bacterial community compositions and consequently, how lake communities influence outlet stream communities.

There have been few microbial sediment and rock biofilm investigations in stream ecosystems (Battin 2000, Hullar et al. 2006, Anderson-Glenna et al. 2008) and no epilithic examinations in Alaskan stream ecosystems. One reason for the paucity of arctic biofilm studies may be due to the difficulty in attaining ample microbial biomass material for genetic DNA amplification from these ultraoligotrophic systems.

1.9 Research Goals

The study described in Chapter 2 of this thesis was carried out within the context of a larger project supported by the Arctic Network (ARCN) of the National Park Service Inventory and Monitoring Program. The goals of ARCN were to establish a baseline for the physical, biological, and chemical characteristics of stream ecosystems in their parklands and to monitor specific metrics pertaining to ecosystem function to assess spatial and temporal variability at the landscape scale and to predict responses to environmental change. The goal of my research was to identify patterns in stream microbial community composition at the landscape and habitat scale and to correlate patterns with stream biogeochemistry. This study contributes to the overarching goals of ARCN by providing insight into patterns of microbial taxonomic structure in arctic streams, in turn, making progress towards understanding the roles of microorganisms and their biogeochemical environment within local and global ecosystems.

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Figure 1.1. The 32 National Park Service (NPS) Inventory and Monitoring (I&M) Networks (taken from nps.gov).

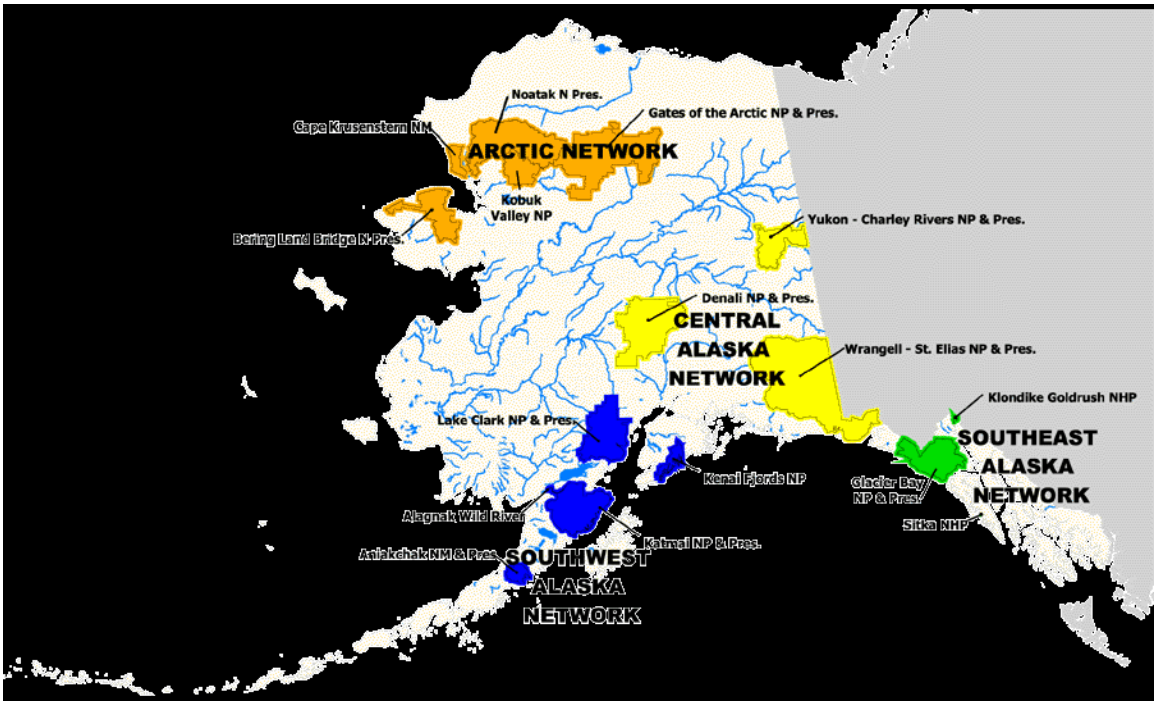

Figure 1.2. The four NPS Inventory and Monitoring Networks in Alaska: Southeast Alaska, Southwest Alaska, Central Alaska and the Arctic (taken from nps.gov).

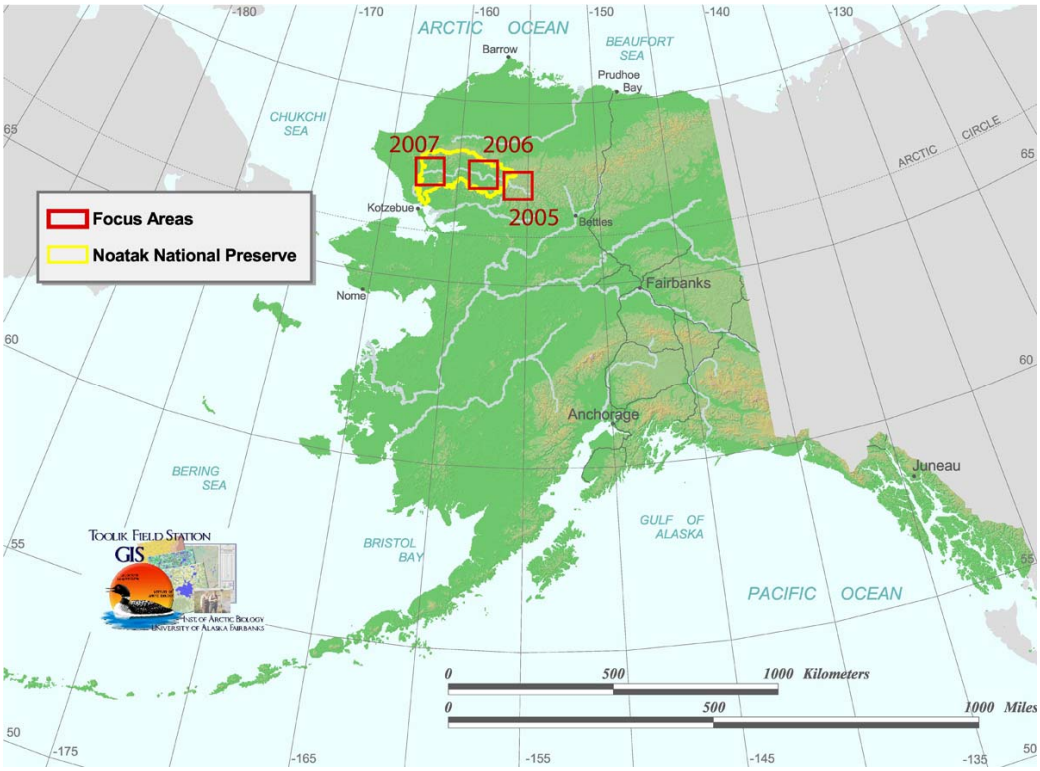

Figure 1.3. The Noatak National Preserve resides in the NPS Arctic Network, Alaska (taken from A. Balser).

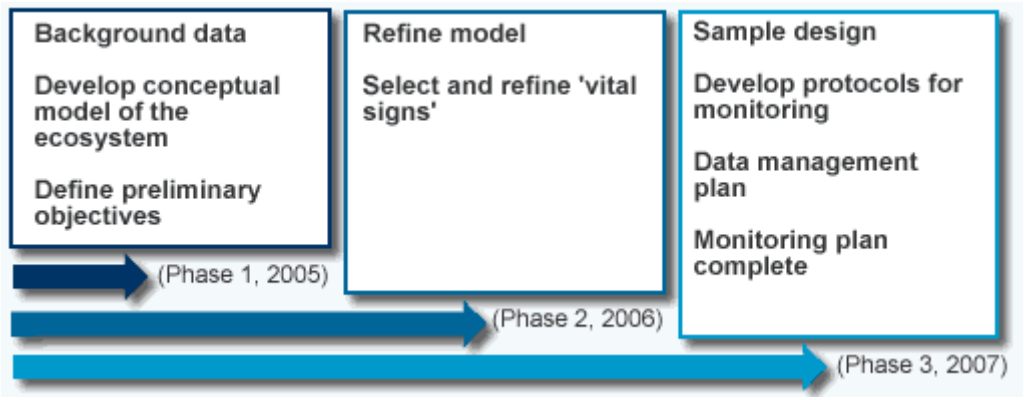

Figure 1.4. Conceptual diagram of the NPS three-phase approach to establish individual network I&M Programs (nps.gov).

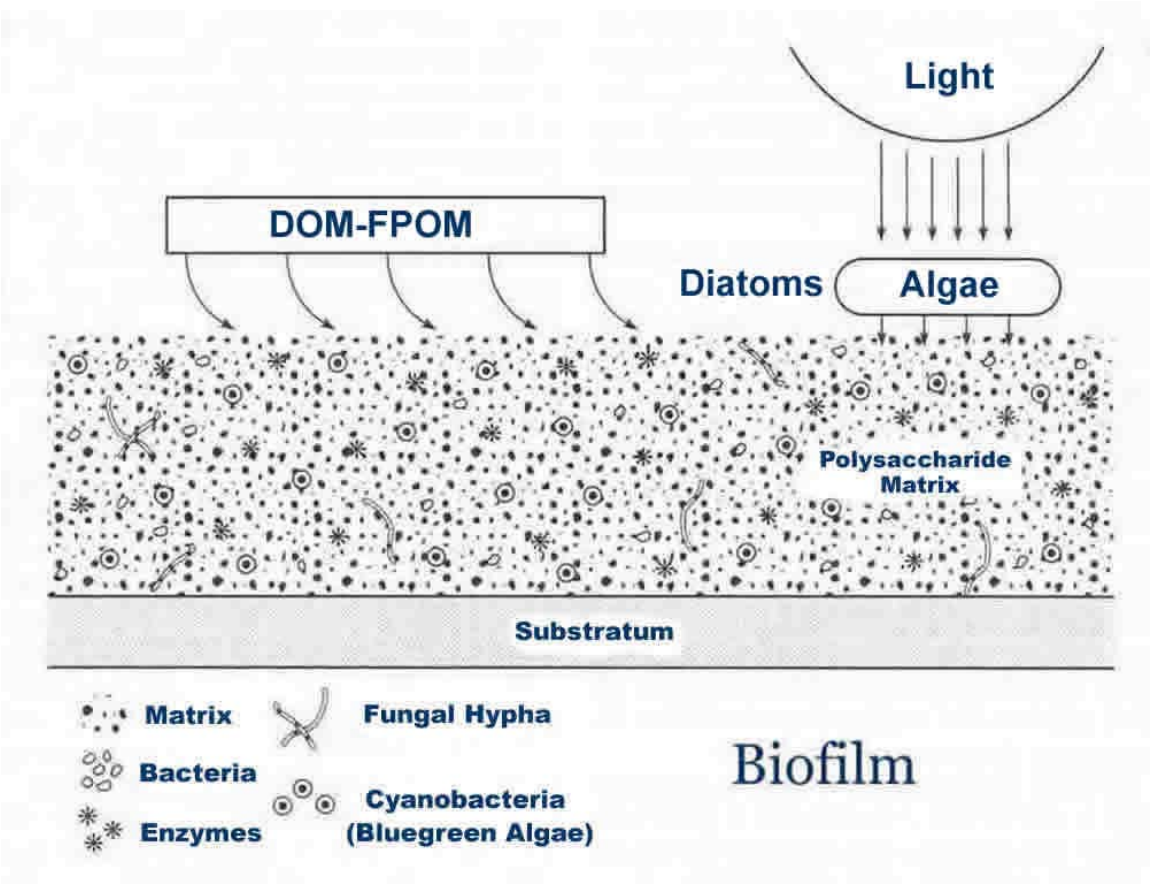

Figure 1.5. A cross-sectional diagram of a microbial biofilm (taken from nps.gov).

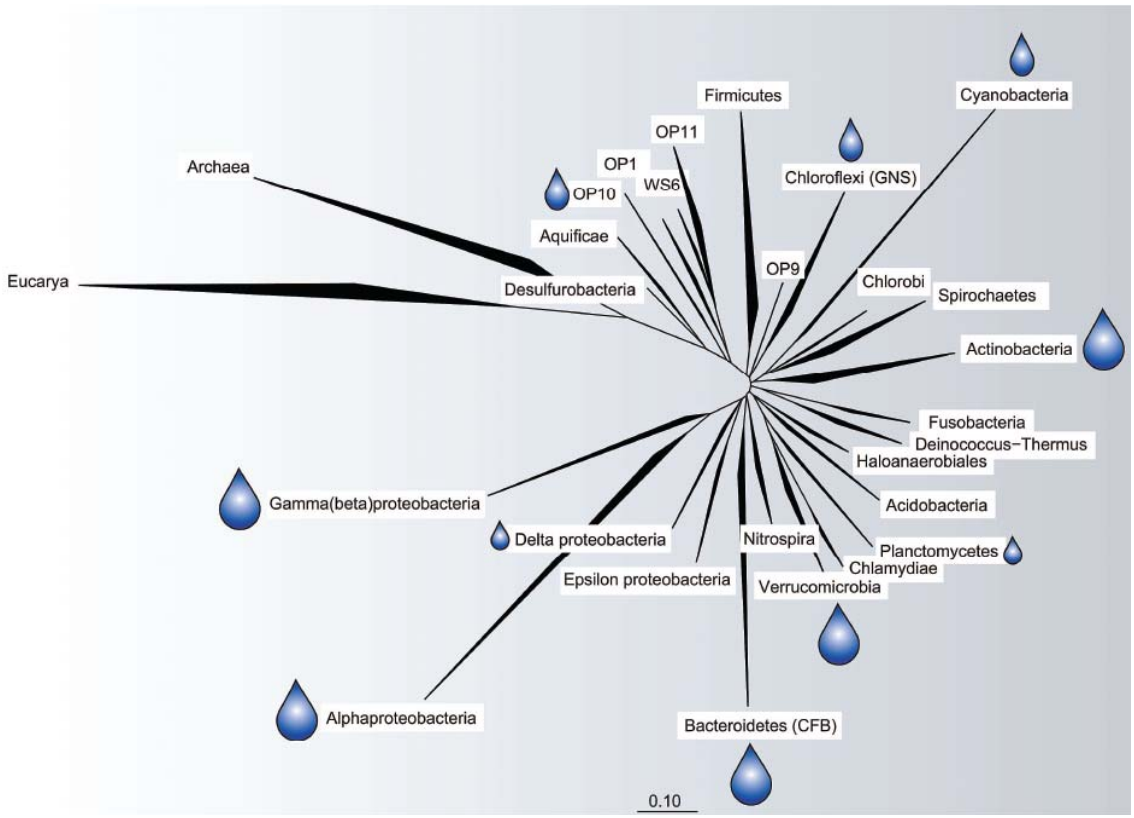

Figure 1.6. Phylogenetic tree based on bacterial small subunit (SSU) rRNA sequences. Large drops indicate typical and dominant groups of freshwater bacteria and small drops represent other groups observed in, but not unique to freshwater (taken from Logue *et al.* 2008).

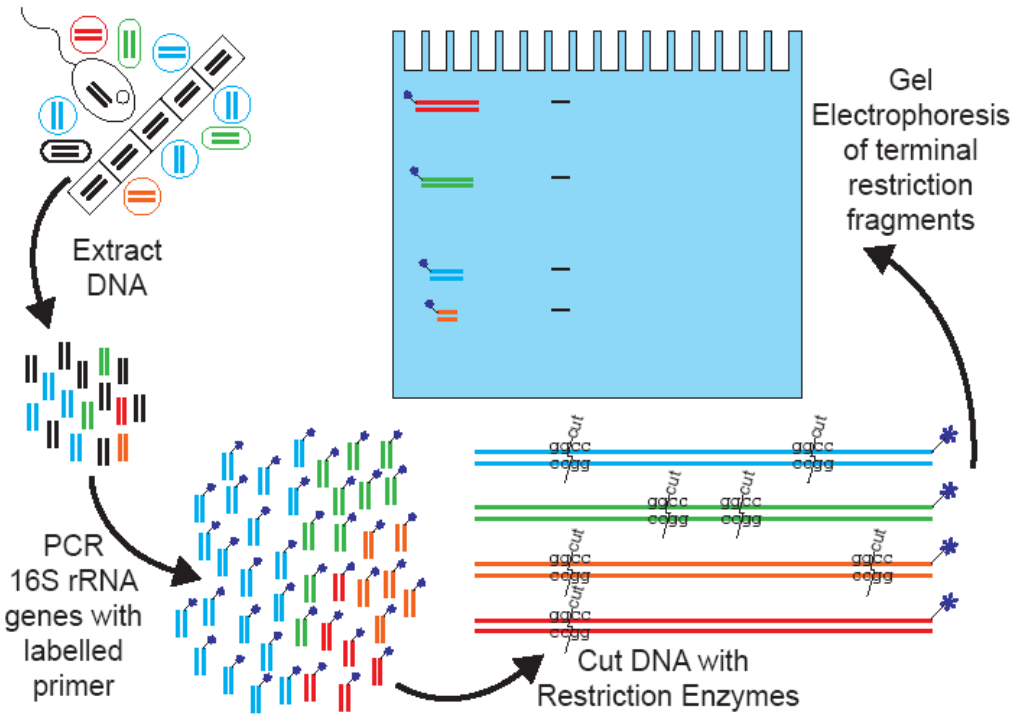

Figure 1.7. Schematic of the terminal restriction fragment length polymorphism (T-RFLP) methodology (taken from Crump, 2005).

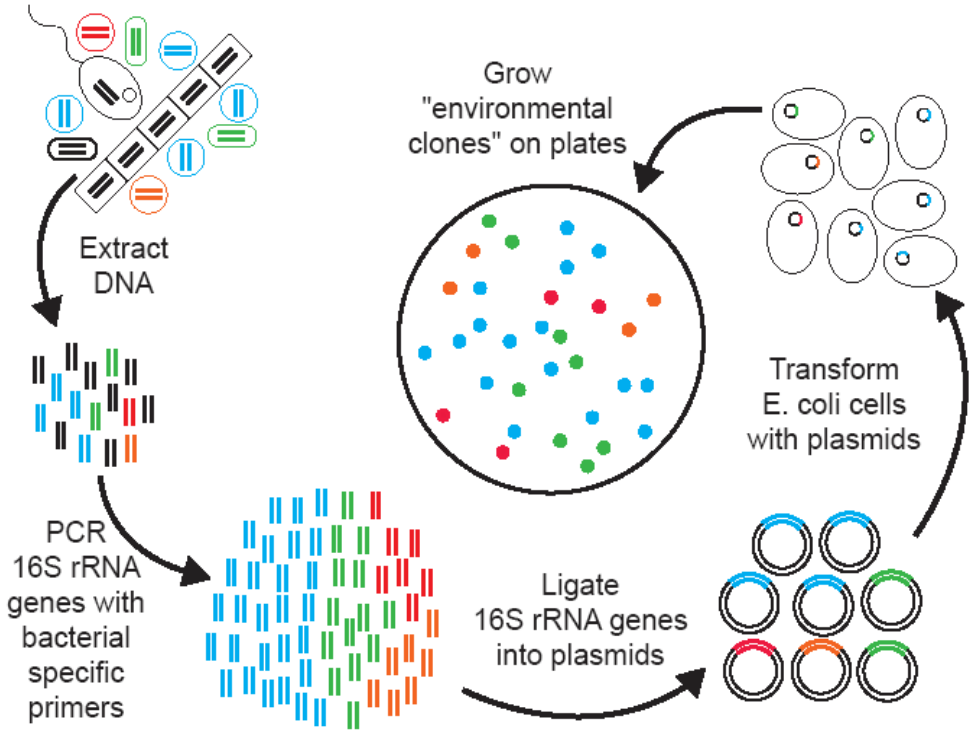

Figure 1.8. Schematic of the 16S rRNA gene cloning from environmental samples (taken from Crump, 2005).

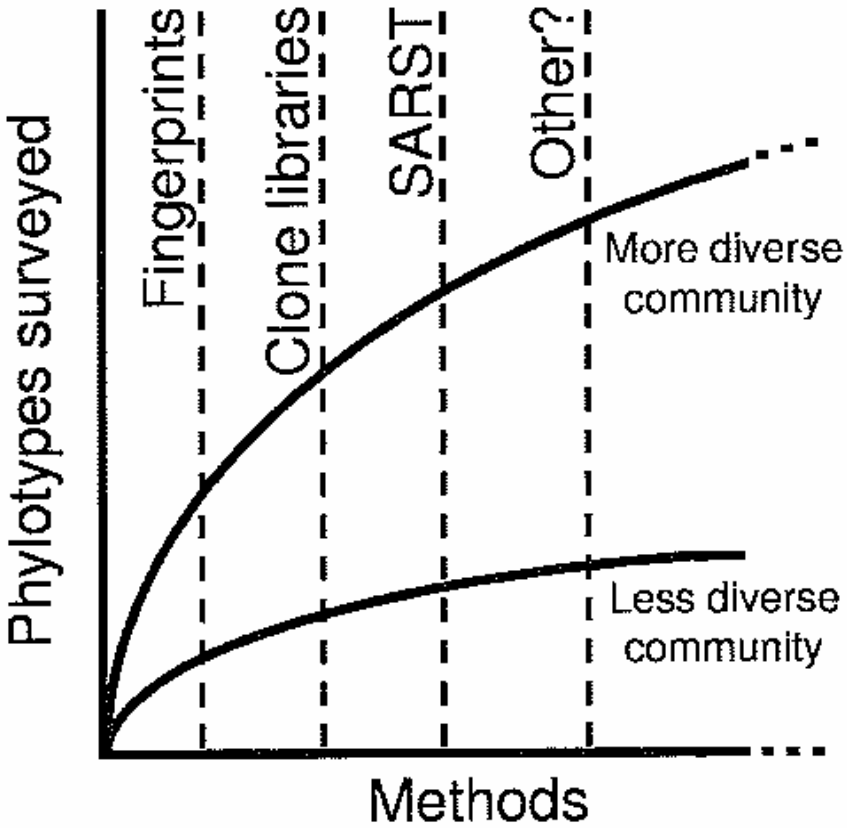

Figure 1.9. Relationship between methodologies (dotted lines) and phylogenetic diversity coverage. Rarefaction curves show theoretical depths of coverage (phylotypes of OTUs observed) for two microbial communities, indicating that the coverage achieved differs by methodology depending on the overall phylogenetic diversity (Stackebrandt 2006). The two methods employed in this study are community fingerprinting and clone libraries.

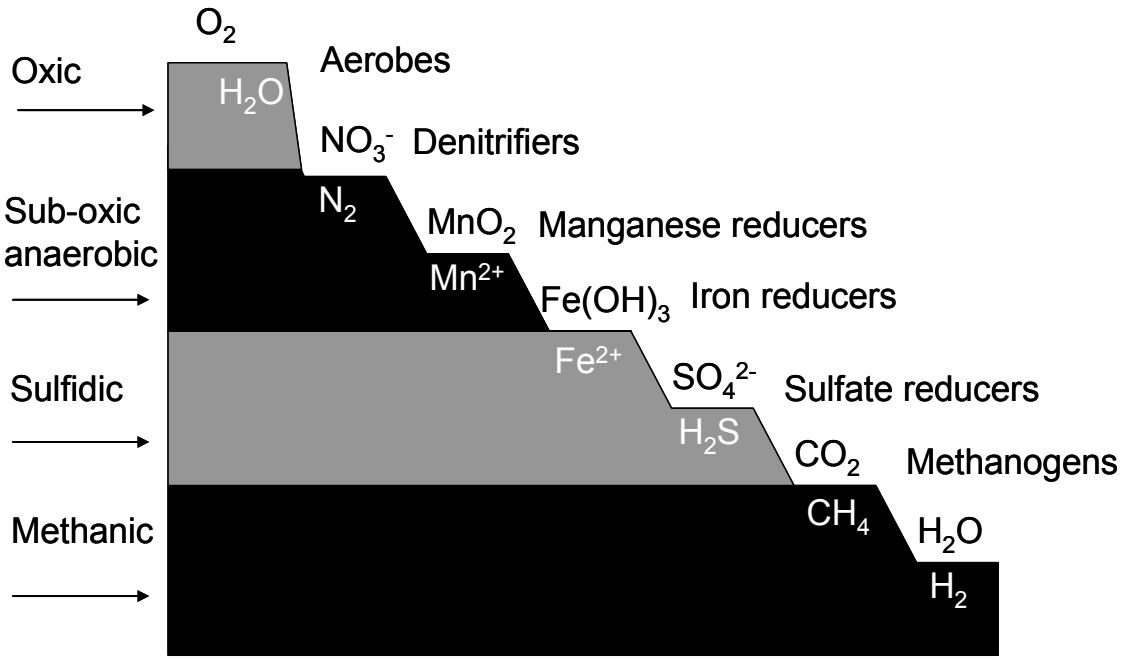

Figure 1.10. The redox ladder showing environmental niches for microbial metabolism (taken from Drever 2002).

CHAPTER 2: MICROBIAL BIOGEOGRAPHY OF ARCTIC STREAMS: EXPLORING INFLUENCES OF LITHOLOGY AND HABITAT

ABSTRACT

Microbes are of critical importance but are a poorly understood component of arctic stream ecosystems. They are responsible for recycling organic matter and regenerating nutrients that are essential to the food webs of aquatic ecosystems. In this work we test the hypothesis that differences in highly contrasting lithologies (non-carbonate and ultramafic) and stream habitat (sediments and rocks) influence the structure of bacterial biofilm communities in arctic streams and whether these patterns can be correlated with stream biogeochemistry. A microbial community fingerprinting method (T-RFLP) and 16S rRNA gene sequencing were used to explore the genetic diversity of microbial communities in biofilms on sediments (epipssamon) and rocks (epilithon) in stream reaches that drain watersheds with contrasting lithologies in the Noatak National Preserve, Alaska. We observed different patterns in bacterial community composition at both the macro-scale (lithology) and micro-scale (stream habitat). Non-metric multidimensional scaling ordination of T-RFLP peaks showed significant separation between the two contrasting lithologies as well as the two habitats. Positive correlations were detected between particular biogeochemical variables (e.g. nutrients, base cations, and dissolved organic carbon) and bacterial community structure unique to each lithology. Although clone libraries indicated high bacterial OTU diversity within and across stream sites, biogeographical patterns were observed that depended on locality type. Our results show relationships at macro- and micro-scales at the landscape level and in ecological niches within a single stream.

2.1 Introduction

The interpretation of results from microbial biogeographical studies in natural environments has become increasingly controversial in recent years (Hughes-Martiny et al. 2006). Typing microbial communities has been acknowledged as an important, yet neglected area of ecological research, especially in reference to the interaction between bacteria, the characteristics of their ecosystems, and their response to environmental changes (Green et al. 2008). The historical view of microbial biogeography as formalized by Baas-Becking (1934), states that "everything is everywhere, but the environment selects". Or as otherwise stated, global microbial diversity is low, consisting of a relatively small number of cosmopolitan species with high dispersal capabilities, and relatively high local diversity shaped by contemporary environmental conditions that act as filters to maintain distinctive microbial assemblages. The pattern emerging from recent molecular studies indicate that biogeographical patterns in microbial distribution and diversity (Fierer et al. 2007, Gray et al. 2007) correlate with taxonomic identity of bacterial taxa and environmental variables (Hughes-Martiny et al. 2006, Fierer et al. 2007), as well as classic patterns of taxa-area relationships (Horner-Devine et al. 2004).

Knowledge of microbial community composition in freshwater and in particular arctic stream ecosystems, is limited compared to what is known about microbial structure and function in terrestrial or marine environments (Logue et al. 2008). The importance of sessile bacteria in aquatic environments has been recognized; an example is the early biofilm research that focused on alpine streams (Geesey et al. 1978, McFeters et al.

1978). Despite the likely importance of their role in arctic stream ecosystems, basic characteristics of the microbial community in arctic streams remain poorly understood.

The few studies that have sought to characterize microbial communities from aquatic ecosystems in arctic Alaska have focused on environments such as lakes (Crump et al. 2003, Crump et al. 2007), sub-arctic sub-glacial streams (Skidmore et al. 2005) and the coastal Arctic Ocean (Galand et al. 2006, Garneau et al. 2006). However, the current understanding of the taxonomic relationships and genetic diversity of microbial communities in arctic Alaska environments and in particular stream ecosystems remains unclear. If microbial communities and their biogeochemical environment influence each other, it is necessary to clearly understand this interaction especially in the arctic, an environment currently responding to a rapidly changing climate.

Microbial activity in stream ecosystems is primarily influenced by the degree of chemical and biological interactions between the terrestrial and aquatic interface (Stanford and Ward 1993, Ward and Stanford 1995, Palmer et al. 2000). Various biotic and abiotic factors likely influence microbial community composition in aquatic ecosystems, including physical variables (i.e. temperature variations, climate, topography, and light availability) (Autio 1998, Kaplan 1989) and biogeochemical variables (i.e. underlying and surrounding lithology, terrestrial vegetation and solute composition including the quality and quantity of carbon sources, inorganic nutrients, and electron acceptors) (Drever 2002, Crump et al. 2003, Eiler et al. 2003).

Few studies have investigated the effect of lithogical and biogeochemical differences on microbial community structure (Takai et al. 2003, Skidmore et al. 2005,

Oline 2006) or the effect of habitat differences within a stream that may vary in terms of organic matter availability and exposure to hydrologic stressors (Hullar et al. 2006). Takai (2003) found that major archaeal organisms present in the groundwater and substratum associated with subsurface Cretaceous rock shifted over small scales (tens of centimeters) to changes in the lithology and geochemical gradients. Skidmore (2005) suggests a reasonable assumption correlating microbial community composition with chemical weathering products controlled by bedrock mineralogy of two geographically distant glaciers. The work of Hullar (2006) suggests the presence of stable seasonal oscillations in bacterial community structure of stream habitats, sediment and epilithon. Hullar's work also determined that these two habitats were composed of both terrestrial and aquatic derived microorganisms, suggesting a close association between headwater streams and their watersheds.

The Noatak River is in the Noatak National Preserve in Alaska (USA). It is the longest continuous river in the U.S. National Wild and Scenic system and the largest mountain-ringed river basin, virtually unaffected by humans (Milner et al. 2005). Jorgenson et al. (2002) studied the relationship between lithology and vegetation composition in the Noatak Basin. The lithology of this area is complex but includes three important and strongly contrasting types that were the focus of this study. Ultramafic rocks (basalt, gabbro, peridotite, pyroxenite, dunite) of the Siniktanneyak mountains tend to be high in iron and magnesium with sparse vegetation. Non-carbonate rocks (glaciolacustrine deposits, conglomerate, sandstone, shales) of the Avingyak Hills support acidic, organic-rich soils and host shrub birch, willow and ericaceous plants.

Complex sedimentary rocks (shale, basalt, limestone and mafic rocks) of the Aniuk mountains support vegetation similar to the non-carbonate lithology (Jorgenson et al. 2002). Conclusions by Jorgenson (2002) stated that vegetation composition differs with lithology type and that the relationship is a consequence of variations in soil pH and possible phytotoxic effects of soluble minerals

We hypothesized that these fundamentally different lithologies not only influence terrestrial productivity but also impart different biogeochemical characteristics to water, which in turn influences the structure and function of the biological communities in stream ecosystems, in particular, the microbial community. Our objectives were to determine: (1) whether stream bacterial community composition differs among streams selected from the three contrasting lithologies, (2) whether these differences are influenced by the biogeochemical characteristics of the host stream ecosystem, and (3) whether microbial community composition differs by stream habitat (sediment vs. epilithon) within each individual stream locality.

We sampled two key habitats, sediment and epilithon, in streams embedded in the three aforementioned lithologies. Two of which consist of starkly contrasting landscapes, the ultramafic (UM) and non-carbonate (NC), as well as an intermediate bedrock, the complex sedimentary (CS). Our conclusions are based on T-RFLP and sequencing of 16S rRNA bacterial clones. Using the former, differences in bacterial communities were detected between different lithologies and stream habitat. While the latter showed differences at the habitat level.

2.2 Material and Methods

2.2.1 Study Area

Samples in this study were collected from headwater stream tributaries arising from uniform and contrasting lithologies within the Noatak National Preserve. We sampled 30 streams located in the Noatak River basin (Figure 2.1) in the vicinity of Feniak Lake (68º14'56.55" N and 158º19'19.90" W, elevation 1411 feet). Fourteen of these streams were used in the microbial community analyses described in this paper.

2.2.2 Sampling Design

A suite of first and second-order streams were sampled within each separate lithology: three in the non-carbonate, five in the ultramafic and six in the complex sedimentary. Sediment and rock samples were collected at each location. The former were obtained in triplicate along a 25-meter reach while the latter were a composite of six rock scrubs from each location (see sample collection details below).

2.2.3 Sample Collection of Benthic Microbial Communities

Sediment samples were collected in sterile 15-ml plastic tubes from the surface to a depth of approximately 3 to 5 cm. Samples were preserved immediately by adding a sucrose lysis buffer (SLB: 20 mM EDTA, 400 mM NaCl, 0.7 M sucrose, 50 mM Tris pH 9.0) in a 1:1 ratio and then frozen on dry ice while in the field and later transferred to a -80ºC freezer for long-term storage.

Biofilm material from the tops of six submerged rocks was obtained from riffle sections of each streambed sampled. Rocks were scrubbed with a nylon brush and the liberated biofilm was collected by squirting filtered (0.22μ) stream water over the rock

surfaces into a sterile plastic container. Water and biofilm material was transferred into a sterile syringe and filtered through a filter capsule with a 0.22μ membrane to trap most of the bacteria. Filter capsules were removed from syringes and 1 ml of DNA extraction buffer (100 mM Tris (pH 8), 100 mM NaEDTA (pH 8), 100mM phosphate buffer (pH 8), 1.5 M NaCl, 1% CTAB) was injected into the filter capsule using a sterile syringe. Filter capsules were kept on dry ice in the field until long-term storage at -80ºC.

2.2.4 DNA Extraction

DNA extractions were conducted using the MoBio Power Soil DNA extraction kit (MoBio Laboratories, Inc. Carlsbad, CA) following the manufacturer's protocol with the following modification: a FastPrep Homogenizer and Isolation System (Thermo Fisher Scientific Waltham, MA) was used to shake tubes at 4.5m/sec for 30 seconds to ensure complete cell lysis of bacteria in sediment and rock biofilm samples. Using sterile conditions, 500-μl subsamples of streambed sediment (1 : 1 sediment : SLB slurry) were placed in the DNA extraction tubes provided with the MoBio kit. Similarly, filters of the rock biofilm samples were removed from their capsules and transferred to DNA extraction tubes. DNA was extracted immediately prior to downstream applications to avoid degradation and bulk DNA was stored at 4ºC.

2.2.5 T-RFLP Profiles

Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis was conducted on all triplicate sediment samples (42 total) and epilithon samples (8 total). The 16S rRNA gene was amplified from both sediment and rock biofilm samples for T-RFLP analysis via polymerase chain reaction (PCR) using the following primers: Bac8f

(5'AGAGTTTGATCCTGGCTCAG, HEX labeled) (Reysenbach et al. 1994) and unlabeled reverse primer, Univ1492r (5'-GGTTACCTTTGTTACGACTT) (Edwards et al. 1989). Forward and reverse primers were obtained from Sigma-Genosys (St. Louis, MO) and Invitrogen (Carlsbad, CA), respectively. PCR reactions were run using Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Life Sciences, Piscataway, NJ). The PCR reaction protocol was as follows: initial denaturation at 94ºC for 4 minutes, followed by 40 cycles of 94ºC for 45 seconds, 54ºC for 20 seconds, 72ºC for 2.5 minutes with a final 4 minutes at 72ºC. Two separate PCR reactions were performed for each DNA sample. Presence of PCR products were confirmed by running 2-µl on a 1% agarose gel at 90 volts for 30 minutes and stained with ethidium bromide. For each sample the two PCR products were pooled and digested separately with three different restriction enzymes: *Msp*I, *Alu*I, and *HinP1*I (New England BioLabs, Beverly, MA). The restriction digest mixture consisted of 10µl of PCR product, 1 unit of restriction enzyme, and 2 µl of 10X reaction buffer 2 (New England BioLabs) brought up to a total volume of 25 µl with Sigma water (Sigma-Aldrich, St. Louis, MO). Reactions were digested overnight at 37 ºC.

Fluorescently labeled terminal restriction fragments (T-RFs) were size separated on an ABI Avant Genetic Analyzer 3100 (Applied Biosystems, Foster City, CA) using an internal size standard (BioVentures MapMarker 1000, BioVentures, Inc. Murfreesboro, TN). To test for variation within each digested PCR product as well as instrument performance, we ran a trial set of ten restriction enzyme digests in duplicates (1-µl aliquots). Even though results from a preliminary assay did not show any substantial

differences across duplicate profiles, we chose to run three replicates for each restriction digest to ensure reliable results.

2.2.6 Analysis of T-RFLP Profiles

T-RFLP electropherograms were analyzed using GeneMapper software version 3.7 (Applied Biosystems, Foster City, CA). GeneMapper software calculates fragment length to 1/100 of a base pair (bp). The error associated with the determination of fragment size can be up to 0.5 bp (Dunbar et al. 2001), therefore T-RF peaks that differed by less than 0.5 bp were considered the same and grouped. Common observations in T-RFLP data include primer-dimer artifact formation in the low base-pair region of T-RFLP profiles and low peak area for T-RFs due to incomplete digestion or excess noise. Given these considerations, only those T-RFs that were sized >80 bp with >50 relative fluorescent units were included in the analysis. The raw data for each profile was examined to ensure that each peak was solely a result of the HEX-green fluorescence. Occasionally false peaks arise due to electrochemical noise from dust or bubbles present in the capillaries of the detector, therefore, careful consideration was taken to report only true peaks in the resulting profiles of each sample. For comparisons between T-RFLP profiles, normalized relative T-RF peak height and allele presence/absence data were considered. Triplicate profiles were collapsed into one average profile by including peaks that occurred in two of the three replicate profiles. T-RFs of different lengths inferred representation of distinct operational taxonomic units (OTUs) but should not be interpreted as specific bacterial species because similar restriction fragment sizes can be produced from different organisms (Liu et al. 1997).

2.2.7 T-RFLP Statistical Analyses

Non-metric multidimensional scaling (NMDS) formalized by Clarke (1993) was used for T-RFLP profile comparisons among different lithologies based on the Bray-Curtis dissimilarity matrix (Bray and Curtis 1957). The purpose of the NMDS is to construct a configuration of the samples, in a specified number of dimensions, while attempting to satisfy all the conditions imposed by the dissimilarity matrix (Clarke 1993). The NMDS recognizes the relative similarities of the samples to each other by configuring the dimension that has the least amount of stress, or goodness of fit, between the similarity rankings (p-dimensional space) and corresponding distance rankings in the ordination plot (k-dimensional space) (McCune and Grace 2002). Stress measures the degree of departure from the relationship between dissimilarity (distance).

Analysis of Similarity (ANOSIM) (Clarke and Green 1988, Clarke 1993) is a nonparametric procedure that evaluates the separation of groups in multivariate space by testing the hypothesis that there is no difference between two or more groups of entities. The ANOSIM test statistic "*R*" indicates the degree of discrimination among groups and usually falls between 0 and 1. $R = 1$ if all samples within a group are more similar to each other than any other samples from different groups. *R* approximates zero if the null hypothesis H_0 is true and there are no differences among groups. ANOSIM is a randomization test that simulates the null hypothesis by randomly reassigning group membership, in which an R^* value is computed for each randomized grouping. The probability (*P*) value calculated is the proportion of the R^* values that are greater or equal to the actual *R* value. Lower *P* value occur for R^* values that rarely exceed *R* which

means that it is less likely that the null hypothesis is true (Minchin P., personal communication).

The NMDS describes relationships within the microbial community data. In addition, higher level relationships can be examined by superimposing corresponding environmental data of each biogeochemical variable on the microbial community ordination by way of a vector analysis. If environmental conditions are responsible for structuring microbial communities, then streams with similar biogeochemistry should have similar species composition. The vector analysis determines which biogeochemical variables are positively correlated with particular groups of samples that represent given microbial community assemblages, as well as determining the strength of the correlation between the two sets of variables. DECODA (Database for Ecological COmmunity DAta) version 3 (Minchin 1990) was used to perform the multivariate analyses (NMDS, ANOSIM, and vector analysis).

2.2.8 Building Clone Libraries for 16S rRNA Gene

The sediment and rock biofilm samples from the stream sites in each lithology that showed the highest degree of variation using T-RFLP were chosen for more detailed phylogenetic analyses. A total of nine clone libraries of the 16S rRNA gene were built from sediment (n=5) and epilithon (n=4) samples from two representative streams within the non-carbonate and ultramafic lithologies. A single clone library was built from a sediment sample in the complex sedimentary lithology. Bacterial 16S rRNA gene sequences were amplified from three sediment samples and the composite epilithon sample taken at each stream site using primers, Bac8f (unlabeled) and Univ1492r

(Invitrogen Carlsbad, CA). PCR reactions protocol was as follows: initial denaturation at 94ºC for 2 minutes, followed by 40 cycles of 94ºC for 30 seconds, 54ºC for 20 seconds, and 72ºC for 1.5 minutes with a final extension of 15 minutes at 72ºC. To minimize the effect of PCR drift in individual reactions during amplification, PCRs were run in triplicate and pooled for each of the DNA extracts from an individual stream site (3 for sediment and 1 for epilithon). The PCR products from individual stream site samples (9 total for sediment and 3 total for epilithon) were run through a 0.75% agarose gel where the corresponding 16S rDNA fragment were visualized by ethidium bromide, excised with a sterile razor blade and cleaned with Zymoclean Gel DNA Recovery kit (Zymo Research, Orange, CA). Gel cleaned PCR products were cloned into pCR®2.1 vectors using the TA cloning kit according to the manufacturer's protocol (Invitrogen, Carlsbad, CA) and transformed into OneShot® Competent Cells (Invitrogen, Carlsbad, CA). Transformants were plated on Luria broth (LB) agar medium containing ampicillin, X-gal and isopropyl-β-d-thiogalactopyranoside (IPTG). Ampicillin-resistant and β-galactosidase-negative clones were randomly selected and grown overnight at 37ºC in LB with ampicillin. Clones were tested for the presence of inserts by PCR amplification and ethidium bromide gel visualization. Clones with inserts were sequenced directly and grown in three mls of LB ampicillin broth for long-term storage at -80 ºC. An excess of 100 clones for each sample were sequenced using primers designed to the pCR®2.1 vector: M13Long Forward (5'-CAGGAAACAGCTATGACCATGATTAC-3') and M13Long Reverse (5'-GTAAAACGACGGCCAGTGAATTGT-3') as well as internal 16S primers custom designed for specific clone groups to ensure complete overlap of

sequence reads in both directions: 16S-A1F (5'-GTGCCAGCAGCCGCGGTAATAC-3'); 16S-A1R (5'-GTATTACCGCGGCTGCTGGCAC-3'); 16S-B1F (5'-GGTGCTGCATGGCTGTCGTCAGC-3'); 16S-B1R (5'-GCTGACGACAGCCATGCAGCACC-3'); 16S-B2F (5'-GGTGGTGCATGGTTGTCGTCAGC-3'); and16S-B2R (5'-GCTGACGACAACCATGCACCACC-3'). One library was sequenced with the following sequencing cycle: initial denaturation at 96ºC for 1 minute, followed by 25 cycles of 96ºC for 10 seconds, 50ºC for 5 seconds, and 60ºC for 4 minutes. Ready to load sequence reactions were run at Vermont Cancer Center, University of Vermont, Burlington, Vermont on an ABI Avant Genetic Analyzer 3100 (Applied Biosystems, Foster City, CA). PCR products of the subsequent eight clone libraries were cleaned and sent to Agencourt Bioscience Corporation, Beverly, Massachusetts for sequencing. A total of 9 clone libraries were constructed from five representative stream sites within each of the three lithologies (streams SNC03 and SNC08 from the non-carbonate lithology, SUM01 and SUM11 from the ultramafic lithology and site SCS07 from the complex sedimentary). Five sediment clone libraries were constructed at each of the five stream sites and four epilithon libraries were constructed from the two representative stream sites in the non-carbonate and ultramafic lithologies.

2.2.9 Processing of Sequence Data

Clone sequences were assembled and edited using Sequencher version 4.6 (Gene Codes, Ann Arbor, Michigan). MacClade version 4.08 (Maddison and Maddison 2002) was used to visualize aligned sequences and positions that varied between clones within

each library were verified in the original contiguous Sequencher files. Sequences were aligned using the Ribosomal Database Project II (RDP) release 9.58 web resource (http://rdp.cme.msu.edu/) by Cole et al. (2007). The RDP makes available a general bacterial rRNA alignment model that uses a modified version of the RNACAD program that takes into consideration rRNA secondary structure in its internal model and is a Stochastic Context Free Grammar (SCFG) based rRNA aligner (Brown 2000, Gutell et al. 2002) We screened for potential chimeric sequences using the RDP's CHIMERA_CHECK program based on the Pintail algorithm by Ashelford (2005). Sequences identified as chimeric were removed from the data. The final data set included a range of 70-95 non-chimeric sequences, approximately 1500 bp long, per clone library. Edited sequences were imported into PAUP (Swofford 2001) where pairwise distances between sequences were computed and Jukes and Cantor (1969) distance matrices were exported for use in subsequent analyses.

2.2.10 Estimations of richness and diversity.

Estimates of richness and diversity of 16S rRNA genes were determined for all clone libraries. Highly similar sequences were considered as part of a single operational taxonomic unit (OTU) determined using the farthest-neighbor criterion of Schloss and Handelsman's (2005) DOTUR (version 1.53; Department of Plant Pathology, University of Wisconsin–Madison [http://www.plantpath.wisc.edu/fac/joh/DOTUR.html]), with a matrix of Jukes-Cantor distances used as input. An OTU was defined as having a 16S rRNA gene sequence similarity of ≥98% (farthest-neighbor distance of 0.02). The "species" level as per convention by Rossello-Mora and Amann (2001) suggests using

≥97% sequence similarity; however we chose a slightly higher cutoff (98%) to increase the stringency of our results and subsequent conclusions. DOTUR was also used to calculate rarefaction curves and diversity estimators, Chao1 (Chao 1984) and ACE – abundance coverage estimator (Chao and Lee 1992, Chao et al. 1993) for each of the 9 clone libraries. Bootstrapping procedures within DOTUR assess the confidence limits of the rarefaction curves and the diversity estimators.

2.2.11 Clone Library Statistical Analyses

We examined the variation in the genetic structure of the bacterial communities in each clone library and among groups of lithology and habitat using analysis of molecular variance (AMOVA) and F_{ST} tests (Arlequin version 3.1; Genetics and Biometry Laboratory, University of Geneva: http://lgb.unige.ch/arlequin). AMOVA uses a hierarchically partitioned matrix of genetic distances to assess, by permutation, the significance of variance component associated with each level of the partitioning (Excoffier et al. 1992). For this analysis, input matrices consisted of distances computed in DNAsp (Rozas and Rozas 1999) and tested as follows: i) all clone libraries considered as distinct groups (n = 9); ii) sediment (n = 5) vs. epilithon (n = 4); and iii) ultramafic sediment (n = 2) vs. non-carbonate sediment (n = 2) and ultramafic epilithon (n = 2) vs. non-carbonate epilithon (n = 2).

F_{ST} tests (Martin 2002) were performed as tests of genetic differentiation among all pairs of samples. F_{ST} can also be considered as a measure of distance between pairs of samples that takes into account both the frequency of identical or closely related sequences and the amount of diversity within the sample pairs. A matrix of pairwise F_{ST}

distances was used as the basis of the NMDS plot in the program PRIMER 5 for Windows (version 5.2.7; PRIMER-E, Ltd: http://www.primer-e.com) to visualize samples that are similar in genetic composition. ANOSIM was also used to determine which samples were most closely associated with patterns of similarity between bacterial communities (Clarke and Green 1988, Clarke 1993) . Additionally, Mantel tests were used to examine the influence of geographic distance (straight-line distance between stream sites) on community composition.

2.2.12 Taxonomic Associations of 16S rRNA Bacterial Clones

Groups of sequences were formed based on their similarities and by identifying their closest genus and species matches using the SeqMatch tool in the RDP, which has its own database as well as being linked to GenBank (Altschul et al. 1990). Identification of unknown clones using GenBank data followed the guidelines established by Goebel and Stackebrandt (1994), which considers a 97-100% match an approximate identification to species level, 93-96% similarity as genus level identification, and 86-92% match a distant yet related organism. The ≥98% sequence similarity criteria concurred with results obtained using DOTUR in that all sequences within an established OTU generally matched to at least the same genus as determined using GenBank.

2.2.13 Nucleotide sequence accession numbers

Sequence data will be submitted to GenBank and accessed using unique accession numbers that will be available upon publication.

2.3 Results

2.3.1 Biogeochemistry of stream study sites

Stream study sites with unique global positioning system location and elevation and a summary of biogeochemical characteristics by lithology are found in Tables 2.1 and 2.2, respectively.

2.3.2 Lithology and Habitat Comparisons using T-RFLP

Using the NMDS ordination of T-RFLP patterns and the ANOSIM (DECODA; (Clarke and Warwick 2001), the 42 samples obtained from the 14 stream sites separated into three general clusters that represented streams arising in the three different lithologies (Global $R = 0.40$; $P < 0.001$) (Fig. 2.2). Pairwise comparisons indicate that ultramafic UM streams were significantly different from non-carbonate (NC) and complex sedimentary (CS) streams (UM vs. CS: $R = 0.50$, $P < 0.0001$; UM vs. NC: $R = 0.55$, $P < 0.001$; and NC vs. CS: $R = 0.06$, $P = 0.2$).

We also observed significant differences when comparing the sediment and rock habitats (Global $R = 0.98$; $P < 0.0001$) (Fig. 2.3). The overall number of phylotypes, or restriction fragments (T-RFs), for sediment samples ranged from 19 to 69 (mean: 51) with an average of 51 for CS, 54 for NC, and 50 for UM. T-RFs for rock biofilm samples ranged from 66 to 99 (mean: 79) with an average of 72 for CS, 86 for NC, and 75 for UM. To detect differences with the T-RFLP data we chose NMDS with the ANOSIM multivariate method rather than diversity indices since it has been suggested that the use of diversity indices on T-RFLP data provides inaccurate estimates of true diversity in microbial communities (Blackwood et al. 2007) and that multivariate methods

(ordination and clustering) have greater sensitivity for detecting microbial community differences (Dunbar et al. 2000, Hartmann and Widmer 2006).

We examined the relationship between bacterial community structure and environmental variables in each of the three lithologies by using a vector analysis. A positive correlation was observed between bacterial communities and measured inorganic chemical variables (Bonferroni-corrected $P > 0.5$ in all cases) (Fig. 2.2). The vectors associated with cation variables (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) were correlated with the complex sedimentary community, while the vectors associated with dissolved organic carbon (DOC) and nutrients (Total dissolved nitrogen – nitrate, (TDN-NO_3^-); Total dissolved nitrogen/Total dissolved phosphorus, (TDN/TDP)) were correlated with the non-carbonate community, and nitrate/total dissolved nitrogen, (NO_3^-/TDN) was correlated with the ultramafic community.

2.3.3 Lithology and Habitat Comparisons using Clone Libraries

Based on the analysis of T-RFLP data, samples that were furthest away in NMDS genotype space stream sites were selected for 16S rRNA gene cloning and sequencing from each of the three lithologies differentiated in Fig. 2.2. Two streams from NC and UM and 1 from CS were selected to further characterize the diversity of the bacterial communities in sediment and epilithon of each lithology as well as to identify specific phylogenetic groups that could be associated with the bacterial community composition pattern shown by the T-RFLP analysis. Subsequent to the removal of chimeric sequences, the number of clones for each library ranged from 77 to 95.

2.3.3.1 Diversity Statistics

Results for the diversity statistics obtained using DOTUR showed that at the 98% similarity level, there were small differences in the Chao1 (from 21 to 58) and ACE (from 22 to 65) diversity estimators calculated for each sample. Moreover, the bootstrapped 95% confidence intervals were large and overlapped between all samples for both estimators (Figure A.5).

2.3.3.2 Analysis by Rarefaction

Using DOTUR a number of the rarefaction curves were generated for each clone library. At the ≥98% sequence-similarity level variation between curves was observed. However, several curves were nearly identical to each other (Figures A.2 and A.3). In general, sediment and rock biofilm curves were at or near plateau, indicating that we were successful in sampling nearly the full extent of bacterial species richness within each of the samples. The rarefaction curves differed when clone libraries were combined by lithology (UM and NC) and habitat (epilithon and sediment) (Fig 2.4; 95% CIs not shown), indicating differences in species richness. The 95% CIs overlap between UM and NC curves, but there was minimal overlap between sediment and rock biofilm curves. Rarefaction curves for both sediment and rock biofilm samples by stream site within the NC and UM lithologies (Fig. 2.5) indicated that streams within the NC were more variable than streams within the UM lithology, suggesting higher bacterial diversity across stream sites within the NC than UM.

2.3.3.3 Comparisons of Clone Libraries using AMOVA

All nine populations had significantly different genetic composition ($P < 0.0001$) relative to the pool of total species in all samples. The AMOVA (Figure 2.6; Table A.3.)

indicated that sediment and rock biofilm samples were composed of genetically different microbial communities ($P = 0.01$). It was not possible to show that microbial populations in contrasting lithologies were genetically different ($P = 1.00$; $P = 0.300$). However, pairwise comparisons of clone library F_{ST} tests (Martin 2002) showed that all were significantly different ($P < 0.00001$), with the exception of EpiSUM01 and EpiSNC03 ($P = 0.11$). A NMDS ordination (Fig. 2.7) using pairwise F_{ST} comparisons, which represents distance in genetic and community composition, segregates samples by sediment and rock biofilm communities.

2.3.3.4 Taxonomic Associations of 16S rRNA Bacterial Clones

Using sequence matches obtained from GenBank and the RDP, we determined the presence of 32 families of bacteria belonging to 14 classes. The most commonly represented bacterial families in sediment samples were as follows: *Enterobacteriaceae* (25.1%), *Paenibacillaceae* (28.3%), *Pseudomonadaceae* (9.4%), and *Xanthomonadaceae* (11.6%) (Fig. 2.8; Table 2.3). Rock biofilm samples were dominated by species of *Cyanobacteria* (43.5%) as well as the following bacterial families: *Flexibacteraceae* (17.7%), *Comamonadaceae* (12.9%), and *Deinococcaceae* (5.5%) (Fig. 2.9; Table 2.3). Sediment clones included 20 families belonging to 11 classes, whereas rock biofilm clones included 16 families representing 11 classes. Sediment samples had four bacterial families that overlapped across all three lithologies. The ultramafic sediment, with 16 bacterial families, had the highest diversity at the family level, while 12 were detected in NC and five in CS. Eight bacterial families were found in both NC and UM sediment samples. Families unique to sediment NC and absent in sediment UM were

Sporolactobacillaceae and *Aeromonadaceae*, while several families were unique to sediment UM: *Microbacteriaceae*, *Hyphomicrobiaceae*, *Carnobacteriaceae*, *Alcaligenaceae*, *Geobacteraceae*, *Flavobacteriaceae*, *Planctomycetaceae*, and *Crenotrichaceae*. Clones in the single CS library consisted of six families represented by three classes. Fifty-seven of the 82 total clones matched at the genus level (98%) to the organism *Paenibacillus borealis*.

For rock biofilm clones, nine of 16 families were shared between the NC and UM samples. In rock biofilm samples, 12 and 13 bacterial families were found in the UM and NC, respectively. Unique families in rock biofilm from NC sites were as follows: *Oxalobacteraceae*, *Cryomorphaceae*, *Planctomycetaceae*, and *Verrucomicrobiaceae*, while those unique to rock biofilm from UM sites were *Bdellovibrionaceae*, *Enterobacteriaceae*, and *Sphingobacteriales*.

A test of isolation of stream site bacterial community composition by geographic distance using the Mantel Test, did not show any structure ($P > 0.3$).

2.4 Discussion

Results from this study indicate that there are high levels of OTU diversity among and within bacterial communities in the streams of the Noatak National Preserve. We used both T-RFLP and 16S rRNA gene sequencing, two methods that differ in their resolution, to determine how the methods would resolve samples collected from our study site. Differing patterns in bacterial community composition at both the small-scale (stream habitat) and large-scale (lithology) were observed. Using a T-RFLP approach we

detected distinct fingerprints of bacterial communities by lithology and habitat, whereas 16S rRNA gene clone libraries differentiated habitats.

2.4.1 Comparisons using T-RFLP

NMDS ordination of T-RFLP peaks show a high degree of separation between UM and NC communities and only partial separation between NC and CS. These trends in bacterial community composition mirror stream biogeochemistry across the three lithologies in that UM and NC have significantly different biogeochemical characteristics, while the NC and CS are similar. Our results are similar to previously reported differences in microbial community composition as influenced by lithology and parent material in soils (Dunbar et al. 2000, Oline 2006), glaciers (Skidmore et al. 2005), groundwater and substratum (Takai et al. 2003). NMDS results of T-RFLP data also showed clear separation by habitat, between sediment and epilithon samples. The average number of phylotypes (T-RFs) detected in sediment samples were similar, suggesting low variation in bacterial diversity across lithologies. However, differences in the number of T-RFs were observed between sediment and epilithon within a lithology, indicating differences in bacterial diversity at the habitat scale.

2.4.2 Relationships between Bacterial Communities and Biogeochemistry

Vector analysis indicates that certain biogeochemical variables explain NMDS ordination of T-RFLP data (Fig. 2.2), although causal relationships can only be inferred. Specifically, we observed a positive correlation between base cations and CS lithology, suggesting bacterial community structure may be influenced by the CS streams' high base cation concentration, whereas the scarcity of cations in NC and UM streams may

constrain the composition of resident bacterial communities. Notably calcium and magnesium are well known to enhance bacterial adhesion to substrates within the exopolysaccharide matrix of biofilms (Geesey et al. 2000), which may facilitate a niche for a more stable bacterial community in the CS lithology. Other constituents such as DOC, TDN-NO_3^-, and TDN/TDP were correlated with NC community composition while NO_3^-/TDN was correlated with the UM community. DOC and nutrient concentrations were lowest in streams of the CS and UM lithologies and highest in streams of the NC lithology. These trends suggest that bacterial community composition may be influenced by the abundance or scarcity of resources. Similar relationships have been observed in other studies that investigated the influence of microbial activity on redox chemistry and mineral processes in natural environments (Nealson and Stahl 1997, Ehrlich 1998). Furthermore, other studies have determined that microbial community composition can be correlated with observed aqueous geochemistry in subglacial chemical weathering (Skidmore et al. 2005), streamwater pH, quality of fine benthic organic matter, and quantity of DOC and nitrogen in stream water (Fierer et al. 2007), and seasonal changes in temperature, nutrient availability and light in estuarine biofilms (Moss et al. 2006).

2.4.3 Comparisons using 16S rRNA Gene Sequencing

The T-RFLP approach used in this study revealed general patterns in community composition indicating differences by lithology and habitat. Sequencing of the 16S rRNA gene offered greater resolution allowing the identification of dominant taxa present in each sample. Although 16S rRNA gene sequencing was only able to distinguish

community composition by habitat, we gained insight into the taxonomic composition of both stream habitat and landscape lithology.

While we are aware that 77-95 clones per library is not a large number compared to the potential microbial diversity of these communities, we nonetheless detected high diversity among samples using 16S rRNA gene sequencing. Similar levels of microbial diversity were detected in studies in which comparable or fewer number of clones were sequenced (Oline 2006, Fierer et al. 2007). In this study, twenty-nine out of thirty possible F_{ST} pairwise comparisons of the 9 samples were significant, suggesting distinct genetic differentiation among all communities with the exception of EpiSUM01 vs. EpiSNC03. Furthermore, there was significant variation in the genetic structure of communities residing in different habitats (AMOVA). This heterogenetity in community composition may be a result of differences in hydrologic stressors and substrate availability in the sediment versus the rock biofilm habitats.

Sequencing of the 16S rRNA gene revealed the presence of genera including gram-negative bacteria that have previously been isolated from aquatic and terrestrial environments. There were distinct differences in taxonomic identities (at the family level) of clones from sediment versus rock biofilm habitats. Only four families are shared between the 20 and 16 families found in sediment and rock biofilm samples, respectively. At the class level, eight of 11 classes were shared between sediment and epilithon. Our results at the class level are similar to those reported by Hullar (2006), who sampled headwater streams in southeast Pennsylvania and found a high degree of similarity in taxa type overlap between sediment and rock biofilm samples. Furthermore, all

representatives of the rock biofilm in our work, including 1 division and 3 classes, were also present in the 13 sediment classes detected in Hullar's study. Our results differ from those of Hullar (2006) in that there are few similar taxons between the sediment and epilithon at the family-level identification in our study and we detected a high abundance of cyanobacteria exclusively in rock biofilm samples (44%), whereas Hullar (2006) found that cyanobacteria clones comprised the majority (40%) of their sediment-derived sequences and a smaller proportion (25%) of the epilithic-derived sequences.

GenBank was used to identify matches to the sediment clones at the genus level. Groups of clones from the sediment samples: *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Aeromonas*, *Enterobacter*, *Xanthomonas*, and *Sporolactobacillus*, known to be heterotrophic bacteria, were also previously isolated from similarly classified pristine stream bed sediments in forested watersheds (Halda-Alija and Johnston 1999). *Aeromonadaceae*, *Pseudomonadaceae*, and *Xanthomonadaceae* species are obligately aerobic, whereas *Enterobacteriaceae* and *Sporolactobacilliaceae* members are facultatively anaerobic with *Enterobacteriaceae* species having the ability to reduce nitrate to nitrite. *Paenibacillus borealis*, a nitrogen-fixing species, also isolated from Norway and Finnish spruce forest humus, was present in sediment samples from all three lithologies, but found to be dominant in the CS clone library.

Unclassifiable *Cyanobacteria* species, the most dominant of the epilithon clones, metabolize via oxygenic photosynthesis. Other dominant epilithic members include the following families: *Flexibacteriaceae*, *Comamonadaceae*, and *Deinococcaceae*, which are all chemoorganotrophic as well as obligately aerobic. Thirteen clones identified as

belonging to the genus *Spingomonas* were found exclusively associated with the epilithic community (3.4%). *Spingomonas* have been isolated from a range of environments, including ultraoligotrophic waters, in which species such as *S. alaskensis* has been shown to possess physiological characteristics adapted to very low carbon substrate concentrations (Eiler et al. 2003). That the detection of *Spingomonas* in our study was restricted to epilithon samples, may indicate lower availability of carbon sources for bacterial metabolism in this habitat. In contrast to the high loads of particulate and dissolved organic matter associated with upwelling areas from the hyporheic zone, associated with stream sediment habitats (Sobczak and Findlay 2002).

2.4.4 Conclusions and Relevance to Microbial Biogeography

In the past decade, studies on the taxonomic, phylogenetic, and physiological diversity of prokaryotes have begun to provide more comprehensive information about microbial communities and their natural environments, and in particular, whether microbes exhibit biogeographical patterns. Structural geographic patterns as detected in microbial communities within stream ecosystems have been attributed to the following factors: geographic distance (<10 km) and connectivity between lakes and streams (Crump et al. 2007); biome-level control in low-order streams (Findlay et al. 2008); variation of chemical characteristics in streams across the southeastern and midwestern U.S. (Gao et al. 2005); and landscape-level controls on streams due to biogeochemical factors (Fierer et al. 2007). In general, very few studies have focused on low-order streams (Hullar et al. 2006, Findlay et al. 2008), as we have done in this study. However,

these types of streams are important because they function as links between the terrestrial environment and the aquatic ecosystem network.

Results obtained by comparing the bacterial OTUs from our study to the GenBank database showed matches with similar bacterial taxa isolated from various locations around the globe, suggesting (as Baas-Becking hypothesized) that bacteria have a cosmopolitan distribution. However, these results should be interpreted with caution given that the 16S rRNA gene sequencing method used in this study can be used as a measure of phylogenetic relatedness which does not necessarily reflect levels of similarity at the physiological level. It is possible that even slight differences in bacterial physiology can be related to biogeochemical processing. Future work that focuses on the metagenomics of environmental samples could be an alternative means to clarify the physiological characteristics of bacteria in a given environment allowing greater discriminatory power in determining bacterial contribution to ecosystem function.

While we observed similarities in bacterial clone types between our study sites and other geographically distant locations, we also detected biogeographic structure of species richness and taxon type. This is shown in the rarefaction curves (Fig. 2.5) by habitat type within NC and UM lithologies, which reveal distinct variation in species richness in the NC landscape, while UM streams track each other. These results indicate that NC streams may be more diverse than UM streams at the landscape scale. Furthermore, sediment and rock biofilm rarefaction curves are also significantly different from one another (Figure 2.4 a). Thus, we conclude that biogeographical patterns in bacterial community composition occur in the Noatak River Basin. With regards to the

controversy related to the Baas-Becking hypothesis (Whitfield 2005), results from our study indicate that whether a given taxon is cosmopolitan or biogeographically restricted will depend on the environment and its biogeochemical specificity. Differences in microbial community composition between sediment and epilithic habitats could result from different hydrologic stressors. For example, varying flow regimes alter sediment structure via erosion and redistribute bacteria, exposing them to different environmental conditions (Hullar et al. 2006). While the epilithic community is not as likely to experience the same degree of disturbance as that found in the sediment, differences in hydrodynamic conditions are known to influence the structure and activity of epilithic biofilms (Battin 2000, Battin et al. 2003).

Biogeographical bacterial patterns as influenced by lithology may be the result of differences in resource availability across different lithologies. The NC lithology is a richer environment, hosting streams with greater nutrient and DOC availability, higher chlorophyll *a* productivity, and concentrations of benthic organic matter, whereas streams of the UM lithology lack the resources abundant in the NC in addition to experiencing a scarcity of base cations. Given these biogeochemical conditions, we infer that the NC landscape may allow for the survival of more varied metabolic types given the abundance of resources, creating opportunities for bacterial diversity to persist across streams. The lack of biogeochemical resources in the UM streams may provide an environment where bacteria that are specialists and thriving on low nutrient and substrate concentrations may competitively exclude others. Thus, the UM lithology displays a predictable species

richness perhaps because it is a highly selective environment where a limited type of organisms can persist.

Our results suggest that there are differences in bacterial community composition across contrasting lithologies that can be related to large-scale linkages between streams and the terrestrial environment and parent material in which they are embedded. In turn, this relationship is reflected in differences in resource availability. Furthermore, the resident microorganisms of sediment and epilithon habitats are composed of significantly different bacterial taxa, indicating the presence of contrasting ecological niches at the small-scale within stream ecosystems. Thus, our study of arctic streams using T-RFLP and 16S rRNA gene sequencing indicates that bacterial community composition is dependent on the physical characteristics of the habitat within a stream as well as the stream location on the lithological landscape.

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Table 2.1. Summary of individual study streams with GPS locations.

Lithology	Stream Site ID	Sample Date	Latitude (DD)	Longitude (DD)	Elevation (m)
Complex Sedimentary	SCS01	7/12/06	68.265361	158.204972	570
Complex Sedimentary	SCS02	7/12/06	68.267917	158.098778	451
Complex Sedimentary	SCS03	7/13/06	68.320300	158.035400	535
Complex Sedimentary	SCS04	7/12/06	68.252083	158.004833	490
Complex Sedimentary	SCS07	7/13/06	68.245472	157.785278	556
Complex Sedimentary	SCS09	7/13/06	68.195222	158.001806	474
Non-carbonate	SNC03	7/11/06	68.266972	158.927500	342
Non-carbonate	SNC05*	7/8/06	68.251750	158.872860	418
Non-carbonate	SNC08	7/8/06	68.198617	158.816683	468
Non-carbonate	SNC10*	7/8/06	68.178950	158.741817	426
Non-carbonate	SNC12	7/11/06	68.139444	158.729750	358
Ultramafic	SUM01	7/9/06	68.327139	158.306750	550
Ultramafic	SUM07	7/10/06	68.392306	158.425778	660
Ultramafic	SUM11	7/10/06	68.386528	158.542361	560
Ultramafic	SUM12	7/10/06	68.382972	158.627056	447
Ultramafic	SUM17	7/9/06	68.303361	158.574611	501

*Sample not used from particular site due to questionable sample preservation.

Table 2.2. Values of biological, chemical and physical parameters for study streams by lithology.

			Concn (mg/liter)				Concn (µM)			Concn (µg/liter)			(µS/cm)		ºC	Concn (mg/liter)		µg/cm^2
Lithology	**n**		**Ca**	**Mg**	**K**	**Na**	**NO_3^-**	**TDN**	**TDP**	**Al**	**Fe**	**Si**	**E_c**	**pH**	**Temp**	**DO**	**DOC**	**Chl *a***
CS	6	mean	38.0	27.4	1.1	5.1	2.6	7.7	0.1	92.3	12.3	1703	502.2	7.7	8.8	10.9	2.8	0.3
		std error	9.0	4.9	0.1	1.5	0.9	0.7	0.0	1.4	1.1	73	140.2	0.1	0.6	0.3	0.2	0.1
NC	5	mean	14.0	9.1	0.9	1.6	1.1	19.9	0.1	102.1	42.3	3176	168.7	7.5	8.5	10.9	8.3	0.3
		std error	2.4	1.8	0.0	0.0	0.1	2.6	0.0	1.8	6.8	608	26.1	0.1	1.2	0.7	0.9	0.1
UM	5	mean	3.8	1.9	0.9	1.3	4.3	7.3	0.1	95.7	33.0	2278	38.8	7.3	7.9	10.9	2.1	0.3
		std error	0.8	0.6	0.0	0.0	1.4	1.2	0.0	2.7	10.4	134	4.4	0.1	0.3	0.2	0.2	0.0

Table 2.3. Phylogenetic affiliation of clones amplified from sediment and epilithon samples.

Habitat	GenBank Identifications		No. of clones			% of clone library			% of total
	Class (Domain*)	Family	NC	UM	CS	NC	UM	CS	clone library
Sediment	*Acidobacteria*	*Acidobacteriaceae*	1	3	0	0.6	1.9	0.0	1.0
n = 5	*Actinobacteria*	*Microbacteriaceae*	0	1	0	0.0	0.6	0.0	0.2
	Alphaproteobacteria	*Unclassifiable Alphaproteobacteria*	2	0	0	1.2	0.0	0.0	0.5
		Hyphomicrobiaceae	0	3	0	0.0	1.9	0.0	0.7
		Rhizobiaceae	1	5	0	0.6	3.1	0.0	1.5
	Bacilli	*Carnobacteriaceae*	0	1	0	0.0	0.6	0.0	0.2
		Paenibacillaceae	30	28	57	18.3	17.4	69.5	28.3
		Sporolactobacillaceae	25	0	0	15.2	0.0	0.0	6.2
	*Bacteria**	*Unclassifiable Bacteria*	1	0	0	0.6	0.0	0.0	0.2
	Betaproteobacteria	*Alcaligenaceae*	0	7	0	0.0	4.3	0.0	1.7
		Comamonadaceae	4	5	0	2.4	3.1	0.0	2.2
	Deltaproteobacteria	*Aeromonadaceae*	22	0	6	13.4	0.0	7.3	6.9
		Geobacteraceae	0	1	0	0.0	0.6	0.0	0.2
	Flavobacteria	*Flavobacteriaceae*	0	2	0	0.0	1.2	0.0	0.5
	Gammaproteobacteria	*Enterobacteriaceae*	49	46	7	29.9	28.6	8.5	25.1
		Pseudomonadaceae	18	17	3	11.0	10.6	3.7	9.4
		Shewanellaceae	4	2	4	2.4	1.2	4.9	2.5
		Xanthomonadaceae	6	36	5	3.7	22.4	6.1	11.6
	Planctomycetacia	*Planctomycetaceae*	0	1	0	0.0	0.6	0.0	0.2
	Sphingobacteria	*Crenotrichaceae*	0	3	0	0.0	1.9	0.0	0.7
Epilithon	*Alphaproteobacteria*	*Sphingomonadaceae*	5	8	n/a	2.6	4.2	n/a	3.4
n = 4	*Bacteria**	*Unclassifiable Bacteria*	7	7	n/a	3.7	3.7	n/a	3.7
	Betaproteobacteria	*Betaproteobacteria*	4	8	n/a	2.1	4.2	n/a	3.2
		Burkholderiales	4	1	n/a	2.1	0.5	n/a	1.3
		Comamonadaceae	14	35	n/a	7.4	18.5	n/a	12.9
		Oxalobacteraceae	6	0	n/a	3.2	0.0	n/a	1.6
	*Cyanobacteria**	*Unclassifiable Cyanobacteria*	107	58	n/a	56.3	30.7	n/a	43.5
	Deinococci	*Deinococcaceae*	1	20	n/a	0.5	10.6	n/a	5.5
	Deltaproteobacteria	*Bdellovibrionaceae*	0	1	n/a	0.0	0.5	n/a	0.3
	Flavobacteria	*Cryomorphaceae*	1	0	n/a	0.5	0.0	n/a	0.3
		Flavobacteriaceae	3	2	n/a	1.6	1.1	n/a	1.3
	Gammaproteobacteria	*Enterobacteriaceae*	0	2	n/a	0.0	1.1	n/a	0.5
	Planctomycetacia	*Planctomycetaceae*	1	0	n/a	0.5	0.0	n/a	0.3
	Spingobacteria	*Flexibacteraceae*	36	31	n/a	18.9	16.4	n/a	17.7
		Sphingobacteriales	0	16	n/a	0.0	8.5	n/a	4.2
	Verrucomicrobiae	*Verrucomicrobiaceae*	1	0	n/a	0.5	0.0	n/a	0.3

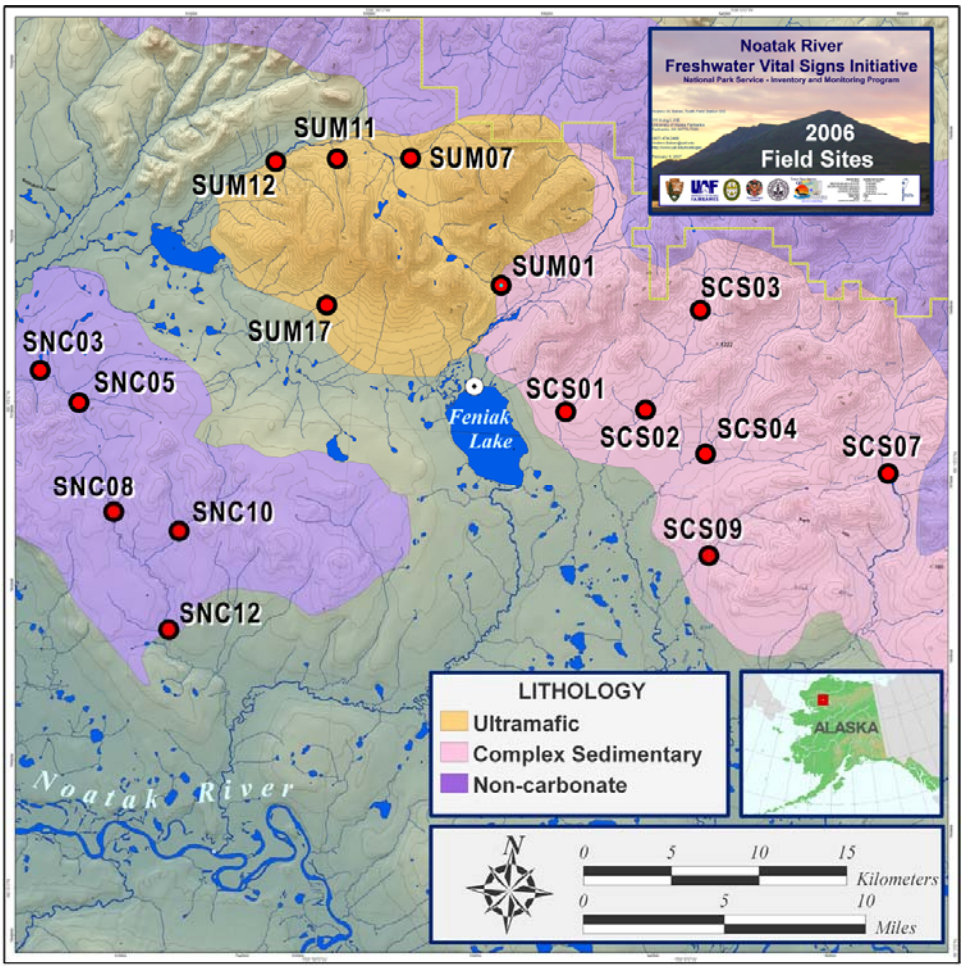


Figure 2.1. Study area of Feniak Lake region with stream site locations across contrasting lithologies in the Noatak National Preserve, Alaska.

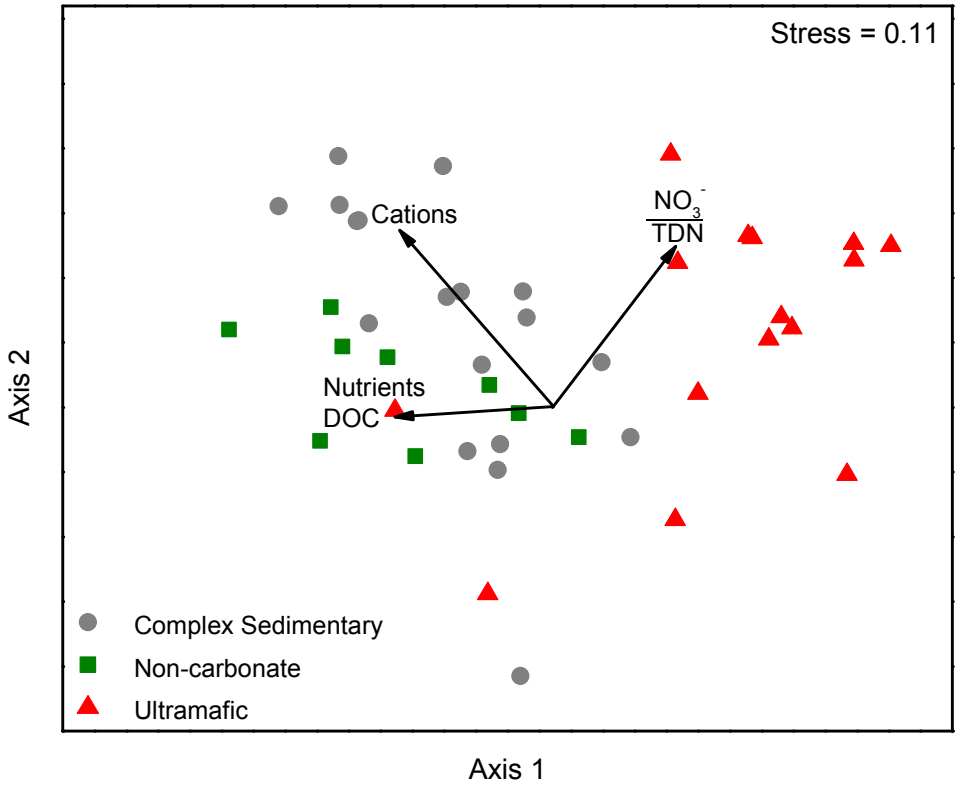


Figure 2.2. Non-metric multidimensional scaling (NMDS) ordination (1st & 2nd of the 3-dimensional solution) of stream sediment bacterial communities based on pairwise similarity estimates (Bray-Curtis). Points that are close together represent sediment biofilm communities with similar bacterial community composition based on the T-RFLP (terminal restriction fragment length polymorphism) method. The associated normal stress value of the ordination is 0.11, indicating a good approximation of the overall structure of the data in multivariable space. Significant ($p<0.005$), Bonferonni adjusted biogeochemical variables were overlaid (arrows) showing the degree of correlation with microbial data. Abbreviations: Nutrients = TDN-NO_3^-, total dissolved nitrogen – Nitrate and TDN/TDP, total dissolved nitrogen/total dissolved phosphorus; DOC, dissolved organic carbon; Cations = Ca^{2+}, Mg^{2+}, K^+, and Na^+; NO_3^-/TDN, nitrate/total dissolved nitrogen.

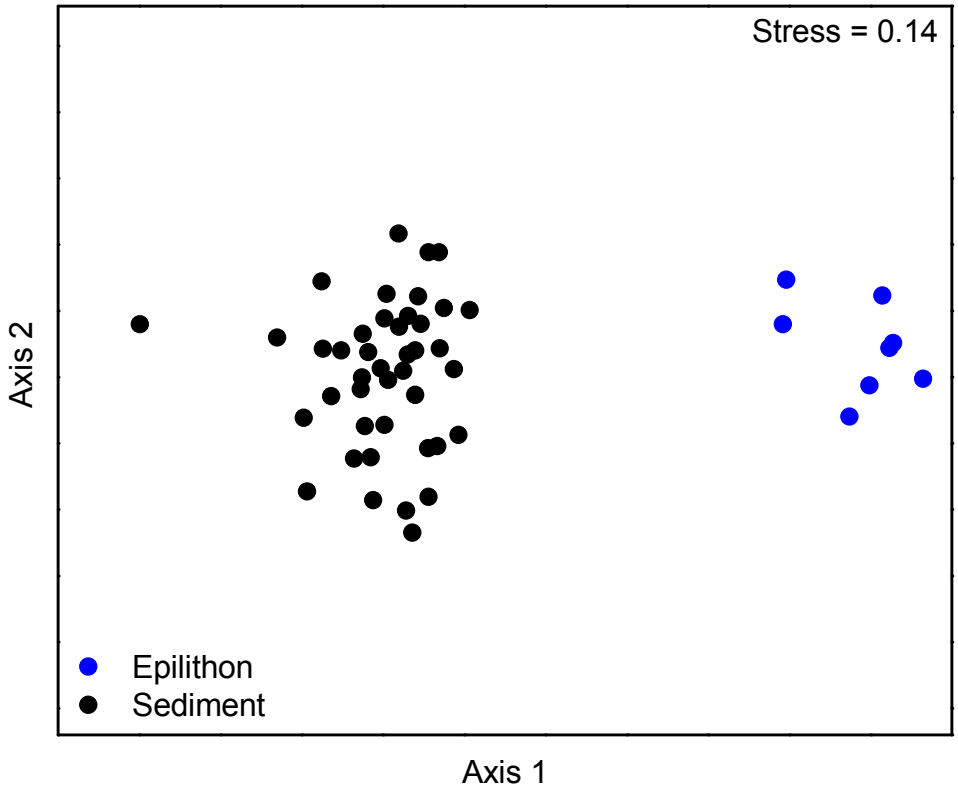
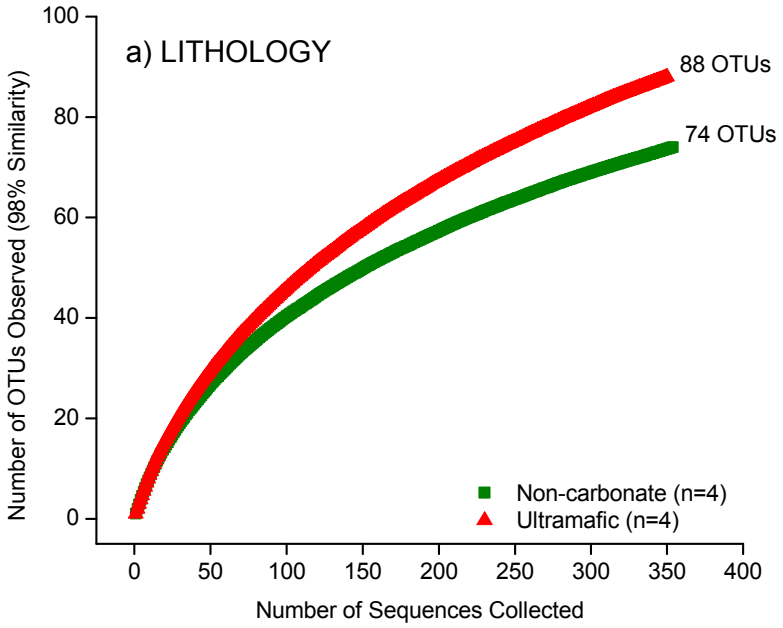


Figure 2.3. Non-metric multidimensional scaling (NMDS) ordination (2-dimensional solution) of stream sediment and epilithon bacterial communities based on pairwise similarity estimates (Bray-Curtis). Points that are close together on the right side of the ordination represent epilithon samples (n = 8) and those points close together on the left side of the ordination represent sediment samples (n = 42).



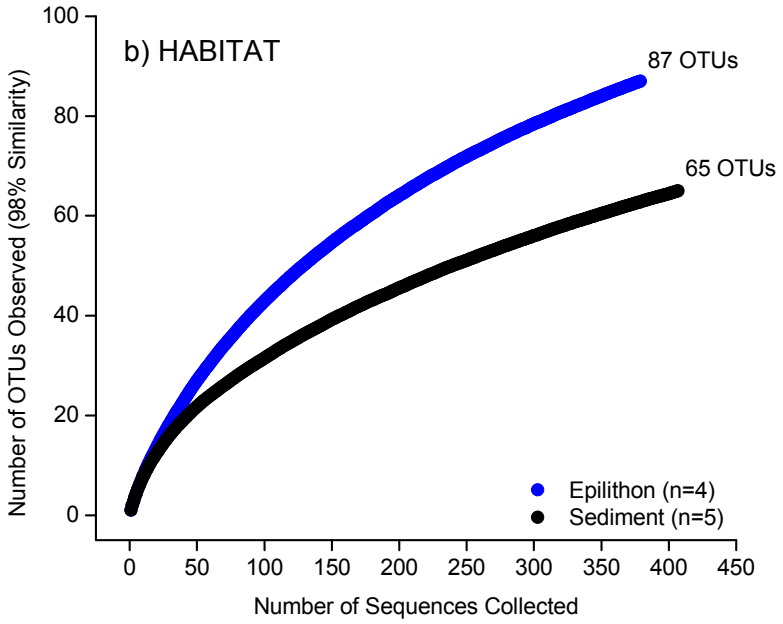


Figure 2.4. a) Rarefaction curves of observed OTU richness in sediment and epilithon samples within each lithology (non-carbonate and ultramafic). b) Rarefaction curves of observed OTU richness in sediment and epilithon samples regardless of lithology. The variance of the number of OTUs drawn in 100 randomizations at each sample size was calculated as 95% CIs (not shown).

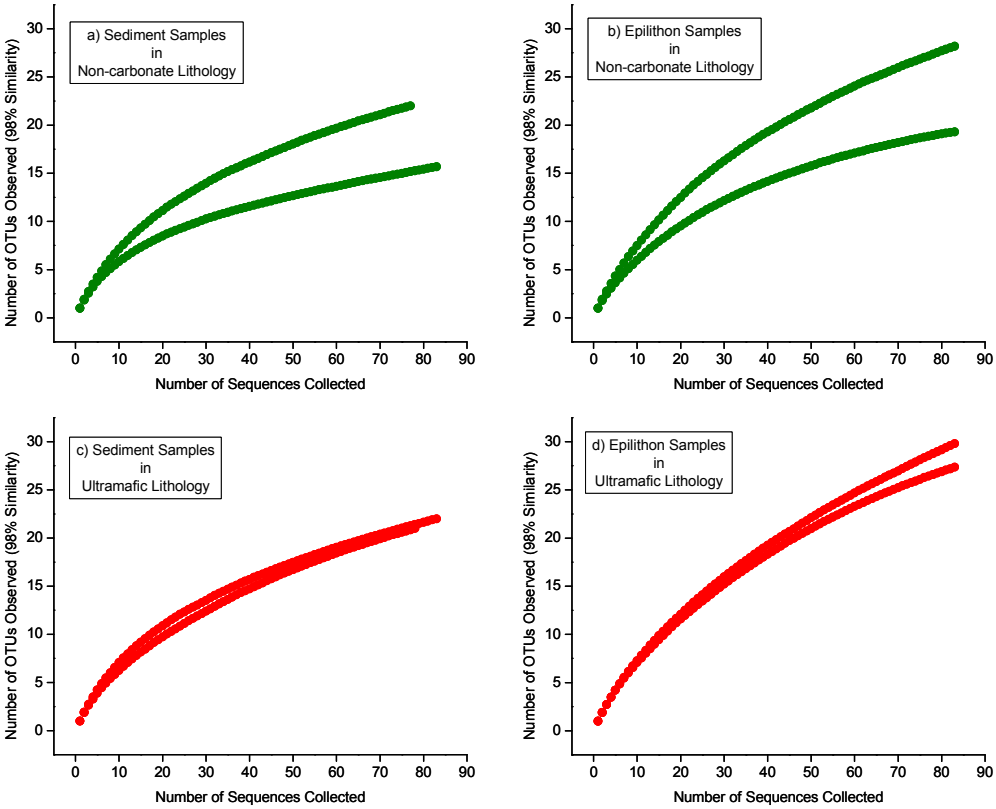


Figure 2.5. Rarefaction curves of observed OTU richness in individual stream and stream habitat comparisons (a) Sediment non-carbonate streams; (b) Epilithon non-carbonate streams; (c) Sediment ultramafic streams; (d) Epilithon ultramafic streams.

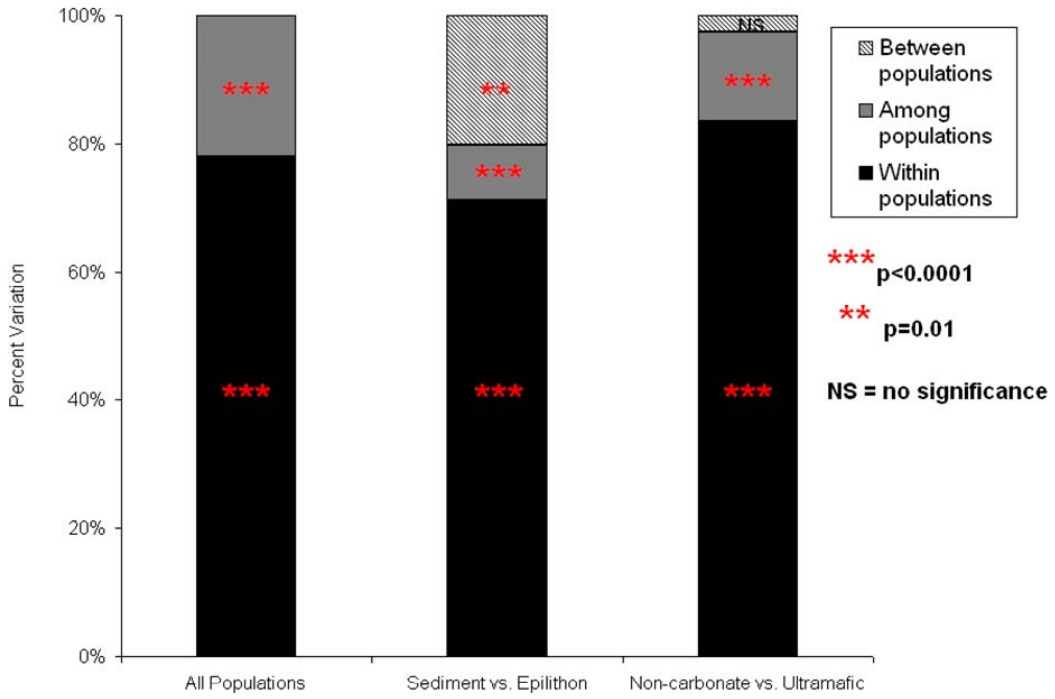


Figure 2.6. Percent variation from analysis of molecular variance (AMOVA) analysis at each level of partitioning: a) all populations includes the 9 clone libraries; b) sediment clone libraries (n = 5) versus epilithon clone libraries (n = 4); and non-carbonate clone libraries (n = 4) versus ultramafic clone libraries (n = 4).

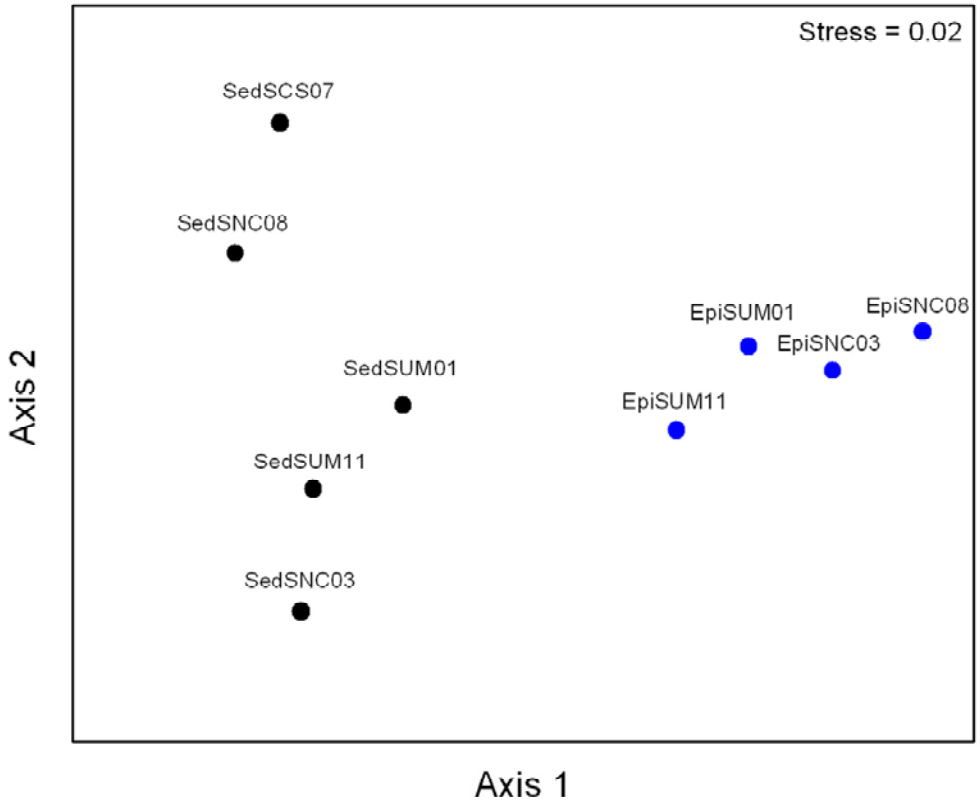


Figure 2.7. Non-metric multidimensional (NMDS) scaling plot of genetic and community structure for sediment and epilithon samples. Distances between points are based upon the F_{ST} statistic for all samples pairs.

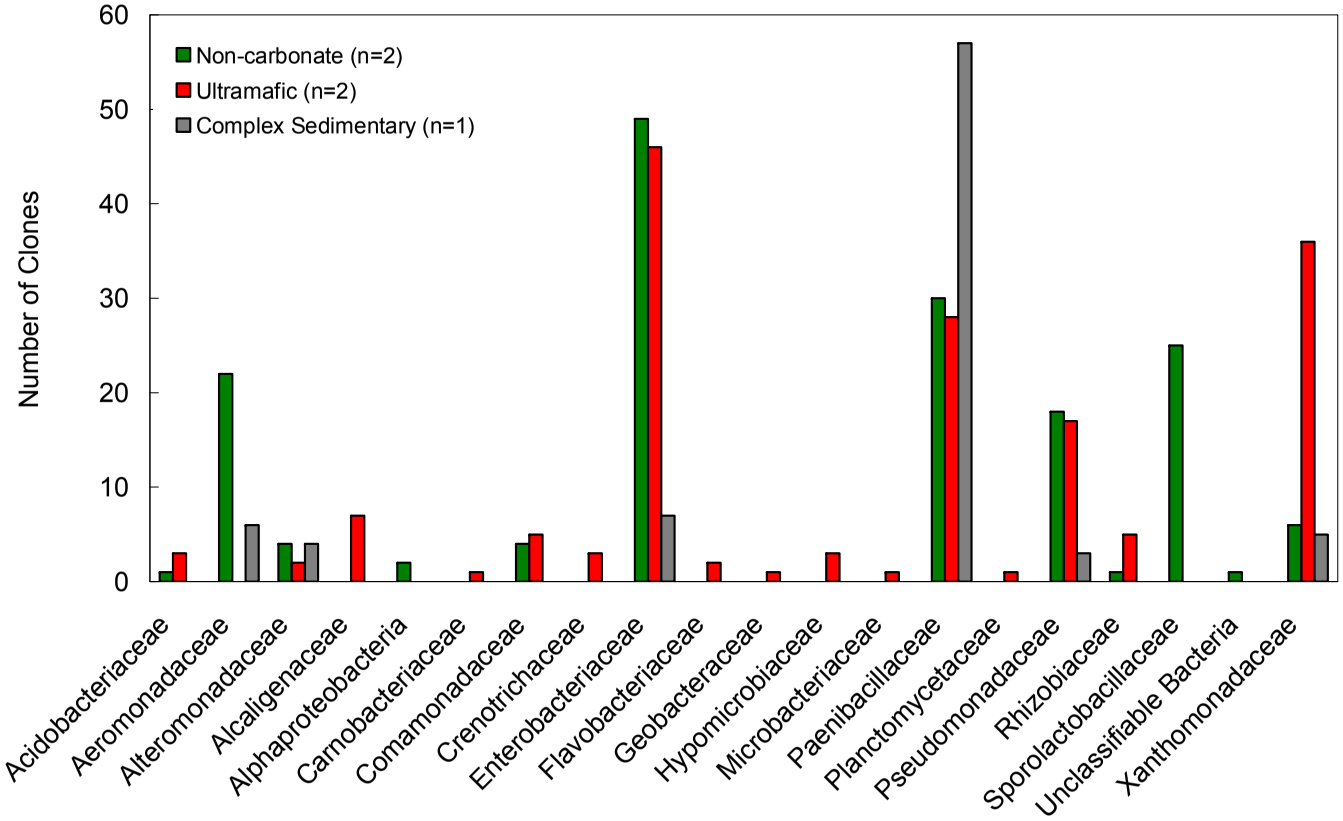


Figure 2.8. Distribution of represented bacterial divisions and families detected in the sediment clone libraries in each lithology.

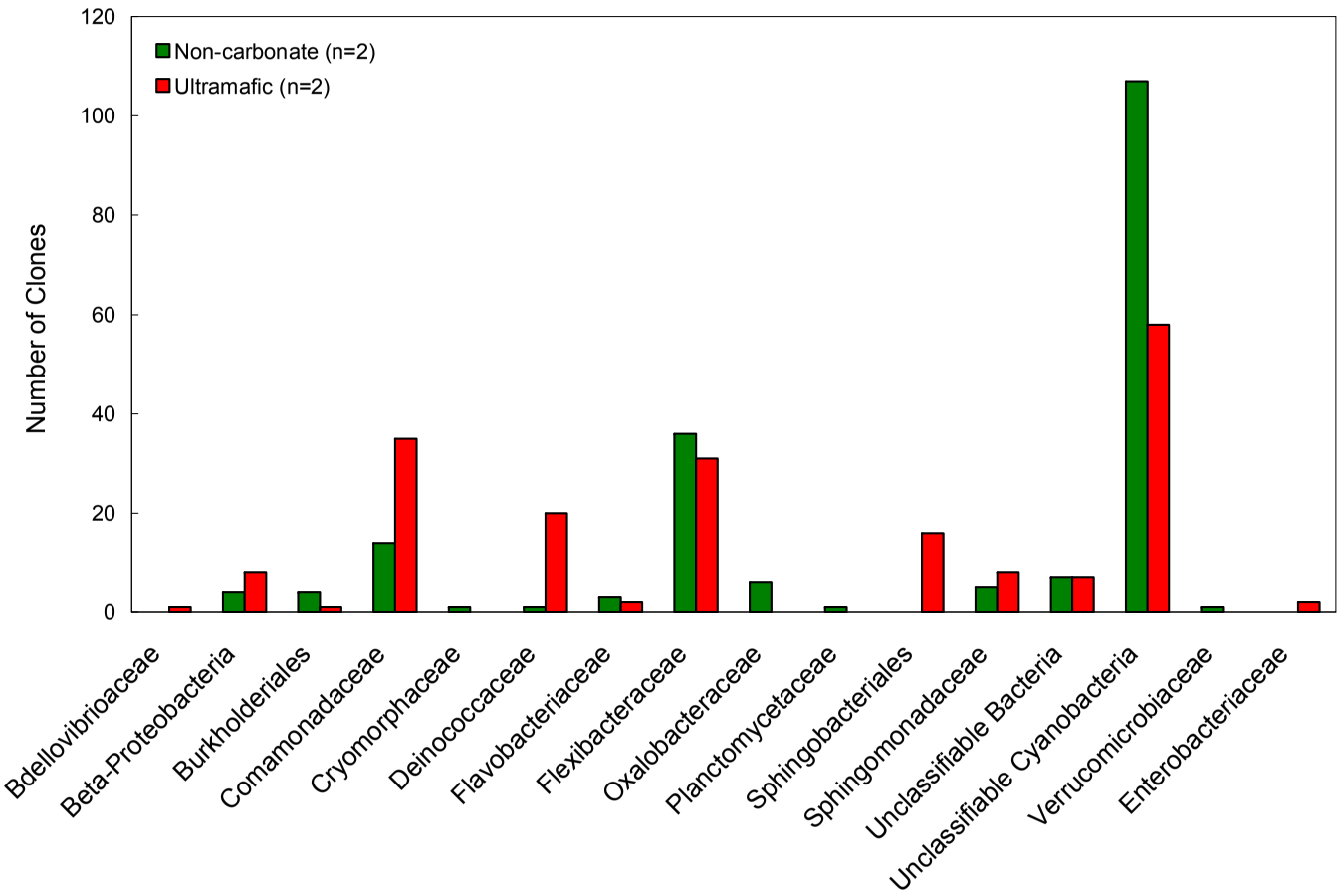


Figure 2.9. Distribution of represented bacterial divisions and families detected in the epilithon clone libraries in each lithology.

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Table A.1. Phylogenetic affiliation of clones from sediment and epilithon samples.

Habitat	Phylogenetic affiliation		Representative	No. of	Closest Relative (cultured or uncultured)			
	Class (Domain*)	Family (*other)	clone	clones	GenBank descriptor	Accession no.	Similarity (%)	Habitat
Sediment n = 5	*Acidobacteria*	*Acidobacteriaceae*	sedSUM11 gi|41|	4	Ellin7137	AY673303	97	soil
	Actinobacteria	*Microbacteriaceae*	sedSUM01 gi|97|	1	Plantibacter sp. NJ-81 strain	AM396918	99	Antarctica
	Alphaproteobacteria	*Unclassifiable Alphaproteobacteria**	sedSNC08 gi|70|	2	clone Elev_16S_1146	EF019620	99	trembling aspen rhizosphere
		Hyphomicrobiaceae	sedSUM01 gi|76|	3	SM1E02 strain	AF445680	98-99	travertine depositional facies
		Rhizobiaceae	sedSUM01 gi|72|	6	IAM 13558 strain	D13943	98-99	n/a
	Bacilli	*Carnobacteriaceae*	sedSUM11 gi|6|	1	ES-11 strain	AM269906	99	deep sea sediment east Pacific
		Paenibacillaceae	sedSNC08 gi|88|	115	KM8 strain	AJ011321	92-98	humus bacteria of finnish spruce stands
		Sporolactobacillaceae	sedSNC08 gi|75|	25	JCM 3417 strain	AB374519	89-99	spoiled OJ lactic acid bacteria
	*Bacteria**	*Unclassifiable Bacteria**	n/a	1	n/a	n/a	>80	
	Betaproteobacteria	*Alcaligenaceae*	sedSUM11 gi|25|	7	strain CBMAI 709	DQ413030	98	n/a
		Comamonadaceae	sedSNC03 gi|77|	9	isolate 5G35/clone SIB2 4D	DQ62893	99	subglacial water or ice or sediment
	Deltaproteobacteria	*Aeromonadaceae*	sedSNC08 gi|15|	28	RBE2CD-51 taxon 415049	EF111230	88-100	Bogota River
		Geobacteraceae	sedSUM01 gi|49|	1	strain PLY 4	EF527234	96	n/a
	Flavobacteria	*Flavobacteriaceae*	sedSUM01 gi|17|	2	strain WB 2.4.44	AM167565	99	hard water creek Westharz Mountains, Germany
	Gammaproteobacteria	*Enterobacteriaceae*	sedSNC03 gi|87|	102	strain Nj-54	AM491461	94-100	Antarctica
		Pseudomonadaceae	sedSUM01 gi|85|	38	strain Nj-55	AM409368	99-100	Antarctica
		Shewanellaceae	sedSNC08 gi|67|	10	strain ML-S2	AF140016	99-100	lake sediments, California USA
		Xanthomonadaceae	sedSUM01 gi|95|	47	strain N11	EF423369	95-100	Antarctica
	Planctomycetacia	*Planctomycetaceae*	sedSUM01 gi|73|	1	clone Elev_16S_1885	EF020316	97	trembling aspen rhizosphere
	Sphingobacteria	*Crenotrichaceae*	sedSUM11 gi|60|	3	clone AKYG1020	AY921733	97	farm soil Minnesota USA
Epilithon n = 4	*Alphaproteobacteria*	*Sphingomonadaceae*	sedSUM11 gi|15|		clone Dolo_14	AB257639	95	subsurface dolomite rock in Alps
	*Bacteria**	*Unclassifiable Bacteria**	n/a	14	n/a	n/a	>80	n/a
	Betaproteobacteria	*Betaproteobacteria**	epiSUM11 gi|77|	12	clone 124ds10	AY212575	94	water with fecal contamination
		*Burkholderiales**	epiSNC03 gi|1|	14	clone Spb132	AJ422163	99	biofilm of polluted river, Spittelwasser River
		Comamonadaceae	epiSUM11 gi|31|	49	clone ANTLV1_B06	DQ521474	99-100	lake ice cover, Antarctica
		Oxalobacteraceae	epiSNC03 gi|27|	6	strain FXS9	AY315179	90	subglacial sediment southern hemisphere
	*Cyanobacteria**	*Unclassifiable Cyanobacteria*	epiSNC08 gi|100|	165	strain p126	AJ536453	97-98	*Fragilaria striatula* chloroplast 16S rRNA gene
	Deinococci	*Deinococcaceae*	epiSUM11 gi|102|	21	strain 6A4-2	EU029136	89	radionuclide contaminated soil
	Deltaproteobacteria	*Bdellovibrionaceae*	epiSUM01 gi|7|	1	clone LR A2-27	DQ988308	96	A2 reactor
	Flavobacteria	*Flavobacteriaceae*	epiSNC08 gi|66|	5	strain WB 2.1-3	AM167557	98	hard water creek Westharz Mountains, Germany
	Gammaproteobacteria	*Enterobacteriaceae*	epiSUM11 gi|96|	2	strain PTB2092	DQ862543	99	maple sap tubing biofilm
	Planctomycetacia	*Planctomycetaceae*	epiSNC03 gi|71|	1	strain JW10-3f1	AF239695	99	Australian lake
	Spingobacteria	*Flexibacteraceae*	epiSUM11 gi|19|	67	isolate GWF20A	AJ011696.1	96-97	oligotrophic cave water system
		*Sphingobacteriales**	epiSUM01 gi|72|	16	clone AKYG1727	AY921801	97	farm soil Minnesota USA
	Verrucomicrobiae	*Verrucomicrobiaceae*	epiSNC03 gi|90|	1	DEV005	AJ401105	96	Elbe River biofilm, Germany

Table A.2. Diversity Indices (Chao1, Shannon, ACE, and Simpson) for 16S rRNA clone libraries.

sediment vs. epilithon					non-carbonate vs. ultramafic				
Site	**Chao1**	**ACE**	**Shannon**	**Simpson**	**Site**	**Chao1**	**ACE**	**Shannon**	**Simpson**
EpiSNC03	48.20	46.52	2.92	0.08	EpiSNC03	48.20	46.52	2.92	0.08
EpiSNC08	21.25	23.44	2.26	0.20	EpiSNC08	21.25	23.44	2.26	0.20
EpiSUM01	57.50	64.89	2.85	0.10	SedSNC03	33.25	34.87	2.65	0.09
EpiSUM11	36.33	41.53	2.77	0.10	SedSNC08	26.50	25.12	2.19	0.15
average	**40.82**	**44.10**	**2.70**	**0.12**	**average**	**32.30**	**32.49**	**2.50**	**0.13**
std error	**7.83**	**8.52**	**0.15**	**0.03**	**std error**	**2.92**	**2.66**	**0.09**	**0.01**
SedSCS07	24.50	21.64	1.51	0.41					
SedSNC03	33.25	34.87	2.65	0.09	EpiSUM01	57.50	64.89	2.85	0.10
SedSNC08	26.50	25.12	2.19	0.15	EpiSUM11	36.33	41.53	2.77	0.10
SedSUM01	30.00	40.42	2.38	0.14	SedSUM01	30.00	40.42	2.38	0.14
SedSUM11	37.00	33.06	2.61	0.09	SedSUM11	37.00	33.06	2.61	0.09
average	**30.25**	**31.02**	**2.27**	**0.18**	**average**	**40.21**	**44.98**	**2.65**	**0.11**
std error	**2.26**	**3.39**	**0.21**	**0.06**	**stdev**	**5.98**	**6.90**	**0.10**	**0.01**

Table A.3. AMOVA results at each level of partitioning.

Source of Variation	df	Sum of Squares	Variance Components	% Variation
A) All Clone Libraries (n = 9)				
Among populations within streams	8	10873.665	14.967***	22.0
Within populations	777	41322.302	53.182	78.0
Total	785	52195.967	68.149	
B) Sediment (n = 5) vs. Epilithon (n = 4)				
Between habitat	1	6587.309	15.202**	20.3
Among populations within habitat	7	4286.357	6.42***	8.6
Within populations	777	41322.302	53.182***	71.1
Total	785	52195.967	74.803	
C) Sed. UM (n = 2) vs. Sed. NC (n = 2)				
Between lithologies	1	699.291	2.305	2.8
Among populations within lithology	2	2138.35	12.281***	14.7
Within populations	321	23650.942	73.679***	88.1
Total	324	26488.582	83.655	
D) Epi. UM (n = 2) vs. Epi NC (n = 2)				
Between lithologies	1	866.928	2.838	3.8
Among populations within lithology	2	658.282	2.733***	3.6
Within populations	375	26310.203	70.161***	92.64
Total	378	26310.414	75.731	

*** $p < 0.0001$
** $p = 0.01$

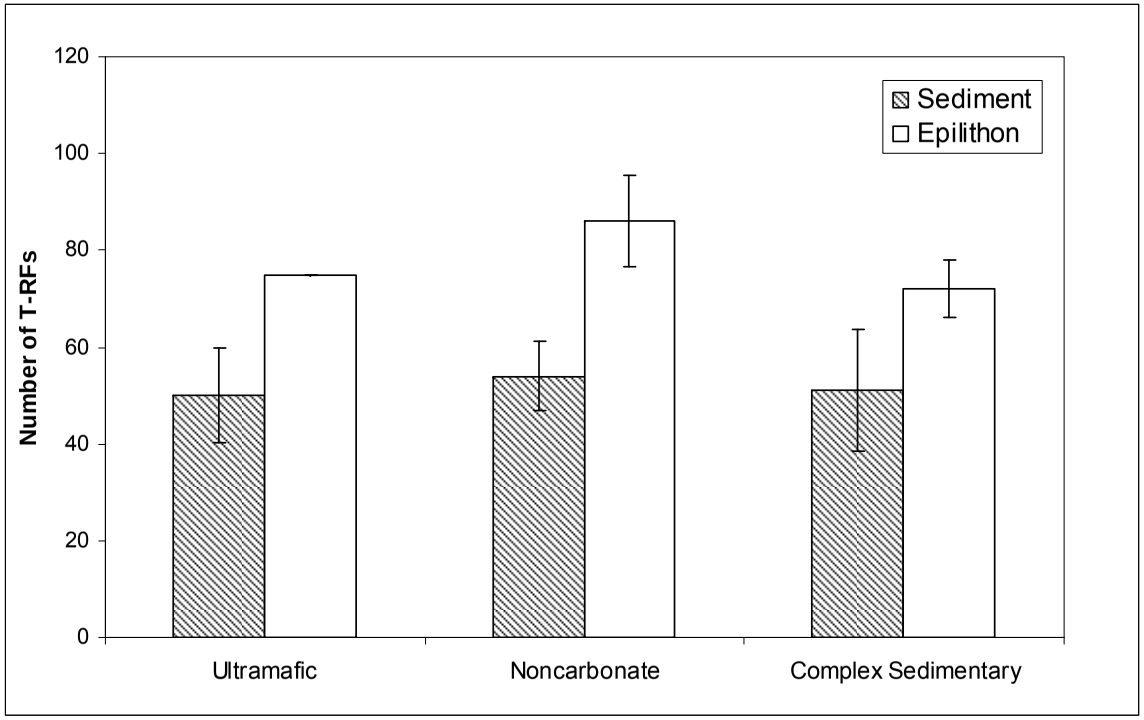

Figure A.1. Distribution of T-RFs by habitat within each lithology.

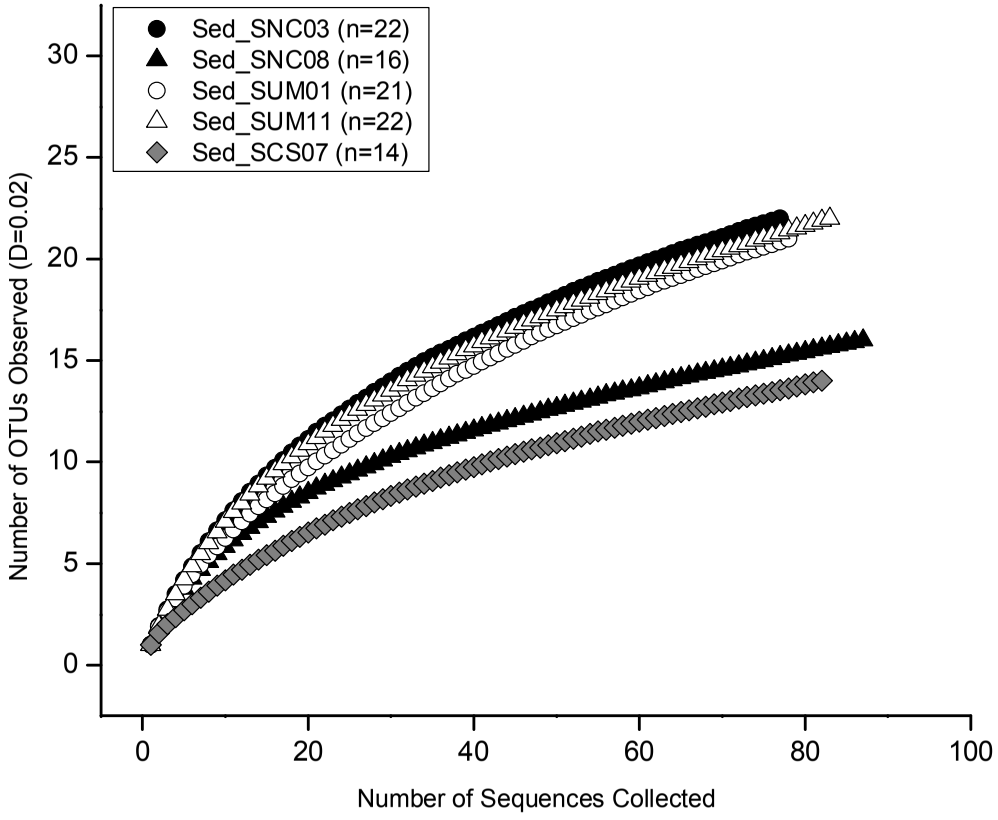

Figure A.2. Rarefaction curves for the five sediment clone libraries (n = number of OTUs observed).

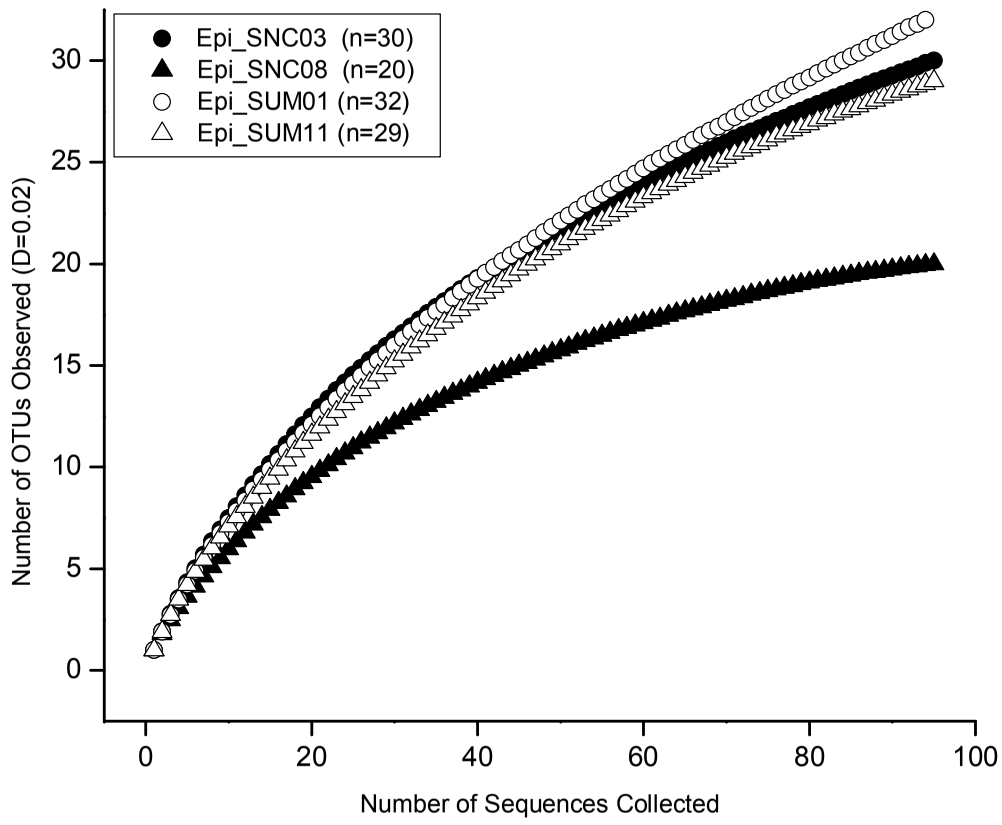

Figure A.3. Rarefaction curves for the four epilithon clone libraries (n = number of OTUs observed).

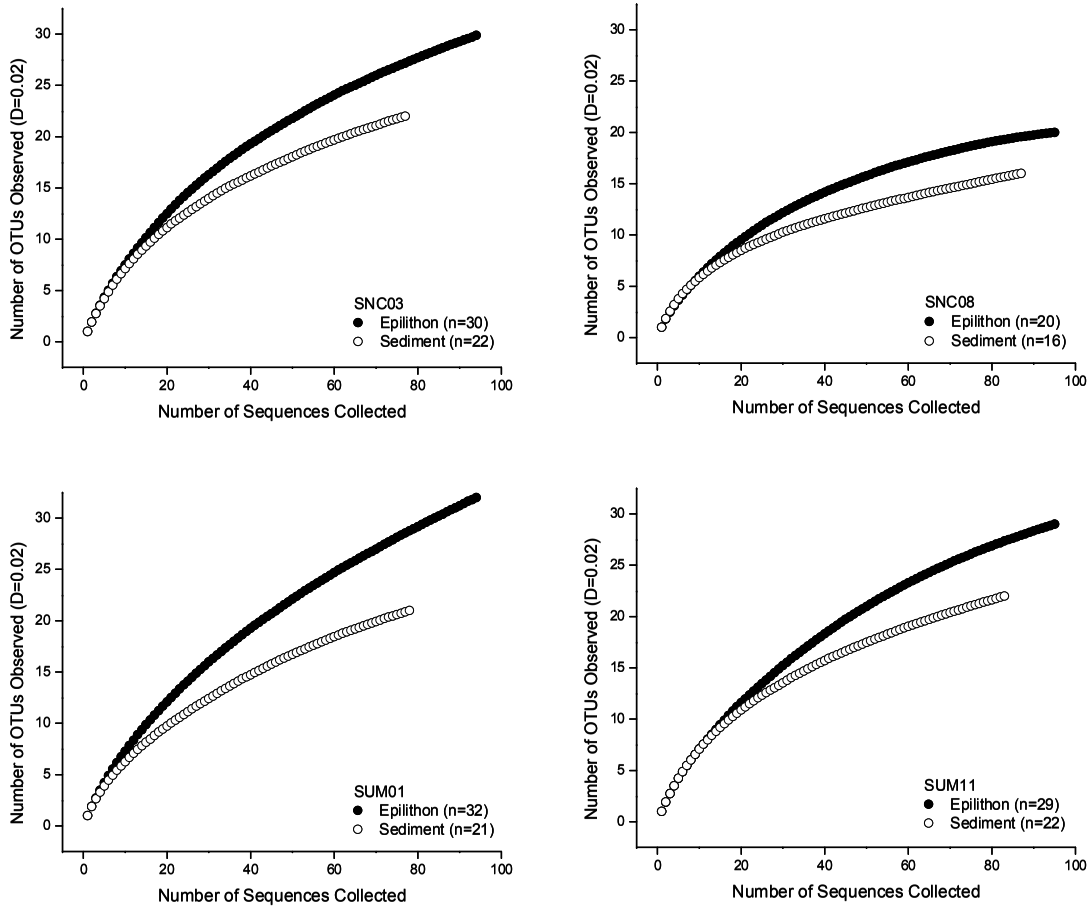

Figure A.4. Rarefaction curves for sediment and epilithon clone libraries at individual stream sites (n = number of OTUs observed).

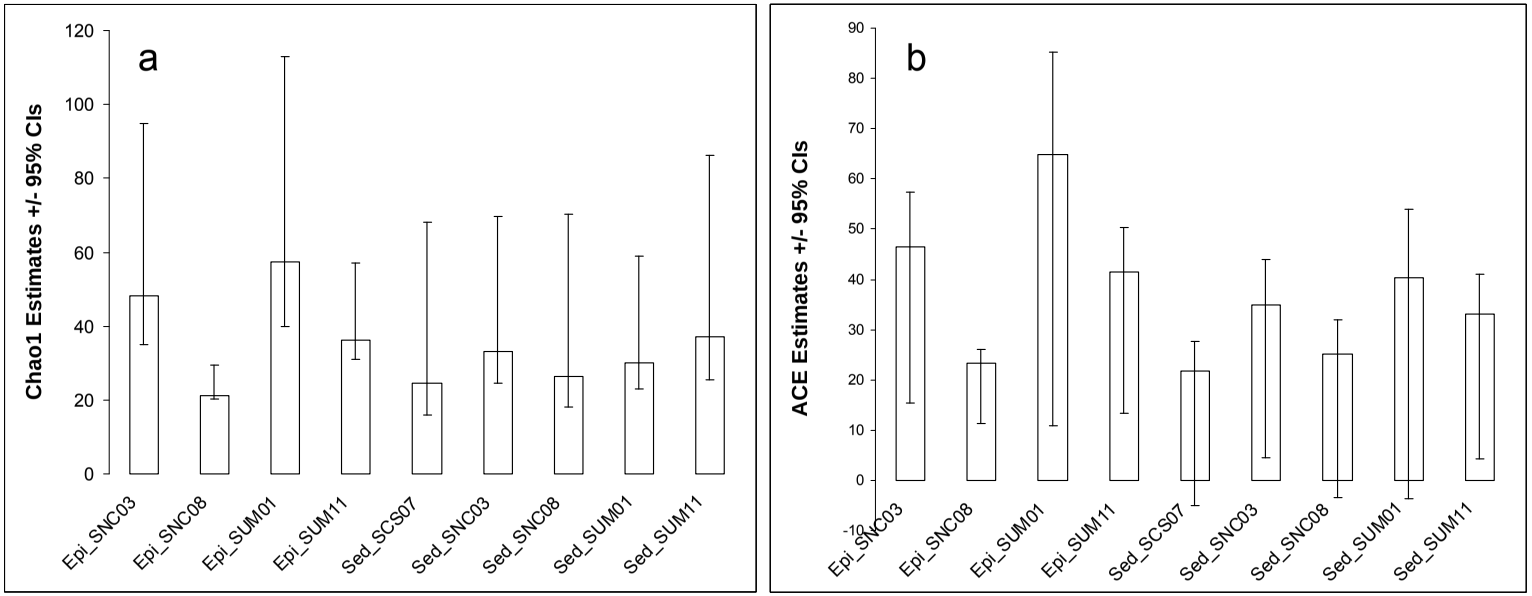

Figure A.5. Diversity richness estimators, (a) Chao1 and (b) ACE, with 95% confidence intervals.