HIGH AND DRY: SPACED OUT MICROBES IN ARID ALPINE ENVIRONMENTS

by

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ABSTRACT

Periglacial ecosystems can be found on every continent and represent life’s coldest terrestrial foothold, yet their ecology is rarely studied. These areas are subject to the harsh environmental stressors of a short annual photosynthetically active period, high solar radiation, high temperature variability, low soil development, and low humidity. This research takes advantage of these environmental stressors to address one central thesis: sampling across multiple spatial scales and environmental gradients will quantify the relative strength of biological and environmental factors shaping periglacial ecosystems. The results demonstrate that periglacial soils harbor the lowest microbial biomass and dissolved nutrient levels of any terrestrial environment that supports life. In general, the abundance of microbes in these soils is positively correlated with soil water content and water holding capacity. However, tropical periglacial soils from Peru had higher average microbial biomass than temperate zone soils from Colorado, Nepal, Argentina, and Alaska despite the relatively dry soil conditions and high altitude of the Peruvian soils. Periglacial soil microorganisms also persist at a surprisingly high microbial biomass C:N ratio in spite of low soil dissolved C:N ratios, which is suggestive of a soil stoichiometry that is greatly divergent from that observed in vegetated systems. This divergence is likely due to microorganisms accumulating high concentrations of drying/freezing protection oligosaccharides in order to survive in the harsh periglacial environment. Soil bacterial communities in the periglacial soils of Green Lakes Valley, Colorado showed strong spatial autocorrelation in community composition up to a distance of 240 meters that was driven by changes in the relative abundance of specific bacterial clades across the landscape. Analysis
of clade habitat distribution models and spatial co-correlation maps identified soil pH, plant abundance, and snow depth as major variables structuring bacterial community composition across this landscape and revealed an unexpected and important oligotrophic niche for the Rhodospirillales in soil. Furthermore, the global analysis of periglacial soils from Argentina, Colorado, Nepal and Alaska shows that habitat distribution models for bacteria have strong predictive power across the entire globe. This multi-scale approach yields important insights into the fundamentals soil microbiology and, ultimately, a greatly improved definition of periglacial ecology.
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1.1 Overview of the Periglacial Ecosystem

The periglacial ecosystem exists at both the latitudinal and altitudinal edges of the world and exerts uniquely extreme demands on life’s ability to survive, yet periglacial ecology is rarely studied explicitly. Traditionally the term periglacial encompasses all soils that show morphology characteristic of frequent frost and ice, i.e. patterned ground, glacial moraines, and permafrost (Karte, 1983). While periglacial processes can occur from the lower edges of the nival zone well into the tundra and subalpine forests, here I employ an operational definition for the periglacial ecosystem: all ice shaped soils above where plants continuously cover the landscape (Figure 1.1). These distinct areas are subject to the harsh environmental stresses of a short annual photosynthetically active period, high solar radiation, high temperature variability, low soil development, and low humidity. As a result, periglacial regions can be extremely arid either seasonally (Ley et al., 2004) or constitutively (Cary et al., 2010) despite their dependence on frost and ice processes. Although periglacial systems are extreme they are one of the only terrestrial ecosystem types (the other being tundra) that is found at all latitudes of the Earth (Figure 1.2). Visually, periglacial soils often appear devoid of life and, as a result, have been thought to be detrital ecosystems that subsist solely on the aeolian deposition of organic material (Swan, 1963). However, the growing body of research on periglacial soils has demonstrated that these soils harbor a surprisingly active and diverse community of microorganisms (Ley et al., 2002, 2004; Nemergut et al., 2007; Freeman et al., 2009a, 200b; Schmidt et al., 2010; Fierer et al., 2010; Cary et al., 2010).
Figure 1.1 The periglacial ecosystem as distinguished from alpine tundra and nival systems. Note the sporadic patches of plant cover within the periglacial zone that are ecotone type communities. Photo taken from above Kangshar Village, Annapurna Conservation Area, Nepal.

Figure 1.2 The global distribution of periglacial soils (pink area) as estimated for all areas excluding Antarctica using a mean temperature range of 2-6.7 °C for the warmest quarter of the year (temperature data from worldclim 1.3, Hijmans et al., 2005). Periglacial area in Antarctica is estimated from Cary et al.’s (2010) survey data.
The diverse microbiology of periglacial soil systems results not only from a largely microbially dominated system but also from the high degree of topographic relief characteristic of ice shaped systems which creates a multiplicity of microclimatic conditions (Burns and Tonkin, 1982; Birkeland et al., 1989). Environmental change exerted by the warming of the global climate further adds to this topo-climatic heterogeneity and manifests in particularly spectacular changes in snow and ice presence across periglacial regions (Beniston, 2003; Zemp et al., 2006; Radic and Hock, 2011). As a result of the combined influence of climate and topography, gradients of survivability in periglacial regions span from extremely harsh conditions with persistently zero plant cover to comparatively mild conditions that manifest in patches of 100% tundra-like plant cover (Figure 1.1). These gradients in environmental conditions make periglacial soils ideal for addressing questions of environmental influence on soil microbial community dynamics and biogeochemistry.

The large environmental variation of periglacial soils leads to the central question of this thesis: *How do gradients in environmental and soil biogeochemical factors influence soil microorganisms?* I approach this question using a nesting of sampling scales from fine scale sampling distances less than 1 m, to an entire periglacial landscape, and finally to a maximum scale of globally distributed sites. My thesis is that sampling across multiple spatial scales and environmental gradients will identify the dominant biological and environmental factors structuring periglacial ecology. Ultimately, this multi-scale approach builds upon the disparate array of studies of periglacial systems in glacier successions (Nemergut et al., 2007; Sattin et al., 2009; Skidmore et al., 2005; Tscherko et al., 2003b), periglacial snowfields (Ley et al., 2002, 2004; Williams et al., 1997; Hood et al., 2002; Freeman et al., 2009a, b) Antarctic dry valleys (Pointing et al., 2009; Cary et al., 2010), polar fellfields (Sjursen et al., 2004), and cold deserts
(Jones et al., 2000; Bahl et al., 2011) in order to create a more complete description of the periglacial ecosystem.

1.2 Jenny’s State Factor Theory for Describing an Ecosystem

The use of environmental gradients to study the interactions of biotic and abiotic components of an ecosystem fits readily within the larger context of soil state factors developed by Hans Jenny (1941). Jenny’s soil state factors span climatic, biotic, topographic, lithographic, and temporal (abbr. clorpt; Jenny, 1941), and comprehensively describe the dominant forces shaping a system. In his 1958 revision, Jenny explicitly broadens the applicability of the state factor schema and describes in detail the interaction between soil formation and landscape ecology. He also points to two ways of studying the effects of clorpt on ecosystem structure: 1) measure all gradients and use statistics to isolate the relative contribution of each factor; 2) examine the correlation between one state factor and soil development with all the others held constant. Importantly, Jenny suggests a workaround for the second approach wherein, if the scale of analysis is coarse enough, one can assume that the variation in specific factors is unimportant in shaping soil processes. Thus, for convenience many studies have taken a reduced interpretation of the state factor model, with plants as the primary biotic component and only broad scale measurements of climate and parent material. These shortcuts are necessary, for example in creating country-wide soil maps, but are known to be problematic for landscape and finer detailed analysis (Johnson and Johnson, 2010).

Other applications of the simplified approach for studying Jenny’s state factors rely on the somewhat serendipitous identification of naturally occurring regions that vary only in one or two factors. A historically important realization of this effect is volcanoes which create successive lava flows with low variation in parent material that occur in known time series, have
similar topography, and are in a narrow enough climatic region (Aplet and Vitousek, 1994; Cutler et al., 2008). Specifically, the studies of the well-characterized Hawaiian lava flow chronosequences have taken advantage of the state factor schema to identify how variation in clorpt affects ecosystem development (Aplet and Vitousek, 1994). Examples such as the Hawaiian chronosequences highlight the utility of the state factor schema for targeted studies of environment-ecosystem interactions.

The periglacial ecosystem with its strong abiotic and biotic gradients over short distances provides a system with similar utility within the Jenny framework. Indeed, studies of glacial recession chronosequences have been conducted with similar effectiveness to lava flow chronosequences, although the oldest time-steps sometimes deviate from the assumed constants of climate and parent material (Wardle et al., 2004). However, periglacial ecosystems are also excellent candidates for study under the approach of simultaneous measurement of multiple state factor effects. This utility is a direct result of the short distances over which state factors vary in periglacial landscapes and allows the application of both the statistical decomposition of the strength of factors as well as the comparison of local sites that only vary significantly in a reduced number of factors. Moreover, because of the broad geographic distribution of periglacial regions, gradients in every factor are possible (Table 1.1). In order to provide the reader with a context for my individual chapters regarding the scale and magnitude of particular state factors in periglacial systems, I summarize the current extent of knowledge regarding each state factor’s influence in the following sections.
<table>
<thead>
<tr>
<th>Ecosystem State Factor</th>
<th>Periglacial Gradient</th>
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<tbody>
<tr>
<td>Climate</td>
<td>Spans all latitudes and desert/monsoon precipitation areas</td>
</tr>
<tr>
<td>Organisms</td>
<td>Zero plant cover to continuous tundra</td>
</tr>
<tr>
<td>Relief (topography)</td>
<td>Flat 0° shelves to 90° Cliffs</td>
</tr>
<tr>
<td>Parent material (lithography)</td>
<td>All major rock types</td>
</tr>
<tr>
<td>Time</td>
<td>Proglacial zones to weathered snowbanks</td>
</tr>
<tr>
<td>Anthropological (added by Amundson &amp; Jenny; 1991)</td>
<td>Ski-slopes to remote peaks</td>
</tr>
</tbody>
</table>

Table 1.1 The extent of variation in Jenny’s (1941) *clorpt* state factors within the periglacial ecosystem.

*Climate*

Because ice and frost are what create periglacial ecosystems, these systems can be found at all latitudes on the Earth (Figure 1.2) and, despite regional differences in elevation, they experience relatively similar annual cycles between snow-covered and snow-free seasons (e.g. Figure 1.3 adapted from Ley *et al.*, 2004). Thus, the global variation in precipitation patterns and the latitudinal gradient in solar energy inputs mainly alter the altitude at which periglacial ecosystems are found (Troll, 1973; Yao *et al.*, 2006; Radic and Hock, 2011); e.g. periglacial ecosystems are at sea level in polar regions, 2500 m.a.s.l. (meters above sea level) in the Bavarian Alps, 4000 m.a.s.l. in the Rockies of central United States, 4700-5000 m.a.s.l. in the Andes of southern Perú, and 5000-5600 m.a.s.l. in the Himalayas (Troll, 1973).
Still, there are some finer differences across the globe. In temperate and polar latitudes, snow-cover is a result of winter snowfall and summers can be dry or wet depending on the region’s precipitation pattern (Fountain et al., 1999; Ley et al., 2004; Concienne, 2010; Sattin et al., 2009). Conversely, in the tropics although daily temperatures do not change significantly throughout the year, precipitation is strongly seasonally, so the wet, snow-covered, summer season conditions are similar to the temperate winter and the dry, snow-free, winter season conditions are similar to the temperate summer. Daily temperature cycling also varies by latitude with the high altitude climate of tropical systems allowing for soil temperatures that reach 20°C during the day and -20°C during the night year-round (Schmidt et al., 2008), whereas diurnal soil temperatures fluctuate over increasingly narrow ranges as the altitude of periglacial ecosystems decreases towards the poles (e.g. Figure 1.3).
Local topo-climatic variation creates landscape-scale heterogeneity in soil microclimate that ranges from highly sheltered areas that accumulate enough snow to stay covered year round, to highly exposed areas that never accumulate snow in significant amounts (Burns and Tonkin, 1982; Erickson et al., 2005; Liator et al., 2008; Molotch et al., 2008). Due to the interaction of temperature with aspect and shelteredness, mountain periglacial landscapes have strong local variation in soil temperature and snowmelt timing (Liator et al., 2008). The additional contribution of melt water from primary glacial mass as well as stranded ice masses (e.g. hanging glaciers, rock glaciers, and snowfields) further structures the heterogeneity of soil microclimate across the periglacial landscape and is important even after the snow has melted out completely (Leopold et al., 2011). As a result of this hydrological heterogeneity, periglacial soils within a landscape can vary from barren and desert-like mineral soils, to wet relatively well developed organic rich protosols (Burns, 1980). The hydrology of periglacial systems has been a subject of intensive study locally by the Williams research group in the University of Colorado’s Geography department (e.g. Williams et al., 1996a, 2009; Hood et al., 2002; Liu et al., 2004; Molotch et al., 2008; Hill, 2009; Leopold et al., 2011) and more broadly by various other researchers (Molotch et al., 2004; Huth et al., 2004; Sickman et al., 2001; Soulsby et al., 2006); however, the relationship between landscape hydrology and the ecology of periglacial organisms is still poorly understood.

Interannual variation in climatic factors such as temperature and water is also important to periglacial systems. Historically, the global extent of periglacial soils has changed on the time scale of global climate cycles (Yao et al., 2006; Radic and Hock, 2011), which would have likely resulted in both decadal and geologic timescale advance and retreat dynamics. A period of colder and/or higher precipitation than average years results in the expansion of the area that is
covered year-round with snow and, in glacial areas, weathering of the soil surface by the advance of glacial ice. These disturbance factors have been essential in shaping periglacial soils and in determining what organisms can survive in periglacial areas. While periglacial areas have persisted in this state of quasi-equilibrium as barren icy soils for hundreds to thousands of years, the majority of the areas have been pushed into an accelerated warming/melt-out trajectory by climate change (Nemergut et al., 2007; Sattin et al., 2009; Zemp et al., 2006; Radic and Hock, 2011); however, parts of the Rockies and Himalayas have experienced periods of increased snowfall and little change in mean temperature in recent time (Williams et al., 1996a; Hoffman et al., 2007; Raina, 2009). Thus, while most periglacial areas over the past 50 years can be thought of as on a primary successional trajectory, periglacial systems are likely more heterogeneous in their response to climate change than might be expected (Hoffman et al., 2007).

**Biotic**

Variation in microclimate and, in particular, the length of time soils are exposed to sunlight across the periglacial landscape creates a gradient in soil biota from plant-free, microbially dominated patches to completely plant-covered patches (Burns and Tonkin, 1982). This gradient in microclimate and plant abundance also has a major effect on the landscape variation in soil nutrients, particularly as plant abundances approach zero. Indeed, soil total organic C, N, microbial biomass C and N levels in the unvegetated soils of temperate Green Lakes Valley, Colorado, are an order of magnitude lower than those seen in nearby tundra, while soil dissolved N are similar to tundra levels (Williams et al., 1997; Ley et al., 2004). Globally, periglacial soils display similar nutrient patterns to Green Lakes Valley, and are consistently lower than even desert soils (Table 1.2).
Table 1.2 A summary of previously reported soil nutrients, microbial biomass, and pH for desert and periglacial soils. Only measurements of microbial biomass via chloroform fumigation are included.

Previous work in the Green Lakes Valley (Brooks et al., 1996; Ley et al. 2001, 2002, 2004) has begun to outline the soil environmental and biological factors which differentiate between seasons and between the end points of plant dominance. In general, plant dominated periglacial soils are wetter, snow free longer, and have ten times as much soil microbial biomass as microbially dominated soils (Ley et al., 2004). However, soil microbial biomass peaks during spring snowmelt and summer and winter biomass is half of the spring levels. Functional guild activities also differ in their seasonal abundance and this effect varies with plant abundance (Ley...
et al., 2004). While these differences are evident, landscapes are not divisible into a dichotomous matrix of plant-free versus plant-covered. Contiguous patches of plant cover are rarely larger than a few square meters and characterizing the role of gradients in microclimate and plant dominance in structuring the abundance of soil microorganisms in periglacial soils is a primary unknown addressed by this thesis research.

Soil C has its source both from in situ autotrophic fixation and from aeolian deposition of pollen (Freeman et al., 2009b) and plant debris (Ley et al., 2004). Although, early studies theorized that heterotrophy would be the dominant process in microbially dominated periglacial soils (Troll, 1973), recent evidence has shown substantial levels of photoautotrophic C fixation and a diverse abundance of phototrophs (Nemer gut et al., 2007; Sattin et al., 2009; Freeman et al., 2009a). The rates of autotrophic C fixation in unvegetated areas are quite low compared to tundra systems (Freeman et al., 2009a; Cary et al., 2010) and heterotrophic activity is likely extremely limited as a result. Similar rates of autotrophic C fixation are also observed in Antarctic periglacial soils (Freeman et al., 2009a; Cary et al., 2010). These levels support the idea of a highly oligotrophic nutrient status in periglacial soils.

Similar to soil C, periglacial N is either fixed in situ, deposited in dust or dissolved in precipitation. While N deposition sources are primarily anthropogenic (Williams et al., 1996b; Hood et al., 2003), N fixation is energetically costly and requires sufficient supplies of phosphorus, iron, and molybdenum (Vitousek et al., 2002). In remote periglacial areas with low N deposition such as the Dry Valleys of Antarctica (Bate et al., 2008) or in the nascent soils of proglacial areas (Nemer gut et al., 2006; Tscherko et al., 2003b), N levels are low enough that N may limit both autotrophic and heterotrophic microbial activity. However, nitrogen levels in periglacial regions near industrialized areas can receive a substantial amount of anthropogenic N
deposition and may have reached saturation levels due to high levels of anthropogenic N deposition (Williams et al., 1996b). The support for the saturation hypothesis comes from the Green Lakes Valley, where high yearly concentrations of NO₃⁻ have been observed in streams draining periglacial catchments despite the high levels of NH₄⁺ dominated wet and dry deposition (Williams et al., 1996b; Hood et al., 2003). Periglacial soils should be particularly sensitive to the effects of N deposition because high N loads can acidify soils and the undeveloped periglacial soils have relatively low buffering capacity (Molotch et al., 2008).

Nutrient limitation of microbial communities is often assayed by measuring the relative increase in soil respiration when nutrients are added. Surprisingly, in a remote periglacial region of the Peruvian Andes the addition of solely C or N to periglacial soils triggered only a slight respiration response to C addition and no response from N cycling rates to N addition (Schmidt et al., 2009). This low response suggests simultaneous limitation of the microbial community by multiple nutrients. In a similar experiment, C and N added in concert to microbial crusts at an early successional glacier foreland in Svalbard caused an increase in soil respiration rates, but neither C nor N alone had an effect (Yoshitake et al., 2007). These results are consistent with the theory that many soil nutrients are only marginally abundant in periglacial soils.

Soil phosphorus is not theorized to be limiting in newly developing soils, based both on standard ecological models (Walker and Syres, 1976) and measurements of soil nutrient ratios in Antarctic Dry Valleys (Bate et al., 2008). However, no experimental evidence of phosphorus addition to periglacial soils has been reported and Tscherko et al. (2003b) have shown that soil microbial biomass accumulation is highly correlated with phosphatase activity in two glacier recession chronosequences. Thus, the very new soils of the periglacial zone may experience P
limitation not because the parent material is lacking in phosphorus, but because weathering has not released sufficient amounts.

Trace mineral abundances such as sulfur, molybdenum, and iron are also likely to be important in periglacial areas. The unweathered parent materials abundant in periglacial areas are often high in reduced forms of sulfur, iron, and manganese which are used by microbial chemolithotrophs as the sole election source for microbial metabolism (Mapelli et al., 2011, Sharp et al., 1999; Skidmore et al., 2005). Mapeli et al. (2011) suggest that these chemolithotrophic organisms prime periglacial soils for further colonization of successional microbial and plant communities. As periglacial soils continue to develop, the availability of elements such as iron, molybdenum, and magnesium becomes important and potentially limits N-fixation (iron, molybdenum; Vitousek et al., 2002) and photosynthesis (iron, magnesium; Scanlan et al., 1989). These mineral limitations will determine the upper limits on the abundance of associated microbial guilds such as N-fixing bacteria or phototrophic bacteria and algae.

Topographic

In periglacial ecosystems, the ice-driven geomorphic processes of glacial scouring, frost weathering, slope stability, and cryoturbation interact to create gradients in topography from flat, frost sorted shelves to near vertical moraines (Karte, 1983; Barsch, 1993). The broadest scale periglacial geomorphic process is glacial activity. Throughout the Earth’s colder climatic periods, glaciers have flowed over thousands of kilometers and carved large valley and lake systems (Benn and Evans, 1998). The eventual retreat of those glaciers has left behind moraines of ground parent material in a well-mixed jumble that vary in size from glacial flower to many
ton glacial erratics. Successive periods of advance and retreat create overlapping moraines that further increase the topographic heterogeneity within the periglacial ecosystem.

In areas of exposed parent material, the freezing of liquid water that has invaded fractures within the parent material turns cliffs into regolith at rates depending on the friability and chemical makeup of the parent rock (Blum et al., 1998; Brady and Carol, 1994; Caine, 1979; André, 2002; Jaboyedoff et al., 2004). This results in topographic heterogeneity which is dependent on the parent material. Therefore, periglacial areas overlying the softest bedrocks have an abundance of scree slopes while those overlying the hardest materials form a disjointed cliff/blockslope landscape.

Once parent material has been weathered into moraines or block, talus, and scree slopes, slope stability and cryoturbation begin to play important roles in shaping landscape topography (Barsch, 1993). An impressive result of these factors is solifluction related mass wasting, wherein failures of the surface layer cause the rapid downslope movement of sizable portions of periglacial landscapes. Such mass wasting events are often the result of high moisture content soils slipping along top of the frozen permafrost layer and, with global climate change, these events are increasing in incidence (Matsouka, 2001). A less dramatic effect of solifluction is the downslope creep of soils in similar conditions to the previous example. Thus, both mass wasting and creep result in soil erosion. However, in flat periglacial areas, cryoturbation or the density-dependent upward movement of saturated, frozen, silty materials within the sediment profile results in patches of fine grained soil surrounded by a moat of inhospitable rocky terrain (Barsch, 1993). Topography of the periglacial zone is primarily a result of erosional processes, but effects such as cryoturbation can sometimes accelerate soil development.

*Parent Material*
As mountain height and latitude are the main determinants of the presence or absence of periglacial ecosystems, parent materials span the gamut of all major rock types. Moreover, the geologic activity inherent in mountain-building metamorphoses portions of the original parent material and creates fractures and faults which may then fill with igneous intrusions. This can create obvious, landscape-scale, heterogeneities in geologic formation age and chemistry that further increase the inherent global variation in parent material. For example, the well-studied periglacial area within the Green Lakes Valley, Colorado has three major formations (Figure 1.4) and a variety of minor cross-cutting dikes within its 4 km$^2$ area. Thus, parent material heterogeneity is a characteristic feature of periglacial ecosystems.

![Figure 1.4](image.png)

**Figure 1.4** A map of the Green Lakes Valley’s major geologic units adapted from the USGS 1950 survey by Lovering and Goddard and the CaO percent composition for subsections of the slope (overlay). Note the wide variation in chemistry across the Idaho Springs formation (see Appendix A for major oxide chemistry by map polygon), which suggests the presence of at least 2 separate dikes. Greater detail on the geology of this area can be found in the master’s thesis by Gallagher (1978).

Parent material is weathered both chemically and physically, and the rate of weathering is a function of rock chemistry, soil pH, water availability, temperature cycles, and salt concentrations (Barsch, 1993; Blum *et al.*, 1998; Brady and Carol, 1994; Caine, 1979; André, 2002). Physical weathering is mediated by freeze-thaw and is greatest over large temperature cycles, with friable parent materials, and with high solute concentration in the infiltrating water.
Chemical weathering is mediated by acidic conditions (dissolved CO$_2$ or organic acids) which primarily dissolve carbonate, silicate, and phosphate minerals as well as oxidation reactions, wherein H$_2$O and O$_2$ weather reduced elements and acidify the soil solution (Schlesinger, 1991). Generally, the relatively high abundance of unoxidized material in igneous bedrock means that derived periglacial soils trend toward increasing acidic conditions as they weather over time (the buildup of organic acids is the other large contributor to acidifying soil pH). Conversely, the relatively high abundance of carbonates in sedimentary bedrock buffers the acidification of derived periglacial soils and requires greater buildup of organic acids before soils acidify appreciably (Schlesinger, 1991).

As water is important both for freeze-thaw mediated physical weathering and for dissolution of parent material minerals, the extreme heterogeneity in periglacial water is a key limiting factor for weathering (Birkeland et al., 1989; Caine, 1979; Herz et al., 2003; Jaboyedoff et al., 2004). Although parent materials may break down during freezing events and biologically mediated oxidation may occur during warm, snow-free conditions, the mobilization of weathering products and exposure of fresh unweathered substrate may not happen until snowmelt events flush large amounts of water through the periglacial soils (Hill, 2008). Moreover, weathering rates should show heterogeneity at the landscape scale because snow depth is dependent on landscape topography (Erickson et al., 2005; Liator et al., 2008). As a result, regional and landscape variation in precipitation are likely as important in determining landscape patterns in the extent of periglacial parent material weathering.

**Time**

The discussion of time as a state factor is inherently integrated with the other soil state factors of Jenny (1941). Any initial patterns in the heterogeneity of biotic, parent material, or
topography can either be exacerbated (Cutler, 2010) or evened (Chesworth, 1973) by time and its interaction with climate. That is not to say that climate does not change over time, as there have been many large shifts in the global climate, but that these effects occur over large enough time-scales that the discretization of climate effects into specific periods is often applied effectively (Benn and Evans, 1998). Therefore, over time, parent material weathers according to the prevailing climatic conditions. This weathering can level mountains and fill valleys with those weathering products in times of warm temperatures, or carve deeper valleys as glaciers grow in response to colder, wetter climates. However, while these processes may take hundreds of years to cause obvious effects, the effect of time on biotic processes is observable over very short timescales.

Interannual variation in climatic factors such as temperature and water is also important to periglacial systems. Long term research has been conducted on soil invertebrate abundance in the McMurdo Dry Valleys and surrounding landscape in Antarctica (Doran et al., 2002; Barrett et al., 2008). This research has shown strong responses of periglacial invertebrate abundance to both warming and cooling periods on an interannual timescale. An even stronger directional response to changing environmental conditions is the well demonstrated community succession following glacial recession (Nemergut et al., 2007; Sattin et al., 2009; Sigeler and Zeyer, 2002; Noll and Wellinger, 2008) which is increasing in prevalence due to the current global warming trend. These studies show significant changes in a suite of soil factors including pH, phosphorus, sulfate, organic C/N ratios, and soil water content as well as soil microbial community composition. Thus, both decadal climate variation and the current global warming trend have strong influences on periglacial biotic communities.
A final note on time is that although glacial recession results in the exposure of new periglacial soils, these proglacial areas can be warmer and at lower elevations than periglacial soils in the surrounding landscape. This is due the fact that the presence of glacial ice below the snow-melt equilibrium line altitude (ELA) is a result of snow accumulation in the nival zone above the region’s ELA (Benn and Evans, 1998) and when glaciers recede the exposed substrate may be subject to a much warmer climate than an nearby periglacial areas (e.g. Sattin et al., 2009). However, the landscape surrounding both actively retreating glaciers and less active ice fields experiences a more stable climate, persisting for long periods under conditions that sustain microbially dominated soil communities with reduced or stagnated succession rates. Thus, as is the case of the periglacial soils underlying late-melting snowbanks, many periglacial areas have persisted in a microbially dominated state for hundreds of years and should reflect the time dependent influence of local ecological state factors in a distinctly different manner from successional proglacial environments.

1.3 Project Overview

These strong periglacial gradients in ecosystem state factors provide a test for the applicability of established biogeochemical theory in an extreme soil environment and an opportunity for novel landscape-scale studies of soil microbiology. Their seasonally prevalent environmental extremes in temperature, solar radiation, and water availability exert unique demands on the periglacial biotic community (Nemergut et al., 2005; Schmidt et al., 2008) that should significantly impact resource allocation (Tilman, 1985) yet the effect on periglacial biogeochemistry is unknown. In addition, the patchiness in soil state factors across the landscape should create similar scale patterns soil microbial diversity (Symstad et al., 2003) and offers an opportunity for the disruption of the first landscape scale microbial clade habitat distributions in
any terrestrial ecosystem. This oligotrophic environment with its short photosynthetically active period, low nutrient inputs (Ley et al., 2004; Freeman et al., 2009a; Cary et al., 2010) and unique microbial diversity (Freeman et al., 2009a,b; Schmidt et al., 2011) is an exciting system for testing our basic understanding of microbial ecology at an ecosystem scale.

The current paradigm of largely descriptive studies of soil microbial ecosystems is akin to the beginnings of macrobiotic ecology 100 years ago (Fierer, 2008b). Indeed, with the recent advances in analytical and molecular techniques (Sinsabaugh, et al., 2008; Hamady et al., 2010; Fierer and Jackson, 2006; Sogin et al., 2006), soil microbiology is at a stage where scientists are both pushing forward ecological theory as well as trying to test the applicability of established models. Microbial ecologists are coarse scale defining trends in microbial occurrence patterns (Bru et al., 2011; Lauber et al., 2009; Fierer and Jackson, 2006) as well as extending models of biogeochemical stoichiometry to soil microbial communities (Cleveland and Liptzin, 2007; Sinsabaugh et al., 2008; Fierer et al., 2009; Taylor and Townsend, 2010). The Jenny soil state factor schema offers a comprehensive framework for organizing the recent inundation of new findings in soil ecology and for testing this newly minted theory at an ecosystem scale.

Here, I explicitly investigate lithographic, climatic, and biotic state factors and implicitly investigate topographic and temporal state factors for periglacial soils. My individual chapters span questions that both push the boundaries of what is known about microbial biogeographic processes as well as to critically examine the predictive power within periglacial soils of models of climate and soil nutrient stoichiometry developed in vegetated systems. To study how these factors integrate to create the periglacial ecosystem I follow the approach of initially characterizing the state factors and then examining the strength of their interaction using controlled experiments in combination with statistical separation of their individual influence in
areas with overlapping gradients and, finally, testing the extent of the universality of these interaction via replication across globally distributed sites. This approach relies on the use of intensive plot-scale and landscape-scale surveys in multiple, globally distributed periglacial regions.

I executed this multi-scale investigation of periglacial ecosystems by initially surveying late-melting snowbank soils in Green Lakes Valley, Colorado for microbial biomass C, DOC, and extracellular enzyme activity (Chapter 2). I then used this initial study to design a landscape-scale study of periglacial soils in the same Green Lakes Valley (Chapter 4) wherein I examined soil bacterial diversity for its biogeography and its dependence on an extensive suite of biogeochemical factors; these relationships were subsequently tested for their predictive ability across globally distributed periglacial soils. I further implemented this same sampling scheme in smaller slopes from a diverse array of globally distributed sites (Nepal: Chapter 4; Denali National Park & Preserve, Argentina, and Peru: Chapter 5) in order to test hypotheses about the dominant state factors developed in previous chapters. This allowed me to identify global trends in periglacial soil biogeochemistry (Chapter 5) and to uncover factors unique to comparisons at this scale. My multi-scale approach framed within the clorpt schema yields important insights into the fundamentals soil microbiology and, ultimately, garners an improved description of the factors defining periglacial ecology.
CHAPTER 2
HIGH LEVELS OF MICROBIAL BIOMASS AND ACTIVITY IN UNVEGETATED TROPICAL AND TEMPERATE ALPINE SOILS

2.1 Summary

Barren high-altitude soils are among the most extreme terrestrial environments on Earth. The present study was undertaken to quantify broad-scale patterns of total microbial biomass in unvegetated soils in the periglacial zone of the Colorado Front Range and the high Andes of Perú. In order to better understand the factors and substrates limiting growth of these soil communities, I measured microbial biomass C, dissolved organic carbon (DOC), total organic carbon (TOC), soil gravimetric water content, and extracellular enzyme activity. To further investigate substrate limitation in these alpine soils, respiration after substrate addition was measured for samples from three sites in Colorado. In general, the abundance of microbes in these soils is positively correlated with soil water content. However, Perú talus soils had higher average microbial biomass than Colorado soils despite the Perú soils being higher in altitude and drier than the Colorado sites. Furthermore, the activity of the heterotrophic portion of the microbial community appears to be limited first by carbon and then by phosphorus as indicated both by results from extracellular enzyme assays and substrate addition experiments.

2.2 Introduction

Barren high-altitude soils are common in high mountain ranges on Earth and occur above the zone of continuous vegetation (e.g. above tundra in the Colorado Rockies) and below the “nival zone” (zone of year-round snow or ice). These barren areas have variously been called the “periglacial zone” (Europe), “puna brava” (Central Andes) or “high mountain desert” and “frost-debris zone” in general terminology (Troll, 1973) and “periglacial” in geomorphology literature (Karte, 1983). Barren periglacial soils occur at much higher elevations in drier
mountain ranges such as the Andes and Rockies as compared to the well-studied Alps. For example the periglacial zone of the Bavarian Alps starts at about 2000 meters in elevation compared to 4700-5000 meters in the Andes of Southern Perú and Northern Bolivia (Troll, 1973). The global extent of the periglacial zone has increased significantly in recent years due to rapid retreat of glaciers and snow fields at high elevations (Zemp et al., 2006), yet we know very little about the organisms that inhabit these seemingly barren areas.

Barren high elevation soils are also of interest because they are among the most extreme terrestrial environments on Earth. The periglacial zones of the Rockies and Andes are characterized by low oxygen pressure, cold temperatures, low humidity, low levels of liquid water, high levels of solar insolation and UV-B, and extreme temperature cycling across the freezing point. These physical extremes in turn lead these high altitude systems to be low in nutrients and have been presumed in the past to be almost devoid of measurable life.

While high altitude environments are not necessarily hard to study, unvegetated periglacial soils have received surprisingly little attention (Ley et al., 2004). These systems consist of boulder fields and scree slopes interspersed with deceptively young unweathered soils and are extensive at high elevations in the Rocky Mountains of Colorado (Shroba, 1977). Although many of these surfaces have been exposed for thousands of years, the accumulation of deep, late melting snowpack creates an environment that inhibits colonization by lichen and plants and presumably inhibits soil development beyond the barren mineral soil stage. Despite the barren appearance of these soils, recent studies indicate that they have unexpectedly high levels of carbon and nitrogen cycling (Bieber et al., 1998; Ley et al., 2001).

Efforts to characterize the microbial communities in the same soils studied by Bieber et al. (1998) and Ley et al. (2001) have yet to yield an understanding of the variation in abundance
and diversity of microbes in these soils. Recent work conducted in by Steve Schmidt's research group stands as the most comprehensive study to date (Ley et al., 2004), and describes marked seasonal patterns of heterotrophic microbes that mineralize labile (e.g. amino acids) and more recalcitrant compounds (e.g. phenols). In addition, Ley and Schmidt (2002) showed that fungi dominated the mineralization of organic compounds during snow melt, whereas bacteria dominated mineralization processes in the summer. While the focused studies of Ley et al. (2002, 2004) yielded proof that dynamic microbial communities exist in these soils, we still do not have a robust estimate of the overall abundance and diversity of microbes in these extreme soils. The present study was undertaken to quantify broad-scale patterns of total microbial biomass in the same soils previously studied by Ley et al. (2002, 2004) and in other barren high-elevation soils of Colorado and the high Andes of Perú. In order to better understand the factors and substrates limiting growth of these soil communities, I measured microbial biomass C, dissolved organic carbon (DOC), total organic carbon (TOC), soil gravimetric water content, and extracellular enzyme activity.

2.3 Methods

Sampling Scheme

My goal was to investigate this understudied environment at both large and small scales through extensive sampling. At each study area, samples were collected in a nested triangle scheme at distances of 0.01 m, 0.1 m, 1 m, 10 m, 100 m, and 1000 m (Figure 2.1). While this scheme does not cover the entire landscape, it does help us to understand how the variability in microbial activity is correlated with scale.
Study Sites

In order to expand upon previous work in the periglacial zone I used the same sites studied by Ley et al. (2004), two other sites in the Colorado Front Range and sites in the Sibinococha Basin, Perú. All of these sites are located on the eastern slope of their respective ranges. They are united in that they are all in the periglacial zone as defined by Troll (1973), have similar dry season rain event dependence, prevailing dry season wind direction, snow pack dependence, and lack of vegetation. There are also some significant differences between the sites in their climate, dry season rain frequency, altitude, parent material, and time since
glaciation. Detailed descriptions of all sites can be found in Williams et al. (1997) and Seimon et al. (2007).

**Colorado Sites**

In September of 2002, samples were collected from three south-facing talus slopes in the Colorado Front Range at an average elevation of 3800m. Sample were collected from two sites in the Green Lakes Valley (GLV), part of the City of Boulder watershed (Site 1: 40°03′25″N, 105°37′27″W; Site 2: 40°03′07″N, 105°37′56″W), and from one site 1 km north of GLV in the South St. Vrain Valley (Lake Isabelle Valley) (Site 3: 40°04′18″N, 105°38′04″W), part of Longmont watershed. The Green Lakes Valley and the Lake Isabelle Valley respectively flank the south and north sides of Niwot Ridge, Boulder County, Colorado. Both valleys are on the eastern slope of the North American continental divide in the Rocky Mountains and were last glaciated in the early Pleistocene (Outcalt and MacPhail, 1965). Henceforth these valleys will be collectively referred to as the Colorado sites. Snowmelt begins in these valleys in May; however, my sites remain snow-covered until late June or July. The late timing of snowmelt appears to prohibit vegetation in these soils, which are all classified as pergelic cryumbrepts. The bedrock differs between the three sites, however, the difference is mainly metamorphic and the general chemistry is similar (Lovering and Goddard, 1950). All previous microbial work has been restricted to the eastern GLV site (Ley et al., 2004). Sampling sites were arranged in a triangle of side length 1km (Figure 2.1).

**Perú Sites**

In August of 2003, samples were collected from the talus and glacial till of the Puca Glacier Valley at an average elevation of 5000 m. The glacier lies in the Laguna Sibinococha Basin, in the Cordillera Vilcanota range in the Peruvian Andes (Site 1: 13°46′41″S,
71°04'57"W; Site 2: 13°46'17"S, 71°04'28"W; Site 3: 13°46'47"S, 71°04'17"W). Henceforth these sites will be referred to as the Perú Sites. The soils and bedrock of the Sibinococha Basin have not been classified; however, the soils contain high quartz and calcite content and are likely cryosols. The Sibinococha Basin, like the Niwot Valleys, is also glacial in origin, though the study area was still glaciated in the late 1800s. The forelands of the Puca glacier are largely unvegetated at distances up to 1000 m from the receding snout of the glacier. Past 1000 m there is a significant amount of vegetated soil. The only previous microbial work done at this site was conducted by Nemergut et al. (2007). Samples were collected at 2 sites on the western talus slope and 1 site on the eastern talus slope of the valley, also arranged in a triangle of side length 1km (Figure 2.1).

Soil Parameter Measurements

Soil water content, microbial biomass carbon, and dissolved organic carbon (DOC) were measured via the chloroform fumigation method (Cleveland et al., 2003) for a total of 117 samples from the Colorado sites and 21 samples from the Perú Sites. Total organic carbon (TOC) was measured via loss on ignition for the 117 Colorado samples but was not measured for the Peruvian soils. Microbial extracellular enzyme activity was measured for 89 of the 117 Colorado sites samples and for all 21 samples from the Perú Sites. Due to high autocorrelation at the 1m sampling scale at the Colorado sites, 28 of the 81 samples taken in 1m triangles were deemed unnecessary characterizing local extracellular enzyme activities. Microbial biomass carbon is indicative of the total size of the microbial community, while extracellular enzyme (exoenzyme) activity measures the activity of enzymes metabolizing large, marginally soluble, organic compounds. Enzyme activity was assayed for N-acetylgalactosaminidase, β-glucosidase, α-Glucosidase, β-Xylosidase, Leucine amino peptidase, Cellulobiosidase, and Acid Phosphatase.
activity using a modification method of (Weintraub et al., 2007) that uses double the weight of soil substrate to compensate for the low activities of periglacial soils.

Soil Respiration Measurements

Soil respiration experiments were conducted to assess nutrient limitation in the Colorado soils. Treatments were sucrose, phosphate, sucrose and phosphate, and distilled water as a control. Sucrose was chosen to serve as a labile source of carbon and has been used successfully in past experiments to stimulate soil microbial respiration (Jonasson et al., 1999). Soil samples were collected from three spatially separated locations in the Colorado study area and analyzed separately. For each treatment, 35 g of soil (dry weight equivalent) from each sampling location was added to a separate 250-ml flask. Sucrose was added to obtain concentration of 100 µg C/g dry soil. Phosphate (Na$_2$HPO$_4$) was added to obtain a concentration of 75 µg P/g dry soil. The necessary amount of substrate was dissolved in enough distilled water so that the final soil moisture level was at 50% of field capacity, which was determined gravimetrically. Each flask was closed with rubber stoppers containing two valves. An EGM-4 CO$_2$ analyzer (PP Systems, Amesbury, MA) was attached to the valves before they were opened and the CO$_2$ concentration in the headspace was non-destructively assayed for approximately 30 seconds. Measurements were taken on an approximately log$_2$ timescale (Powers of 2 e.g. $\frac{1}{2}$, 1, 2, 4, 8, 16, 32 hrs). Curves of CO$_2$ accumulation were fit using the non-linear regression function of KaleidaGraph software (Synergy Software, Reading, PA, USA) and the integrated Logistic equation (Schmidt et al., 2004).

Statistical Analyses

Correlation analyses and t-tests were performed using MVPStats (MVP Programs, Vancouver, WA, USA) using type two error tolerance of 0.05. All tests were two tailed with the
exception of the respiration experiment which was one tailed. The Colorado sites were separated into the nine sub-sites based on the 10 m triangle groups (Figure 2.1) for further analysis. For each sub-site, microbial biomass C was plotted against soil water. An ANOVA was used to compare linear models in R 2.12 (2011) for the soil water content: microbial biomass relationship with and without division into sub-sites.

2.4 Results

Microbial biomass levels across the Colorado sites were higher than previously estimated (Ley et al., 2004), with a mean of 82 µg C/g dry soil (N=112, SD=45). Microbial biomass was significantly higher (p<0.001) in Peruvian soils with a mean of 140 µg C/g dry soil (N=21, SD=52). Upon further analysis, eight of the nine 10m sites in Colorado display the same correlation between microbial biomass and soil water. However, Colorado site 2A (one of the three 10m subsites at site 2) was significantly different from the other Colorado sites (Figure 2.2). In addition to differences in the biomass to water correlation, site 2A has higher TOC and soil water content, but lower DOC than the rest of the Colorado talus soils (p<0.001). Summary statistics for the soil parameters measured for Perú, Colorado site 2A, and the remaining Colorado samples are listed in Table 2.1. Of the environmental variables measured in both Perú and Colorado environments, percent soil water most strongly correlated with biomass levels across both Perú and Colorado (r=0.270, p=0.009). Colorado site 2A, the remaining Colorado samples, and Perú when regressed separately yield strikingly better fit to the data (Figure 2.2). In turn soil water levels correlated with total organic carbon in the Colorado soils including site 2A (r=0.672, p<0.001.).
Figure 2.2 Correlation between soil water content and microbial biomass regressed separately for Colorado site 2A (open squares, $r = 0.673$, $p < 0.001$), the remaining Colorado sites (filled triangles, $r = 0.687$, $p < 0.001$), and the Perú site (open circles, $r = 0.899$, $p < 0.001$). Dividing the data into these categories significantly improved model fit (ANOVA, $p < 0.001$).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Perú Sites</th>
<th>Colorado Site 2A</th>
<th>Colorado Sites w/o 2A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>N</td>
<td>Mean (SD)</td>
<td>N</td>
</tr>
<tr>
<td>%H₂O</td>
<td>5.3 (1.8)</td>
<td>12 (2.1)</td>
<td>7.4 (2.5)</td>
</tr>
<tr>
<td>DOC</td>
<td>17 (4.0)</td>
<td>24 (4.1)</td>
<td>51 (16)</td>
</tr>
<tr>
<td>%TOC</td>
<td>n.d.</td>
<td>3.3 (0.58)</td>
<td>1.9 (0.68)</td>
</tr>
<tr>
<td>MBC</td>
<td>140 (57)</td>
<td>76 (18)</td>
<td>83 (49)</td>
</tr>
<tr>
<td>NAG</td>
<td>0.22 (0.21)</td>
<td>1.6 (0.96)</td>
<td>3.4</td>
</tr>
<tr>
<td>CBH</td>
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<td>3.4 (1.7)</td>
<td>6.2 (4.7)</td>
</tr>
<tr>
<td>AG</td>
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<td>0.22 (0.22)</td>
<td>0.64 (0.61)</td>
</tr>
<tr>
<td>BG</td>
<td>1.1 (1.1)</td>
<td>15 (6.6)</td>
<td>23 (13)</td>
</tr>
<tr>
<td>LAP</td>
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<td>n.d.</td>
<td>0.18 (0.37)</td>
</tr>
<tr>
<td>BXYL</td>
<td>0.18 (0.28)</td>
<td>0.61 (0.43)</td>
<td>1.9 (1.5)</td>
</tr>
<tr>
<td>PHOS</td>
<td>20 (9.1)</td>
<td>13 (4.5)</td>
<td>27 (14)</td>
</tr>
</tbody>
</table>

Table 2.1 Soil parameter summary statistics for Colorado and Perú Soils. %H₂O: Soil water content (g H₂O/g wet soil). DOC: Dissolved organic carbon (µg C/g dry soil). %TOC: Total organic carbon (g C/g dry soil). MBC: Microbial biomass carbon (µg C/g dry soil). NAG: N-acetylglucosaminidase. PHOS: Acid Phosphatase.

To complement previous work that had shown relatively high heterotrophic activity in Colorado talus soils (Ley and Schmidt, 2002), I measured a standard array of exoenzyme activities in both the Colorado and Perú soils. Most of the enzymes measured showed very low activities (Table 2.1), with the exception of peptidase and phosphatase in the Perú soils and β-glucosidase and phosphatase in the Colorado soils. In addition, Colorado site 2A had significantly lower exoenzyme activities than the remaining Colorado sites across all enzymes measured (p<0.001). In Colorado, β-glucosidase showed correlation with microbial biomass C (r = 0.231 p=0.032), while phosphatase showed correlation with DOC (r =0.503 p<0.001) and with microbial biomass C (r =0.306 p=0.004) but neither enzyme was significantly correlated with soil water content. In Perú, peptidase and phosphatase were not significantly correlated with any soil carbon measurement or with soil water content.

To determine if the relatively high phosphatase activity in these soils indicates phosphorus limitation of microbial activity, I carried out incubation studies on the Colorado soils.
to see if P stimulates microbial respiration. Addition of P alone significantly stimulated microbial respiration as did the addition of C (Figure 2.3). However, the addition of C and P had the greatest effect on respiration (Figure 2.3). In contrast nitrogen additions to these soils either decreased respiration or had no effect (data not shown).

![Figure 2.3](image)

**Figure 2.3** Effects of added P and C on the kinetics of CO₂ production in Talus soils from Colorado. Data points represent mean values of three replicates for all treatments (± S.E.). Curve fits are from the integrated logistic equation (Schmidt et al., 2004). R values for curve fits were 0.993, 0.994, 0.981, and 0.994 for control, +P, +C and +P,C, respectively. There was a significant difference between CP and C at 30 and 40 hrs (1 tailed t-test). There was a significant difference between P and control at 15, 20, and 30 hrs (p<0.05, 1 tailed t-test).

2.5 Discussion

*Microbial Biomass Levels*

I found unexpectedly high levels of microbial biomass in unvegetated periglacial zone soils in both Colorado and Perú. The microbial biomass in the alpine talus from this study was
30 times that estimated by previous work on alpine talus (Ley et al., 2001, 2004). Despite being higher than anticipated, the values I obtained were similar to some of the harshest environments worldwide; for example, the average microbial biomass in Alpine and Antarctic glacial moraines is 60 μg C/g soil and 100 μg C/g soil respectively (Tscherko et al., 2003a,b) while both arctic polar desert and temperate desert average 150 μg C/g soil (Jones et al., 2000; Bailey et al., 2002). In comparison, the alpine tundra in close proximity to the Colorado sites displays biomass levels of 500-2000 μg C/g soil (Lipson et al., 1999; Oline and Grant, 2002). Wardle’s meta-analysis of ecosystems worldwide (1998) helps put my data in a global context. He estimates that microbial biomass is on average 800 μg C/g soil for grasslands and 750 μg C/g soil for forests. Clearly, the soils of harsh environments harbor significantly lower biomass than soils of more mesic, plant dominated systems.

The consistently lower biomass levels observed in the wide variety of unvegetated soils described above lends strong credence to the idea that carbon inputs from plants determine the size and activity of soil microbial communities. It should be pointed out, however, that there are too few detailed studies of plant-free soils to make broad generalizations about the controls on microbial biomass levels in barren soils. Indeed, some barren soils are early successional and will eventually support plants (e.g. glacial chronosequences: Ohtonen, 1999; Tscherko et al., 2003a,b), whereas others experience conditions that are simply too extreme for plants to establish (e.g. some deserts: Bailey et al., 2002; Jones et al., 2000). While glacial recission chronosequences clearly demonstrate a buildup of higher microbial biomass associated with increasing soil organic matter and plant cover (Ohtonen, 1999; Tscherko et al., 2003a,b), permanently plant free soils exist in a semi-equilibrium state with microbial heterotrophic activity hypothetically balancing ecosystem carbon inputs. I contend that my sites are in the
latter category based on the fact that they have been deglaciated for thousands of years (Ives, 1953), yet, due to the extreme duration of the snowpack, still do not support vegetation.

The closest analog to my unvegetated periglacial system is found in small snow-free patches of Antarctic and Arctic barren soils (Gajananda, 2007; Jones et al., 2000). For example, the Antarctic soils studied by Gajananda (2007) receive substantial amounts of snow and, like my periglacial zone soils, are not limited by precipitation or soil age, so much as by the long duration of the snowpack. Deep late melting snowpack is what excludes plants in both Antarctic and periglacial barren soil environments, limiting carbon availability and, ultimately, microbial growth. However, in light of global warming trends, these systems may be pushed out of their equilibrium state onto a successional trajectory similar to classical glacial recession (Zemp et al., 2006).

*Implications of Biomass Underestimation using Heterotrophic Stimulation*

While unvegetated soils do indeed contain lower biomass than vegetated systems, the talus soil biomass levels are only lower than alpine tundra by a factor of 5 not 150 as suggested by Ley et al. (2004). This unexpectedly higher estimate of periglacial zone microbial biomass stems from the use of the chloroform fumigation (CF) approach to estimate biomass levels. Previous workers at the Colorado site (Ley et al., 2001, 2004) used a substrate induced growth response (SIGR) method that had been developed to measure microbial biomass levels in tundra soils (Colores et al., 1996; Lipson et al., 1999) but had never been used for barren soils. The SIGR method strongly correlates with the chloroform fumigation method in tundra soils (Lipson et al., 1999) and, though it underestimates microbial biomass levels, the Ley et al. (2001, 2004) results were calibrated based on the CF to SIGR ratio for alpine tundra soils (e.g. 7:1 in the fall of the year). However, my new data indicate that the SIGR approach may greatly underestimate
total microbial biomass in unvegetated alpine soils and that such a method should be recalibrated to better agree with chloroform fumigation measurements.

There are two possible reasons for why the SIGR approach so grossly underestimates microbial biomass in barren soils. The first reason is that the SIGR approach and related SIR methods are based on heterotrophic respiration of added carbon sources, whereas unvegetated soils likely have large populations of photoautotrophic and chemoautotrophic microbial communities. Indeed, molecular phylogenetic analyses of periglacial talus soils in Colorado (Freeman, 2009a) and of unvegetated early successional soils in Perú (Nemergut et al., 2007) indicate that these soils have a significant number of autotrophic microbes that would not be assayed by the SIGR method.

An additional reason for this underestimation of the microbial biomass is that SIGR measures the microbes that are immediately responsive to substrate additions, while the chloroform fumigation method I employed measures both active and inactive biomass. Therefore, there may exist a population of dormant microorganisms that was only detectable via the CF method. These microbes may be relict from the spring peak of heterotrophic activity during snowmelt as observed by Ley et al. (2004) and have become dormant during the hot, dry summer conditions. Alternatively, there may be a population of warm temperature adapted microbes that are only active for short periods under sporadically favorable summertime conditions such as after rain events.

*Controls on Microbial Biomass in Periglacial Soils*

Whether high altitude talus soil biomass is dominated by autotrophs, ephemeral heterotrophs, or a combination of the two, it is intriguing that microbial biomass was significantly higher in the dry, high altitude, tropical sites of Perú than in the wetter, lower
altitude, temperate sites of Colorado (Figure 2.2). Moreover, while DOC was higher in Colorado than Perú (46 vs. 17 μg C/g soil), DOC plus microbial biomass is higher in Perú than Colorado (160 vs. 127 μg C/g soil). There are a number of factors that could potentially explain these differences but the present study provides only glimpses into the possible reasons. It may be that the Colorado community is under a higher predator pressure resulting in a lower standing biomass. There is ample evidence of active microphageous protozoa, mites, nematodes, and rotifers in the Colorado soils (Kristen Freeman and Michael Robeson, personal communication 2007), but, thus far, attempts to amplify eukaryotic DNA out of the Peruvian samples have failed (Diana Nemergut, personal communication 2007). It is also possible that the low pH of the Colorado soils could help explain their relatively low levels of biomass. The pH of the Colorado sites averages about 4.5 (Ley et al., 2001) compared to 7.5 for the Peruvian soils (Nemergut et al., 2007). Whatever the reason, the microorganisms of the Peruvian soils appear to incorporate carbon more efficiently than those of the Colorado soils, achieving a higher microbial biomass with lower DOC.

Although the Perú soils display a larger microbial biomass under significantly drier conditions, both the Perú and Colorado soils have a significant correlation between soil water and microbial biomass (Figure 2.2). This water dynamic was somewhat expected given that the low water content of the Colorado summertime talus soils as reported by Ley et al. (2004) suggested that microbial biomass levels might show a dependence on water availability. The correlation between microbial biomass and soil water was clearly demonstrated in the present study even when all samples from Perú and Colorado were analyzed together. On the other hand, the Colorado sites with similar conditions group to the exclusion of my wettest site (2A), and to the exclusion of the Perú samples (Figure 2.2). Thus, while there is a general trend
toward higher biomass with higher soil water, this trend is much stronger when the data are
separated into these three groups.

What then is it that makes these three sites so different? Snow depth studies in the
Colorado talus indicate that the position of site 2A at the base of a cliff face leads to a deeper
snowpack than at the other Colorado sites (Erickson and Williams, 2005). The deeper snowpack
may cause 2A to remain frozen later into the summer and, therefore, to have a shorter active
period than the other sites. This idea is supported by the fact that 2A, in addition to having lower
than expected microbial biomass, has the highest TOC and lowest DOC and exoenzyme levels of
the Colorado sites, suggesting impeded breakdown of soil carbon. The Peruvian sites, on the
other hand, appear to accumulate a much smaller snowpack, to the point that snowmelt does not
result in the formation of any visible seeps. It may be that microbial biomass is correlated with
the size of the late melting snowfield in all periglacial barren soils, and that areas with similar
size snowpack have similar trends in the correlation between soil water content and microbial
biomass. Snowpack depth may in turn regulate the period of microbial activity and/or microbial
predator abundance by either restricting the length of the growing season or by excluding
predators in the driest, earliest melting environments. The persistence of the water to biomass
correlation through the dry season appears to reflect not just a seasonal but a yearlong influence
of water on site fertility. Although the dynamics of microbial biomass have not been extensively
studied in unvegetated polar soils, it has been show that microbial activity is related to soil water
content and TOC (Gajananda, 2007; Tscherko, 2003a). Obviously more work is needed to better
understand microbial population dynamics in snowpack structured systems, but this study
provides an informative first look into this little understood world.
Substrate Limitation in Barren Soils

In an effort to characterize the types of substrates that may fuel microbial activity in talus soils I carried out a standard array of enzyme assays. Most of these enzymes showed very low activities, but the few that showed significant activity (Table 2.1) provide a glimpse of the types of substrates that may be fueling and/or limiting microbial activity in the talus. The specific exoenzyme results suggest that cellulose and organic phosphate are important substrates in Colorado talus, while protein and organic phosphate are important in Perú. The level of activity of phosphatase was especially striking; on a unit biomass basis (activity divided by microbial biomass) the talus soils of Colorado showed phosphatase activity twice as high as those in well-developed nearby forest soils (Weintraub et al., 2007). In order to determine if these high phosphatase levels indicate P limitation of microbial activity I carried out a substrate incubation experiment on the Colorado soils (Figure 2.3). Interestingly, the microbial community appears to be limited primarily by labile carbon and then by phosphorus in the presence of excess carbon.

This carbon limitation and the higher microbial biomass than DOC suggest that by late summer the labile carbon sources are gone from the talus soil. While carbon limitation is not unexpected in barren soils, phosphorus limitation is normally predicted to occur only in very young or in old, highly weathered soils. In addition, microorganisms of most other barren soil types (temperate and polar deserts, glacial moraines) have been shown to have nitrogen limitation in addition to carbon limitation, but none of those environments have shown phosphorus limitation (Gallardo and Schlesinger, 1992; Hopkins et al., 2006; Yoshitake et al., 2007). Though many theories predict that phosphorus availability decreases over time due to leaching and to sorption to Al and Fe oxides (Walker and Syers, 1976; Lajtha and Schlesinger, 1988), the Late Pleistocene origin of the Colorado talus (Ives, 1953) implies that these soils are
too young for this to be a feasible explanation for the observed microbial phosphorus limitation. Available phosphorus in these specific soils has not been measured, but work by Hood et al. (2002) has shown strong ecosystem retention of phosphorus in waters draining the Colorado talus sites, with dissolved organic phosphorus levels averaging just 4.5µgP/l. Thus, it is more likely that the observed phosphorus limitation is the result of either the parent rock having been originally poor in phosphorus or the soils having been insufficiently weathered despite their intermediate age. Periglacial soils of Colorado appear to have a suite of nutrient limitations unique among barren soil systems.

**Broader Implications**

The Sibinococha Basin was created by the continuing recession of the Puca glacier, which has been linked to global warming (Seimon et al., 2007). Moreover, snowmelt in the Niwot Valleys has occurred earlier in recent years, and it has been observed that the barren sites used in this study have shown an increase in plant colonization. If these trends continue, it is expected that many of these environments will lose the protection of the late melting snow and these interesting microbial communities will become plant dominated communities as seen in faster melting polar oases (Jones et al., 2000). However, the current altitude of the periglacial zone is still lower than many of the mountaintops worldwide. Thus, these dynamic and intriguing barren soil microbial communities may soon become restricted to only the most extreme heights of the Earth.

**2.6 Conclusions**

The small amount of information known about the microbial communities in barren soil areas tells us that they are all carbon limited, largely due to the exclusion of plants. Barren alpine soils harbor higher than predicted levels of microbial biomass but these levels are still low in
comparison to plant dominated systems. Although only a small subset of the community in Colorado talus soils have been shown to be immediately responsive to substrate induced respiration (Ley et al., 2004), the remaining portion of the microbial community may be composed of autotrophic organisms or ephemerally active heterotrophs. In general, the abundance of microbes in barren periglacial soils of both Colorado and Perú is correlated with soil water content. However, there are exceptions, which, in the case of the Perú soils, cause higher than expected microbial biomass in spite of drier soils. The size of the snowpack and timing of its meltout may explain such phenomena, and certainly helps explain the lower microbial biomass in the wettest Colorado site. Furthermore, the activity of the heterotrophic portion of the community appears to be substrate limited and exoenzyme activities are predictive of this limitation. The barren soils of the alpine periglacial zone show surprising levels of microbial biomass and activity, but their dependence on snowpack melt rates makes them an endangered ecological system in the face of global climate change.

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CHAPTER 3

BIOGEOGRAPHY AND HABITAT MODELLING OF HIGH-ALPINE BACTERIA

3.1 Summary

Soil microorganisms dominate terrestrial biogeochemical cycles, however, we know very little about their spatial distribution and how changes in the distributions of specific groups of microbes translate into landscape and global patterns of biogeochemical processes. Here I use a nested sampling scheme at scales ranging from 2 to 2000 meters to show that high-alpine, periglacial bacteria have significant spatial autocorrelation in community composition up to a distance of 240 meters and that this pattern is driven by changes in the relative abundance of specific bacterial clades across the landscape. Analysis of clade habitat distribution models and spatial co-correlation maps identified soil pH, plant abundance, and snow depth as major variables structuring bacterial communities across this landscape and revealed an unexpected and important oligotrophic niche for the Rhodospirillales in soil. Furthermore, my global analysis of high elevation, periglacial soils from the Andes, Rockies, Himalayas and Alaska ranges shows that habitat distribution models for bacteria have strong predictive power across the entire globe.

3.2 Introduction

Despite the known importance of microorganisms to the maintenance of Earth’s biogeochemical cycles (Falkowski et al., 2008; Houghton, 2004), the relationship between the ecological niche of microbial groups and the culture-independent identification of their presence in the environment is poorly understood (Ettema and Wardle, 2002; Green et al., 2008; Martiny et al., 2006). This is due to lack of resolution in data collected using traditional methods, which obscures the identification of potentially important groups across a landscape (Ettema and Wardle, 2002). However, Ettema and Wardle (2002) point out that given enough data, “spatial
variability is the key, rather than the obstacle, to understanding the structure and function of soil biodiversity.”

Previous studies have shown that there are spatial patterns to microorganisms (Green et al., 2004; Homer-Devine, et al., 2004; Ramette and Tiedje, 2007; Robertson and Freckman, 1995; Rodrigues et al., 2009) and that some coarse-scale taxonomic groups (at the phylum or division level) show strong correlation with environmental parameters; e.g. Acidobacteria abundances are negatively correlated with soil pH while Actinobacteria and Bacteriodetes abundances are positively correlated with pH and the diversity of all three groups as well as many Proteobacteria lineages strongly varies with pH (Lauber et al., 2009). However, groups such as the Acidobacteria and Proteobacteria are extremely large and functionally diverse; for example the Proteobacteria encompass almost all known microbial physiologies ranging from phototrophs to heterotrophs to chemoautotrophs and recent studies indicate that the Acidobacteria may be equally metabolically diverse (Bryant et al., 2007). Thus, we gain very little information about the biogeochemistry of a specific system or the global biosphere by knowing the spatial distribution of such large taxonomic groups. Likewise, we still have only a rudimentary understanding of the local spatial scale at which soil microbes and soil biogeochemical parameters are distributed. Matching the relative abundance patterns of specific microbial taxa and biogeochemistry at both local and global scales has remained an elusive task.

This study makes use of recent advances in high throughput sequencing, bioinformatics and biogeochemical methods (Jones et al., 2009; Monson et al., 2006; Sinsabaugh et al., 2005; Hamady et al., 2008; Lozupone and Knight, 2005; Sogin et al., 2006) to map the co-occurrence of microbial groups with biogeochemical soil properties across a highly heterogeneous, high-elevation, periglacial landscape near the continental divide in the Rocky Mountains of Colorado,
USA (Chapter 2; Ley et al., 2004; Nemergut et al., 2008). Based on my previous study of the spatial autocorrelation of soil biogeochemical properties (Chapter 2), I collected 160 soil samples in a nested sampling scheme (Figure 3.1). This sampling scheme allowed me to determine spatial variation in microbial diversity (a random subset of 85 samples was pyrosequenced for the 16S gene) and its relationship to 21 soil biogeochemical properties at scales from 2 to 2000 m in Colorado. These analyses were essential for obtaining spatially explicit, landscape habitat distributions (models based on covariation of relative abundance with biogeochemistry) for bacterial community members, which were tested at the global scale by sampling similar soils in the Colorado Rockies, Himalayas, Andes and Alaska Ranges (Sanger clone libraries of the 16S gene).

[Figure 3.1] Satellite image of the sampling area in Green Lakes Valley, Colorado (40°3’24”N 105°37’30”). Black dots indicate sampling locations and the red dots indicate the samples that were sequenced for bacterial community composition. The distance between the farthest two samples was 2 km.
Here I show that periglacial bacterial communities have significant spatial autocorrelation at distances up to 240 meters, but beyond that distance community composition does not display significant spatial autocorrelation. In addition, the dominant bacterial clades from the landscape-scale survey display strong covariation with biogeochemical parameters such that their relative abundances across the globe are predictable using habitat distribution models.

3.3 Methods

Sampling Scheme

A total of 160 soil samples were collected from a continuous landscape on the south side of the Green Lakes Valley Watershed (GLV), CO. I sampled a distinct and well-defined landscape unit within the GLV that is bounded on the east by tundra, on the south by alpine lakes, glaciers and meadows, on the west by the continental divide and on the north by steep cliffs. There is a large cliff face in the center of the GLV landscape, along the base of which exists a narrow 75m wide corridor that connects the upper and lower parts of the landscape (Figure 3.1). The sampled landscape is composed of a matrix of block slope, late-melting snow banks overlaying unvegetated gravel soils, fellfields, and small patches vegetation (Chapter 2; Ley et al., 2004; Hood et al., 2002). However, even in the most developed soils, the soil texture is high in sand content and the total soil depth is minimal. The valley receives the majority of its precipitation during winter months (Jones et al., 2009) and many snow-banks do not completely melt until late July/early August. Our sampling was conducted from Sept. 4 through Sept. 8, 2007 in order to minimize to effects of localized variation in soil water due to snowmelt subsidies.

The main goal of my sampling effort was to construct spatially explicit landscape models. Such models require a subset of samples to be collected at a small enough scale in order to
establish a baseline for the spatial autocorrelation (Legendre and Fortin, 1989). My preliminary study of GLV soils (Chapter 2) spanning sampling distances from 10 cm to 1 km was used to determine an optimum sampling interval of 50 m. However, in order to be able to generate accurate spatial models, I selected three locations for smaller spaced sampling, which were performed in 5 m grid over a 30 m x 30 m plots. At each sampling location, a 10cm diameter section of soil to 4cm depth in the approximate center of the soil patch closest to the predetermined grid point was mixed and approximately 75 g placed in a sterile conical tube. The location of each sampling point was recorded with a Garmin eTrex Vista gps unit (Garmin International Inc., KS, USA). Soils samples were stored at 4°C for a maximum of 1 week while soil dissolved organic carbon and total dissolved nitrogen (DOC and DN) measurements were conducted. Afterwards, soils were stored at −20°C until processing. Soils for soil texture analysis were collected from each location in September of 2008.

**Biogeochemical Measurements**

Dissolved organic C/N, microbial biomass C/N, and analysis of extracellular enzymes N-acetylglucosaminase, β-glucosidase, α-glucosidase, β-xylase, cellobiosidase, leucine amino peptidase, organic phosphatase, and lignin oxidase/peroxidase were performed using the methods of Weintraub et al., (2007). Soil pH was measured after the addition of 2 ml water to 2 g soil and shaking for 1 hr. Soil water content and soil water holding capacity were measured gravimetrically. Soil texture (clay/sand/silt) was measured by South Dakota Soil Laboratory (South Dakota State University, Brookings, SD). Plant diversity and abundance were measured by identifying and recording all vascular plants within a 1 m radius of a sampling location. Snow depth values at each point were obtained by averaging the snowpack depth from the kriging interpolations of snow surveys in the Green Lakes Valley from 1997-2003 (Niwot
LTER database, [http://culter.colorado.edu/exec/Database/gis_layer_query.cgi](http://culter.colorado.edu/exec/Database/gis_layer_query.cgi). Degree of slope was calculated based on a 10 m digital elevation model also available from the Niwot LTER website.

**DNA Extraction and Amplification for Pyrosequencing**

Microbial diversity data for the Green Lakes Valley samples was obtained by pyrosequencing 85 randomly selected samples (out of 160 total) (Figure 3.1) for the 16S gene using the method of Fierer *et al.* (2008a) and resulted in 16,945 sequences with an average length of 230 nucleotides. DNA was extracted from 1 gram of each soil sample using MO BIO PowerSoil™ DNA Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). Partial 16S gene sequences were amplified using fusion primers (Integrated DNA Technologies, Inc., Coralville, IA, USA) similar to those described by Hamady *et al.* (2008), targeted toward the 27-338 region. Each reverse primer consisted of a 454® adapter sequence, a unique 12 nt. error-correcting barcode, a two base pair linker sequence, and the 338R bacterial 16S primer as described elsewhere (Fierer *et al.*, 2008; Li and Godzik, 2006). Forward primers consisted of a 454® adapter sequence, a two base pair linker sequence, and the 27F bacterial 16S primer. Polymerase chain reactions were performed using a reaction mix consisting of 7 µl nuclease free water, 13 µl HotStarTaq® Matster Mix (Qiagen, Valencia, CA, USA), 1.5 µl of each primer (5 µM), and 3 µl DNA extract. For samples that would not amplify using this formulation, 2 µl of DNA extract and 1 µl BSA were used in place of 3 µl extract. The samples were initially denatured at 94° C for 3 minutes, followed by 35 cycles of 94° C for 45 seconds, 50° for 30 seconds, and 72° for 90 seconds. Finally, samples were held at 72° for ten minutes to promote complete amplification of the target sequence. PCR was performed in triplicate for all samples, and the PCR products for each sample were pooled prior to clean-up and normalization.
PCR products were cleaned and concentrations standardized to an approximate concentration of 1 ng/μl using the SequalPrep™ Normalization Kit (Invitrogen Corp., Frederick, MD, USA). Equal volumes (5 μl) of all samples were pooled, and 100 μl of the pooled product was sent to the Consortium for Comparative Genomics pyrosequencing facility at the University of Colorado in Denver.

**Sequence Analysis and Tree Building**

The raw pyrosequencing data were preprocessed as described in Hamady et al. (2008), assigning each sequence to the sample it came from using an error-correcting barcode. Sequences removed from the analysis include: sequences of length < 200 nt. or >300 nt., with low quality scores (<25), containing ambiguous characters, or that were from samples containing fewer than 50 sequences. The remaining sequences were clustered using cd-hit (Li and Godzik, 2006), an alignment was built from the resulting representative set of sequences using NAST (DeSantis et al., 2006), a tree was then built from this alignment using FastTree (Price et al., 2009), and Fast UniFrac (Hamady et al., 2010) was used to generate the UniFrac distance matrix of all pairs of samples. Taxonomy assignments were made using the RDP classifier (Wang et al., 2007) with a minimum support of 60%. For clade specific UniFrac analyses, these taxonomy assignments were used to prune all but members of the clade of interest from the full tree before applying Fast UniFrac.

**Phylogenetics and Habitat Modeling**

Clades were defined by selecting all nodes on the full community tree that aggregated at least 100 sequences. Semivariograms, correlation matrices and correlation significance tests were performed in R (R Development Core Team, 2009) with the aid of the spatial statistics add-on package geoR. Semivariogram models were fit in R for a spherical model (Bailey and
Gatrell, 1995) using a Nelder–Mead non-linear algorithm (Nelder and Mead, 1965). Mantel tests for spatial autocorrelation models (Mantel, 1976) were evaluated from 0 m to the modeled distance of autocorrelation for each semivariogram in R using the statistics add-on package ade4 using 1000 iterations. Moran’s I tests for spatial autocorrelation (Anselin, 1995) in clade relative abundance were performed in R using in spatial statistics add-on package ape. Clade habitat distribution models were constructed in the Spatial Analyses in Macroecology (SAM; Rangel, 2006) program using the Akaike information criterion (AIC; Buckland et al., 1997) to select environmental variables and a generalized least squares spatial partial regression to add the spatial component. Maps were generated in ArcGIS 9.3 (ESRI, Redlands, CA) using cokriging of the relative abundance for each clade on 85 sequenced samples in combination with the three most significant environmental variables in each habitat distribution model for all 160 samples (Bailey and Gatrell, 1995). Cokriging was chosen to generate the maps of the distribution of the clades because it creates a continuous map surface using a linear least squares model similar to my SAM models and has an estimation error that is dependent on the spatial the autocorrelation distance for the variable of interest (relative abundance). Thus, the error is relatively low for estimations at distances less that the clade’s autocorrelation distance from sample locations (Bailey and Gatrell, 1995).

Global Scale Sampling

In order to test the applicability of the habitat distribution models developed for bacterial clades from the Green Lakes Valley periglacial soils, 6 samples were collected from each of four globally distributed periglacial sites during the regional dry season using the same methods as for the main Colorado Rocky Mountains dataset. The sites were Green Lakes Valley, CO (GLV), Denali National Park & Preserve, AK, USA (DNP&P); Annapurna Conservation Area, Nepal;
and Llullaillaco Volcano, Argentina. Samples from outside the USA were frozen in the field, kept frozen during transportation from the field, shipped frozen via express airmail and stored at -20 °C until processed. A restricted set of biogeochemical properties was measured for each site using the same methods as for the primary Colorado dataset (Soil Water Content, Soil Water Holding Capacity, all eight extracellular enzymes, Forb Abundance, Soil pH, Microbial Biomass C&N, Total Dissolved Nitrogen, Dissolved Organic Carbon).

**Sanger Clone Library Comparisons**

DNA was extracted from samples from Green Lakes Valley of the Colorado Rocky Mountains (GLV), Denali National Park & Preserve, AK, USA (DNP&P), Annapurna Conservation Area, Nepal (ACA), and Llullaillaco Volcano, Argentina. The GLV sequences were obtained from six samples collected in late July, 2006 from unvegetated soils underlying late melting snowbanks; these samples were collected at the same locations as the depth separated samples from the current study (Freeman et al., 2009a). The DNP sequences were obtained from six samples collected in from a glacial recession chronosequence at the Middle Fork Toklat Glacier (63°23′50″N, 150°07′50″E) at 1350 m. a. s. l. in July of 2008 and represent an average of 25 yrs exposure. The DNP&P site is located on shale bedrock. The ANC samples were collected in October, 2008; three from a lower elevation site and three from a higher elevation site within the region. The low-elevation samples in the ACA are from unvegetated, south-east facing slopes (28°43′N, 83°55′E) at 5122 m above sea level (m.a.s.l.) and approximately 8 km east of the northern edge of Tilicho Lake. The high-elevation samples in the ACA are from unvegetated south-east facing slopes from 5503 to 5516 m.a.s.l., 1 km north of Thorong Pass (28°48′N, 83°56′E). The Thorong Pass crosses the divide separating the Marsyangdi River to the east and the Kali Gandaki River to the west. All of the ACA sites are
located on shale bedrock. The Llullaillaco sequences were collected from two elevations on the Llullaillaco Volcano in Argentina (24°42’S, 68°31’E). Three samples were collected at 6034 m.a.s.l. and two samples were collected at 6330 m.a.s.l. The Llullaillaco sites are located on basalt bedrock.

DNA was extracted from the soils using the MO BIO Ultra-Clean Mega Prep Soil DNA kit (MO BIO Laboratories, Carlsbad, CA, USA). Bacterial community SSUrDNAs were amplified using the Bacteria-specific primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1391R (5′-GACGCGCGGTGCGGTRCA-3′) and primers 4Fa (5′-TCCGGTTGATCCTGCRG-3′) and 1492R (5′-GGTTACCTTGTTAGACTT-3′). PCRs were performed with 2.5 mM MgCl2, 0.2 mM each deoxynucleoside triphosphate (dNTP), 0.4 mM each primer, 1 U Taq polymerase (Promega) and buffer supplied with the enzyme using a range of template concentrations. Gradient thermal cycling was carried out for 25 cycles to minimize PCR bias. Amplicons from six different reactions, with different annealing temperatures (±3°C of optimal), were pooled and gel purified using isolated bands from agarose gels and QIAquick gel purification columns (Qiagen, Valencia, CA, USA). Purified products were ligated into the pCR 2.1 vector (Invitrogen, Carlsbad, CA, USA) and transformed into Escherichia coli following the manufacturer’s instructions. Transformants were inoculated into a 96-well deep-dish plate containing 1.5 ml of TB Dry nutrient broth (MO BIO Laboratories, Carlsbad, CA, USA). Cultures were shaken at 200 r.p.m. for 16 h at 37°C and then centrifuged. Cell pellets were sent to Functional Biosciences (Madison, WI, USA) for plasmid extraction and were sequenced bidirectionally using sequencing primers T7 and M13R. For each sample, a clone library of 192 sequences was constructed.
Sequences were edited (vector-trimmed and assembled into contigs) using Sequencher 4.1 (Gene Codes, Ann Arbor, MI, USA). Bacterial sequences were aligned using the NAST alignment tool (Li and Godzik, 2006; DeSantis et al., 2006b) and preliminary classifications were obtained using the Greengenes Database (DeSantis et al., 2006a). This alignment was then imported into a 16S rRNA ARB database (Ludwig et al., 2004). All sequences were chimera checked using Bellerophon (Huber et al., 2004) and Mallard (Ashelford et al., 2006).

Global Scale Habitat Model Testing

After the initial (Niwot) models were used to predict the relative abundance, I looked for additional predictor variables that showed a broader range of variation and had significant correlation with the residuals (predicted relative abundance – actual relative abundance) on the global scale. Clades with significant correlation between residuals and environmental variables (Acidobacteria G4 and Saprospirales) were error corrected by using ordinary least squares regression to predict residual error across all samples (analyses performed in SAM). The reason these variables were not adequately weighted in the original model is because the DNP&P and Annapurna soils are formed from calcareous shale bedrock while the GLV (and Llullaillaco) site is formed from igneous bedrock. The shales of the DNP&P and Annapurna site create soils with a significantly more basic pH (7.5 vs. 4.5, t-test, p<0.001). This difference in soil pH is known to have a significant effect on extracellular enzyme activity, particularly for Leucine peptidase which like most peptidases has its activity optimum in basic pH solutions (Sinsabaugh et al., 2009; in the present dataset: 11 vs. 0.01, t-test, p<0.001). In addition, DNP&P and Annapurna soils had lower water holding capacities than found in the main GLV dataset (0.27 vs. 0.46, t-test, p=0.016). As a result, the habitat models for the Acidobacteria G4 and the Saprospirales are error corrected by adding leucine amino-peptidase activity, which was only appreciably active at
pH>7, as a model parameter as well as reweighting the contribution of water holding capacity and soil pH. In essence I had to broaden the range of predictability of the models once I had data across a wider range of pH values.

\[
\text{Corrected}_\text{AG4}=\text{Predicted}_\text{AG4} - 6.39 + 0.194 \times \text{PH} + 14.334 \times \text{WHC} - 0.317 \times \text{LAP}.
\]

\[
\text{Corrected}_\text{Sapro}=\text{Predicted}_\text{Sapro} - 1.789 + 3.722 \times \text{WHC} - 0.172 \times \text{SoilWater}
\]

Relative abundances were rescaled for Figure 3.4 due to a bias in Sanger vs. pyro sequencing that I observed in the 6 samples from Colorado that were analyzed using both sequencing approaches. Similar effects have been observed in previous comparisons of Sanger vs. pyrosequencing, although the cause of this bias is still the subject of debate (Jones et al., 2009; Morgan et al., 2010). However, the fact that these rough rescalings enabled the accurate prediction of Sanger relative abundances based on models of pyrosequencing data suggests that these biases are consistent across samples and, given absolute abundance estimates derived from a method such as FISH, similar types of correction factors could be used in future to estimate actual abundances of microbial clades in soil samples.

**Online Sequence Depository Information**

Sequences have been deposited in NCBI’s Genbank database under the accession numbers: HM780503-HM797396.

### 3.3 Results and Discussion

**Landscape Patterns in Bacterial Community Relatedness**

The first step in assessing habitat distributions for bacteria was to determine if there was a significant spatial pattern to their distribution across the landscape (see Figure 3.1 for sampling design). I used UniFrac phylogenetic analysis (Lozupone and Knight, 2007; Sogin et al., 2006) to show that there was a significant decrease in community relatedness with increasing distance
between any two samples across the landscape (n=85, p = 0.001, Mantel Test) up to a maximum autocorrelation distance of 240 meters (Figure 3.2). However, the decrease in community relatedness up to this scale was somewhat small (change in UniFrac distance over 240m = 0.03), perhaps indicating that only a subset of the total bacterial community was changing across the landscape. In contrast, beyond 240 meters there was a random scatter of the data around the plateau value equal to the average community relatedness among all samples (see inset to Figure 3.2); in other words, at distances greater than 240 meters it is just as likely to find a closely related community as it is to find a distantly related community.
Figure 3.2 A semivariogram plot of the decay in bacterial community similarity (as measured by the UniFrac community dissimilarity metric on the y-axis) with increasing distance between samples. A UniFrac value of 1 indicates no shared community members between 2 samples and a value of 0 indicates 100% shared community members. The solid line is the variogram model fit, which tracks the predictable change in shared community membership with distance. The vertical dashed line is the distance of spatial autocorrelation (range), which is the maximum distance, according to the model, that similarity in community composition between samples is correlated (240 meters in this case). The horizontal dashed line represents the “nugget,” which is the proportion of the change in community composition not explained by the spatial model. The inset demonstrates that past the autocorrelation distance there is no predictable change in community composition with distance.

To determine which, if any, subset of the bacterial community was changing across the landscape I examined the spatial autocorrelation in genetic relatedness and relative abundance for major bacterial clades containing more than 100 sequences across all sampling sites (30 clades in all). Genetic relatedness and/or relative abundance of clades may account for community biogeographic patterns (Martiny et al., 2006); however, across the high-alpine
landscape only the relative abundance of specific clades contributed to the community spatial pattern across the landscape (6 clades, p≤0.002, Moran’s I for relative abundance, Appendix B). These analyses show that high-alpine bacterial clades have distinct landscape-scale patterns in distribution, suggesting that bacterial clade relative abundance may be structured by patterns in biogeochemical parameters at the landscape scale.

*Habitat Distribution Modelling for Bacterial Clades*

Given the spatial patterning of microbial clades, I next determined which, if any, biogeochemical parameters underlie these patterns (Green *et al.*, 2008). I used habitat distribution models (van Buskirk, 2005; Guisan and Zimmermann, 2000) to analyze the habitat distributions of the 30 clades and identify the major landscape-scale structuring factors. The models characterize the relationship between the relative abundance of bacterial clades and an extensive set of biogeochemical parameters across the landscape (21 different factors including soil pH, plant cover, average annual snow depth, soil texture, and extracellular enzyme activities, see Appendix B). These independent analyses identified some of the same clades as the spatial autocorrelation analyses, including the Rhodospirillales, Rhizobiales, Acidobacteria G4, and Saprospirales as having the highest levels of correlation with soil biogeochemical parameters (Appendix B). This approach yielded strong model fits with r² values between 0.53 and 0.62 (Table 3.1), equivalent to the best r² values for models of plant and animal abundances at the landscape scale (van Buskirk, 2005; Guisan and Zimmermann, 2000).
Table 3.1 Model fit components for each clade with strong relative sequence abundance predictive habitat models. Components include model fit based on spatial autocorrelation in relative abundance alone, environmental predictor variables with spatial effects within the variables factored out, environmental predictor variables with spatial effects not removed, and the full model combining the environmental predictor model and the pure spatial model. The distances of spatial autocorrelation in relative abundance for the residuals of the OLS regression based on environmental variables (spatial effects within environmental variables not removed) are also shown. With environmental effects removed, the averaged distance of spatial autocorrelation in relative abundance is equal to the distance of autocorrelation in whole community composition overlap (240m).

To visualize how these clades are related to soil biogeochemical parameters I mapped the habitat distributions for each of the three most abundant clades across the landscape (Figure 3.3); each mapped model describes the relative abundance of a clade based on its response to the biogeochemical parameters at any one location in combination with a kriged (Bailey and Gatrell, 1995) spatial component that is a proxy for the influence of unmeasured biogeochemical parameters. In addition, out of the 21 biogeochemical parameters measured I identified three parameters as the primary factors shaping microbial distribution in this environment (Table 3.2). These parameters were soil pH, snow depth, and forb abundance (forbs are broadleaved flowering plants, e.g. not grasses). Although spatial studies have been conducted for microorganisms at many scales (Green et al., 2004; Horner-Devine et al., 2004; Ramette and
Tiedje, 2007; Robertson and Freckman, 1995; Rodrigues et al., 2009), these models represent the first successful description of predictive habitat distributions for bacteria.

Figure 3.3 Maps of the sequence relative abundance as predicted by habitat distribution models for the three most abundant clades with strong correlation with environmental variables. Each relative abundance map is depicted with 4 dimensions, the length and width representing geographic space, the vertical dimension representing forb abundance for the upper two maps and soil pH (hash mark scale on right), and color representing the relative abundance of each of the clades (scale on left, red=high; blue=low). The clades are a) Rhizobiales, b) Rhodospirillales, and c) Acidobacteria G4. The clade with the fourth highest relative abundance, the Saprospirales, had a distribution very similar to the Acidobacteria G4 and is not shown. The maps were created by cokriging (Bailey and Gatrell, 1995), an interpolation method that uses the 85 relative abundance measurements in combination with the environmental predictors from our model at all 160 sample locations to create a continuous map of relative abundance in the sampling area. The bottom topographic map shows the two-dimensional extent of the landscape.
Table 3.2 Standardized model coefficients, which indicate the relative importance of each variable to the models. Landscape-scale habitat models are given as the first number and the revised global-scale models are given in parentheses (global models were revised due to lack of data for some biogeochemical parameters). Global-scale model predictive power is also given for each clade. For the global models, clades with significant correlation between residuals (predicted relative abundance – actual relative abundance) and environmental variables were error corrected by using ordinary least squares regression to predict residual error (Acidobacteria G4 and Saprospirales). The error correction involved adding leucine amino-peptidase activity, which was only appreciably active at pH>7, as a model parameter as well as reweighting the contribution of water holding capacity and soil pH due to the fact that these parameters have a much greater range in the worldwide samples (WHC 0.15-1.3; pH 3.5-7.8) versus Colorado (WHC 0.208-1.3; pH 3.5-6.2).

The predicted ecological niches based on my habitat distribution models confirm what is already known about some groups and suggests an unexpected dominance and new niche for another large group of bacteria. For example, the Rhizobiales are known plant-root symbionts (Garg and Geetanjali, 2006), so it was not a surprise that their relative abundance was most highly correlated with plant abundance across the landscape. In contrast, my results pointed to the unexpected importance of the Rhodospirillales (my most abundant clade) across this alpine landscape. These organisms are mostly found in aquatic habitats where many of them fill a phototrophic niche, although they have extreme metabolic versatility encompassing photoheterotrophic, chemoorganotrophic and photoautotrophic lifestyles (Madigan and Jung,
While, pH was the best predictor of Rhodospirillales relative abundance, the mechanism of this control for this and broader groups of microorganisms remains unknown (Fierer and Jackson, 2006; Fierer et al., 2009). However, although we do not yet know the Rhodospirillales’ function in high alpine soils, their negative correlation with plant abundance (the next strongest correlate) may indicate that they are out competed for light by plants in the alpine landscape and may be previously undocumented phototrophs in this environment. The Rhodospirillales were also negatively correlated with soil nutrients and total microbial biomass (Appendix B), which in combination with their metabolic versatility suggests that these alpine microorganisms are well adapted to extremely oligotrophic areas. Regardless of their exact function, this is the first report of the widespread occurrence of the Rhodospirillales in soil and their importance across large expanses of the alpine landscape would not have been predicted without detailed models of their habitat distribution.

_Landscape Patterns in Biogeochemical Factors_

The recurring significance of forb abundance, snow depth, and pH in shaping clade habitat distributions suggests that these environmental factors are particularly important in the periglacial landscape (Table 3.2). However, while forb abundance appears to be a strong metavariable and shows significant linear relationships to many measures of soil biogeochemistry (microbial biomass, soil dissolved nutrients, extracellular enzyme activity, soil texture, soil water content) neither soil pH nor snow depth showed significant association with the other variables (Appendix C). Thus, the few hotspots of plant abundance areas within the landscape (Figure 3.3) appear to have a very strong, direct influence on both soil biogeochemistry and the distribution of microbial clades.
The lack of association between snow depth and the other biogeochemical variables is surprising because snow depth is a known driver of landscape structure in alpine environments (Chapters 2; Ley et al., 2004; Seastedt et al., 2004; Nemergut et al., 2008). Moreover, in contrast to previous reports of non-linear response of plant communities to snow-depth (Seastedt et al., 2004) with either too deep or too shallow snowpack negatively affecting plant survivorship, plants neither display a significant linear or non-linear response to snow depth in this periglacial landscape. This is likely due to the subsurface variation in hydrology that creates a difficult to predict landscape pattern of springs and seeps (Hill, 2008) whose moisture subsidies are likely the primary determinant of plant abundance in this landscape. Thus, the role of snowpack in structuring the periglacial landscape is less straightforward that previously hypothesized (Chapter 2) and merits further study.

Similarly, the lack of interactions between soil pH with other model variables suggests that pH, like snow depth, also measures a distinct landscape process; e.g. the composition of bedrock weathering products as discussed by Jacobson et al. (2002). Indeed, soil pH patterns show strong correspondence to parent material CaO content (Figure 3.4), which readily weathers to the high buffering capacity CaCO₃ (MgO also does this, Schlesinger, 1991). Additionally, in agreement with previous studies at continental scales (Fierer and Jackson, 2006; Fierer et al., 2009; Jones et al., 2009), soil pH was the only model variable that showed a strong effect on the distribution of all four clades, suggesting that soil pH is an important driver of microbial community composition at both small and large scales.
Figure 3.4 The CaO content for 1 rock sample collected within each polygon (top) overlayed on the USGS 1950 survey by Lovering and Goddard of major geologic units in the area. Sampling locations for soils in this study are also shown. Notice that the kriged map of soil sample pH (bottom) predicts lower pH in areas where the bedrock has low CaO content.

Global-Scale Predictive Power of Habitat Distribution Models

In order to estimate global-scale applicability of bacterial habitat models to geographically separate high-elevation environments, Sanger clone libraries were constructed from six samples in each of four of the highest mountain ranges on Earth. These locations represent tests of my models against the extreme environmental limits of high-alpine systems (newly deglaciated soils near Mt. Denali, late-melting snowbanks near the continental divide in the Colorado Rockies and alpine deserts in the high Andes and Himalayas, Table 3.3). Taken together, the four clades identified in my pyrosequencing study made up a significant portion of the Sanger library
microbial community in most of my sites, representing 26% of all bacteria in the Colorado Rockies, Himalayas, and Alaska Range, but only 8% in my most extreme site, the high Andes.

<table>
<thead>
<tr>
<th>Range</th>
<th>Site</th>
<th>UTM coordinates (zone)</th>
<th>Elevation (m.a.s.l.)</th>
<th>Number of Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rocky Mountains</td>
<td>Green Lakes Valley, Colorado USA</td>
<td>446772E, 4434307N (13)</td>
<td>3740</td>
<td>1052 (6 samples)</td>
</tr>
<tr>
<td>Alaska Range</td>
<td>Denali National Park &amp; Preserve, Alaska, USA</td>
<td>354260E, 7032590N (6)</td>
<td>1300</td>
<td>949 (6 samples)</td>
</tr>
<tr>
<td>Himalayas</td>
<td>Annapurna Conservation Area, Nepal</td>
<td>787000E, 3188900N (44)</td>
<td>5450</td>
<td>1040 (6 samples)</td>
</tr>
<tr>
<td>Andes</td>
<td>Llullaillaco, Argentina</td>
<td>547606E, 7266160S (19)</td>
<td>6330</td>
<td>388 (5 samples)</td>
</tr>
</tbody>
</table>

Table 3.3 Sampling sites used in this study. Sequence counts are from Sanger clone libraries. UTM: Universal Transverse Mercator coordinate system; m.a.s.l.: meters above sea level.

My habitat distribution models correctly predicted the relative abundance of the four major clades from my pyrosequencing study across the entire global dataset (Figure 3.5). The models had the highest predictive power in cases were the biogeochemical variables closely matched the Colorado Rockies environment, however, the models fit less strongly in areas with extreme differences in environment. In my most extreme global location, the volcanic soils of the high Andes with almost no snowpack and no plant cover, the Rhodospirillales were, predictably, the group with highest relative abundance whereas the other three groups were absent or had very low relative abundances. This suggests that while extreme habitats result in lower accuracy of habitat modelling for alpine bacteria, these same major clades are predictable in their importance globally. This conclusion is supported by recent findings that fungal communities are very similar in plant-free soils of the Rocky Mountains, Himalayas and Antarctica (Freeman et al., 2009b). Thus, high elevation and high latitude environments appear to harbour globally distributed microbial clades and are proving to be ideal environments to test hypotheses about the biogeography of soil microbial community diversity and function.
Figure 3.5. The relative abundance of Colorado’s four major clades across high-alpine soils at a global scale (Acido: Acidobacteria Gp4; Rhizo: Rhizobiales; Rhodo: Rhodospirillales; Sapro: Saprospirales; A, Rocky Mountains; B, Alaska Range; C, Himalayas; and D, Andes). Actual relative abundance: open; predictive habitat model relative abundances: shaded; error bars represent standard error; * indicates nonsignificant difference between actual and predicted, t-test, n=6, p>0.05. Ordinary Least Squares (OLS) predictive habitat models using a restricted parameter set (see model parameters, Table 3.2). Acidobacteria Gp4 and Saprospirales had significant correlation between residuals (predicted relative abundance – actual relative abundance) and environmental variables and are error corrected by using OLS to predict residual error. Sanger relative abundances were rescaled due to the previously described biases in Sanger vs. pyro sequencing (Jones et al., 2009); Sanger relative abundance * factor: Acidobacteria G4 *0.667; Rhizobiales *2.85; Rhodospirillales *6.54; Saprospirales *0.204; Raw relative abundances are given in Table 3.4.

<table>
<thead>
<tr>
<th></th>
<th>Acidobacteria Gp4</th>
<th>Rhizobiales</th>
<th>Rhodospirillales</th>
<th>Saprospirales</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niwot</td>
<td>4.29 (1.87)</td>
<td>1.24 (1.19)</td>
<td>0.76 (0.17)</td>
<td>11.84 (3.95)</td>
</tr>
<tr>
<td>Nepal</td>
<td>15.86 (5.87)</td>
<td>1.45 (1.14)</td>
<td>0.86 (0.79)</td>
<td>7.60 (5.67)</td>
</tr>
<tr>
<td>Llullaillaco</td>
<td>0</td>
<td>0</td>
<td>3.53 (4.03)</td>
<td>3.53 (2.82)</td>
</tr>
<tr>
<td>Denali NP&amp;P</td>
<td>9.06 (3.78)</td>
<td>1.16 (1.23)</td>
<td>0.63 (0.69)</td>
<td>14.97 (3.72)</td>
</tr>
</tbody>
</table>

Table 3.4 Means for raw Sanger clone-library relative abundances uncorrected for biases between Sanger and pyro sequencing. Standard deviations are in parentheses.
3.5 Conclusions

While other studies have shown that 1: spatial patterns exist in soil microorganisms (Green *et al*., 2004; Horner-Devine *et al*., 2004) and 2: steep gradients in soil chemistry are correlated with phylum-level changes in microbial community composition (Ley *et al*., Nemergut *et al*., 2008), this study is the first to successfully link spatial autocorrelation in microbial communities to the distribution of individual clades and to demonstrate that these distributions can be modelled with strong predictive power across the landscape and the globe. I did this by examining the relationship between narrowly defined bacterial clades and soil environmental and biogeochemical patterns, which affords greater power to identify ecological patterns than previous OTU diversity (Green *et al*., 2004; Horner-Devine *et al*., 2004) or phylum level (Fierer and Jackson, 2006; Fierer *et al*., 2009) studies. By examining narrowly defined clades, I was able to provide the first environmental-sequencing based description of ecological niches for bacteria and to identify unexpectedly important bacterial clades such as the Rhodospirillales. In addition, the groups that showed the highest level of spatial structuring across the landscape have predictable distributions in high-elevation soils across the globe, suggesting that these groups are easily dispersed and are of significant importance to alpine biogeochemistry and bacterial community dynamics. These findings are evidence that soil microorganisms are not homogeneously distributed across landscapes but rather occur in patches whose composition is related to the landscape distribution of biogeochemical properties. This approach is uniquely ecosystem focused and greatly expands our ability to link changes in community diversity with the relative abundance of individual bacterial clades and understand the ecology of soil organisms across the landscape and the Earth.
Acknowledgements

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CHAPTER 4
MICROBIAL BIOMASS AND ACTIVITY IN HIGH ELEVATION (>5100 METERS) SOILS FROM THE ANNAPURNA AND SAGARMATHA REGIONS OF THE NEPALESE HIMALAYAS

4.1 Summary

High elevation periglacial-zone soils are increasing in spatial extent in the Himalayas due to glacial retreat and grazing pressures. These seemingly barren soils actually harbor significant microbial diversity but have remained mostly unstudied in all of the major mountain ranges of the Earth. Here I describe a preliminary survey of periglacial-zone soils and one vegetated high-elevation soil of the Annapurna and Sagarmatha regions of the Nepalese Himalayas. I examined microbial biomass and activity as well as key microclimatic and edaphic variables that may control microbial activity in these soils. Microbial biomass carbon levels are the lowest ever reported for any soil, whereas microbial nitrogen and soil enzyme activities are similar to levels measured in previous studies of periglacial-zone soils of Peru and Colorado. This initial study also indicates that soil water availability is the primary limiting factor for life in these high-elevation soils.

4.2 Introduction

On the highest mountain ranges of the Earth, between the upper zone of year-round snow and ice (the nival zone) and the zone of continuous vegetation (the alpine zone), exists a stark expanse of seemingly bare rock and barren soils called the “periglacial zone” or “mountain desert” (Figure 4.1a; Nagy and Grabherr, 2009; Troll, 1973). Yet, upon close inspection the periglacial zone is a landscape mosaic in which soil development and plant colonization are related to local variation in microclimate (Burns and Tonkin, 1982). This variation results in a patchy environment with barren soils underlying high snow areas, sparse plant communities in moderate accumulation areas, and further barren soils in wind-scoured locations. When not
snow-covered, the soils of this highly exposed environment are subject to extreme fluctuations in temperature, solar radiation, and soil moisture. This effect is particularly pronounced during the fall season when 40 degree C diurnal soil temperatures fluctuations result in nighttime conditions below freezing and surprisingly hot daytime temperatures upwards of 30°C (Figure 4.2). These factors create a harsh environment for periglacial organisms and, deservedly, one of the most barren looking ecosystems on Earth. It is presently unclear if, given enough time, plants can colonize the upper-elevations of the periglacial zone, however, abundant and surprisingly diverse microorganisms persist even the highest elevation soils sampled to date (Costello et al., 2009; Schmidt et al., 2009, 2010).
Figure 4.1
a) Photograph of the Chulu range taken (10/19/2008) from above Kangshar village during fieldwork in the ACA. The labels indicate the distinct zonation of the alpine, periglacial, and nival zones. The boundary between alpine and periglacial zones is defined as the upper extent of continuous plant cover. The boundary between periglacial and nival zones is defined as the lower extent of continuous ice and snow cover.

b) An elevational map of Nepal. The red area represents the extent of the periglacial zone as estimated by area having an elevation of 5000-5600 m.a.s.l. Sampling locations are in the black circles.

Figure 4.2 Soil temperature measurements from two days near the 4824 m elevation Kobresia dominated site in the ACA region. Closed circles represent the soil surface temperature and open triangles represent the soil temperature at a depth of 4 cm.

Due to the dependence on snow accumulation, the area defined as the periglacial zone occurs at much higher elevations in drier mountain ranges such as in the Andes and in the inner ranges of the Himalayas than in more humid mountain ranges such as the Alps. The periglacial zone of the Bavarian Alps starts at about 2500 m above sea level compared to 4700-5000 m in the Andes of southern Perú and 5000-5600 m in the Himalayas (Chang, 1981; Rawat and Pangtey, 1987; Engholm et al., 2009). Owing to their extremely high elevation and historical inaccessibility, much less is known about periglacial ecosystems in ranges such as the Himalayas than in the comparatively well-studied Alps. Globally, the periglacial zone is thought to have expanded downwards in recent years due to overgrazing in the upper alpine zone (Ahmad et al.,
1990; del Valle et al., 1998) and upwards, due to the retreat of glaciers and icepacks at high elevations (Byers, 2007) which is predicted to increase over the next century (Zemp et al., 2006). In Nepal, the periglacial zone currently occupies about 6% of the land area (Figure 4.1b), yet we know almost nothing about the organisms that inhabit these environments and their how they drive biogeochemical cycling.

It is still unclear how life forms that survive in the periglacial zone obtain the nutrients and energy needed to sustain life. Swan (1963, 1990, 1992) contended that life at these extreme elevations subsisted mainly upon aeolian-deposited organic matter; that is, organic matter blown from lower elevations to higher elevations. However, recent studies of microbial communities at high elevations (Chapters 2,3; Freeman et al., 2009a,b; Schmidt et al., 2009, 2010) have given cause for reevaluating how life is sustained at these elevations. The Schmidt research group has been studying microbial life in soils up to 6000 m above sea level in the high Andes of South America and the southern Rocky Mountains of the United States for the past ten years. Although we have observed pockets of aeolian-supported life (Ley et al., 2004; Freeman et al., 2009b), we have found much larger areas of wind-swept lands that do not accumulate high amounts of organic debris from lower elevations but are nonetheless teeming with previously unreported microbial life (Chapters 2,3; Freeman et al., 2009a; Schmidt et al., 2009). These studies have shown that in many periglacial soil microorganisms obtain their sustenance not from wind-blown organic matter but primarily from atmospheric gases through the processes of microbial photosynthesis and nitrogen fixation (Chapter, 3; Freeman et al., 2009a; Schmidt et al., 2008, 2009) and that the buildup of microorganisms may be largely limited by soil water availability (Chapter 2). Thus, although we have pushed our understanding of high-elevation life beyond the pioneering efforts of Swan (1963, 1990, 1992), the question remains as to whether these new
discoveries made in the Andes and Rocky Mountains apply to even higher mountain ranges of the world.

The large area occupied by periglacial soils in the Nepalese Himalayas (Figure 4.1b) makes it particularly important to understand the activity and abundance of high elevation microorganisms there. Here I examine microbial biomass and extracellular enzyme activity in periglacial soils from the Annapurna and Sagarmatha (Everest) regions of the Nepalese Himalayas. This study describes microbial life in four periglacial substrates: plant-covered soil, eroded previously vegetated soil, unvegetated shale-derived mineral soil, and unvegetated granite-derived mineral soil. The findings presented herein are the first report of an ongoing research effort to characterize the microbial activity and diversity of the periglacial soils of the Himalayas.

4.3 Methods

Study Sites and Sample Collection

My sampling sites were in the Annapurna Conservation Area (ACA, Panthi et al., 2007; Shrestha et al., 2007) and the Sagarmatha National Park (SNP, Byers, 2007) of the Himalayan Mountains in Nepal (Figure 4.1, Table 4.1). In each region, samples were obtained from mineral soils from sites just above the highest plants and from sites as high as were attainable due to prevailing conditions (e.g. presence of ice and snow) at the time of sampling. Sampling was conducted in October and November of 2008 in order to take advantage of the seasonal lack of precipitation. Low precipitation seasons are ideal for this type of descriptive study because it minimizes short term variability due to individual precipitation events when comparing across sampling sites and allows access to the highest possible soils due to the relatively snow-free conditions. In addition to the unvegetated soils from of ACA, samples were also obtained from patches of soil dominated by the sedge Kobresia cf. pygmaea (C. B. Clarke) C. B. Clarke as well
as soil from eroded areas adjacent to patches of *Kobresia*. These sites were located just below the lowest-elevation plant-free site. Soil samples were collected to a depth of 5 cm and placed in sterile zip-lock bags. Samples were frozen in the field by packing sample bags in a cooler along with snow and ice collected from the landscape and transported back to the laboratory over about a week. Samples were immediately extracted for $\text{K}_2\text{SO}_4$ dissolvable C and N (Weintraub *et al.*, 2007) and then stored at $-20^\circ\text{C}$ for further analysis.

<table>
<thead>
<tr>
<th>Site</th>
<th>UTM coord. (zone)</th>
<th>Elevation (m.a.s.l.)</th>
<th>Parent Material</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zun Tal (ACA)</td>
<td>784700E, 3180600N (44)</td>
<td>5101 - 5289</td>
<td>shale</td>
<td>7.2</td>
</tr>
<tr>
<td>Kobresia sward &amp; eroded soils (ACA)</td>
<td>786770E, 3176125N (44)</td>
<td>4824</td>
<td>shale</td>
<td>6.1</td>
</tr>
<tr>
<td>Thorong La (ACA)</td>
<td>787000E, 3188900N (44)</td>
<td>5482 - 5516</td>
<td>shale</td>
<td>7.3</td>
</tr>
<tr>
<td>Gokyo Lake 5 (SNP)</td>
<td>468350E, 3097500N (45)</td>
<td>5094 - 5111</td>
<td>granite</td>
<td>5.3</td>
</tr>
<tr>
<td>Chola Pass (SNP)</td>
<td>493650E, 3092850N (45)</td>
<td>5376 - 5387</td>
<td>granite</td>
<td>5.3</td>
</tr>
<tr>
<td>Island Peak (SNP)</td>
<td>493170E, 3089600N (45)</td>
<td>5272 – 5291</td>
<td>granite</td>
<td>5.3</td>
</tr>
</tbody>
</table>

**Table 4.1** Sampling sites used in this study. ACA, Annapurna Conservation Area; SNP, Sagarmatha National Park

The low-elevation sites in the ACA are unvegetated, south-east facing slopes at 5122 m above sea level (m a.s.l.) and approximately 8 km east of the northern edge of Tilicho Lake.

Samples were also collected from patches of *Kobresia* covered soil and from eroded areas adjacent to the patches of *Kobresia* on a south-south-west facing slope at 4824 m a.s.l. The high-elevation sites in the ACA are unvegetated south-east facing slopes from 5510 m a.s.l., 1 km north of Thorong Pass. The Thorong Pass crosses the divide separating the Marsyangdi River to the east and the Kali Gandaki River to the west. All of the ACA sites are located on shale bedrock.

The sampling sites in the SNP receive significantly more precipitation than do the ACA sites (Figure 4.1c). Four samples were collected from each of three unvegetated south-east facing slopes in this region, Chola Pass at 5380 m a.s.l., Gokyo Lake 5 at 5100 m a.s.l., and Island Peak 5280 m a.s.l. All of the SNP sites are located on granite bedrock.
Soil Temperature

Soil temperature measurements were conducted in order to measure diurnal climatic variability. Temperature was recorded using HOBO data loggers (Pendant temp/light, UA-002-64, Onset Computer Corp., Bourne, MA) every five minutes from October 16th to 19th 2008 at the ACA Kobresia dominated site. One data logger was placed flush with the soil surface and another at a depth of 4cm.

Microbial Biomass Carbon and Nitrogen

Soil dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and microbial biomass carbon (MBC) and nitrogen (MBN) were determined using the methods described in Weintraub et al. (2007). For soil DOC and TDN, 5 g of each soil sample was shaken with 25 ml of 0.5 M K$_2$SO$_4$ for 1 hour. For microbial biomass C and N, 5 g of soil was added to a 250 ml glass flask with 2 ml of chloroform, sealed and fumigated for 24 hours, and then vented for 1 hour; 25 ml of 0.5 M K$_2$SO$_4$ was added to each flask, and then they were shaken for 1 hour. Solutions were pre-filtered using a 1 μm Pall glass fiber filter (Pall Corporation, East Hills, NY). Solution C/N analysis was performed using a Shimadzu total organic carbon analyzer (TOC 5000) equipped with a total dissolved nitrogen (TDN) module (Shimadzu Scientific Instruments, Inc., Columbia, MD).

pH Determination

In order to determine soil pH, 2 g of each soil was placed to an individual 15 ml conical tube to which was added 2 mL of distilled water. Conical tubes were placed horizontally on a shaker table and shaken for 1 h at 175 rpm. Soil pH was measured using a glass Fisher pH probe (Fisher Scientific, Pittsburgh, PA).
Extracellular Enzyme Activity

Microbial extracellular enzyme activities were assayed using the modification from Chapter 2 of the method of Weintraub et al. (2007). Enzymes assayed were: N-acetylglycosaminase, cellulase (β-glucosidase), α-glucosidase, β-xylase, cellobiosidase, leucine aminopeptidase and organic phosphatase. For each sample, 2 g of soil was added to 150 ml of buffer at the average pH for the soil type (0.5M Acetate at pH 5 for granitic, 0.5M Acetate at pH 6 for eroded and vegetated, and 0.5M Bicarbonate at pH 7.3 for shale derived soils) and homogenized at 3000 rpm for 1 minute using a Ultra-Turrax homogenizer (IKA Works Inc., USA). Soil slurries were assayed using the same controls, fluorescent substrates, and solution volumes as in Weintraub et al. (2007). Soils were incubated with substrates for 20 hours at 14°C.

Water Holding Capacity

Tubes for assaying water holding capacity (WHC) were constructed by cutting the bottom off of a 1 cm diameter 15 ml conical tube and covering the opening with 1-mm gauge plastic mesh. The mesh was wetted with deionized water prior to the addition of soil to the tube so that particles less than 1 mm in size would clump together at the bottom of the tube. For each sample I added ~4 g of soil to a tube and then added ~2 ml of H$_2$O. Wetted sample tubes were placed in 50 ml conical tubes, which were drained periodically. When an individual sample stopped dripping, the mass of the sample was recorded. Samples were then dried at 100 °C for 24 hours. Water holding capacity is reported as the g H$_2$O at soil saturation divided by g dry soil.
Statistical Analyses

Tukey’s honestly significant difference tests (Devore, 2004) were performed in R (version 2.8.1, 12/22/2008, R Foundation for Statistical Computing http://www.r-project.org/index.html). A correlation test (Devore, 2004) between microbial biomass C (MBC) and water holding capacity was also performed in R.

4.4 Results

Microbial biomass and extracellular enzyme activity are extremely low in the shale-derived soils from the ACA (Figures 4.3,4.4). The granitic soils of the SNP have significantly higher biomass and activity than the shale-derived soils (Figures 4.3,4.4). As expected, soils of the lower-elevation eroded area have higher microbial biomass than either of the two higher-elevation mineral soils, and the Kobresia soils had the highest microbial biomass C and N (Figure 4.4). Surprisingly, while both the eroded soils and the Kobresia soils have higher enzyme activity than the mineral soils, the eroded soils have significantly higher extracellular enzyme activity than the vegetated soils (Figure 4.4). The biomass trends are mirrored by the water holding capacity measurements (Figure 4.5). Finally, there is a significant correlation between MBC and WHC for all samples (Figure 4.6, Pearson $r^2 = 0.426$, correlation test: $p < 0.001$). However, this trend is primarily driven by the increase of WHC and MBC with plant colonization.
Figure 4.3 Extracellular enzyme activities for the four soil types. Enzymes activities shown are N-acetyltriacosaminidase (NAG), cellulase (β-glucosidase) (BG), organic phosphatase (PHOS) and leucine amino peptidase (LAP). α-glucosidase and β-xylanase activities are at similar levels to NAG and are not shown. Significant differences are designated by letters grouping similar levels of activity for an individual enzyme (Tukey’s Test, p < 0.05). Error bars are standard error.

Figure 4.4 (A) Dissolved organic carbon (DOC) and microbial biomass carbon (MBC) for the four soil types. (B) Total dissolvable nitrogen (TDN) and microbial biomass nitrogen (MBN) for the four soil types. Significant differences are designated by letters grouping similar levels of activity for an individual enzyme (Tukey’s Test, p < 0.05). Error bars are standard error.
Figure 4.5 Water holding capacity (g H$_2$O/g dry wt of soil) for the four soil types. Significant differences are designated by letters grouping similar levels of activity for an individual enzyme (Tukey’s Test, p < 0.05). Error bars are standard error.

Figure 4.6 Microbial biomass C versus water holding capacity for all samples (Pearson r$^2 = 0.426$, correlation test; p < 0.001). It is apparent that this trend is driven by the increase in WHC and MBC with plant colonization. Taken individually the only sample group with a significant positive association between WHC and MBC is the granitic soils (r$^2$=0.301, p<0.05). Error bars are standard error.
4.5 Discussion

The periglacial soils examined in this study are subject to some of the most extreme environmental conditions of any soils on Earth (Troll, 1973), yet even the most visually barren mineral soils had small amounts of measureable microbial biomass and enzyme activity (Figures 4.3, 4.4). At an average of 20 µg C/g soil, the shale-derived mineral soils of the ACA have the lowest microbial biomass levels ever reported for periglacial or recently deglaciated soils. Previously, the lowest microbial biomass numbers had been reported in alpine and Antarctic glacial moraines, which harbor 60 and 100 µg C/g soil, respectively (Tscherko et al., 2003a,b). In less polar soils Periglacial biomass levels have been shown to be even higher, with the temperate periglacial soils of Colorado at 80 µg C/g soil and the tropical periglacial soils of Perú at 140 µg C/g soil (Chapter 2). Thus, it is fitting that the periglacial soils of the region that has been described as earth’s “third pole” (Dyhrenfurth, 1955) may represent the upper boundary of suitable conditions for sustaining microbial life and highlights the need to understand what proportion of this biomass represents endemic communities versus dormant, wind-blown, transients.

The correlation between soil water holding capacity and microbial biomass (Figure 4.6) lends credence to the idea that high-Himalayan microorganisms are indeed active and supports the hypothesis that the primary limiting factor for microbial biomass in periglacial soils is water availability. Indeed, the plant-free soils of the Nepal’s most arid region (the ACA, Figure 4.1c) had the lowest water contents and the lowest measured levels of microbial biomass of any of the soils measured. Furthermore, although the granitic soils of the SNP had similar microbial biomass levels to the previously examined periglacial granitic soils from Colorado, the shale-derived plant-free soils from the Andes in Perú had higher microbial biomass than either the
granitic soils (Chapter 2), which is a reverse of the Himalayan trend. This relationship is also mirrored by the lower extracellular enzyme activities and DOC content of the shale-derived soils versus the intermediate, granite-derived soils from the Nepalese Himalayas and the high activity Andean soils.

The discrepancy in microbial biomass between sites as delineated by parent material suggests that parent material is not the main factor determining microbial biomass levels in unvegetated periglacial soils. Instead it is likely that differences in climate are driving soil development across the barren periglacial ecosystems of the ACA and the SNP. As I discussed in the introduction to this dissertation, the influence of climate on the soil environment originates from variation in topography, bedrock type, and the prevalence of frost (Barsh, 1993; Gabet, 2004). While SNP and ACA are similar in their age, temperature regime, and ruggedness of the landscape, the SNP receives greater precipitation than does the ACA (Figure 4.1c) likely resulting higher rates of physical and chemical weathering which should accelerate soil formation. Thus, these data support the idea that climatic differences in water availability are a primary determinant of periglacial processes.

The Kobresia-dominated swards of the ACA, with an average microbial biomass of 325μg C/g soil, are low in soil nutrients for an alpine dry meadow community and suggestive of a low productivity system; microbial biomass C in *Kobresia myosuroides* dominated soils from Niwot Ridge, Colorado averages 1260μg C/g soil (King et al., unpublished data). Conversely, the ACA’s *Kobresisa* soils had relatively high microbial N content (~54μg N/g soil) which is very similar to *Kobresia* soils in Colorado (75μg N/g soil, Fisk et al., 1998). Once again, the low precipitation of the arid ACA region may be causing low soil microbial biomass C even in more
developed soils by directly limiting plant photosynthesis and indirectly limiting the amount of carbon available to soil heterotrophs.

The relatively high microbial N levels despite low soil C when viewed from the perspective of the late-fall time may indicate an adaptation of the plant community that fosters N retention in this ecosystem over the winter. A similar retention mechanism occurs in other ecosystems via microbial growth on senescing plant material after the plant growing season resulting in immobilization of N, especially in seasonally cold (Jaeger et al., 1999; Zak et al., 1990) or seasonally dry systems (Singh et al., 1989; Vitousek and Matson, 1984). This explanation is further supported by the low extractable nitrogen levels of the bulk soils from the Kobresia soil patches of the ACA (10 µg N/g soil). Regardless of the mechanism for N storage, the data suggest that the Kobresia soil communities of the ACA have high N retention in the face of low productivity.

Surprisingly, although Kobresia sward soils had the highest biomass of periglacial Nepalese soils, the eroded soils adjacent to the Kobresia soils had the highest enzyme activity. This is likely a result of the eroded soils losing their structure, allowing the microbial community greater access to sequestered organic matter. This effect would be compounded by cessation of rhizodeposition of labile carbon sources, which could cause the microbial community to shift towards breaking down more recalcitrant organic matter by increasing extracellular enzyme activity (Sinsabaugh et al., 2008). In addition, the lower nitrogen availability in the eroded soils relative to the Kobresia sward may be responsible for the concurrent spike in N scavenging enzymes such as NAG and LAP (Figure 4.3). In general, shifting nutrient limitations have been theorized to explain the shift in dominance of extracellular enzyme production in nutrient limited environments (Wallenstein and Weintraub, 2008), but the majority of evidence for this
phenomenon comes from studies of microorganisms in culture (Harder and Dijkhuizen, 1983). The soil biogeochemical measurements from periglacial eroded soils in Nepal support the long held hypothesis that high microbial enzyme activity should be higher in soils that have low amounts of labile nutrients relative to complex organic substrates.

Although globally soil bedrock type is a poor predictor of periglacial microbial biomass, soil pH varies in accordance with bedrock type as well as degree of plant colonization. Significantly, Lauber et al. (2009) have shown that microbial community composition changes predictably with soil pH and Sinsabaugh et al. (2008) have demonstrated that this pH variation can result in different rates of extracellular enzyme activity. Indeed, the periglacial soils in this study show strong associations between extracellular enzyme activity and pH. Cellulase (β – glucosidase) activity is very low in the unvegetated shale-derived soils (high pH) while it is one of the predominant enzymes in the unvegetated granitic (low pH). The opposite trend was observed with protease (leucine aminopeptidase) activity, wherein activity was high in the shale soils and barely detectable in the granitic soils. Furthermore, unlike the microbial biomass patterns, the same dichotomy was observed between soils from Colorado and the Andes (Chapter 2). As a result, the decomposition rate of particular organic substrates may vary between periglacial ecosystems as a function of parent material.

In the more developed Kobresia dominated soils, however, the ratio between the activity of cellulase, protease, and phosphatase extracellular enzymes shifted toward that global ratio reported for vegetated systems (Figure 4.3) in concert with a shift in soil pH to a slightly acidic level (6 in Kobresia soils vs. 7.3 in nearby unvegetated, periglacial soils). This suggests that in the past as soil development and ecosystem succession preceded the differences in nutrient cycling between the unvegetated soils on different parent materials may often disappear. But, if
climate change results in the predicted increase of periglacial areas (Radic and Hock, 2011), the aridness of the Nepalese Himalayas may result in plant colonization rates that lag behind the growth in the extent periglacial zone and, ultimately, increase the total effect of the parent material based differences in soil microbial activity I have observed.

4.6 Conclusions

The levels of microbial biomass and activity I observed in the periglacial zone soils of the ACA and SNP regions of the Nepalese Himalayas are some of the lowest ever observed for this, or any, ecosystem type. It is likely that the extent of the periglacial zone will expand upwards as a result of climate change and in some areas expand downslope due to overuse of alpine grazing ranges. The evidence from my work suggests that newly exposed areas of the periglacial zone will be characterized by low levels of microbial biomass that increase gradually with water holding capacity and soil development. However, specific activity levels will be dependent on nutrient availability, soil pH, speed of soil development, and regional precipitation patterns. This preliminary survey of microbial biomass and activity in the periglacial zone of the Nepalese Himalayas reinforces the notion that the organisms that live in this precarious ecosystem are subjected to some of the most extreme environmental conditions on earth and further study of these areas has the potential to uncover novel microbial communities in these fascinating soils from the highest mountain range on Earth.

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CHAPTER 5
GLOBAL PATTERNS OF SOIL MICROBIAL BIOMASS AND STOICHIOMETRY OF SOIL NUTRIENTS IN HIGH-ALPINE PLANT-FREE PERIGLACIAL SYSTEMS

5.1 Summary

Periglacial soils are found from the tropics to the poles and are the coldest terrestrial system in which organisms are able to grow. Here I examine carbon and nitrogen dynamics in soil microbial biomass and dissolved nutrients across the most extensive set of high-alpine plant-free periglacial soils sampled to date. I show that periglacial soils harbor the lowest microbial biomass and dissolved nutrient levels of any terrestrial environment that supports life. Moreover, the surprisingly high microbial biomass C:N ratios but low soil dissolved C:N ratios are suggestive of a soil stoichiometry that is greatly divergent from that observed in vegetated systems. This divergence hypothesized to be due to microorganisms accumulating high concentrations of oligosaccharides that are dual cryo/osmoprotectants in order to survive in the dual environment stresses of frequent freezing and drying events unique to periglacial areas. Thus, the traditional models of soil stoichiometry must be modified when applied to periglacial ecosystems to reflect the fact that carbon requirements are high and carbon inputs are low.

5.2 Introduction

Soil microorganisms are important drivers of ecosystem services and nutrient cycling and are the dominant mode of life in the periglacial zone (soils subjected to frequent freezing cycles with weathering and erosion by ice; Karte, 1983). The periglacial zone is one of two terrestrial ecosystems that can be found at almost all latitudes excluding the polar ice caps (the other being tundra) and includes the glacial forelands, long-lasting snow beds, and cold-deserts of Arctic, Antarctic and Alpine regions. A conservative estimate based on regional average temperatures for the warmest three months at just above freezing yields a global distribution for the periglacial
zone of at least 4% of the Earth’s total terrestrial area (Figure 5.1), an extent that is likely growing in area due to the melting of glaciers and ice packs worldwide (Beniston, 2003; Zemp et al., 2006; Radic and Hock, 2011). The soils of this globally well-distributed ecosystem are highly oligotrophic due to harsh environmental stressors such as low temperatures, high winds, low water availability, and low productivity (Chapters 2-4; Freeman et al., 2009a; Ley et al., 2004). While, plant-free periglacial soils have been studied for many decades in polar regions (see Cary et al., 2010 for a comprehensive review), we are just beginning to understand alpine periglacial soils (Chapters 2-4; Ley et al., 2004; Freeman et al., 2009a,b; Schmidt et al. 2009). Thus, the extreme environmental stress exert unique demands on the periglacial biotic community (Nemergut et al., 2005; Schmidt et al., 2008) that should significantly impact resource allocation and soil stoichiometry (Tilman, 1985), however, the specific effects on periglacial biogeochemistry is unknown. This study examines the applicability of current models of soil nutrient cycling in these high-alpine, plant-free, periglacial areas.

Figure 5.1  The global distribution of periglacial soils (pink area) as estimated for all areas excluding Antarctica using a mean temperature range of 2-6.7 °C for the warmest quarter of the year (temperature data from worldclim 1.3, Hijmans et al., 2005). Periglacial area in Antarctica is estimated from Cary et al.’s survey data (2010). Dots indicate locations included in this analysis.
In order to understand how available soil nutrients and the microbial biomass are related to nutrient cycling, it is important to understand the stoichiometry of soil processes and nutrient pools (Cleveland and Liptzin, 2007; Walker et al., 2010). Recently, developments in this field have resulted in a more exact description of the stoichiometric relationships displayed by soil microorganisms and soil organic matter (Cleveland and Liptzin, 2007). This work has established constraints on C:N ratios in microbial biomass (8.7C:1N) and total soil organic matter (14.3C:1N) for plant dominated systems. Accordingly, deviations from these Redfield-like elemental ratios are thought to reflect nutrient limitations or excess nutrients (Cleveland and Liptzin, 2007).

In order to compare periglacial soil processes to results from vegetated soils, we focused on periods for which periglacial environmental conditions support photosynthetic activity (hereafter referred to as “growing season”). The periglacial growing season is dependent on the extent of time that the soils are not snow covered and on the availability of water during this time period (Chapter 2; Freeman et al., 2009a). Despite the favorability of conditions implied by the term growing season, conditions are actually harsher than the snow-covered periods because the lack of insulating snow cover exposes these soils to frequent freeze-thaw stress (Chapter 4; Ley et al., 2004; Schmidt et al., 2009). In addition to these environmental pressures of temperature and a short growing season, periglacial soils also suffer from minimal water holding capacity due to low organic matter content and minimal weathering of the parent material. Thus, periglacial soils dry out rapidly, especially in the low humidity of high alpine environments. Together, these extreme environmental pressures make periglacial soils one of the harshest soil environments for life on Earth.
It is well known that soil nutrient cycling rates are dependent upon the demands of the soil community (Fisk and Fahey, 2001; Hobbie and Vitousek, 2000; Vance and Chapin, 2001; Schimel et al., 2007), however, little is known about periglacial nutrient stoichiometry. Previous work by Steve Schmidt’s research group has demonstrated that, overall, periglacial soils are oligotrophic with significantly less microbial biomass carbon, dissolved organic carbon, and total carbon than vegetated soils (Chapters 2,4; Ley et al., 2004). In addition, like most soils (Allen and Schlesinger, 2004; Cleveland et al., 2003; Johnasson et al., 1999), periglacial soils respond rapidly to labile C additions (Chapter 2, Schmidt et al., 2009). We have also shown that there is an increased respiration response when periglacial soils from the Rocky Mountains in Colorado are amended with phosphorus but not with nitrogen and that these responses are reflected in the extracellular enzyme activity of these soils (Chapter 2). In contrast, tropical periglacial soils from Peru have a relatively low respiration response to C additions (Schmidt et al., 2009) in association with both high phosphatase and protease activity (Chapter 2). Thus, it may be that the generally oligotrophic nutrient status of periglacial soils results in co-limitation of the soil microbial community by nutrients other than carbon but that the stoichiometry of periglacial nutrient demand may be site dependent.

Here I report on soil microbial biomass and soil dissolved nutrient pools from unvegetated periglacial soils sampled across nine globally distributed sites. I demonstrate that C:N ratios consistently deviate from the soil stoichiometric norms for vegetated systems, indicating that plant-free microbial ecosystems are subject to a unique, environmentally driven, nutrient stoichiometry.
5.3 Methods

Study Sites and Sampling

Nine globally distributed, plant-free, high elevation ecosystems were analyzed in this study. In the United States I collected samples from on and in front of the debris-covered Middle Fork Toklat Glacier (Denali National Park and Preserve, Alaska; Concienne, 2010), from in front of the Mendehall glacier (Alaska; Sattin et al., 2010), and from periglacial slopes in the Green Lakes Valley, Colorado (Chapter 3). In Nepal, samples were collected from three periglacial slopes in the Annapurna Conservation Area and three in Sagarmatha National Park near Mt. Everest (Chapter 4). In Perú, samples were collected from three periglacial slopes in the Laguna Sibinacocha Valley (Chapter 2). Finally, in Argentina and Chile samples were collected from periglacial slopes Socompa and Llullaillaco Volcanoes. Due to lack of data, samples from the Mendenhall glacier and Laguna Sibinacocha Valley are included only for the soil pH to MBC comparisons. For comparison, samples were collected from adjacent vegetated (>10 plants per square meter) periglacial soils at the Denali, Annapurna, and Green Lakes Valley sites. A summary of the environmental and biogeochemical attributes for each site can be found in Table 5.1.
<table>
<thead>
<tr>
<th>Location</th>
<th>Rock Type</th>
<th>n</th>
<th>pH</th>
<th>WHC</th>
<th>Soil Water</th>
<th>MBC</th>
<th>DOC</th>
<th>MBN</th>
<th>TDN</th>
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<td>51(17)</td>
<td>14(7.3)</td>
<td>19(13)</td>
<td>7.7(7.9)</td>
<td>2.1(2.1)</td>
<td>0.48(0.25)</td>
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<td>-</td>
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<td>7.2(4.8)</td>
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<td>0.06(0.1)</td>
<td>0.06(0.12)</td>
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<td>8.8(11)</td>
<td>2.8(2.1)</td>
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</table>

Table 5.1 Study site environmental variable means and standard deviations (in parentheses). WHC and soil water are given in %g H2O/g soil; MBC, DOC, MBN, and TDN are given in µmol/g soil

All samples were collected during the snow-free season at each site. In order to obtain sufficient spatial replication, I implemented a nested sampling scheme with distances between samples increasing logarithmically from 0.10m to 1km for Perú and Colorado (Chapter 2). Based on those results I determined that a maximum sampling distance between samples for adequate spatial replication was 50m. Samples from all other locations were collected with at most 50m between any sample and its nearest neighbor and with the greatest distance between samples at any site up to 2 km. Soils were homogenized in situ to a depth of 4 cm and placed in individual plastic ziplock bags. Samples were frozen and stored on ice during transport to Colorado, with a maximum transport time from my most remote field site (Annapurna Region of Nepal) of one week. Samples were thawed (a maximum of 3 days) at 4°C and extracted for dissolved organic C (DOC) and total dissolved nitrogen (TDN). Samples were then stored at -20°C and all further analyses were performed on these frozen samples. These manipulations were deemed not to
drastically affect the soil microbial community because soils from most of these zones were undergoing daily freeze-thaw cycles during the period they were sampled (Chapter 4; Schmidt et al., 2009).

**Soil DOC, TDN, and Microbial Biomass C & N**

Soil DOC, TDN, and Microbial Biomass C & N (MBC, MBN) were determined using the methods described in Weintraub et al., 2007. Briefly, for soil DOC and TDN, 5 g of each soil sample (unsieved) was shaken with 25 ml of 0.5 M K₂SO₄ for 1 hr. For microbial biomass C & N, 5 g of soil was added to a 250 ml glass flask with 2 ml of chloroform, stoppered and fumigated for 24 hrs, and then vented for 1 hr. 25 ml of 0.5 M K₂SO₄ was added to each flask, and then they were shaken for 1 hr. Solutions were prefiltered using a 1μm Pall glass fibre filter (Pall Corporation, East Hills, NY). Solution C/N analysis was performed using a Shimadzu total organic carbon analyzer (TOC 5000) equipped with a total dissolved nitrogen (TDN) module (Shimadzu Scientific Instruments, Inc., Columbia, MD). For Calculation of C:N ratios, samples with outlying C and N concentrations less than three times the detection limits of the analyser (0.1 μmol C/g soil; 0.001 μmol N/g soil) were excluded to avoid artificially influences on the C:N estimation. All nutrient levels are reported as μmol nutrient/g dry soil.

**pH Determination**

2 g of soil was shaken with 2 ml of distilled water for 1 hr and the first stable pH measurement was recorded. Soil pH measurements were made using a glass Fisher pH probe (Fisher Scientific, Pittsburgh, PA).

**Soil Water Content**

1 g of field condition soil was dried at 60°C and the change in mass divided by the remaining mass was recorded.
Soil Water Holding Capacity

Tubes for assaying water holding capacity (WHC) were constructed by cutting the bottom off of a 1 cm diameter 15 ml conical tube and covering the opening with 1-mm gauge plastic mesh. The mesh was wetted with deionized water prior to the addition of soil to the tube so that particles less than 1 mm in size would clump together at the bottom of the tube. For each sample we added ~4 g of soil to a tube and then saturated the soil with water H$_2$O. Wetted sample tubes were placed in 50 ml conical tubes, which were drained periodically. When an individual sample stopped dripping, the mass of the sample was recorded. Samples were then dried at 100 ºC for 24 hours. Water holding capacity is reported as the g H$_2$O at soil saturation divided by g dry soil.

Statistical Analyses

Pearson correlation tests and t-tests were performed in MVPStats (MVP Programs, Vancouver, WA). Model fitting was performed using standardized major axis regression for C:N comparisons (Cleveland and Liptzin, 2007) and least squares for soil nutrient to environment comparisons in R 2.12 (2011) with the package lmodel2 (Legendre, 2008). The Spatial Analyses in Macroecology (SAM; Rangel, 2006) program using the Akaike information criterion (AIC; Buckland et al., 1997) was used to select environmental variables for general linear model of microbial biomass C in order to estimate the relative explanatory power for the periglacial soil biogeochemical variables measured in this study.

5.4 Results

Soil Nutrients and Stoichiometric Ratios

Soil dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) levels are extremely low in unvegetated and vegetated periglacial soils compared to the global Redfield-
like ratio for vegetated soils (Cleveland and Liptzin, 2007), but DOC levels are especially low (Figure 5.2). The global mean for unvegetated periglacial DOC is 1.61 µmol C/g soil with a range of 0-7.07 and the mean for TDN is 0.21 µmol N/g soil with a range of 0-1.36. This results in an average DOC:TDN ratio of 8:1 (mol:mol) that is significantly lower (t-test, p<0.0005) than the average C:N ratio in vegetated soils of 14.3:1 (Cleveland and Liptzin, 2007), but indistinguishable from vegetated periglacial soils (8.2:1; t-test, p=0.81). In addition, the average value for DOC:TDN in periglacial soils is significantly lower (t-test, p<0.0005) than the ratio reported for forests (12:1) and grasslands (30:1) (Jones et al., 2006; Smolander and Kitunen, 2002). A strong correlation of DOC with TDN is also observed with an r² of 0.67, and a Pearson’s correlation test p<0.0005 (Figure 5.2). Most periglacial samples fall below the global mean for DOC:TDN (Figure 5.2), which, suggests widespread C limitation/demand for periglacial soils.
Figure 5.2 Disolved Organic Carbon versus Total Dissolved Nitrogen in globally distributed periglacial soils. The Pearson $r^2$ for this regression is 0.67 (thin line). The bold line represents the global average C:N ratio of 14.3:1 reported by Cleveland and Liptzin (2007). The dashed line represents periglacial soils with plant abundance $>10$ individuals per square meter ($r^2 = 0.82$).

Unvegetated periglacial microbial biomass carbon (MBC) and nitrogen (MBN) levels are also extremely low in all of the soils examined. However, MBN levels are especially low compared to the global mean based on extensive previous work in vegetated systems (Figure 5.3) and even compared to adjacent vegetated periglacial soils. The global mean for periglacial MBC is 4.83 µmol C/g soil with a range of 0-23.6 and the mean for MBN is 0.11 µmol N/g soil with a range of 0-0.52. This results in a C:N ratio of 27:1 (mol:mol), which is substantially higher (t-test: $p<0.0005$) than the average of 8.7:1 for microbial biomass C:N in vegetated soils (Cleveland and Liptzin, 2007) as well as higher than the average of 12:1 for vegetated periglacial soils ($p<0.0005$). The C:N ratio for vegetated periglacial soils is also significantly greater than the global average ($p<0.0005$). Most periglacial samples are above the global C:N ratio for vegetated soils (Figure 5.3), which, in contrast to the DOC:TDN relationship, suggests widespread over-abundance of C in periglacial microbial biomass.
**Figure 5.3** Microbial Biomass Carbon versus Microbial Biomass Nitrogen in globally distributed periglacial soils. The Pearson $r^2$ for this regression is 0.10 (thin line). The bold line represents the global average of 8.7:1 reported by Cleveland and Liptzin (2007). The dashed line represents periglacial soils with plant abundance >10 individuals per square meter ($r^2 = 0.74$).

**Interaction of Soil Nutrients with Abiotic Factors**

In agreement with previous findings (Chapter 4), soil water holding capacity (WHC) and to a lesser extent soil water content (SWC) displayed significant correlation ($p<0.005$) across the measured soil nutrient parameters (Table 5.2, Figure 5.4). Surprisingly, soil pH also has strong predictive power for soil nutrients (Table 5.2, Figure 5.5) and this power appears to be linked to site differences in soil parent material type and its associated carbonate content. AIC selection of an integrated model of microbial biomass levels revealed TDN and pH as the two most important variables predicting MBC ($r^2 = 0.29$; relative model weightings: $pH^* - 0.27$, TDN$^* 0.38$) and pH, SWC, and DOC as the three most important variables predicting MBN ($r^2 = 0.42$; relative model
weightings: pH* -0.25, SWC*2.9, DOC*0.29). This suggests that soil nutrient status and pH are the most important determinants of global variability in microbial biomass.

**Figure 5.4** The relationship between microbial biomass C and soil water content (A, $r^2$ is 0.07) or water holding capacity (B, $r^2$ is 0.07).

**Figure 5.5** The relationship between microbial biomass C and soil pH; $r^2$ is 0.04 for MBC (the regression line on the plot is 0.32 and represents the exclusion of the high pH/high biomass samples from the tropical Peru site; the plot for MBN vs. pH looks the same and is not shown). Filled circles are soils from sites with granitic bedrock, open circles are from basalt bedrock, filled squares are from calcareous shale bedrock, and open triangles are from the tropical Peru site also on calcareous shale bedrock.
Table 5.2 Pearson r values, * indicates a significant correlation at p<0.01.

5.5 Discussion

This most extensive survey of periglacial soils to date firmly places the periglacial ecosystem among the most oligotrophic environments on Earth. Many of these individual sites (Table 5.1) have nutrient levels comparable to the most extreme Arctic and Antarctic periglacial areas (Jones et al., 2000; Ball et al., 2009; Cary et al., 2010), with both microbial biomass and dissolved nutrient levels often approaching detection limits (Table 5.1; Figures 5.2, 5.3). Both high-alpine and polar periglacial areas have on average an order of magnitude lower microbial biomass C and N levels than vegetated systems (e.g. average periglacial microbial biomass 4.83 µmol C/g soil vs. 67 µmol C/g soil for grasslands; Wardle et al., 1998) and a third as much as found in desert systems (12.8 µmol C/g soil; Bailey et al., 2002). Similarly, periglacial dissolved organic C levels at 1.61 µmol C/g soil are half as much as is found in desert soils (3 µmol C/g soil; Bastida et al., 2006). Thus, the low nutrient status of periglacial soils is consistent with other extreme soil environments and provides a natural test for the applicability of biogeochemical theory to ecosystems functioning under extreme environmental conditions.

Divergent Redfield-like C:N Ratios for Periglacial Soils

In addition to the extremely low levels of microbial C and N, high-alpine periglacial soils have soil nutrient C:N ratios that diverge strongly from those for vegetated soils.
(Figures 5.2, 5.3). Dissolved organic C to total dissolved N ratios are lower than would be predicted by C:N ratios of vegetated systems (Figure 5.2), however, the opposite trend is observed in the microbial biomass, with consistently high MBC to MBN ratios (Figure 5.3). In fact, periglacial microbial C:N ratios are so divergent that only a handful of samples had nutrient ratios near the average for vegetated systems, resulting in an average C:N ratio three times that of vegetated soils (Figure 5.3) and double the largest C:N ratio reported for desert soil (approximately 14:1; Schade and Hobbie, 2005).

Current discussions of soil stoichiometry (Cleveland and Liptzin, 2007; Sinsabaugh et al., 2008, 2009) state that deviations from the global average should indicate nutrient limitation/demand and that early successional soils should be primarily N limited (Stevens and Walker, 1970; Wardle, 1998; Chapin et al., 1994; Vitousek, 2004; Walker et al., 2010).

Therefore, theory predicts that the high microbial biomass C:N ratio for periglacial soils indicates strong N limitation. On the other hand, theory also predicts that the observed low dissolved soil nutrient C:N ratio for periglacial soils indicates C limitation. This discrepancy between nutrient limitation predictions based on microbial and dissolved C:N does not readily fit into the current paradigm of soil nutrient stoichiometry.

Together, the low dissolved C:N and the high microbial biomass C:N may be indicative of a divergent carbon and nitrogen use stoichiometry for periglacial soils that is related to the harsh environmental conditions of periglacial environments. It is well known that in order for cells to survive desiccation or freezing they must alter the concentration of solutes in their cytoplasm (Potts, 1984; Conska, 1989; Panoff et al., 2000). In osmotically stressed conditions, adaptations for survival require increasing the abundance of osmoprotectant compatible solutes such as K+, amino acids, and oligomeric sugars in the cytoplasm to concentrations greater than
the surrounding solution in order to keep water inside the cell (Potts, 1984; Csonka, 1989). However, in soils which are subject to near total drying, osmotic stress becomes replaced with the stress of complete desiccation. Cells have a different mechanism to survive this “air drying,” which involves the production and concentration of polyhydroxyl sugars such as trehalose within the cytoplasm (Potts, 1984, Crowe et al., 1998). These molecules serve as water replacements, both maintaining the folding of proteins as well as converting the cytoplasm into a “glass-like” suspension (Potts, 1984; Crowe et al., 1998; see Browne et al., 2002, for a description of a protein in anhydrobiotic animals that stabilizes non-polyhydroxyl sucrose to achieve a similar bioglass).

Importantly, there is a property of oligosaccharide cryo/osmoprotectants (e.g. trehalose) that confers protection against both complete drying as well as freezing (Potts, 1984; Panoff et al., 2000; Crowe et al., 2002). This suggests that the only way for periglacial soil microorganisms to survive the frequent complete desiccation and freezing events of this environment (Chapter 4, Schmidt et al., 2009) is to maintain high concentrations of cryo/osmoprotectant sugars. Conversely, warm desert soil microorganisms may be able avoid excessively high C cost of this mechanism by also accumulating nitrogen based osmoprotectants, which would explain why the most extreme microbial biomass C:N ratio for a desert soil is still half as large as that of periglacial soils. In a similar manner, vegetated areas within the periglacial zone should be indicative of less frequent desiccation stress (Chapter 3) and also allow microorganisms from these soils use of nitrogen based cryoprotectants, which is agreement with their significantly lower microbial biomass C:N ratios. Thus, the unique physiological constraint imposed by the periglacial environment in unvegetated areas should result in a soil biomass stoichiometry of increased C requirement relative to vegetated systems and explain the
higher microbial biomass C:N ratio than predicted by the Cleveland and Liptzin’s (2007) Redfield-like model of soil. Moreover, the C limitation/demand suggested by the low dissolved C:N ratios is not paradoxical but expected if the microbial biomass C:N ratio is indeed indicative of an environmental constraint on cellular C:N ratios.

The fact that across unvegetated periglacial soils the pattern of high microbial C:N and low dissolved nutrient C:N is so widespread suggests that periglacial microorganisms maintain a high cytoplasmic concentration of cryo/osmoprotectants over long time periods. This is counter to the temporary physiological response observed in vegetated systems (Schimel et al., 2007) wherein after a rewetting or thaw event soil microorganisms have been observed to lose their stress resistance osmolytes into solution, resulting in a loss of up to 50% of a soil’s biomass (Schimel et al., 2007). With drying and freezing events occurring frequently in unvegetated periglacial soils (Ley et al., 2004; Chapter 4; Schmidt et al., 2009), maintaining such a leaky stress response would be prohibitively costly. Periglacial microorganisms must therefore either remain dormant during the summer or have evolved to retain their osmolytes. The functionality of cells with high osmolyte concentrations is supported by Potts (1984) who highlights the fact that intracellular enzymes may need as little as a monolayer of water covering their surface to be biologically active. Furthermore, evidence showing active metabolism and respiration in high alpine periglacial soils during the growing season (Ley et al., 2004; Freeman et al., 2009a; Schmidt et al., 2009) confirms that microorganisms are indeed active. A consistently high cytoplasmic concentration of oligosaccharides explains why such a high microbial biomass C:N ratio is widely observed in periglacial soils.

The unusually high stoichiometric C requirement for periglacial microorganisms is fundamentally different from the expectations of an all-encompassing Redfield-like model for
soil. Although the C:N ratio of aquatic microorganisms can vary in a similar fashion, aquatic 
microbial stoichiometry it is related almost exclusively to the nutrient conditions of the 
environment (Geider and La Roche, 2002). Soil systems present an added complexity to our 
ability to predict microbial stoichiometry, with both temperature and humidity exerting extreme 
pressure on the availability of life’s most essential compound, water. The results of this study 
suggest that although it is indeed realistic to infer demand from soil nutrient ratios, it is essential 
to also account for the effect of environmental conditions on the nutrient requirements for 
resident organisms. While most terrestrial ecosystems are vegetated and obey a strong Redfield-
like ratio (Cleveland and Liptzin, 2007), extreme environmental conditions such as those of 
periglacial regions result in an altered microbial stoichiometry. This stoichiometry is indicative 
not only of different nutrient demands, but also cellular elemental ratios that are not interpretable 
within the framework of the general Redfield-like trend for soil. In short, periglacial soil 
microorganisms have a unique Redfield-like ratio.

*Environmental Influences on Periglacial Soil Stoichiometry*

The hypothesized link between a periglacial-specific Redfield-like ratio and severe 
drying/freezing stress suggests that environmental conditions should be predictive of periglacial 
soil nutrient levels. While soil water content (SWC) is only significantly correlated with MBC, 
soil water holding capacity (WHC) is significantly correlated with DOC, TDN, and MBC. 
However, neither of these factors have particularly tight correlations with soil biogeochemistry at 
this global scale (Table 5.2), which is in contrast to the much stronger in individual site based 
studies (Figure 5.4; Chapters 2,3,4; Concienne, 2010). Thus, it is likely that soil water variability 
is most influential in determining absolute nutrient levels landscape scale, whereas the
stoichiometry of soil carbon and nitrogen for globally distributed periglacial areas suggests that exist above threshold for continuous maintenance of soil desiccation stress protection.

Surprisingly, the multivariate models for microbial biomass C and N had soil pH as their most important variable, with soil dissolved nutrients also contributing, and soil water content contributing only weakly. While dissolved nutrient pools are a known indicator of soil fertility and water holding capacity (Fierer et al., 2009; Cleveland and Liptzin, 2007), it was unexpected that microbial biomass decreased strongly with increasing soil pH (Figure 5.5) as low soil pH is usually linked with low microbial biomass across equivalent ecosystems (Wardle, 1992). This reverse trend for periglacial soil pH dependence in combination with the lack of strong within-site effects hints at the possibility it is not pH limiting microbial growth but possibly some other associated property (e.g. phosphorus availability as suggested in Chapter 2). The relationships between biogeochemical soil parameters and soil nutrients revealed by this study further support the hypothesis put forth in Chapter 2 that soil water plays a key role in structuring periglacial soil biogeochemistry, but that soil processes are best characterized when accounting for both climatic and geologic factors.

5.6 Conclusions

Periglacial soils represent an environment where standard models of soil stoichiometry are a poor fit. The disparate stoichiometry of microbial biomass and soil nutrients suggests that new theories are needed to explain the periglacial ecosystem. Because of the extreme harshness of the periglacial environment, microorganisms likely require high concentrations of drying/freezing protection oligosaccharides to survive. This requirement reflected both by the high C:N ratio of the microbial biomass and the low C:N ratio of the dissolved nutrient pool. Moreover, the unusual nutrient requirements of periglacial microorganisms suggest that these
areas operate under a stoichiometry of nutrient demand that is highly divergent from most vegetated soils. These features make periglacial areas a unique ecosystem type with great potential to study soil stoichiometry under conditions of very high microbial C demand.

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CHAPTER 6
CONCLUSIONS

My examination of periglacial ecosystems aims to provide a comprehensive description for the microbial ecology of a poorly understood environment that is extreme in climate and topography, highly heterogeneous in parent material, and strongly structured by time. The central thesis of this research was: sampling across multiple spatial scales and environmental gradients will identify the major biological and environmental factors shaping periglacial ecosystems. In order to integrate the wide range of topics addressed by this approach into and understanding of ecosystem-scale processes I employed Hans Jenny’s state factor schema (1941). Thus, I will summarize the novel results of my research as they relate to the climotfactors for soil formation and ecosystem definition.

Climate is a strong determinant of periglacial process and my research identifies multiple mechanisms through which climate influences periglacial ecology. While deep, late-melting snowpack creates patches of plant-free microbially dominated soil, it can depress the growth of even the microbial communities adapted to these conditions when snow accumulation is high enough (Chapter 2). In more exposed areas, however, it is not the overabundance of snowcover but the lack thereof that creates a separate, distinct niche within the periglacial zone. I found that the highly exposed soils at the upper reaches of the Green Lakes Valley contain an abundance of endemic Rhodospirillales bacteria and that this pattern is applicable to arid periglacial soils distributed across the globe (Chapter 3). These occurrence patterns suggest that climatic and microclimatic controls on water availability are the most important factors in determining the relative levels of microbial biomass regardless of differences in parent material (Chapter 2,4,5). As a whole, periglacial microorganisms are so strongly structured by the climatic stresses of desiccation and freezing that their cellular carbon and nitrogen stoichiometry is greatly divergent.
from that of vegetated systems (Chapter 5). Thus, I have identified both temperature and water availability as dominant factors driving periglacial soil ecology.

The microbially dominated biotic community of the periglacial zone is diverse, but displays striking coherence in its landscape and global distributions. Bacterial community composition displays coherent spatial structuring across the periglacial ecosystem of the Green Lakes Valley, with significant spatial autocorrelation to distances of 240m. Such strong spatial patterning is surprising given that relative to the size of a bacterium, traveling 240m is equivalent to a human flying six times around the globe. Although climate is a strong determinant of total microbial biomass levels (Chapters 2, 4, 5), the interaction between plant abundance, pH, snow depth, and the abundance of individual bacterial clades creates striking patterns at the landscape scale (Chapter 3). Thus, I was able to show that groups such as the Rhizobiales and Rhodospirillales display strongly predictable habitat distributions in relation to plant abundances across the Green Lakes Valley’s periglacial landscape (Chapter 3), while the Acidobacteria Group 4 and the Saprospirales have distributions that are most strongly shaped by soil pH. That these habitat distribution models based on landscape occurrence patterns scale globally (Chapter 3) supports the argument for the periglacial ecosystem as an environment distinct from other systems and that the microbially dominated biotic state factor is well constrained for periglacial ecosystems.

Topography is important in periglacial ecosystems because it interacts with climate to determine how snowpack accumulates as well as which areas receive subsidies from the melting of late-persisting snowbanks. As I discussed above, my landscape-scale study of microbial habitat distributions (Chapter 3) showed a strong relationship between the abundance of Rhodospirillales and the arid soils of exposed windblown locations. Conversely, I showed in
Chapter 2 that microbial biomass levels were inhibited in the most sheltered, high snow accumulation locations within the Green Lakes Valley. These patterns demonstrate that topography interacts with climate to structure soils in periglacial ecosystems.

The effect of parent material on periglacial ecology was often obscured in my study by interactions with other state factors. Although, climate appears to be the main determinant of the microbial biomass level across most sites, the low biomass levels for my comparatively wet shale-derived Denali and Mendenhall sites disobey the general trend as shaped by the high biomass of my tropical, shale-derived Andean site, moderate biomass in the intermediate Sagarmatha (Everest Area, Nepal) and Green Lakes Valley granitic sites, and the low biomass levels for my dry, shale derived Annapurna site and basalt derived Llullaillaco and Socompa sites (Figure 5.4, Chapter 5). On closer inspection, however, parent material appears to cause consistently lower microbial biomass in shale-derived soils regardless of climate (assuming that the Andean site is an outlier due to the divergent temperature dynamics of the tropic). Moreover, when comparisons of extracellular enzyme activities across sites are examined while controlling for microbial biomass differences (this also controls for climate indirectly) it is obvious that soil pH and, therefore, parent material exerts a strong influence on microbial activity (Appendix D). Similarly, my global scale analysis of the relative abundance measurements of bacterial clades from Chapter 3 should have been unaffected by divergence in microbial biomass levels between sites. As a result, I was able to demonstrate that clades which are strongly structured by environment (Chapter 3) have predictable variation in their abundances based on parent-material-dependent soil pH differences. The dependency of microbial activity and bacterial relative abundances on parent material chemistry indicate that it is a strong factor shaping periglacial ecosystems.
Time has been shown to be an important factor determining periglacial ecology in glacial chronosequence studies (Nemergut et al., 2007; Tscherko et al., 2003b; Sattin et al., 2009; Wardle et al., 2004). Implicit in these studies is the idea that landscape heterogeneity in climate, parent material, and topography exert minimal influence on biological succession. However, my work shows that the influence of landscape level variation in these factors is not insignificant and that they play a large role in structuring the environment even before plants are able to colonize periglacial soils (Chapters 2-5). One consequence of this landscape heterogeneity should be that the relative effect of other state factors over time will differ across even the initial stages of a chronosequence and argues for a more integrated approach to studying succession in periglacial biotic communities.

The research I have described herein validates my postulated thesis, demonstrating that sampling across multiple spatial scales and environmental gradients can identify the major biological and environmental factors shaping periglacial ecosystems. I have identified temperature and water availability as dominant controls on microbial biomass, with parent material also taking a significant but subsidiary role in controlling microbial biomass (Chapters 2,4). The relative abundance of periglacial soil microbiota is determined by multiple factors, and strongly by plant abundance, topographically regulated snow depth, and soil pH (Chapter 3). Finally, as a result of the extreme climatic pressures of freezing and desiccation, the stoichiometry for periglacial ecosystems across the globe requires significantly greater microbial demand for carbon than observed in vegetated systems (Chapter 5). These climatic, biotic, lithographic, and topographic factors govern the microbially dominated soil ecology of the periglacial zone.
COMPREHENSIVE BIBLIOGRAPHY


Concienne B, 2010. Microbial succession within a pro-glacial zone: The Middle Fork Toklat
Glacier, Denali National Park & Preserve, Alaska, USA. Master’s Thesis, University of
Colorado at Boulder, Boulder, Colorado.
Rev* 53:121-147.
Costello EK, Halloy SRP, Reed SC, Sowell P, Schmidt SK, 2009. Fumarole-supported islands of
biodiversity within a hyperarid, high-elevation landscape on Socompa Volcano, Puna de
Physiol* 60:73-103.
Crowe JH, Oliver AE, Tablin F, 2002. Is there a single biochemical adaptation to anhydrobiosis?
Cutler NA, 2010. Long-term primary succession: a comparison of non-spatial and spatially
del Valle HF, Elissalde NO, Gagliardi DA, Milovich J, 1998. Status of desertification in the
Patagonian region: Assessment and mapping from satellite imagery. *Arid Soil Res Rehab
12:95-121.
DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P,
Andersen GL, 2006a. Greengenes, a chimera-checked 16S rRNA gene database and
DeSantis TZ, Hugenholtz P, Keller K, Brodie EL, Larsen N, Piceno YM, Phan R, Andersen GL,
2006b. NAST: a multiple sequence alignment server for comparative analysis of 16S
Doran PT, Priscu JC, Lyons WB, Walsh JE, Founain AG, McKnight DM, Moorhead DL,
Virginia RA, Wall DH, Clow GD, Fritsen CH, McKay CP, Parsons, AN, 2002. Antarctic
Dyhrenfurth, GO, 1955. To the third pole: the history of the high Himalayas. London, UK:
Werner Laurie.
distribution of snow in rugged mountain terrain, Colorado, United States. *Water Resour
Falkowski PG, Fenchel T, Delong EF, 2008. The microbial engines that drive Earth’s
Fierer N, Hamady M, Lauber CL, Knight R, 2008a. The influence of sex, handedness, and


Sattin SR, Cleveland CC, Hood E, Reed SC, King AJ, Schmidt SK, Robeson MS, Ascarrunz N, Nemergut DR, 2009. Functional shifts in unvegetated, perhumid, recently deglaciated


APPENDIX A

MAJOR OXIDE COMPOSITION OF PARENT MATERIAL FROM GLOBALLY DISTRIBUTED PERIGLACIAL AREAS

At 11 representative locations in Green Lakes Valley, Colorado, a softball-sized rock was collected (A). At 5 locations in ACA, 2 locations in SNP and 1 location at Socompa, racquetball-sized rocks were collected (B). Rocks were analyzed for major oxide composition at the University of Colorado’s LEGS Laboratory. For NWT5, 6, 11, ACA-ZunTal, and ACA-ThrongLa 1, measurements were adjusted to sum to 100% by assuming missing percentages were SiO$_2$ lost in the digestion preparation step.
APPENDIX B

SIGNIFICANCE OF GREEN LAKES VALLEY’S BACTERIAL CLADE SPATAIL DISTRIBUTIONS AND CORRELATIONS WITH SOIL BIOGEOCHEMICAL FACTORS

P-values (*=p<0.002 Šidák correction) for the spatial autocorrelation in the relative sequence abundance (Moran’s I test) and Pearson r correlation values (*=p<0.002 Šidák correction, correlation test,) are given for all 30 clades identified in Chapter 2. The maximum distance of spatial autocorrelation for the most is given for each group and each biogeochemical parameter. Clades with a 0/2000 autocorrelation distance indicate a slight change in relative abundance across the entire landscape with a high amount of background variability in relative abundance. † indicates a parameter that was used in the habitat distribution model for a specific clade. Ф indicates a parameter that was excluded from the global habitat distribution model due to lack of data. Pearson r^2 are also give for each clade for the Colorado habitat model and the global model.
<table>
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<th>Clade</th>
<th>Relative Abundance (p)</th>
<th>Moran's I</th>
<th>Autocorrelation Distance</th>
<th>N-acetylglucosaminase</th>
<th>Cellobiosidase</th>
<th>α-Glucosidase</th>
<th>β-Glucosidase</th>
<th>α-Glucosidase</th>
<th>β-Glucosidase</th>
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APPENDIX C

CORRELATION MATRIX FOR GREEN LAKES VALLEY BIOGEOCHEMICAL FACTORS

Pearson r correlation values across the biogeochemical variables measured in Chapter 2. * Indicates significance at p<0.01.

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| | | | | | | | | | | | | | | | | | | |
| | 0.063 | 0.808* | 0.736* | 0.734* | 0.746* | -0.115 | 0.058 | 0.431* | 0.293* | -0.081 | | | | | | | |
| Cellobiosidase | 0.141 | 0.788* | 0.835* | 0.776* | 0.831* | -0.069 | 0.066 | 0.466* | 0.349* | -0.087 | | | | | | | |
| α-Glucosidase | 0.176* | 0.742* | 0.813* | 0.754* | 0.803* | -0.054 | 0.064 | 0.443* | 0.357* | -0.079 | | | | | | | |
| β-Glucosidase | 0.203* | 0.792* | 0.819* | 0.825* | 0.842* | -0.018 | -0.08 | 0.477* | 0.354* | -0.059 | | | | | | | |
| β-Xylase | 0.160* | 0.760* | 0.799* | 0.832* | 0.842* | -0.026 | 0.049 | 0.468* | 0.316* | -0.067 | | | | | | | |
| Phosphatase | 0.161* | 0.747* | 0.811* | 0.799* | 0.824* | 0.026 | 0.072 | 0.507* | 0.416* | -0.076 | | | | | | | |
| Soil Water | -0.034 | 0.571* | 0.546* | 0.475* | 0.519* | -0.083 | 0.052 | 0.355* | 0.319* | 0.006 | | | | | | | |
| Water Holding Capacity | 0.206* | 0.624* | 0.610* | 0.640* | 0.624* | 0.054 | 0.106 | 0.443* | 0.318* | -0.071 | | | | | | | |
| Total Plants | 0.794* | 0.316* | 0.413* | 0.417* | 0.390* | 0.068 | 0.058 | 0.134 | 0.036 | -0.16 | | | | | | | |
| Forb Abundance | 0.490* | 0.379* | 0.440* | 0.476* | 0.435* | 0.052 | -0.05 | 0.217* | 0.131 | 0.171* | | | | | | | |
| Monocot Abundance | 0.116 | 0.234* | 0.186* | 0.191* | 0.071 | 0.052 | -0.04 |-0.133 | -0.097 | | | | | | | | |
| Total Dissolved N | 0.116 | 0.577* | 0.882* | 0.669* | 0.292* | -0.05 | 0.618* | 0.482* | -0.093 | | | | | | | | |
| Microbial N | 0.234* | 0.577* | 0.660* | 0.837* | 0.071 | 0.106 | 0.325* | 0.271* | -0.038 | | | | | | | | |
| Dissolved Organic N | 0.186* | 0.882* | 0.660* | 0.709* | 0.022 | 0.077 | 0.633* | 0.503* | -0.08 | | | | | | | | |
| Microbial Biomass C | 0.191* | 0.669* | 0.837* | 0.709* | -0.057 | 0.05 | 0.447* | 0.326* | -0.109 | | | | | | | | |
| Dissolved C:N | 0.071 | 0.292* | 0.071 | 0.022 | -0.057 | 0.093 | -0.13 | -0.133 | -0.051 | | | | | | | | |
| Microbial Biomass C:N | -0.052 | -0.05 | -0.106 | -0.077 | 0.05 | 0.093 | -0.048 | -0.066 | -0.002 | | | | | | | | |
| Total Dissolved P | -0.04 | 0.618* | 0.325* | 0.633* | 0.447* | -0.13 | 0.048 | 0.878* | -0.121 | | | | | | | | |
| Dissolved Inorganic P | -0.133 | 0.482* | 0.271* | 0.503* | 0.326* | -0.133 | 0.066 | 0.878* | -0.061 | | | | | | | | |
| Microbial Biomass P | -0.097 | -0.093 | -0.038 | -0.08 | -0.109 | -0.051 | 0.002 | -0.121 | -0.061 | | | | | | | | |
| Sand | -0.05 | -0.169 | 0.269* | 0.203* | 0.270* | 0.194* | 0.107 | 0.054 | 0.017 | -0.009 | | | | | | | |
| Clay | 0.013 | 0.224* | 0.264* | 0.192* | 0.224* | 0.03 | 0.067 | -0.135 | -0.038 | 0.012 | | | | | | | |
| Silt | 0.007 | -0.122 | -0.151 | -0.062 | -0.107 | 0.163* | 0.113 | -0.108 | -0.068 | 0.05 | | | | | | | |
| Snow Depth | 0.356* | -0.024 | 0.175* | 0.159* | -0.018 | -0.129 | 0.11 | 0.085 | 0.061 | 0.234* | | | | | | | |
| Soil pH | 0.295* | 0.018 | 0.233* | 0.118 | 0.164* | 0.296* | -0.08 | 0.171* | 0.259* | 0.157 | | | | | | | |
| Altitude | 0.233* | -0.143 | -0.145 | 0.206* | 0.290* | -0.096 | 0.022 | -0.13 | 0.008 | 0.119 | | | | | | | |
| Slope | 0.081 | -0.051 | -0.016 | -0.029 | -0.012 | 0.158* | 0.053 | 0.055 | 0.067 | -0.085 | | | | | | | |
| Morr-an's I (p) | 0.000 | 0.467 | 0.928 | 0.001 | 0.024 | 0.688 | 0.223 | 0.889 | 0.116 | 0.382 | | | | | | | |</p>
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| glucosaminase   | 0.273*   | 0.264*   | -0.124   | -0.001     | 0.08    | -0.098   | 0.013 |
| Cellobiosidase   | 0.280*   | 0.266*   | -0.138   | -0.059     | 0.099   | -0.147   | -0.027 |
| α-Glucosidase    | 0.281*   | 0.220*   | -0.106   | -0.091     | 0.1     | -0.137   | -0.007 |
| β-Glucosidase    | 0.319*   | 0.267*   | -0.104   | -0.126     | 0.179*  | 0.179*   | -0.011 |
| β-Xylase         | 0.208*   | 0.259*   | -0.147   | -0.133     | 0.157*  | 0.193*   | -0.01  |
| Phosphatase      | 0.353*   | 0.262*   | -0.102   | -0.114     | 0.101   | -0.145   | -0.01  |
| Soil Water       | -0.084   | 0.312*   | 0.224*   | 0.002      | 0.022   | 0.116    | -0.088 |
| Water Holding Capacity | -0.182   | -0.085   | 0.007    | -0.105     | -0.011  | 0.187*   | 0.026  |
| Total Plants     | -0.116   | -0.003   | 0.041    | 0.368*     | 0.283*  | 0.298*   | 0.086  |
| Forb Abundance    | -0.139   | -0.013   | 0.054    | 0.296*     | 0.215*  | 0.276*   | 0.071  |
| Moncot Abundance  | -0.05    | 0.013    | 0.007    | 0.356*     | 0.295*  | 0.233*   | 0.081  |
| Total Dissolved N| -0.169   | 0.224*   | -0.122   | -0.024     | 0.018   | -0.143   | -0.051 |
| Microbial N      | 0.269*   | 0.264*   | -0.151   | 0.175*     | 0.233*  | -0.145   | -0.016 |
| Dissolved Organic N | 0.203*   | 0.192*   | -0.062   | 0.159*     | 0.118   | 0.206*   | -0.029 |
| Microbial Biomass C | 0.270*   | 0.224*   | -0.107   | -0.018     | 0.164*  | 0.290*   | -0.012 |
| Dissolved C:N    | 0.194*   | 0.03     | 0.163*   | -0.129     | 0.296*  | -0.096   | 0.158* |
| Microbial Biomass C:N | -0.107   | 0.067    | 0.113    | 0.11       | -0.08   | -0.022   | 0.053  |
| Total Dissolved P| 0.054    | -0.135   | -0.108   | 0.085      | 0.171*  | -0.13    | 0.055  |
| Dissolved Inorganic P | 0.017    | -0.038   | -0.068   | 0.061      | 0.259*  | 0.008    | 0.067  |
| Microbial Biomass P | -0.009   | 0.012    | 0.05     | 0.234*     | 0.157*  | 0.119    | -0.085 |
| Sand             | 0.739*   | 0.965*   | 0.054    | -0.098     | 0.210*  | 0.087    |       |
| Clay             | 0.739*   | 0.802*   | -0.011   | -0.069     | -0.091  | -0.026   |       |
| Silt             | 0.965*   | 0.802*   | -0.069   | 0.095      | 0.218*  | -0.085   |       |
| Snow Depth       | 0.054    | -0.011   | -0.069   | 0.372*     | 0.157*  | 0.135    |       |
| Soil pH          | -0.098   | -0.069   | 0.095    | 0.372*     | 0.190*  | 0.009    |       |
| Altitude         | 0.210*   | -0.091   | 0.218*   | 0.157*     | 0.190*  | -0.013   |       |
| Slope            | 0.087    | -0.026   | -0.085   | 0.135      | -0.009  | -0.013   |       |
| Morran's I (p)   | 0.560    | 0.876    | 0.088    | 0.000      | 0.000   | 0.000    | 0.810 |
Extracellular enzyme activities for globally distributed periglacial soils show a strong dependency on pH (activity rates normalized by soil microbial biomass C). Sinsabaugh et al. (2008) explain that different microorganisms can produce either acid or alkaline adapted phosphatases, which is the cause for the lack of pH dependence for phosphatase activity.