Microbial Succession in Glacier Foreland Soils

by

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ABSTRACT

Alpine glaciers have been retreating since the Little Ice Age, leading to exposure of foreland soils. Microorganisms are the primary below ground biological influence on nutrient cycling in recently deglaciated soil and are linked to down valley vegetation colonization. Previous studies demonstrate high turnover rates of bacterial communities within the first 50 years following glacier retreat, coinciding with plant colonization. It thus remains unclear whether turnover occurs as a result of changing conditions after glacier retreat, or from the effects of plant colonization. Using high throughput sequencing of 16S rRNA genes and standard soil chemistry analysis, I examined the trends in both bacterial diversity and soil chemistry to address my central hypothesis: bacterial community turnover will be linked to glacier retreat in newly deglaciated soils and plant colonization in more developed soils. Changes in bacterial community structure were examined in 42 samples collected from two chronosequences within the foreland soils of Duke River, located in Kluane National Park Reserve, Yukon. The chronosequences contain up to 220 years of non-vegetated soils before an appreciable grassline, therefore allowing extended assessment of bacterial succession in bare soils before analyzing changes following plant colonization. I determined the existence of three successional groups within both chronosequences; an “early” group in soils of less than approximately 50 years since deglaciation; an “intermediate” group within bare soils after deglaciation but before the grassline; and a “grassline” group following plant colonization. These results suggest the high turnover after glacier retreat occurs as a result of glacier

retreat itself, and the later colonization by plants is associated with a second period of turnover linked with changes in soil chemistry properties. This work elucidates and provides further insight into the processes of microbial succession, which may serve to enhance our understanding of ecological development following environmental disturbances.

Preface

Acknowledgments

I would like to thank Dr. Brian Lanoil for accepting me into his lab as a largely unproven and inexperienced undergraduate student. Dr. Lanoil recognized my passion for learning and determination to always improve and allowed me to join the lab and prove my merit. The experience and knowledge I have gained while working under his guidance for the past five years has transformed and shaped who I am and enabled me to begin to realize my full potential. For this, I am extremely grateful. I would also like to thank my parents for their advice and steadfast support, my fiancée Danielle for ensuring I always stay balanced, and my fellow lab members Ann Hammad and Ido Hatam for their substantial assistance in the lab. And thank you to my examining committee, Sylvie Quideau and Yan Boucher for guiding this project.

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CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

Succession is the change in a biological community that occurs over time following a disturbance (Horn 1974; Walker *et al.* 2010). Glacier foreland soils provide an ideal model to study succession as the retreat of glaciers results in a chronosequence, a space-time proxy where increasing distance from the glacier terminus is used as a proxy for increased time since glacier retreat (Schütte *et al.* 2009; Göransson *et al.* 2011). The study of plant succession in these systems has been well characterized, while microbial succession is in the earliest stages of investigation (Chapin *et al.* 1994; Fastie 1995; Stocklin & Baumler 1996; Willis *et al.* 1997; Sigler *et al.* 2002; Tscherko *et al.* 2003; Nicol *et al.* 2005; Deiglmayr *et al.* 2006; Kandeler *et al.* 2006; Nemergut *et al.* 2007; Schütte *et al.* 2009, 2010; Zumsteg *et al.* 2012; Brown & Jumpponen 2013). However, recent advances in high throughput sequencing methods have allowed greater insight into these processes, and a series of studies have begun to detail microbial succession in these systems (Schütte *et al.* 2010; Brown & Jumpponen 2013). In this literature review, current and past studies of proglacial microbial succession are examined along with essential related background context including biodiversity, biogeography, and previous studies of succession in plants. Knowledge of these concepts is critical in understanding the various factors at play when examining microbial succession in glacial foreland soil systems.

I will begin with discussion of biodiversity, which can be defined as the number of species in a system along with their function in that system (Nannipieri

et al. 2003). Understanding how an ecosystem will respond to disturbances is strongly associated with its biodiversity (Nannipieri *et al.* 2003). For example, a large number of different species in an ecosystem will likely result in a more stable system that can better respond to disturbances due to the functional redundancy incurred with more species; this is referred to as the insurance hypothesis (Nannipieri *et al.* 2003). Therefore, determining how the biodiversity of a microbial community changes over time in response to the disturbance of glacier retreat will enable a more comprehensive understanding of microbial community dynamics following disturbance.

Next, I will discuss the concept of biogeography, which is the study of spatial and temporal distributions of species (Green *et al.* 2008). Biogeography investigates how and why organisms in an area are where they are and can be categorized into two main areas: ecological biogeography and historical biogeography (Martiny *et al.* 2006). Ecological biogeography examines present day factors involved in a species distribution (Martiny *et al.* 2006). Ecological biogeography answers questions such as: *Why is a species currently confined to its location? What enables species to live where they do? What is preventing species from expanding into other areas? What roles do environmental parameters play in species distribution?* On the other hand, historical biogeography examines past events responsible for a species current distribution (Martiny *et al.* 2006). Historical biogeography answers questions such as: *How did a species come to be present in its current location? When did a species become restricted to that area? How have geological or climatic events*

influenced distribution? In glacier foreland soils, examining the distribution of bacterial communities over the foreland following glacier retreat and determining the complex underlying variables that shape these distributions will lead to greater insight and a more holistic view into the successional processes occurring within the communities over time.

This literature review will also highlight and evaluate past studies of plant succession in glacier foreland systems. Plant succession has been well studied and several prominent studies have emerged detailing these processes in glacier foreland soils, particularly at Glacier Bay, Alaska (Chapin *et al.* 1994; Fastie 1995; Stocklin & Baumler 1996; Willis *et al.* 1997). I will explore the mechanisms and pathways of plant succession in glacier forelands, such as whether succession is deterministic or stochastic and the significance of specific functional groups on the community as a whole. These insights will help with interpretation of microbial succession pathways.

Lastly, I will discuss microbial succession in glacier foreland soils. This is the central focus of this literature review and will be discussed in depth. To fully understand the succession processes in glacier forelands, we must first acknowledge that bacteria are present and active in subglacial environments (Skidmore *et al.* 2000, 2005; Lanoil *et al.* 2009). Therefore, a brief overview of subglacial microbiology will be discussed before reviewing proglacial successional processes.

1.2 Biodiversity

Biodiversity is an important concept in microbial ecology and ecology generally. It is a broad term that encompasses the species richness (i.e. the number of species present, (18)), species evenness (i.e. the relative abundance of each species (17)), community composition (i.e. the identity of the species present (19)), as well as interactions between species, such as trophic and symbiotic interactions (Chapin *et al.* 2000). Alpha diversity describes the within-site diversity of a system whereas beta diversity compares diversity between sites (Shmida & Wilson 1985). Each of these components of biodiversity is crucial in influencing ecosystem processes.

Describing biodiversity has typically involved analyzing phylogenetic diversity (i.e. describing the evolutionary history of a community), however this information typically does not provide much information about the functional roles organisms perform and how this impacts communities and ecosystems (Petchey & Gaston 2006). This is an important distinction because organisms that are phylogenetically similar (i.e. share similar evolutionary history) may have different functionality; thus, when species are operationally defined according to such definitions as 16S rRNA gene sequence similarity, which provides extensive taxonomic information but little information about functionality, their grouping into operational taxonomic units may be misleading (Torsvik & Øvreås 2002). There are many approaches used to link phylogenetic diversity with function; in molecular ecology these typically include analyzing rRNA genes and genes encoding for enzymes involved in functional processes with environmental

parameters (Torsvik & Øvreås 2002). Using these methods, researchers have been able to acquire knowledge about key groups of bacteria involved in fundamental physiological processes (Torsvik & Øvreås 2002).

1.2.1. Role of Biodiversity in Ecosystems

The role of biodiversity in ecosystems is a fundamental question in ecology and has been the focus of extensive literature (Johnson *et al.* 1996; Finlay *et al.* 1997; Ohtonen *et al.* 1997; Loreau *et al.* 2001; Bell *et al.* 2005; Langenheder *et al.* 2010). There is not space here to exhaustively review the biodiversity literature. Therefore, in this section I will explore two specific roles of biodiversity in ecosystems: increased stability and increased ecosystem function and efficiency.

The insurance hypothesis is that the role of biodiversity is to increase stability of ecosystem function (Yachi & Loreau 1999; Wittebolle *et al.* 2009). The overall concept of the insurance hypothesis states that high biodiversity allows ecosystems to maintain high-level functioning despite changing conditions (Yachi & Loreau 1999). This is because high biodiversity allows for the coexistence of many species, therefore producing a community with high functional redundancy due to multiple species performing the overlapping functions (species redundancy) and high relative abundance of those species (species evenness). Thus, when conditions change, there will be a greater likelihood, or insurance, of a species performing a particular function to be resistant to that change, and therefore that function will not be lost (Yachi & Loreau 1999; Wittebolle *et al.* 2009). A subsequent consequence of high biodiversity therefore is an increase in

the resistance and resilience of an ecosystem to changing conditions (Chapin *et al.* 2000; Wertz *et al.* 2007). Resistance is an ecosystem's ability to cope with a disturbance while resilience is an ecosystem's ability to recover following a disturbance. A higher species richness, for instance, should increase the resistance and resilience of an ecosystem since an increased number of species can therefore perform a greater range of functions and thus decrease the chance that key functional processes are not lost following a disturbance (Pfisterer & Schmid 2002). For example, soil microcosms with lower species richness had lower ecosystem functioning (reduced denitrification and nitrite oxidation activity) than those with higher species richness following a heat treatment (Wertz *et al.* 2007). Thus, decreased species richness is associated with higher sensitivity to disturbance. Species evenness is also an important determinant of ecosystem stability and thus of ecosystem functioning; when stressors are introduced to an environment, high species evenness usually allows for resistance to that particular stressor and preservation of the function, as there is a greater chance that a resistant species is present in a high enough abundance to have a significant influence in resisting the change (Wittebolle *et al.* 2009). However, when species evenness is low, and the community is dominated by one or a few species, the probability that these few species are resistant to the stressor is much lower (Wittebolle *et al.* 2009). For example, microcosms containing microbial communities with lower evenness (with identical species richness) had decreased net denitrification (used as measure for ecosystem functioning)

following a salt stress disturbance (Wittebolle *et al.* 2009), indicating that these low evenness systems are more sensitive to disturbance.

An inherent problem in determining the influence of biodiversity on the stability of an ecosystem is the definition of stability itself. Ives and Carpenter discuss this problem and describe six different interpretations of stability based on the intrinsic dynamics of an ecosystem, including alternative stable states, nonpoint attractors, pulse perturbation, press perturbations, extinctions, and invasions (Ives & Carpenter 2007). For example, a pulse perturbation describes a system whereby a system only a single equilibrium point; when an environmental perturbation disturbs this established equilibrium, the diversity changes in response. Thus, if these disturbances occur rarely, one way to measure the stability of this system would be to measure the rate at which the system returns to equilibrium, with faster rates indicating increased stability (Ives & Carpenter 2007). In a press perturbation on the other hand, an environmental perturbation causes a permanent change in the biodiversity of a system; thus, in a more stable system, biodiversity changes minimally in response to the disturbance, or, can endure a greater press perturbation before the biodiversity begins to change (Ives & Carpenter 2007). However, ecosystems are complex and finding a single model that accurately describes the dynamics involved is unlikely; rather, most ecosystems are described by several different models of stability. This is an important detail to consider when examining studies of stability.

Another role of biodiversity is in increasing ecosystem functioning (Loreau

et al. 2001; Bell *et al.* 2005; Langenheder *et al.* 2010). The complementarity and selection mechanisms are two competing hypotheses used to understand the relative impact of biodiversity on ecosystem functioning (Bell *et al.* 2005). The selection mechanism states that communities with relatively high species richness are more productive on average because they are more likely to contain species that have a large impact on ecosystem functionality (Bell *et al.* 2005). Meanwhile, the complementarity mechanism states that, since each species in a community occupies a slightly different niche, and thus has subtly different resource utilization, as species richness increases the community as a whole is more productive because resources are being utilized at a higher efficiency (Bell *et al.* 2005). Thus, in the complementarity mechanism, increased species richness affects ecosystem functionality linearly if species are completely complementary, and has a decelerating impact on ecosystem functioning if species are complementary but, to some degree, functionally redundant (Bell *et al.* 2005). For example, increasing species richness (independent of the composition of the communities) in microcosms leads to an increasing daily respiration rate (used as a proxy for ecosystem functioning) (Bell *et al.* 2005). The relationship was decelerating, but did not reach an asymptote even after all 72 species used in the microcosm were included, indicating further increases in richness still would increase ecosystem functioning (Bell *et al.* 2005). Thus, the complementarity mechanism appears to be a primary means by which increased richness leads to increased ecosystem functioning and efficiency.

It is important to keep in mind the complementarity and selection

mechanisms are not as pronounced in natural microbial systems as in microcosm experiments due to the extremely high overall functional redundancy in natural environments; i.e. since bacterial communities are already so diverse and saturated, the addition or subtraction of species will not have as significant an effect on the overall ecosystem functionality in natural microbial communities (Bell *et al.* 2005). Overall, however, the complementarity and selection mechanism hypotheses demonstrate the effect of biodiversity on ecosystem functioning, and imply that increasing biodiversity of an ecosystem will, at least to a certain point, increase the functionality of the ecosystem (Bell *et al.* 2005).

Biodiversity studies have indicated that ecosystem function and stability are dependent upon maintenance of high overall diversity (Yachi & Loreau 1999; Loreau *et al.* 2001; Bell *et al.* 2005; Wittebolle *et al.* 2009; Langenheder *et al.* 2010). However, these studies and the underlying theory do not explain why specific species are found in some areas and not others and therefore does not address a major question that arises regarding bacteria: is there a microbial biogeography?

1.3 Biogeography

Biogeography is important in understanding the distribution of organisms, including understanding why certain species are present in a particular geographic area, how species respond to changes in their environment, and how different species coexist with one another (Green *et al.* 2008). There are two main subsets of biogeography: historical biogeography and ecological

biogeography (Martiny *et al.* 2006). Historical biogeography examines how historical events influence contemporary biogeographical patterns whereas ecological biogeography examines present day factors influencing species' distributions (Martiny *et al.* 2006). An example of historical biogeography is the formation of Gondwana, a supercontinent existing approximately 510 to 180 million years ago (Queiroz 2005). The formation of Gondwana allowed organisms normally confined to a single continent to disperse among a number of continents, thus influencing present day biogeographical distributions of those organisms (Queiroz 2005); indeed, researchers now refer to certain species' as having Gondwanan distribution (Donoghue & Moore 2003). Ecological biogeography, on the other hand, focuses on the contemporary environmental or habitat features that influence the distribution of organisms (Hanson *et al.* 2012). In a meta analysis of studies investigating the relationship between environmental variables and microbial community structure, 92.6% of the studies showed significant correlations between environmental variables and microbial community composition, thus demonstrating the important role of contemporary influences on community composition (Hanson *et al.* 2012).

The vast majority of literature examining biogeography has focused on plant and animal species and only recently, due to the rise of culture-independent microbial diversity tools (Muyzer & Smalla 1998; Shokralla *et al.* 2012), have studies examining microbial biogeography arisen (Crump *et al.* 2004; Green *et al.* 2008; Pagaling *et al.* 2009). This chapter discusses two main hypotheses of microbial biogeography: 1) cosmopolitan distribution (i.e. everything is

everywhere – the environment selects) and 2) endemic distribution (i.e. geographic barriers limit microbial dispersal) (Cho & Tiedje 2000; Fierer & Lennon 2011).

1.3.1 Cosmopolitan Distribution Hypothesis

A cosmopolitan distribution of microbes is commonly associated with the long-standing theory that 'everything is everywhere – the environment selects' (O'Malley 2007). This theory was proposed by Lourens Baas-Becking and was founded on work by Martinus Beijerinck who endorsed the notion that there are small or no barriers for transport of microorganisms (41) and, therefore, environments with similar conditions have similar microbial communities (O'Malley 2007). In this section I will discuss two prominent explanations of why microbes should theoretically be distributed everywhere: dispersal capacity and metabolic plasticity.

Dispersal capacity is the maximum distance an organism can travel in its lifetime (Martiny *et al.* 2006). Owing to their small size, microbes have a high dispersal capacity and as such, may not be restricted by geographic barriers due to their susceptibility of being transported large distances by stochastic environmental events such as wind, water and biological vector dispersal (Finlay & Fenchel 2004). This mechanism of dispersal capacity is referred to as passive dispersal, and occurs when an organism is propelled by external forces such as ocean or wind currents and is in contrast to active dispersal which occurs when an organism is able to propel itself, such as with flagella (Martiny *et al.* 2006). For example, studies have demonstrated the ability of microbes to be passively

transported from high temperature subsurface petroleum environments to cold, Arctic marine sediments through seabed flow (Hubert *et al.* 2009), and have linked super-volcano eruptions to the cosmopolitan distribution of certain thermophilic microbes (Herbold *et al.* 2014). Furthermore, geographic distance has been demonstrated as having less impact on microbial community composition than environmental conditions; for example, the type of carbon source input was the only determinant of microbial species enriched from differing soil types collected 1km apart (Wawrik *et al.* 2005) and likewise the relative abundance of certain microbes was identical when collected from ecologically similar but geographically distinct grasslands on a global scale (Finlay *et al.* 2001). Several studies have also indicated that pH is a better determinant of bacterial community composition than geographic distance in soils, thus providing support that local edaphic factors select for specific bacterial taxa (Fierer & Jackson 2006; Lauber *et al.* 2009). Overall, high dispersal capacity provides strong evidence behind the cosmopolitan distribution of organisms; evidence indicates microbes can disperse on a global scale and that environmental conditions rather than geographic distance determines microbial community composition. An important consideration of cosmopolitanism is that is organisms may appear to have cosmopolitan distribution at lower phylogenetic resolution but endemic distribution at higher levels; for example the genus of a species may have a global distribution, but the individual species can have an endemic distribution (Papke *et al.* 2003). Thus the observed biogeographical patterns may differ depending on the phylogenetic resolution used.

The metabolic plasticity of microorganisms is another factor that could contribute to a cosmopolitan distribution of microbes. Through mechanisms such as horizontal gene transfer, along with plasmid and genomic island acquisition, microbes are able to display a high range of functional and metabolic plasticity (Dobrindt *et al.* 2003). This therefore may allow microbes the ability to colonize a wide variety of environments regardless of harsh conditions; for example studies have demonstrated that certain microbes have the genetic ability to transition from fast growth in nutrient-rich conditions to slow growth in nutrient-poor conditions (Yooseph *et al.* 2010) thus allowing adaptation to unfavorable novel environments encountered during dispersal.

Overall, based on theoretical mechanisms such as high dispersal capacity and metabolic plasticity that imply ease of dispersal, along with direct evidence that environment rather than geographic location can determine the types of microbes present, there is considerable support for the cosmopolitan distribution hypothesis and the theory that 'everything is everywhere - the environment selects.'

1.3.2 Endemic Distribution Hypothesis

Despite the strong theoretical arguments and evidence for cosmopolitanism in microorganisms, the concept creates difficulties. For example, speciation is usually thought to occur as the result of genetic isolation due to geographical or environmental barriers (Douady *et al.* 2003; Yesson *et al.* 2009; Casteleyn *et al.* 2010); such barriers are difficult to account for with complete cosmopolitanism. Furthermore, construction of communities of

interacting organisms often requires adaptations by individual members (Douady *et al.* 2003); such adaptations would be difficult if communities were assembled stochastically, as is implied by complete cosmopolitanism. These difficulties raise the question: could there be a biogeography of microorganisms? In other words, could microorganisms have an endemic distribution after all?

The principle behind the endemic microbial distribution hypothesis is that microbes are constrained by geographical and environmental barriers. Endemic distribution implies that, as there are barriers to distribution, speciation can occur through an allopatric mechanism (Douady *et al.* 2003; Yesson *et al.* 2009; Casteleyn *et al.* 2010). Thus, microbial communities can be dependent upon the source of organisms such as different land use types (Bowers *et al.* 2011) or by highly specific environments that select for discrete microbial species. The latter is most prominently observed in extremophiles; for example, *Sulfolobus* strains are genetically distinct even when isolated from geochemically indistinguishable hot springs (Whitaker *et al.* 2003) and sea ice microbes may be uniquely found in either Arctic or Antarctic sea (Mock & Thomas 2005).

Thus, both cosmopolitan and the endemic distribution are well supported. It is possible that endemism may be found in a range for microorganisms, from global cosmopolitanism to small-scale local endemism and that individual environments may be more or less selective for endemic organisms. Thus, the microorganisms in some environments, such as acidic hot springs (Whitaker *et al.* 2003) may be entirely endemic, while in other environments, the organisms may have entirely cosmopolitan distribution (Chu *et al.* 2010); yet others may

have a mix of organisms with differing levels of endemism (Herbold *et al.* 2014). This complexity creates conceptual difficulties for successional studies: how does the changing community assemble? Do new species present arise via invasion (i.e. cosmopolitanism) or speciation (i.e. endemism)? Is the assembly of the community stochastic and dependent upon which global microbes are deposited in the system, or is it highly directional and specifically dependent upon the starting community composition? In other words, is the change observed in communities over time due to changes in the environment leading to a new "environment selects" and any organism from anywhere could be selected for, or is it due to a highly ordered "hand-off" of functional duties from one species to the next, i.e. succession? Thus, analysis of glacier foreland soil microbial communities must be interpreted in the same way – there may be a mix of both cosmopolitan organisms that are hallmarks of these systems as well as endemic species (possibly originating in subglacial ice) that are unique to that particular foreland or region.

1.4 Succession

The previous discussions of biodiversity and biogeography have assumed that microbes are in a steady state. However, what happens when microbes are disturbed (i.e. when the environment that microbes are colonizing is altered) and how does this influence community composition and distribution? How does the magnitude or type of disturbance affect communities? And more specifically, is glacier retreat a disturbance that affects microbial communities (thus implying the

existence of microbial communities beneath glaciers)? These ideas are explored in this discussion of succession.

Succession is defined as the change in species composition and/or structure over time following a disturbance (Horn 1974; Walker *et al.* 2010). Progressive succession occurs when biomass, nutrient availability and vegetation increase over time, and retrogressive succession occurs when these parameters decrease over time (Walker *et al.* 2010). Primary microbial succession is ecosystem development following a disturbance leaving no trace of life and occurs relatively infrequently (Nemergut *et al.* 2007) whereas secondary microbial succession is ecosystem development following a disturbance leaving life intact and is more common (Horn 1974). A notable example of microbial primary succession is ecosystem development occurring after a volcanic eruption. Lava flow from the Mount St. Helens eruption of 1980, for example, destroyed all biotic components in the surrounding environment, and therefore development of the ecosystem progressed from colonizing organisms such as pioneer microbes, plants and insects (Bishop 2002). Examples of microbial secondary succession, on the other hand, are more prevalent and include ecosystem development resulting from forest fires (Romme & Knight 1981), slash and burn agriculture practices (Uhl & Murphy 1981), and mining (Titlyanova & Mironycheva-Tokareva 1990).

1.4.1 Succession in Glacier Foreland Soils

Subglacial ecosystems were initially assumed to be abiotic (Skidmore *et al.* 2005). However, detailed examination of these systems revealed abundant

and active microbial communities in subglacial environments, causing reclassification of these systems as biotic (Skidmore *et al.* 2005). With the discovery of subglacial microorganisms, glacial foreland soils are now recognized as secondary successional systems (Schütte *et al.* 2009), in contrast to the previous assumption that deglaciated foreland soils represented primary successional systems (Nicol *et al.* 2005; Schmidt *et al.* 2008).

Glacier forelands provide an ideal model to study succession as the retreat of glaciers results in chronosequences, a space-time proxy where increasing distance from the glacier terminus is used as a proxy for increased time since the disturbance of glacial retreat (Schütte *et al.* 2009; Göransson *et al.* 2011). These systems have been used for extensive studies of plant ecological succession pathways and have begun to receive more focus for studies of microbial ecological succession pathways and mechanisms.

1.4.2. Plant Succession in Glacier Foreland Soils

A prominent plant successional study conducted in the foreland of a 230 year old chronosequence at Glacier Bay, Alaska demonstrated that facilitation was the primary mechanism of plant successional patterns (Chapin *et al.* 1994). Facilitation occurs when colonized species modify the surrounding environment and consequently place themselves at a competitive disadvantage, which thereby facilitates the colonization of later successional species that can better utilize the improved environment (Chapin *et al.* 1994). Plant succession at Glacier Bay was characterized as having four stages: *Epilobium latifolium* (pioneer), *Dryas drummondii*, alder, and spruce stages (Chapin *et al.* 2000).

The *Epilobium* stage occurred within the first 30 years since deglaciation, while the *Dryas* stage occurred between 30 year and 50 years (Chapin *et al.* 1994). *Epilobium* and *Dryas* were together referred to as the early successional species. The soil environment at these stages was characterized by high pH, low organic carbon, low cation exchange capacity and high bulk density (Chapin *et al.* 1994). Later, the presence of *Dryas* was associated with an increase in N accumulation in the soils due in part to the nitrogen fixation ability of microbes associated with *Dryas*. There were several mechanisms involved in establishment of the early successional species at Glacier Bay. A long 100 km glacial foreland meant an extensive distance between newly-exposed soils and the seed sources of plants. Therefore, long-distance seed dispersal ability was critical for successful colonization, and the smaller seeds of *Epilobium* and *Dryas* were fundamental in ensuring broad seed dispersal (Chapin *et al.* 1994). Further mechanisms including life history traits such as younger age at first reproduction, short life-spans, and short plant heights were also found to be important for colonization of young soils by early-successional plants (Chapin *et al.* 1994). Further characteristics that contribute to the success of early-colonizing plants include the quantity of seeds produced by a plant, as well as the adaptation of the seeds to wind dispersal and the ability to growth on low-nitrogen soils is another attribute of pioneer plants (Stocklin & Baumler 1996). A general successional trend observed in these systems is an increase in both alpha and beta diversity of plants within the first 50 years since deglaciation (Erschbamer *et al.* 2008). For example, Jones et al., found richness increased over time in a 44

year old chronosequence, which they attributed to the amount of open space available for colonization and an overlap between the establishment of early pioneer species (0-30 years) and intermediate species (20-40 years) (Jones & Henry 2003). After 50 years, however, both alpha and beta diversity stabilize, likely due to increased competition (Raffl *et al.* 2006; Erschbamer *et al.* 2008).

After 50 years since deglaciation, alder replaced *Dryas*. The mechanisms of succession from *Dryas* to alder involved competitive displacement, in part due to shading of the shorter *Dryas* by the taller alder; burial of *Dryas* by alder litter; and chemical inhibition effects (Chapin *et al.* 1994). From the *Epilobium* to alder stage, significant changes were found in soil parameters including increases in organic matter, soil moisture and total N, and decrease in soil pH (Chapin *et al.* 1994). Like *Dryas*, N-fixing microorganisms associate with alder, thereby continuing to increase the total soil N (Chapin *et al.* 1994)

Finally, after 100 years since deglaciation, spruce replaced alder. Shading by spruce was the primary successional mechanism accounting for the change from the mid-successional alder to the late-successional spruce stage, as the height at maturity of spruce is much greater than that of alder (Chapin *et al.* 1994). The oldest stages of succession consist of slow growing, long-lived plants, which suggests that longevity is an important characteristic in successional processes (Jones & Henry 2003). From alder to the spruce stage, declines in nutrient availability were apparent, including decreased soil ammonium, nitrate, exchangeable cations, phosphate, net primary production, and N and P accumulation (Chapin *et al.* 1994). The decline in soil N over time was in direct

conflict with previous studies of plant succession, and the authors attributed this to N becoming bound to soil minerals, dead soil organic matter, and vegetation. The decline in soil P was attributed to P becoming bound in vegetation, soil organic matter, changing to an unavailable form due to acid digestion, or loss due to leaching (Chapin *et al.* 1994). Plant biomass, however, increased throughout the entire chronosequence, which is a trend observed in almost all glacier foreland studies of plant succession (Chapin *et al.* 1994; Frenot *et al.* 1998).

Overall, studies of plant succession provide a number of mechanisms that fit into the broader field of successional ecology. For example, it is clear that an early, transitional period exists within approximately the first 50 years after deglaciation during which richness and biomass increase rapidly. Furthermore, the soil environment in the most recently deglaciated sites is oligotrophic and relies on biological processes such as carbon fixation to increase nutrient pools. As we will see, these trends are also clearly apparent when examining microbial succession in glacier foreland systems.

1.4.3 Microbial Succession in Glacier Foreland Soils

Increasing ambient temperatures during the twentieth century have led to glacial retreat across the northern hemisphere (Haeberli *et al.* 2007).Due to the existence of viable microbial communities beneath glaciers that have reclassified glacier retreat as a secondary successional process (Skidmore *et al.* 2005; Lanoil *et al.* 2009), it is crucial to investigate and understand this “seed source” of microbes in glacier foreland soils, which is thought to be at least partially

comprised of organisms originating from subglacial ecosystems (Skidmore *et al.* 2005; Nemergut *et al.* 2007; Lanoil *et al.* 2009). This topic will be discussed next and is followed by examination of the subsequent successional processes occurring in proglacial foreland soils.

1.4.3.1 Subglacial Microbiology

A necessary condition for life to exist is liquid water, which is required for cellular biochemical processes, electrochemical gradients, protection from freezing, and as an important source of nutrients (Sharp *et al.* 1999; Yde *et al.* 2011). Due to insulation of glaciers by overlying ice, liquid water from basal and surface melting is present in subglacial sediments and is supplied with particulate matter, dissolved gasses, and nutrients including nitrate, ammonium, and iron and silicon (Sharp *et al.* 1999). Furthermore, organic carbon, supplied from bedrock and soils, is also present in subglacial environments (Sharp *et al.* 1999). Enzymatic processes, however, are generally slowed as a consequence of the cold and nutrient-limited subglacial environment causing microorganisms inhabiting subglacial environments to be characterized as oligotrophs, thriving in low nutrient conditions (Yde *et al.* 2011). Subglacial ecosystems around the world are dominated by β-*Proteobacteria*, and also include α-*Proteobacteria*, *Bacteroidetes* and *Actinobacteria* (Miteva *et al.* 2004; Skidmore *et al.* 2005; Gaidos *et al.* 2009; Yde *et al.* 2010). The closest relatives of these bacteria include heterotrophs, iron and sulfate reducers, denitrifiers, sulfate oxidizers, and methanogens (Yde *et al.* 2011). Indeed, as sunlight cannot penetrate the thick glacial ice, microorganisms in subglacial environments survive using chemical

energy likely from weathering of bedrock, such as from pyrite oxidation (Mitchell *et al.* 2013). It should be noted, however, the presence of these organisms in subglacial environments is not always consistent; some abundant phylogenetic groups, for example, have been observed in subglacial environments in Alaska but not Nunavut and vice versa (Skidmore *et al.* 2005). Despite their hindered ability to perform metabolic functions, these microbes have been demonstrated to have a strong influence on biogeochemical cycling and mineral weathering rates in subglacial environments, and are therefore likely important contributors to solute flux from glaciers (Sharp *et al.* 1999; Skidmore *et al.* 2005).

1.4.3.2 Proglacial Microbiology

As demonstrated by the abundance and activity of subglacial microorganisms, we must redefine foreland soil maturation following glacial retreat as secondary microbial succession, which leads to more complex transitions than primary succession (Sharp *et al.* 1999; Skidmore *et al.* 2005; Schütte *et al.* 2009). Following glacier retreat in foreland soils, there are a number of variables that may impact bacterial communities, including temperature fluctuations, solar irradiation, water content variation as a result of precipitation and drying, and influences from geomorphical features on glacier forefields including neighboring moraines and stream beds (Bekku *et al.* 2004; Chiri *et al.* 2015). The proglacial foreland is also susceptible to aerial deposition (i.e. allochthonous inputs) of biological matter including insects, spores, lichens, and microbial invasion that influence microbial community assembly after retreat (Hodkinson *et al.* 2002; Řeháková *et al.* 2010; Schulz *et al.* 2013).

The drastic change in conditions from subglacial to proglacial environments is linked to high turnover of bacterial communities immediately after retreat (Tscherko *et al.* 2003; Bekku *et al.* 2004; Nemergut *et al.* 2007; Schütte *et al.* 2010; Hahn & Quideau 2013). For example, the highest rate of turnover (i.e. change in species composition) was observed to occur within the first 5 to 19 years since deglaciation at the Midre Loven Glacier (Schütte *et al.* 2010; Brown & Jumpponen 2013). Biomass, richness, evenness, and diversity also increase after retreat up to approximately 50 years after deglaciation (Tscherko *et al.* 2003; Bekku *et al.* 2004; Schütte *et al.* 2010; Brown & Jumpponen 2013). Additionally, plant colonization occurs within this period, establishing within 16 years at the Midre Loven Glacier (Hodkinson *et al.* 2003), 20 years at the Rotmoosferner Glacier (Tscherko *et al.* 2003, 2004), and 35 years at the Lyman Glacier (Brown & Jumpponen 2013). Following this 50 year period after retreat, biomass, diversity and turnover stabilize as a result of maturation of the bacterial communities (Tscherko *et al.* 2003; Schütte *et al.* 2010; Brown & Jumpponen 2013). Together, these data may indicate a transition from r-strategists, preferring rapid reproduction rates and unstable conditions dominating in low-density recently deglaciated sites, to K-strategists, those that have slow growth rates, and prefer more stable and competitive environments dominating in high-density, more developed soils (Deiglmayr *et al.* 2006).

Nutrient concentrations in glacier foreland soils mirror the patterns observed in bacterial community structure, as immediately after glacier retreat

nutrient pools are in flux in the developing forefield until an eventual stabilization (Tscherko *et al.* 2003; Bardgett *et al.* 2007; Brankatschk *et al.* 2011). Soil development is influenced by both allochthonous (arrival of nutrients from off-site) and autochthonous (accumulation within soils) processes (Brown & Jumpponen 2013). Allocthonous inputs include arrival of particulate organic matter and plant and insect matter via wind dispersal (Hodkinson *et al.* 2002) while autochthonous processes include on-site biogeochemical cycling, including carbon and nitrogen cycling (Tscherko *et al.* 2003; Bardgett *et al.* 2007; Brankatschk *et al.* 2011).

Carbon cycling in initially involves consumption of ancient organic carbon by heterotrophic bacterial communities (Tscherko *et al.* 2003; Bardgett *et al.* 2007); this carbon is more recalcitrant than carbon found in more developed soils and is likely sourced from organic matter in sub-glacial soils and from wind-blown organic matter deposited into cryoconite holes (Kastovská *et al.* 2005; Bardgett *et al.* 2007). Following the eventual depletion of carbon in these soils by heterotrophic bacteria, autotrophic bacteria capable of carbon fixation then support the heterotrophic community (Bardgett *et al.* 2007). Similar patterns are observed with nitrogen. For example, N mineralization has been demonstrated to most significantly contribute to N cycling in 10-year-old soils, likely as a result of decomposition of deposits of old, recalcitrant organic matter while nitrogen fixation is more prominent in soils between 50 and 70 years since deglaciation when organic matter nitrogen stores are depleted (Brankatschk *et al.* 2011). For these reasons, carbon and nitrogen initially limit bacterial growth in newly

deglaciated soils, after which their concentrations increase exponentially over time until an eventual stabilization (Göransson *et al.* 2011).

Microbially-mediated bedrock weathering releasing inorganic nutrients is also a mechanism by which nutrient pools are increased in glacier forefields (Puente *et al.* 2004; Lazzaro *et al.* 2009). For example, phosphorus, a key chemical parameter in glacier foreland soils, is supplied almost exclusively by parent material (Walker & Syers 1976). Microbes can release phosphorus from bedrock via production of organic acids that dissolve insoluble phosphates (Puente *et al.* 2004). For these reasons, phosphorus concentrations are typically highest in most recently deglaciated soils and decrease over time through leaching and uptake by plants and microbes (Göransson *et al.* 2011).

Soil pH is a fundamental component of soil nutrient dynamics and plant and microbial community structure (Kemmitt *et al.* 2006). For example, biogeochemical processes such as nitrification are highly pH sensitive, with nitrification rates significantly reduced in acidic soils (Kemmitt *et al.* 2006). Plant colonization also decreases soil pH in glacier forelands through litter inputs and root exudates including H+, and organic and inorganic acid release (Badri & Vivanco 2009; Knelman *et al.* 2012)

The activities of proglacial microbial communities may have strong implications for down valley vegetation colonization, development, and succession. Conversely, through the influence of root exudate and litter decomposition, which increase total organic carbon and nitrogen pools (Tscherko *et al.* 2003; Miniaci *et al.* 2007; Duc *et al.* 2009; Hahn & Quideau 2013; Brown &

Jumpponen 2013), plant colonization is thought to have a substantial effect on soil nutrient profiles and consequently on bacterial community structure. However, the impact of bacterial community succession in glacial foreland soils in preparing the soil for plant colonization, and the influence of plant colonization on bacterial community structure, is poorly understood (Nemergut *et al.* 2007). Initial studies suggest that plant colonization influences the metabolism, community structure, and the relative abundance of microbial species (Knelman *et al.* 2012). For example, presence of pioneer plants increases the abundance of nitrogen fixing microbes (Duc *et al.* 2009) while both the type of plant (Knelman *et al.* 2012) and even the presence of a plant regardless of its identity (Brown & Jumpponen 2013) influence bacterial community structure in glacier foreland soils. Thus, overall it is clear that plants have an influence in bacterial communities at glacier forelands. Therefore, an understanding of plant dynamics, including when they colonize foreland soils, is crucial in understanding the overall successional processes in these systems.

1.4.4 Comparison of Plant and Microbial Succession

There are several comparable mechanisms and patterns of succession between bacteria and plants. In both plant and bacterial succession, biomass increases following glacial retreat, reflecting the development and maturation of communities over time (Chapin *et al.* 1994; Hofmann *et al.* 2013). Furthermore, just as nitrogen-fixing plants are part of the early plant colonizers of foreland soils, nitrogen-fixing microorganisms are among the first microbial colonizers of deglaciated soils. The resulting accumulation of soil nitrogen may also facilitate

colonization by later successional species (Chapin *et al.* 1994; Nemergut *et al.* 2007). Moreover, early colonizers in both bacterial and plant successional processes have been demonstrated as having fast growth rates, suggesting that in both bacteria and plants, r-selection (i.e. favoring short life spans as observed with *Epilobium* and *Dryas* at Glacier Bay (Chapin *et al.* 1994)) is favored in the most recently deglaciated soils while K-selection (i.e. favoring long life spans as observed with alder and spruce at Glacier Bay (Chapin *et al.* 1994)) is favored in more developed soils (Chapin *et al.* 1994; Deiglmayr *et al.* 2006). There is also an increase in alpha and beta diversity observed in both plants and microorganisms over the first 50 years of deglaciation, followed by relative stabilization attributed to increased competition over time (Tscherko *et al.* 2003; Raffl *et al.* 2006; Erschbamer *et al.* 2008).

Microbial succession in glacier foreland soils is a dynamic, complex process involving multiple biological and environmental gradients and variables. However, there is a general pattern of succession that is observed in nearly all of these studies. Within the 50 years after deglaciation, microbial community richness and diversity increases rapidly, along with a high rate of turnover (Schütte *et al.* 2009, 2010; Brown & Jumpponen 2013). At the same time, nutrient pools, which are initially oligotrophic, increase rapidly leading to higher carbon and nitrogen concentrations (Göransson *et al.* 2011). An important consideration of these studies, however, is the concurrent establishment of plants within the initial 50-year period after deglaciation (Tscherko *et al.* 2003). Plants have substantial effects on soil dynamics in glacier forelands (Tscherko *et*

al. 2003; Miniaci *et al.* 2007; Duc *et al.* 2009; Hahn & Quideau 2013; Brown & Jumpponen 2013), and thus it remains unclear whether the initial turnover and subsequent stabilization of bacterial communities is because of factors independent of plants (i.e. from the effects of glacier retreat), or from impact of vegetation. In the next chapter of this thesis, I have investigated this question and decoupled the effects of glacier retreat and plant colonization on microbial community succession in glacier foreland soils.

1.4.5 Conclusion

Here, I have described three major concepts relevant to soil microbial communities following glacier retreat: biodiversity, biogeography, and succession. High biodiversity is important in maintaining high ecosystem function and stability; biogeography helps explain why certain species are found where they are; and succession explains how communities change in response to the disturbance of glacier retreat.

In the next chapter, I describe studies of the Duke Glacier valley, Kluane National Park Reserve, Yukon, Canada, where we were able to separately evaluate plant colonization and glacial retreat disturbance effects on microbial community composition in glacier foreland soils due to the presence of bare soils until ~200 years after glacial retreat. I dated two parallel chronosequences and characterized bacterial community composition and basic soil chemical parameters for 42 different samples along those chronosequences. I found that glacier retreat and plant colonization each were a significant disturbance to the microbial communities, of similar magnitude. This led to three separate “zones” in

the glacial foreland: an early, rapidly changing zone in the first 50 years following glacial retreat; an intermediate, stable 150 years where the communities changed slowly; and a rapid change with the colonization of plants.

In my final chapter, I summarize the significance of my data in the context of the field more generally and propose future directions for my research.

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CHAPTER 2: BACTERIAL COMMUNITY SUCCESSION IN GLACIER FORELAND SOILS IS A THREE-STAGE PROCESS

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2.1 Abstract

Alpine glaciers are retreating rapidly, leading to exposure of foreland soils. Soil bacterial communities exhibit high rates of turnover and undergo dramatic shifts in composition within the first 50 years after deglaciation, followed by relative stabilization. This period of microbial development occurs simultaneously with plant colonization and thus it remains unclear whether the changes in the bacterial communities occur primarily as the result of abiotic or biotic forcing factors. Here we decouple the effects of glacier retreat and plant colonization on microbial community structure in glacier foreland soils. We examined bacterial community structure along two independent, parallel chronosequences within the soil foreland of Duke River Glacier, Yukon, Canada; this foreland is unusual in containing over 200 years of relatively bare soils before an appreciable grassline. We observed three successional groups in each chronosequence; an “early” group in soils of approximately 50 years since deglaciation; an “intermediate” group within bare soils after the early period but before the grassline; and a “grassline” group in soils collected after plant colonization. We interpret these findings as fast adaptation of subglacial bacterial communities to glacier foreland conditions; slower development over the next 200 years; and finally, rapid change in response to plant colonization. Our results do not support a model of microbial preparation of soil for plant colonization, although such a model cannot be ruled out.

2.2 Introduction

The rate of glacial retreat has increased due to global climate change and is projected to lead to the disappearance of alpine glaciers by 2050 if warming continues at its current rate (Fitzharris 1995). One consequence of glacial retreat is exposure of subglacial till, which subsequently develops into mineral soil that supports grassland ecosystems (Anderson 1988). This process can be observed in the glacier foreland, with increasing distance from the glacier terminus used as a proxy for time since retreat; a chronosequence (Hämmerli *et al.* 2007). Retreating glacier chronosequences have been extensively studied as models of plant primary succession (Chapin *et al.* 1994; Fastie 1995). However, subglacial ecosystems contain active microbial communities that impact biogeochemical cycling beneath glaciers (Skidmore *et al.* 2000, 2005; Kaštovská *et al.* 2006). Therefore, microbial community development following glacier retreat should be viewed as a process of secondary succession.

In the period immediately following glacier retreat, microbial communities in glacial foreland soils are characterized by low biomass, low bacterial diversity, and nutrient limitation (Hodkinson *et al.* 2003; Bekku *et al.* 2004; Schütte *et al.* 2010; Göransson *et al.* 2011). Succession is driven by nutrient input and cycling as well as the recruitment of new and change in abundance of already present bacterial species (Hodkinson *et al.* 2003; Bekku *et al.* 2004; Hahn & Quideau 2013; Schulz *et al.* 2013; Chiri *et al.* 2015). New nutrient sources include allochthonous input from particulate organic matter and plant and insect detritus

via wind dispersal (Hodkinson *et al.* 2002) and microbially-mediated biogeochemical cycling (Tscherko *et al.* 2003; Deiglmayr *et al.* 2006; Kandeler *et al.* 2006; Bardgett *et al.* 2007; Lazzaro *et al.* 2009; Brankatschk *et al.* 2011). In the first 50 years after glacial retreat, communities increase in biomass, experience high bacterial species turnover (i.e. changes in species composition), and become considerably more diverse with the recruitment of new species and increase in evenness of the distribution of species (Tscherko *et al.* 2003; Bekku *et al.* 2004; Nemergut *et al.* 2007; Schütte *et al.* 2010; Hahn & Quideau 2013).

A notable trend observed within this initial stage following glacier retreat is the concurrent establishment of higher plants within 20 to 35 years after deglaciation (Hodkinson *et al.* 2003; Tscherko *et al.* 2003, 2004; Brown & Jumpponen 2013). Newly exposed glacial till is bare with only a few rare and hardy plants (Chapin *et al.* 1994); however, vegetation cover increases dramatically during this time before eventually forming a less variable, distinct grassline (Tscherko *et al.* 2003; Raffl *et al.* 2006). Plant colonization has substantial effects on nutrient dynamics in glacier foreland soils through the influence of root exudates and litter decomposition, which increase total organic carbon and total nitrogen pools (Tscherko *et al.* 2003; Miniaci *et al.* 2007; Duc *et al.* 2009; Hahn & Quideau 2013; Brown & Jumpponen 2013). Approximately 50 years after glacial retreat, plant communities are established and the microbial community composition and enzymatic activity show also decreased variability (Tscherko *et al.* 2003; Schütte *et al.* 2010; Brown & Jumpponen 2013).

Two hypotheses could explain this stabilization of the microbial community: either plant colonization leads to microbial community stabilization or microbial community stabilization precedes and is independent of plant colonization, potentially helping to prepare the “new” soils for subsequent plant colonization. At most sites, it is impossible to distinguish between these alternate hypotheses because plant and microbial succession occur simultaneously. Here, we characterize microbial community structure in two parallel chronosequences at Duke River Glacier in Kluane National Park, Yukon, Canada. Each chronosequence includes over 200 years of relatively bare soils before the presence of an appreciable grassline. Our results indicate that that stabilization of the bacterial community structure can precede plant colonization by as much as 150 years. Bacterial community succession in these chronosequences is a three-stage process linked to two disturbance events following glacier retreat in early successional systems: (i) a fast adaptation of bacterial communities to glacier retreat; (ii) gradual development and convergence of bacterial communities over time; and (iii) rapid change in bacterial communities associated with the disturbance of plant colonization. The consequences of this model for understanding soil bacterial successional processes are discussed.

2.3 Materials and Methods

Site Description and Sample Collection

The Duke River Glacier (60°57'28.3"N, 138°55'13.3"W) is located in Kluane National Park Reserve, Yukon, Canada. It has an elevation of 2214 m

meters. Soil samples were collected in July 2011; the mean annual temperature in 2011 was -7.9 °C with an average of -7.3 °C from 2006-2013 and the total annual rainfall in 2011 was 155 mm, with an average of 174 mm annually from 2008-2013 (Wheler *et al.* 2014). Surface soil samples (0-10 cm) were collected using a sterile trowel into a sterile bag. Two chronosequences, each 50 m away from the center of the valley on the left ("L") and right ("R") sides of the streambed, were sampled every 400 to 500 m within the 3.3 km foreland (Figure 1); these chronosequences were assumed to be independent from each other due to the small size of bacteria (Schütte *et al.* 2009). Seven sites were sampled within each chronosequence, from the glacier terminus to the visually observable grassline (Figure 1). One site from each chronosequence was located within the grassline itself (L1 at the left chronosequence and R1 on the right); all other sites were located in bare soils.

At each sampling site three subsamples were collected, each 10 m apart from each other. Subsamples were kept separate throughout all stages of the study. Careful attention was made to ensure samples were collected on moraines in each chronosequence so as to avoid influences from reworked streambeds as well as to avoid drainage streams located on the moraines themselves. Soils were sieved on-site using a 2.00 mm Testing Sieve (VWR LabShop, Batavia, IL) to remove large rocks and plant matter and to homogenize the samples. Homogenized soils were kept in a cooler on ice until transport to the laboratory within a week of collection, at which time they were transferred to -80ºC for storage until processing.

Chronosequence Age Determination

Glacier isolines were established from aerial photographs obtained from the National Air Photo Library, Government of Canada showing range of glacier coverage in 1950 (Roll A12852), 1957 (Roll A15739), and 1979 (Roll A25265). Using ArcGIS (v10.2; ESRI, Redlands, CA, USA). Isolines were traced onto a geo-referenced 2008 satellite image of the glacier to illustrate the changing extent of glacier coverage over time. GIS-based sampling sites were superimposed onto the satellite image. In 1950 a patch of relict ice was present between L3/R3 and L4/R4, though this did not influence when these samples were uncovered (Figure 1). Furthermore, due to the quality of the 1950 aerial photograph, the position of the terminus was not fully outlined, and thus was estimated using a dashed line (Figure 1). Sites R6, R7, L6, L7 were beneath the ice in 1950. In 1957, sites L6, L7, R7 were beneath ice and in 1979 sites L6, L7, R6 were beneath ice. The number of years since deglaciation at the sampling sites was calculated by dividing the distance between isolines by the number of years between pairwise photographs to determine the rate of retreat and then estimating the number of years since deglaciation based on distance to the glacier terminus.

Soil Chemistry

All three subsamples at each site were used for soil chemistry analysis, resulting in a total of 42 samples between the two chronosequences. Measured soil chemistry parameters included pH, total phosphorous (TP), total nitrogen

(TKN), ammonium (NH_4^+), nitrate (NO_3^-), total organic carbon (TOC), and total carbon (TC). A standard 2:1 water-to-soil ratio was used to determine pH with an Accumet Basic pH Meter (Fisher Scientific, Waltham, MA, USA). Ammonium and nitrate were extracted using 2N potassium chloride. Ammonium was measured by the Berthelot method (Harfmann & Crouch 1989; Maynard & Kalra 2008) using a SmartChem Discrete Wet Chemistry Analyzer, Model 200 (Westco Scientific, Brookfield, CT, USA, 2007). Nitrate was measured by the diazo coupling method (Maynard & Kalra 1993) on a SmartChem Discrete Wet Chemistry Analyzer, Model 200 (Westco Scientific, Brookfield, CT, USA, 2007). Total nitrogen and total phosphorous were extracted using a Kjeldahl digestion (Rutherford *et al.* 2008) and measured using the SmartChem Discrete Wet Chemistry Analyzer, Model 200 (Westco Scientific, Brookfield, CT, USA, 2007) (Environmental Protection Agency 1993a; b). Total organic carbon was determined by loss on ignition (LOI) (Lim & Jackson 1982) using a Lindberg SB Muffle Furnace (Thermo Fisher Scientific Inc., Waltham, MA, USA). Total carbon was measured by dry combustion (AOAC International 2000) using a Costech 4010 Elemental Analyzer System (Costech International Strumatzione, Florence, Italy, 2003). Raw soil chemistry values are given in Supplementary Table S1.

DNA Extraction and Sequencing

Extraction of DNA from all three subsamples was performed using the FastDNA® SPIN KIT (MP Biomedicals, Solon, OH) following the manufacturer's protocol with 0.5 g of soil. Library preparation included PCR amplification of the V3 variable region of the 16S rRNA gene with 341 forward and 518 reverse

primers and IonTorrent sequencing using a two-step protocol (Bybee *et al.* 2011; Galindo-González *et al.* 2015). The V3 region was used because of the small size of the fragment which enabled sufficient coverage during sequencing, and also because it is among the most popular regions for bacterial community characterization therefore allowing sufficient comparison of our results to other studies (Wang & Qian 2009). The resulting adapter and barcoded 16S rRNA gene was prepared for emulsion PCR using the Ion PGM™ Sequencing 200 Kit v2 with the Ion 316 Chip v2 (Life Technologies, Carlsbad, CA). All subsamples were amplified in triplicate in the first step, combined, and amplified in triplicate and combined in the second step thus helping to minimize intrinsic biases involved in PCR amplification (Acinas *et al.* 2005). PCR amplification conditions were: 95°C for 4 minutes, followed by 10 cycles of 95°C for 15 seconds, 65°C for 30 seconds, and 72°C for 30 seconds; the next 20 cycles included 95°C for 15 seconds, 55°C for 30 seconds, and 72°C for 30 seconds; the final elongation step was performed at 72°C for 10 minutes. Subsamples were mixed in equal concentrations and purified using UltraClean GelSpin DNA Extraction Kit (MO BIO Laboratories, Inc). Sequencing was performed with the IonTorrent Personal Genome Machine (PGM).

Pre-processing and Quality Control of Raw Sequences

All pre-processing and sequence quality control steps were performed using USEARCH (v 7.0.1090 for Windows 32bit) according to the UPARSE pipeline (http://drive5.com/usearch/manual/uparse_pipeline.html; date accessed 1 Oct 2014) (Edgar 2013) and Python v 2.74 for Windows

(https://www.python.org/). OTUs were assigned using the UPARSE greedy algorithm for an OTU definition of 97% sequence similarity. Though this step also removes chimeras it is recommended by the USEARCH pipeline to include a dedicated chimera removal step. Therefore chimeras were additionally detected and removed using UCHIME (Edgar *et al.* 2011). Global singleton OTUs (those that are represented by a single sequence in the entire dataset) were removed due to their unknown nature. The bacterial OTUs were classified to phylum, class, family, and/or genus level using the Ribosomal Database Project classifier (train set 10) with a 60% confidence threshold (RDP; http://rdp.cme.msu.edu/). An 18 strain mock community was used to assess quality of amplification and sequencing (Supplementary Table 2). Our analysis of the mock community demonstrates a one-to-one ratio of OTUs to strains used and OTUs were identified to the same genus and species if possible as the original 18 strains used indicating that our PCR amplification and sequencing methods were satisfactory (Supplementary Table 3).

Community and Statistical Analysis

The processed dataset generated in USEARCH was imported into Mothur (v 1.33.3; (Schloss *et al.* 2009)) where sequences were subsampled to lower than smallest library size to normalize sampling effort for each sample before further analyses. Mothur was also used to calculate alpha diversity for each chronosequence; OTU richness was estimated using Chao1 nonparametric richness estimator, and overall diversity using inverse Simpson's diversity index (Simpson 1949; Chao 1984; Chao & Shen 2003).

Beta diversity was examined using PC-ORD (Version 6; (McCune & Grace 2002)). Bray-Curtis distance matrices of all pairwise comparisons within each chronosequence and between the two chronosequences were generated to assess differences in bacterial composition across time. We tracked how the initial, most recently deglaciated bacterial community at each chronosequence (i.e. L7 and R7) changed over time by plotting all pairwise Bray Curtis dissimilarity distances between these samples and every other sample within each chronosequence as a function of age difference (Figure 2). Based on a three-order polynomial regression (R^2 = 0.93 and 0.99 at the left and right chronosequences, respectively) describing the change from the most recently deglaciated community to the oldest community (i.e. L1 and R1 grassline communities) at each chronosequence, we categorized samples into three successional groups: an “early” group of samples of less than approximately 50 years since deglaciation (termed LE and RE at the left and right chronosequences, respectively); an “intermediate” groups of samples after 50 years but before the grassline (LI and RI); and the two samples in the grassline (L1 and R1) as “grassline” (Table 1; Figure 2). Slope, used as a proxy for change in similarity per year, was calculated at three intervals: between the early and the first intermediate sample; between the first and last samples within intermediate; and between the last intermediate sample and the grassline sample (Figure 2).

Overall change in bacterial community composition across the two chronosequences was visualized with nonmetric multidimensional scaling (NMS) using 100 runs of real data based on Bray-Curtis dissimilarity. To verify that the

axes created in the NMS were significantly better than would be obtained by chance, a Monte Carlo test of 100 runs with randomized data was performed. Distances along axis 1 or axis 2 for triplicate samples for each site were averaged and standard error was calculated to display within-site variability in the NMS plot. Multiresponse permutation procedure (MRPP) was used to determine significant differences in community composition between the previously defined early, intermediate, and grassline successional groups (McCune & Grace 2002). The MRPP delivers three statistical values: the test statistic (T) shows the separation between pre-defined groups, where more negative values indicate stronger separation; the chance-corrected within-group agreement (A), where A=0 when within-group heterogeneity equals that expected by chance, A<1 when within-group heterogeneity is greater than expected by chance, A=1 (max value) when all samples within a group are identical; and finally a probability (p) value given for all comparisons, including multiple comparisons, which indicates the likelihood that the comparison groups are significantly different from each other (McCune & Grace 2002). The pre-defined successional groups were also used to assess differences in soil chemical properties in the chronosequences. The Shapiro-Wilk normality test was used to test for normality within data sets; for non-normal data, the Kruskal-Wallis test was used to examine differences in datasets while unpaired two-tail t-tests (k = 2) were used to determine significant differences in normal datasets (SigmaPlot Version 12.0).

2.4 Results

The average rate of retreat between 1950-1957 was 11.2 m/yr and from 1957-1979, 13.6 m/yr. Sampling sites R1-R5 and L1-L5 fell beyond the 1950 isoline; thus the rates of retreat from 1950-1957 (11.2 m/yr) and 1957-1979 (13.6 m/yr) were used to generate a range of time since deglaciation at these sites (Schmidt *et al.* 2008) (Table 1). Based on our estimates of the rate of glacier retreat, the left chronosequence at Duke River glacier ranged from 7 years to between 245 and 264 years since deglaciation while the right chronosequence ranged from 12 years to between 250 and 267 years (Table 1). Sites L7-L2 (representing 7-215 years since deglaciation) and R7-R2 (12-229 years) are in bare soils while L1 (245-264 years since deglaciation) and R1 (250-267 years) are located within the grassline (Table 1).

Sequencing of the 16S rRNA gene resulted in an average of 22,173 sequences per subsampled site after clean-up; each site was subsequently randomly subsampled to 4500 sequences. Coverage was high, with the lowest subsample having 89% coverage (data not shown); rarefaction analysis (Supplementary Figure S1) confirmed high coverage. The microbial communities in the samples could be grouped into three statistically supported groups: early (L7, L6, R7, R6), intermediate (L5-L2, R5-R2), and grassline (L1, R1) successional stages (Figure 3).

Apart from a significant increase in observed OTUs from L7 to L6, there were no changes in richness or diversity within the early communities (data not shown). There were also no significant changes in any of the measured soil

chemical parameters within the early samples at either chronosequence (Table 2).

Bacterial communities within the intermediate group at each chronosequence appear to stabilize and converge over time. Apart from R4 and R3, all intermediate samples distinctly cluster together despite over 150 years separating the youngest and oldest samples at each chronosequence (Figure 2). The convergence of bacterial communities over time was observed in the increase of OTUs shared over time between the two chronosequences; RE and LE shared 57% of their OTUs while RI and LI shared 73%, indicating that bacterial communities in these stages become more similar with increasing time since glacial retreat (Figure 4). Bacterial community richness and diversity did not change in the intermediate samples for either chronosequence, supporting the concept that intermediate stage bacterial communities are more stable than for early stage (data not shown). Furthermore, the change in similarity within the intermediate period was 4.87E-04 and 1.83E-04 Bray-Curtis distance values per year at the left and right chronosequences compared to 3.38E-03 and 5.32E-03 Bray-Curtis distance values per year at the left and right chronosequences, respectively between the early and intermediate samples and 6.18E-04 and 3.95E-03 Bray-Curtis distance values per year at the left and right chronosequences, respectively between intermediate and grassline samples. Together this also indicates stabilization within intermediate samples, as the rate of change within this 150-year period was the lowest of any other interval (Figure 2). A lack of change in most of the measured soil chemical parameters during

this span further reflects the stabilization occurring in bacterial communities (Table 2). However, we also note a significant increase of TOC and NH_4 and a decrease of TC within LI, but no changes in chemistry within RI, indicating some differences in soil chemistry patterns between the left and right chronosequences.

Each chronosequence contained one site within the grassline, L1 at the left chronosequence and R1 at the right. Site L1 was located closer to the bare soil-grassline border while R1 was further into the grassline. Because there was only one sample at each chronosequence within the grassline, we cannot determine whether there is significant variability in bacterial community composition or soil chemistry within the grassline itself.

In the transition between early and intermediate samples, both richness and diversity started low and increased significantly between succession stages at each chronosequence (Figure 5). The pattern of change in bacterial communities following retreat is at least partly due to the loss of several key taxonomic groups (Supplementary Figure S2). For example, relative abundance of Betaproteobacteria was higher in early samples (24% of all sequences) than in intermediate samples (10% of all sequences) (Supplementary Figure S2). Within the Betaproteobacteria, the family Comamonadaceae were also higher in early samples (data not shown). Measured soil chemical parameters, however, did not change substantially (Table 2), indicating that the environmental variables in this study were likely not responsible for driving the change in bacterial communities between these periods.

There is considerable change in both bacterial community composition and soil chemical properties with plant colonization. Between intermediate and grassline samples, there were significant ($p<0.05$) changes in TN, NH4, TC, and TOC between LI and L1 and in all measured soil parameters except TP between RI and R1 (Table 2), indicating that plant colonization is associated with substantially more change in soil chemistry compared to preceding periods. The presence of vegetation also appears to disrupt the established intermediate communities; for example, the proportion of OTUs shared between L1 and R1 (56%) is dramatically lower than the proportion shared between LI and RI (73%) samples (Figure 4). Furthermore, taxonomic composition suggests that the presence of vegetation was associated with changes in the relative abundance of major taxonomic groups as well as the recruitment of new taxonomic groups. For example, within the Alphaproteobacteria, Rhizobiales increased over time, making up their highest proportion in grassline soils (data not shown). Furthermore, Flavobacteria were not found in early samples or RI and had only 1% relative abundance in LI, but were noticeably higher in R1 (4% relative abundance) and L1 (6% relative abundance), demonstrating differences in composition between intermediate and grassline samples. Although richness does not change from intermediate to grassline within each chronosequence, diversity increases significantly (Figure 5) due to increased evenness (data not shown), further demonstrating differences between intermediate and grassline communities.

2.5 Discussion

There were two, distinguishable mechanisms driving change in microbial communities at the Duke River glacier foreland: 1) succession from a subglacial to proglacial environment and, 2) plant colonization. Our data indicate that the magnitude of each mechanism on bacterial community composition is similar and suggests a decoupling of the effects of glacier retreat and plant colonization on bacterial communities.

Substantial change in bacterial communities after approximately 50 years since deglaciation demonstrates that microbial community succession occurs in Duke glacier foreland soils despite the absence of higher plants, indicating other factors besides plant establishment must be responsible for the change in bacterial communities between the early and intermediate stage at each chronosequence. The most parsimonious explanation is that the shift from subglacial environments to newly exposed substrate introduces previously established subglacial bacterial communities to a novel environment, despite the lack of significant change in measured environmental variables. Microbes conditioned to subglacial systems are likely not well adapted to this new environmental framework, thus glacial retreat initiates a period of transition from subglacial to proglacial communities. Environmental variables differing between subglacial systems and foreland soils include solar irradiation, temperature fluctuations and increased temperature, and water content variation as a result of precipitation and drying (Bekku *et al.* 2004; Chiri *et al.* 2015). Moreover, soils are influenced by neighbouring moraines, streambeds, and other geomorphical

features of glacier forefields that may lead to regular reworking and thus disturbance of soil (Hodkinson *et al.* 2003; Schulz *et al.* 2013).

The makeup of bacterial communities within early samples appears to be at least partly of subglacial origin, and changes over time. For example, as Comamonadaceae have previously been isolated from glacial ice cores (Sheridan *et al.* 2003), their decreased abundance along the chronosequence suggests they may have been initially present in subglacial ice and, once exposed after glacier retreat, were outcompeted in the proglacial environment. Furthermore, the low richness and diversity observed in early samples and their subsequent increase over time is consistent with studies demonstrating that richness and diversity are restricted in cold, dark and nutrient-poor subglacial environments (Skidmore *et al.* 2000) and that they increase dramatically within the first 50 years after deglaciation (Tscherko *et al.* 2003; Schütte *et al.* 2010). In contrast to the drastic change in bacterial community composition, most measured soil chemical parameters were invariable from the early to intermediate periods thus further corroborating the view that the transformation from a subglacial to proglacial environment rather than edaphic properties was responsible for the observed change in bacterial communities.

The approximately 150 year intermediate period following the initial shift in bacterial communities was defined by relatively little change (i.e. stability) in bacterial community composition. Thus, stabilization of bacterial communities occurs after approximately 50 years, which is in line with previous studies (Tscherko *et al.* 2003; Schütte *et al.* 2010) despite the absence of plants during

this period at the Duke Glacier foreland. We attribute this stability to an adaptation of communities to proglacial conditions as well as largely invariable edaphic properties during this period. Furthermore, the increase in shared membership between the left and right chronosequences within the intermediate period demonstrates convergence between the two chronosequences over time and indicates a deterministic successional process over time, which is in line with previous studies (Brown & Jumpponen 2013).

Plant colonization at the Duke River glacier foreland proceeded despite a lack of substantial change in measured soil chemical parameters in the over 200 years since deglaciation. It is therefore uncertain what factors lead to plant colonization. We offer several suggestions for this apparent disconnect. First, there may have been changes in soil physicochemical parameters that were not measured in this study, such as soil moisture content, salinity, and soil texture that may have been required for successful plant colonization and growth. Second, a series of small variations in specific soil chemical parameters may have resulted in an optimal set of conditions required for plant growth that was impossible to delineate using our methods. Together these points indicate that microbial communities may be sufficiently preparing the soil for plant colonization, and therefore that microbial processes are responsible for the structure of plant communities in the foreland; however this remains unclear due to methodological constraints. Lastly, an extensive distance may have been present between the sampled glacier foreland and the seed source of plants capable of successfully colonizing these low nutrient-soils, and therefore the

geographic position of the grassline soils may have been at an optimal distance for successful seed dispersal.

Subsequently, our data do not support a model whereby microbes prepare soil for plant colonization by carbon and nitrogen fixation, as proposed in previous studies (Hodkinson *et al.* 2002; Schmidt *et al.* 2008). However, there may be other factors responsible for preparing the soils for plant colonization not measured in this study; thus, the broad hypothesis that bacterial community succession in glacial forelands prepares the soil for subsequent plant colonization cannot be fully rejected.

The transition from intermediate to grassline was also marked by change in bacterial communities. The change in taxonomic profiles, increased taxon evenness, and decreased similarity between left and right chronosequence samples substantiates the view that colonization of higher plants is associated with turnover of bacterial communities. Although the mechanisms responsible for the change in communities remain unclear, appreciable changes in soil chemical parameters concomitant with plant colonization are the most likely driver. The presence of vegetation induces significant effects on nutrient dynamics in glacier foreland soils through root exudates and plant litter decomposition (Tscherko *et al.* 2003; Miniaci *et al.* 2007; Duc *et al.* 2009; Hahn & Quideau 2013; Brown & Jumpponen 2013); these effects may be a cause of the increased rate of change observed in grassline bacterial communities.

2.6 Conclusion

Overall, our results demonstrate the presence of three successional stages defined by two disturbances at the Duke River glacier foreland. The initial period of turnover within the first 50 years since deglaciation suggests a drastic change in the bacterial communities not linked to the arrival of plants, but to the changing conditions between subglacial and proglacial environments. Communities stabilize after 50 years and remain stable for over 150 years until plant colonization; the stability (i.e. decreased rate of change) is likely due to an absence of change in soil chemistry over this period and adaptation of communities to proglacial conditions by changing composition and membership. Finally, we observed a second period of turnover concurrent with plant colonization; this turnover is associated with the considerable changes in soil chemistry brought on by plant colonization, which suggests that bacterial communities are influenced by the presence of vegetation.

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Table 1. Number of years since deglaciation and successional group designation of each sampling site at the left (“L”) and right (“R”) chronosequences at Duke River glacier.

Table 2. Changes in measured soil chemical parameters within and between left and right chronosequences.

Figure 1. Sampling scheme of the left and right chronosequences at Duke River glacier demonstrating extent of ice coverage. Glacier coverage in 1950, 1957, and 1979 is depicted in blue relative to a 2008 satellite image of the glacier.

Figure 2. Substantial change in community composition from the most recently deglaciated sample over time. Bray-Curtis Dissimilarity values were plotted as a function of age difference between the most recently deglaciated sample at the right (R7) and left (L7) chronosequences and every other sample within the chronosequence. We termed the blue circle “early,” the red circles “intermediate,” and the green circle “grassline.”

Figure 3. Change in community composition demonstrating a three-stage successional process. Nonmetric Multidimensional Scaling (NMS) ordination (stress=0.15) using Bray-Curtis distance measure of 16S rRNA gene data visualized presence of three significantly ($p<0.05$) distinct early, intermediate, and grassline groups. Each point represents an average of three replicates and associated standard error.

Figure 4. Communities become more similar between over time. The percentage of OTUs shared between and within the left and right chronosequences at the Duke River glacier foreland is demonstrated using a heatmap. OTUs were defined at 97% sequence similarity.

Figure 5. Alpha diversity changes according to successional stage. Alpha diversity was measured using observed richness, estimated richness based on the Chao1 estimator, and overall diversity (inverse Simpson’s diversity index). Significant ($p<0.05$) differences between groups within a chronosequence are marked by different letters. OTU definition set at 97% sequence similarity.

Supplementary Table S1. Soil chemistry at the left (L) and right (R) chronosequences of Duke River glacier foreland. Values indicate the average of three replicates collected at each site along with standard error.

Supplementary Table S2. Strains used in mock community analysis.

Supplementary Table S3. Mock community analysis demonstrating similar output of strains as compared to initial input of strains. Brackets indicate percent confidence of RDP classifier.

Supplementary Figure S1. Rarefaction curves across samples collected at the left and right chronosequence at Duke River glacier. All samples were subsampled to 4500 sequences. OTU definition set at 97% similarity.

Supplementary Figure S2. Relative abundance of class-level taxonomy for each successional stage group at the left (L) and right (R) chronosequences of Duke River glacier. All listed classes represented greater than 1% of the overall community for each group. Classes falling below the 1% threshold were grouped into “Other.”

Table 1.

Sampling Site	Years Since Deglaciation	Successional Group
L7	7	Early
L6	24	Early
L5	77-79	Intermediate
L4	117-123	Intermediate
L3	162-173	Intermediate
L2	200-215	Intermediate
L1	245-264	Grassline
R7	12	Early
R6	55	Early
R5	84-87	Intermediate
R4	124-131	Intermediate
R3	165-177	Intermediate
R2	204-229	Intermediate
R1	250-267	Grassline

Table 2.

Sampling Sites	Significant ($p<0.05$) increases (↑) and decreases (↓) in TP, TN, NH_4, NO_3, TC, TOC, and pH
L6-L7 (LE)	No significant changes
R6-R7 (RE)	↑ NH4
L5-L2 (LI)	↑ NH4, TOC; ↓ TC
R5-R2 (RI)	No significant changes
LE-LI	↓ TN
RE-RI	↑ TOC, NO3; ↓ TC
LI-L1	↑ NH4, TOC, TC, TN
RI-R1	↑ NH4, TOC, TC, TN, NO3; ↓ pH

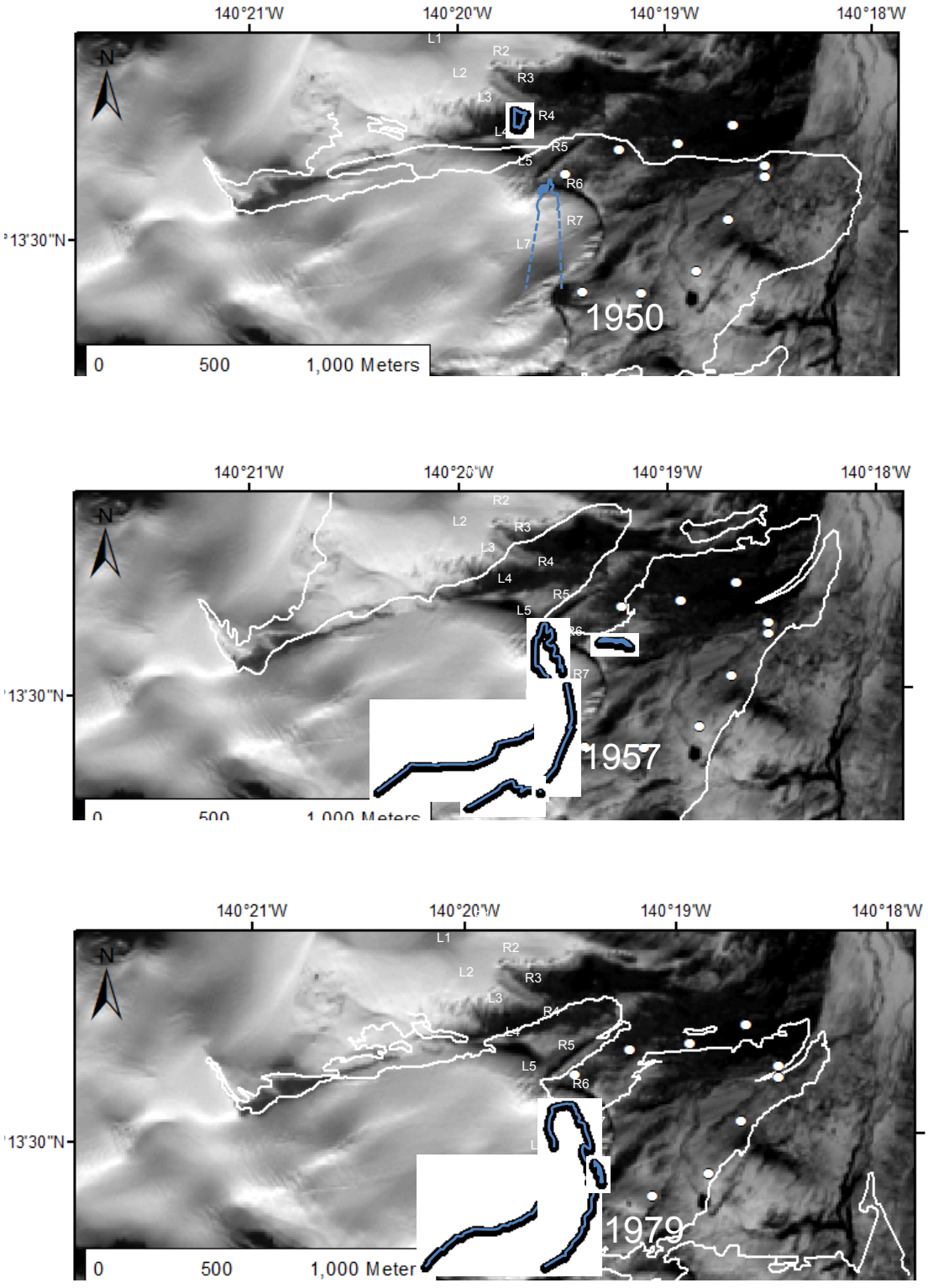

140°21'W
140°20'W
140°19'W
140°18'W
13'30"N
N
L1
R2
L2
R3
L3
R4
L4
R5
L5
R6
R7
L7
1950
0
500
1,000 Meters
1957
1979

Figure 1

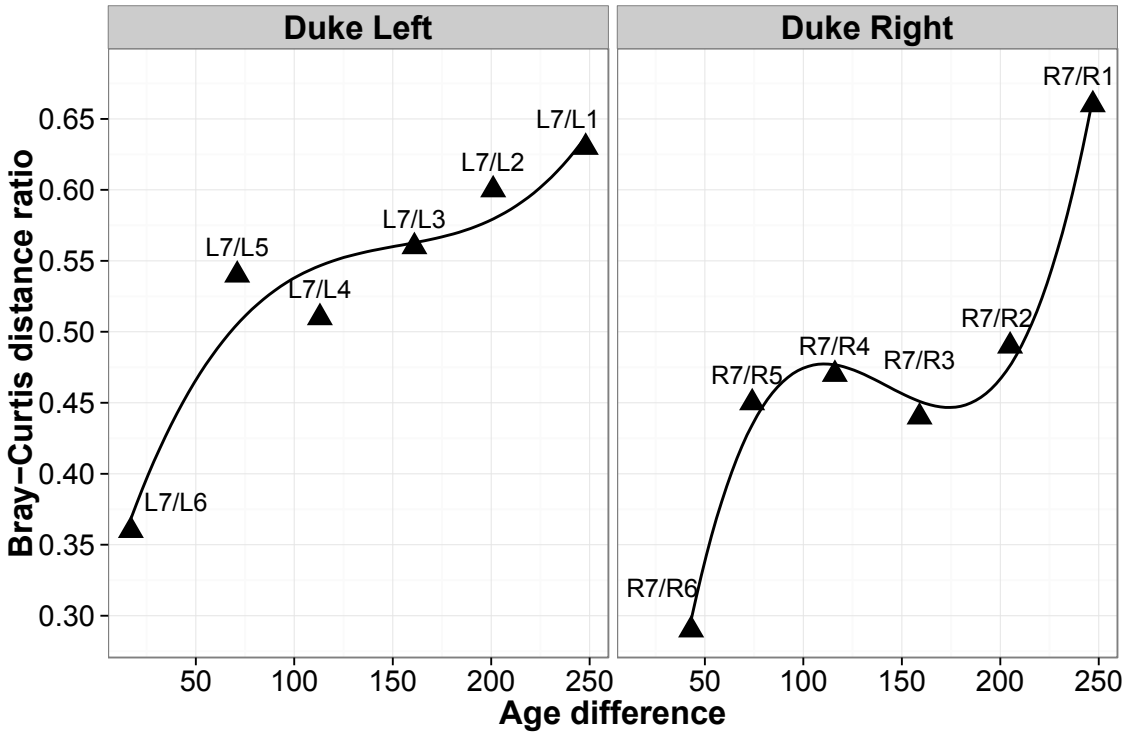

Duke Left
Duke Right
Bray−Curtis distance ratio
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
L7/L1
L7/L2
L7/L3
L7/L5
L7/L4
L7/L6
R7/R1
R7/R2
R7/R4
R7/R3
R7/R5
R7/R6
50
100
150
200
250
Age difference

Figure 2.

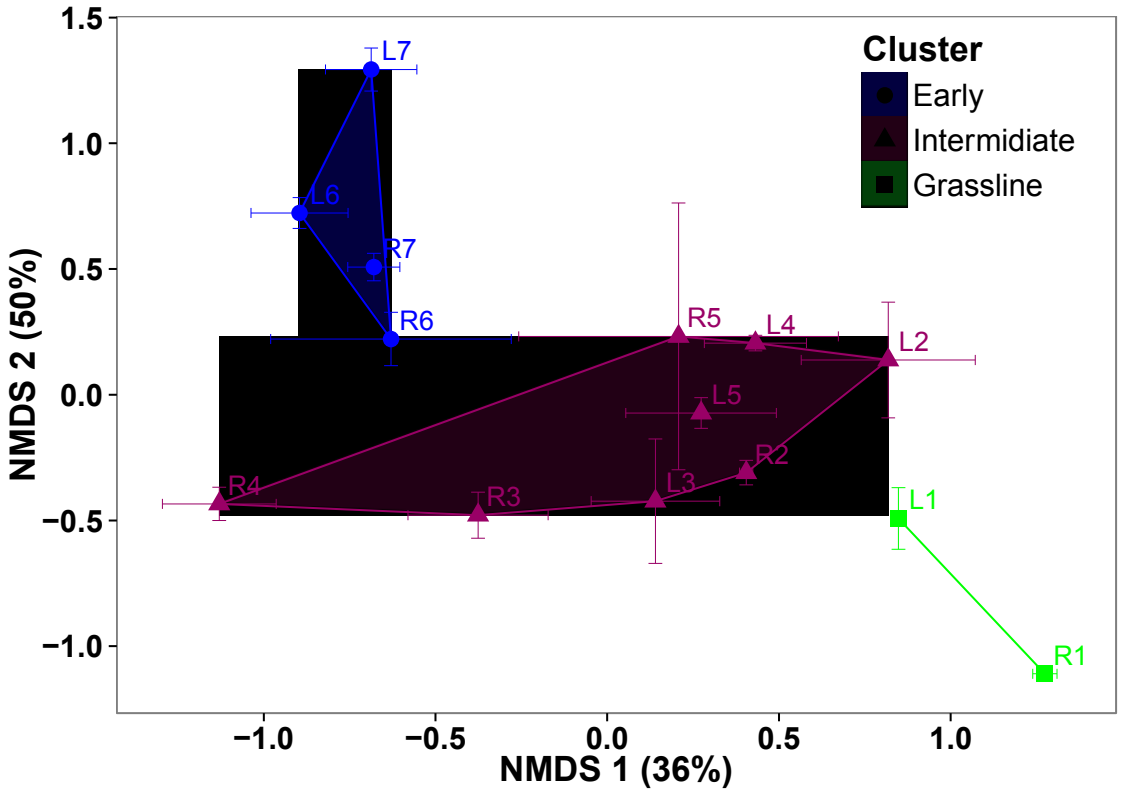

Cluster
Early
Intermidiate
Grassline
L7
L6
R7
R6
R5
L4
L2
L5
R2
R4
R3
L3
L1
R1
NMDS 2 (50%)
NMDS 1 (36%)
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.0
−0.5
0.0
0.5
1.0

Figure 3.

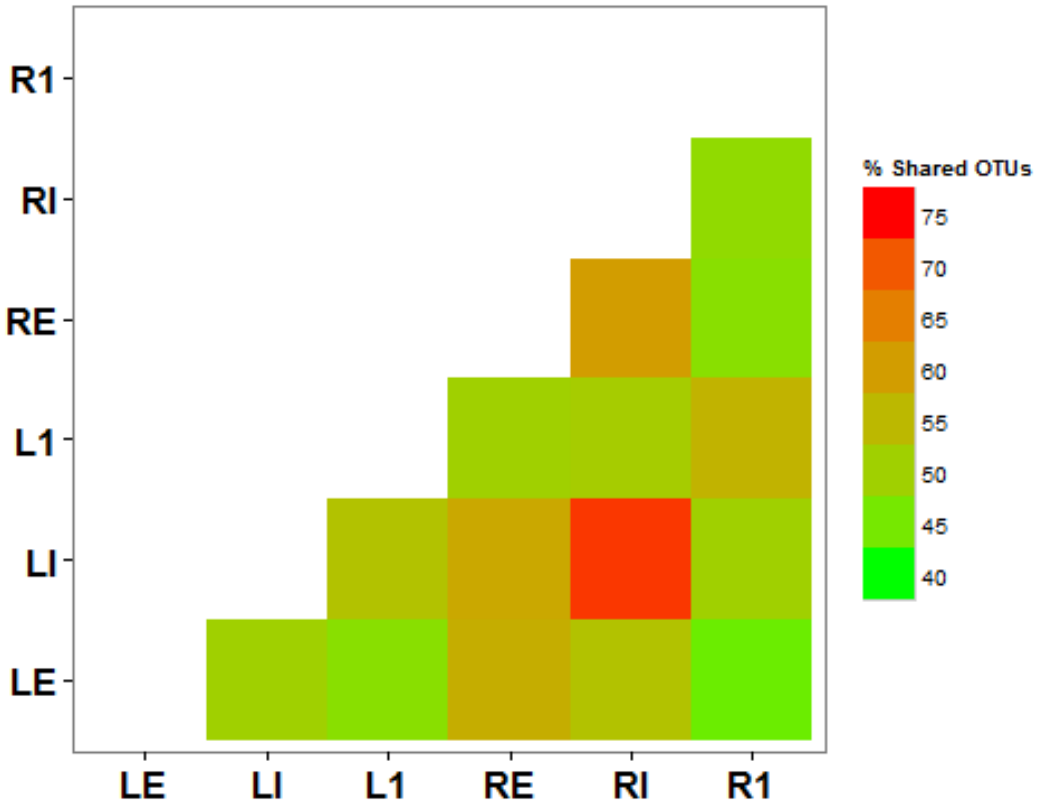

R1
RI
RE
L1
LI
LE
LE
LI
L1
RE
RI
R1
% Shared OTUs
75
70
65
60
55
50
45
40

Figure 4.

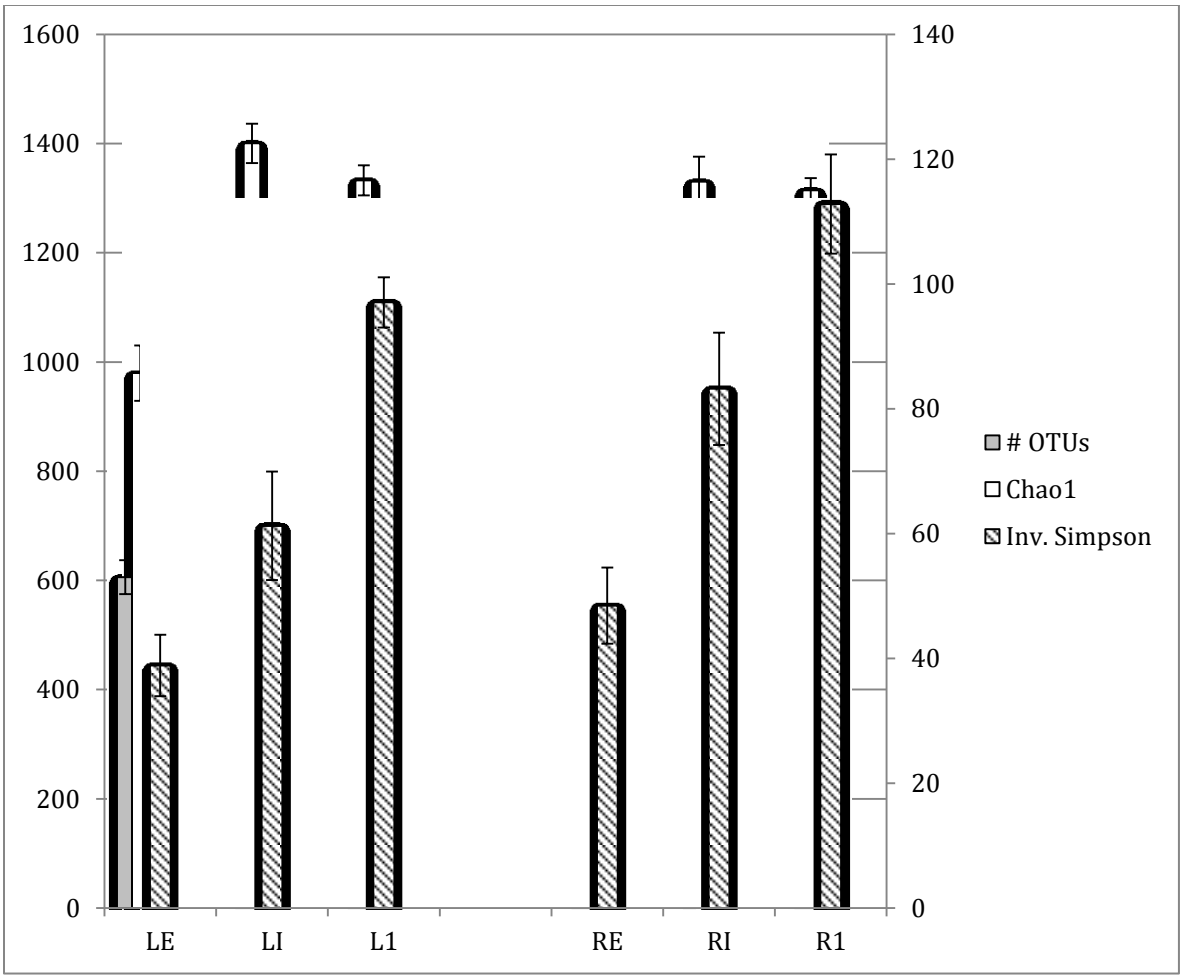

1600
1400
1200
1000
800
600
400
200
0
140
120
100
80
60
40
20
0
LE
LI
L1
RE
RI
R1
OTUs
Chao1
Inv. Simpson

Figure 5.

Supplementary Table S1

	pH	%TP (x10^{-1})	%TN (x10^{-1})	%NH_4 (x10^{-4})	%NO_3 (x10^{-4})	%TC	%TOC (x10^{-1})
L1	8.53±0.04	1.12±0.02	1.66±0.03	5.29±0.14	3.96±0.02	2.14±0.17	5.35±0.04
L2	8.70±0.13	0.83±0.01	0.81±0.03	2.37±0.02	4.11±0.02	1.63±0.07	0.94±0.02
L3	8.54±0.16	1.07±0.02	0.53±0.01	1.65±0.01	4.42±0.04	1.60±0.20	1.06±0.01
L4	8.52±0.13	0.83±0.00	0.72±0.01	1.92±0.05	3.92±0.01	1.87±0.05	1.71±0.02
L5	8.76±0.01	0.95±0.00	0.94±0.03	1.35±0.00	4.25±0.01	1.2±0.05	2.4±0.03
L6	8.83±0.14	0.87±0.01	1.13±0.03	1.42±0.02	3.92±0.02	1.75±0.08	1.89±0.01
L7	8.73±0.30	0.75±0.01	1.18±0.04	1.77±0.01	4.60±0.03	1.58±0.06	2.15±0.01
R1	7.78±0.33	1.25±0.03	16.1±0.15	26.4±0.55	9.15±0.21	18.4±1.55	35.3±0.56
R2	8.35±0.13	1.00±0.01	1.10±0.04	2.52±0.01	5.25±0.04	1.68±0.07	2.03±0.01
R3	8.77±0.03	0.91±0.01	0.89±0.01	1.97±0.02	4.56±0.02	1.74±0.05	1.91±0.03
R4	8.69±0.09	0.91±0.01	0.79±0.03	1.84±0.02	4.99±0.02	1.71±0.03	2.2±0.01
R5	8.75±0.04	0.83±0.00	0.87±0.03	2.07±0.02	5.01±0.02	1.78±0.01	1.87±0.01
R6	8.89±0.05	0.85±0.00	0.97±0.03	2.23±0.02	4.28±0.03	1.88±0.08	1.51±0.04
R7	8.72±0.04	0.90±0.01	0.71±0.02	1.63±0.01	4.47±0.01	1.79±0.06	0.57±0.01

Supplementary Table S2

Strains Used in Mock Community

E. coli

M. capsulatus bath

M. album BG8

M. sporium strain 5

M. trichosporium OB3

E. faecalis

P. vulgaris

E. aerogenes

S. aureus

Thauera*

Desulfomicrobium sp.

N. communis

N. europea*

Thermatoga cell2

T. affectus

Synechocystis

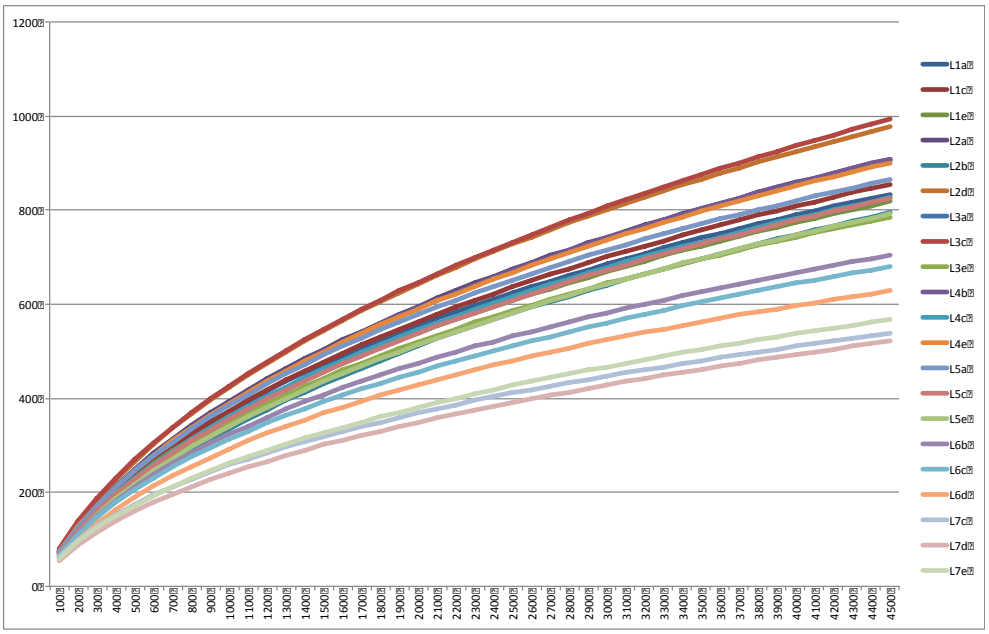
Leptolyngbya*

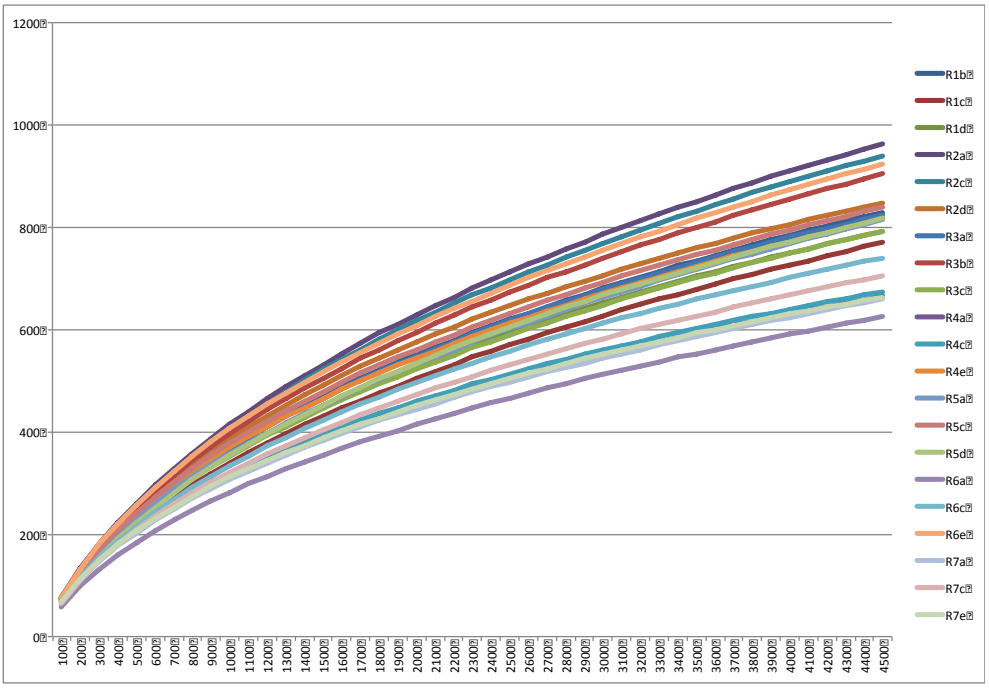
Anabaena

*partial sequence

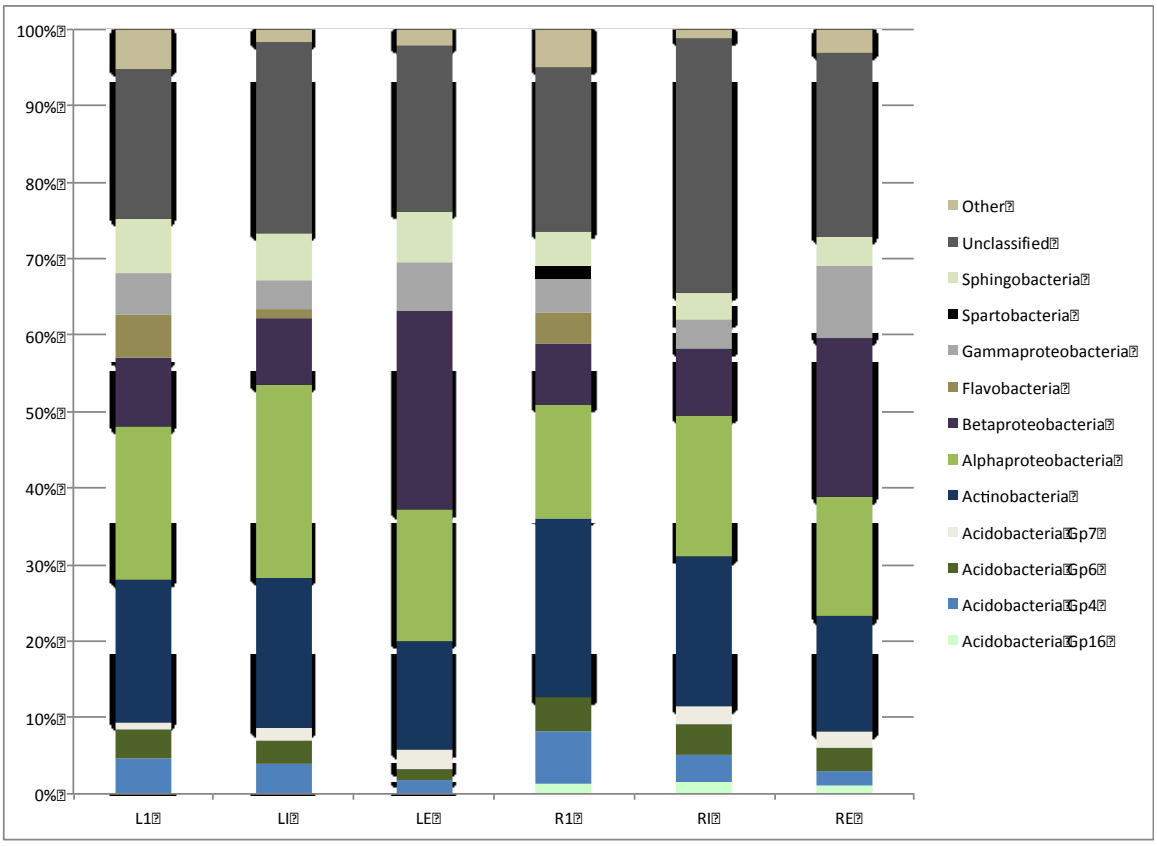
Supplementary Table S3

OTU	Number of seqs	Family based on RDP	Genus based on RDP	Genus based on NCBI blastn
1	3566	Desulfomicrobiaceae(100)	Desulfomicrobium(100)	Desulfomicrobium
2	2619	Enterobacteriaceae(98)	Unclassified	P. vulgaris
3	189	Enterobacteriaceae(100)	Unclassified	E. coli
4	134	Rhodocyclaceae(95)	Thauera(66)	Thauera
5	136	Staphylococcaceae(100)	Staphylococcus(100)	Staphylococcus aureus
6	105	Enterobacteriaceae(89)	Unclassified	Enterobacter
7	87	Cyanobacteria (100)	GpI(100)	Nostoc
8	50	Pseudomonadaceae(98)	Pseudomonas(98)	Pseudomonas aeruginosa
9	49	Enterococcaceae(100)	Enterococcus(100)	E.faeclis
10	2478	Enterobacteriaceae(98)	Unclassified	Enterobacter
11	7	Unclassified	Unclassified	Synechocystis
12	4	Sphingomonadaceae(83)	Sphingomonas(71)	alpha
13	4	Methylococcaceae(81)	Methylosarcina(75)	Methylomicrobium
14	3	Rhodocyclaceae(73)	Unclassified	Nitrosomonas communis
15	6	Methylocystaceae(64)	Methylocystis(64)	Methylosinus (alpha)
16	20	Enterobacteriaceae(66)	Unclassified	Enterobacter
17	29	Enterobacteriaceae(93)	Salmonella(76)	Enterobacter
18	2	Enterobacteriaceae(87)	Unclassified	P. vulgaris





Supplementary Figure S1.



Supplementary Figure S2.

CHAPTER 3: CONCLUSION

3.1 Contribution to the Field

The number of studies examining microbial succession in glacier foreland soils has increased considerably in recent years (Hodkinson *et al.* 2003; Tscherko *et al.* 2003; Nemergut *et al.* 2007; Schmidt *et al.* 2008; Schütte *et al.* 2009, 2010). This is due in part to the advent of next-generation sequencing (NGS) methods allowing the analysis of millions of DNA sequences in parallel in a single run (Shokralla *et al.* 2012; Nikolaki & Tsiamis 2013), thereby providing researchers with an unprecedented depth of information. Furthermore, global warming is leading to heightened rates of glacier retreat across the world (Hock 2014; Hood *et al.* 2015). Glacier ice covers approximately 10% of Earth's terrestrial surface (Hock 2014); it is therefore important to understand the implications of retreat on local ecosystem development. In this thesis, the overarching goal was to use NGS technology to examine microbial succession in glacier foreland soils, and to link these findings to changes in soil development occurring after glacier retreat.

Most studies of microbial succession in foreland soils have sampled soils that are recently deglaciated and unvegetated (Nemergut *et al.* 2007; Schmidt *et al.* 2008), or, soils rapidly colonized by plants, where plant succession coincides with microbial community development (Schütte *et al.* 2010; Brown & Jumpponen 2013). My research utilizes two chronosequences within the Duke River glacier foreland that include over 200 years of relatively bare soils before a grassline. To the best of my knowledge, this is the first study investigating succession in this

region of glaciers fed by the largest ice field outside the poles, as well as at the Duke River glacier. The unique attributes of the glacier therefore allowed me to establish two main findings that were previously impossible to discover at other glacier forelands.

First, due to the extensive period before plant colonization, I was able to examine the development of microbial communities over time without the added influence of plan colonization. Other studies examining microbial succession in glacier foreland soils have observed a trend of high bacterial community turnover immediately after glacier retreat (Tscherko *et al.* 2003; Schütte *et al.* 2010; Brown & Jumpponen 2013). However, this turnover coincides with higher plant establishment and therefore it is impossible to discern whether the turnover would occur if vegetation was not present. In my findings, I determined that bacterial community turnover occurred even without the influence of plant colonization. Thus, the turnover likely occurs because previously established subglacial bacterial communities are introduced to a vastly different environment after glacier retreat; therefore a period of transition occurs whereby microbes initially conditioned to subglacial systems adapt to this new environmental framework.

The second main finding in my research involves the dynamics associated with the arrival of a grassline at Duke River glacier. I determined that the presence of the grassline was marked by another period of high turnover in bacterial communities. This finding is unique as it demonstrates that the initial period of turnover of bacterial communities in other studies is likely due both the

transition from subglacial to proglacial environments, as well as the coinciding plant establishment.

Glaciers are retreating around the globe due to the effects of global warming (Hock 2014; Hood *et al.* 2015). Continued retreat of glaciers will result in rise of global sea levels, impact fresh water availability, change biogeochemical and ecological properties of streams, and liberate significant stores of ice-locked organic carbon which will impact downstream terrestrial and aquatic ecosystems (Solomon *et al.* 2009; Hock 2014; Marzeion *et al.* 2014; Hood *et al.* 2015). Overall, my research provided an important link in the successional framework at glacier foreland soils following glacier retreat and reinforces the idea that microbial processes occurring in foreland soils after glacier retreat are linked to downvalley ecosystem development and must be carefully considered when evaluating the impacts of climate change on glacier ecosystems.

3.2 Improvements to Study

Although I achieved my goal of evaluating microbial successional processes in glacier foreland soils, there were a number of improvements that could have been implemented to strengthen my results. Most of the limitations in the study were due to time constraints of my thesis program and the overwhelming amount of data that would need to be analyzed in a restricted time period. I have outlined two main improvements that would have enhanced the results in this thesis.

3.2.1 Soil Physicochemical Parameters

In my thesis I measured the following soil chemical parameters: pH, carbon (organic and total carbon), nitrogen (ammonium, nitrate, total nitrogen), and total phosphorous. These parameters were chosen because of their well-known importance in soil development, especially in the context of glacier forelands, and which I have discussed in the first chapter. However, there were other soil parameters that are also important that could have been analyzed, including soil moisture and soil texture. These parameters have been demonstrated in previous studies in influencing bacterial community structure, and insight into how these variables changed in my study may have allowed a better understanding of the successional processes occurring in the soils (i.e. why communities change after retreat and plant colonization or remain stable in intermediate soils).

Soil moisture influences bacterial cell physiology and physicochemical properties of soils (Castro *et al.* 2010). For example, low soil moisture causes low bacterial intracellular water potential which can therefore reduce enzyme activity and consequently, microbial activity (Stark & Firestone 1995). In turn, low microbial activity may lead to slowed carbon turnover therefore reducing substrate availability and thus those organism that are able to utilize a broad range of substrates and therefore withstand desiccation and fluctuations in soil moisture content would be favored in dry soils (Castro *et al.* 2010). For example, oligotrophic Acidobacteria have been demonstrated to dominate in dry soils and become outcompeted in wet soils, therefore demonstrating the impact of soil moisture on bacterial community composition (Castro *et al.* 2010). Furthermore,

low soil moisture was found to restrict substrate supply due to the diminished diffusive flow of substrates to the cell surface, causing a decline in the activity of nitrifying bacteria (Stark & Firestone 1995). Glacier foreland soils are initially characterized by soil low moisture content, which increases over time, and is linked to increased organic decomposition rates (Hodkinson *et al.* 2003). The increased soil moisture is likely inked to changes in soil texture over time (Kaštovská *et al.* 2006).

Soil texture is also important component involved in soil development. Recently deglaciated soils often contain course, sandy-silty sediment with barren rocks, and larger boulders (Kaštovská *et al.* 2006; Bernasconi *et al.* 2011). Over time, biological, chemical, and physical weathering processes eventually result in soils with finer, clay sediment, thus increasing the surface area of the soil (Kaštovská *et al.* 2006; Bernasconi *et al.* 2011). This process of change in soil texture has been demonstrated to occur within 108 years since deglaciation (Bernasconi *et al.* 2011). An increased surface area results in more liquid water between grains (i.e. high water retention), and more surface for attachment of microbial cells (Kaštovská *et al.* 2006). This also makes course sediments prone to leaching of nutrients relative to finer sediments (Anderson *et al.* 2006). Furthermore, finer sediments increase the amount of organic matter able to be stored in soils, increase nutrient storage, and, due to the negative charge associated with clay, increase the cation exchange capacity of soils (Anderson 1988). Overall, the course sediments in recently deglaciated soils is less favorable as a microbial substrate, and may hinder development of microbial

communities (Kaštovská *et al.* 2006).

3.2.2 Archaea and Fungi

Much of the focus of succession of microbial communities in glacier foreland soils has been on bacteria. However, fungi and Archaea are also thought to play an important role, and several studies have investigated successional patterns and potential activity of these organisms. For example, anaerobic methanogenic Archaea were found to contribute to methane production in foreland soils of the Rotmoosferner glacier in Austria (Hofmann *et al.* 2013) while a correlation has been found between ammonia-oxidizing archaea and nitrification (Brankatschk *et al.* 2011). Moreover, both Zumsteg et al., and Nicol et al., found various stages of succession in archaeal communities along glacier forefields similar to those patterns observed in bacteria thus supporting that archaeal communities do exhibit successional processes (Nicol *et al.* 2005; Zumsteg *et al.* 2012). However, the study by Zumsteg et al., observed decreasing diversity of archaeal communities over time while Nicol et al., found increasing diversity, pointing to the need for further investigation of archaeal successional patterns (Nicol *et al.* 2005; Zumsteg *et al.* 2012).

Succession of fungal communities in glacial forelands has also been studied. Similar to bacteria, there are distinct fungal communities in the bare and vegetated soils of glacier forelands, with a shift from *Ascomycota* that can live on rocks in the bare soils to *Basidiomycota* in the vegetated soils. However, recent findings also point to major differences between bacterial and fungal successional patterns. For example, unlike the increase in diversity observed in

bacterial successional patterns, fungal community diversity either remains unchanged or decreases along glacier forelands (Jumpponen 2003; Zumsteg *et al.* 2012; Brown & Jumpponen 2013). Jumpponen argued that the decrease in diversity was due to a shift from spore bank in the youngest soils to an active, though less diverse, fungal community in older soils (Jumpponen 2003). Another major difference is that unlike the convergence of bacterial communities along glacier forelands, fungal community development appears to be more stochastic and therefore less deterministic (Jumpponen 2003; Brown & Jumpponen 2013). Schmidt et al., articulate several reasons why this discrepancy might exist (Schmidt *et al.* 2014). Due to their smaller size, bacteria have a larger dispersal range than fungi, thus allowing a consistent input of bacteria in these systems and leading to more deterministic patterns of assembly (Schmidt *et al.* 2014). Also, bacteria possess a much wider range of metabolisms and physiological capabilities, including photoautotrophy, heterotrophy, and chemoautotrophy, of which many can fix nitrogen, allowing more successful colonization of nutrient-poor foreland soils relative to fungi, which are strictly heterotrophs relying on fixed carbon and nitrogen (Schmidt *et al.* 2014). These mechanisms allow bacteria to have more consistent patterns of assembly relative to fungi which rely on nutrient buildup and thus lead to more stochastic distributions (Schmidt *et al.* 2014). Overall, these studies have established preliminary work underlining the successional patterns of Archaea and fungi in glacier foreland chronosequences. However, more studies are needed to fully examine these patterns and to draw similarities to bacterial processes.

3.3 Future Directions

In the future, my research could be expanded upon in a number of ways. Future experiments could include modifications of the sampling design through to changes in sequencing analysis. In this section I will describe three directions my research could be taken in: reciprocal transplant experiments, metagenomic sequencing, and additional sampling.

3.3.1 Reciprocal Transplant Experiments

Reciprocal transplant experiments involve transplanting a soil sample from site a to site b, and a sample from site b to site a. By performing these experiments, we can begin to investigate a critical question in microbial ecology: are microbial communities structured by environmental parameters, or vice-versa? There have been several studies in glacier foreland soils that have performed reciprocal transplant experiments and have provided evidence that microbial communities shift in response to changing soil environments (Zumsteg *et al.* 2013; Meola *et al.* 2014). For example at the Damma glacier, transplanting soils from sites receiving low sunlight to sites receiving higher sunlight resulted in increased microbial activity and high rates of species turnover, demonstrating that soil environment influences bacterial activity and composition (Zumsteg *et al.* 2013). At the Duke River glacier foreland, a future experiment could include transplanting bare soils to sites within the grassline and vice versa; this would determine whether communities moved from bare soils to grassline soils, where soil chemical parameters are substantially different, would shift towards becoming like grassline communities, remain the same, or shift toward a new

type of community entirely, and vice versa. Moreover, performing a reciprocal transplant of younger bare soils to older bare soils, where soil chemical parameters are largely the same, would also determine whether age, rather than environment influences bacterial communities.

3.3.2 Metagenomics

Metagenomics is the sequencing of all of the DNA extracted from a sample (Hugenholtz & Tyson 2008). In practice, metagenomics obtains fragments of genomes from members of microbial communities. This approach is distinct from traditional 16S rRNA gene sequencing, which provides information about a single phylogenetic marker gene; metagenomics details the entire genome and therefore the entire gene inventory of a microbe (Hugenholtz & Tyson 2008). This has substantial implications – by having this information, researchers are therefore able to infer potential functional information such as the presence or absence of physiological pathways (Hugenholtz & Tyson 2008). In my research, performing metagenomic analysis would lead to more detailed examination of successional processes occurring in the soils. For example, determining whether the community shifts from primarily carbon or nitrogen fixing microbes to heterotrophic microbes would establish much greater insight into the shifts occurring in the communities over time following retreat.

3.3.3 Additional Sampling

An improvement in sampling is another way of enhancing this study. Notably, in this study there was only one grassline sample collected within each

chronosequence and therefore within-grassline analysis could not be completed. Collecting samples further into the grassline would enable me to determine whether the communities continue to change or become stabilized with plant establishment. Grassline sampling could also include rhizosphere sampling, which would allow characterization of communities directly associated with plants. Seasonal variability in sampling would also be another way of improving sampling. Comparing samples collected in summer with samples collected in spring, for example, would allow determination of whether seasonal variation influences microbial community successional processes.

3.4 Concluding Remarks

Glaciers are retreating around the globe due to the effects of global warming (Hock 2014; Hood *et al.* 2015). Continued retreat of glaciers will result in rise of global sea levels, impact fresh water availability, change biogeochemical and ecological properties of streams, and liberate significant stores of ice-locked organic carbon which will impact downstream terrestrial and aquatic ecosystems (Solomon *et al.* 2009; Hock 2014; Marzeion *et al.* 2014; Hood *et al.* 2015). Overall, my research provided an important link in the successional framework at glacier foreland soils following glacier retreat and reinforces the idea that microbial processes occurring in foreland soils after glacier retreat are linked to downvalley ecosystem development and must be carefully considered when evaluating the impacts of climate change on glacier ecosystems.

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