THESIS

THE EFFECTS OF TEMPERATURE AND MOISTURE ON ALPINE MICROBIAL

PROCESSES ACROSS A GRADIENT OF SOIL DEVELOPMENT

Submitted by

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ABSTRACT

THE EFFECTS OF TEMPERATURE AND MOISTURE ON ALPINE MICROBIAL

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Alpine ecosystems are being transformed by global change. Climate change and

atmospheric nitrogen deposition are exposing soils to novel temperature regimes,

melting alpine glaciers, altering precipitation patterns, and directly introducing

bioavailable nutrients. Because microbial communities are important drivers of nutrient

cycling and ecosystem function in the alpine, and because temperature, moisture and

nutrient availability are primary controls of microbial abundance and activity, it is likely

that microbial linkages exist between global change and ecosystem-level consequences

of global change in alpine regions. Deglaciation in high-elevation regions incrementally

exposes soils to primary succession, which creates a wide range of soil environments.

Yet, little is understood about these unusual environments' respective microbial

communities or how they respond to the influence of global change.

This research studied the effects of changing temperature and moisture controls

on microbial carbon and nitrate (NO_3^-) processing in a range of alpine soils. The soils

were collected from a watershed that exhibits characteristics of nitrogen saturation as a

result of atmospheric nitrogen deposition. Glacial outwash, talus, and meadow soils

were characterized by physical, chemical and biological properties. Soil temperature

regimes were highly variable in the field, with some soils experiencing great diurnal

fluctuations, while others remained consistently cold. The response of microbial

community size, structure, activity and behavior to warming and changing soil moisture was addressed with laboratory incubations.

Microbial community size and nutrient availability increased with increasing soil organic carbon. Microbial activity in all soils increased with temperature and moisture, as evidenced by total and microbial biomass-specific rates of respiration. However changes in microbial biomass carbon and parameters of community structure and behavior differed among the soils. This indicated that the soils responded using individual mechanisms to changing microclimate conditions during the incubations. The net production of NO_3^- occurred in all soils under all experimental conditions, however the rate at which NO_3^- was produced responded differently to temperature and moisture treatments. This suggests that global change may affect biological controls of NO_3^- availability in the alpine.

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1. INTRODUCTION

Human-caused climate change and atmospheric nitrogen deposition are transforming many high-elevation ecosystems (IPCC 2007; Baron et al. 2009; Hobbs et al. 2010). Record high annual temperatures have been observed in alpine regions of the western US and European Alps since the year 2000 (Diaz & Eischeid 2007; Cannone et al. 2008). In addition to altering temperature regimes, climate warming is shrinking glaciers at an unprecedented rate and modifying alpine precipitation patterns worldwide (Paul 2004; IPCC 2007). Industrial and agricultural anthropogenic activities have artificially increased the emissions of reactive nitrogen into the atmosphere (Galloway et al. 2004). As a result, many alpine regions in North America have received chronic nitrate (NO_3^-) and ammonium (NH_4^+) pollution of growing intensity since ~1950 (Wolfe et al. 2001; Hobbs et al. 2010). Warming trends in temperature are changing hydrological processes and directly introducing bioavailable nutrients from ablating glaciers to high-elevation regions. Together, climate change and atmospheric nitrogen deposition are modifying important controls of ecosystem function.

The alpine is an extreme and nutrient-poor environment, which makes it especially sensitive to the effects of climate change and atmospheric nitrogen deposition (Williams et al. 1998). The depth and duration of seasonal snowpack are dominant controls of alpine ecosystem hydrological cycling, but susceptible to change

under climate warming conditions (Brooks & Williams 1999; Campbell et al. 2000). High-elevation regions also demonstrate acute sensitivity to increases in nutrients due to their natural state of oligotrophy. Previously nitrogen limited alpine systems of the US and Europe have shifted to states of nitrogen saturation as a result of atmospheric deposition since the post-WWII increase in human population and synthetically-available fertilizers (Williams et al. 1996; Rogora & Mosello 2007). We cannot yet predict how alpine ecosystem function will reflect the interactive consequences of climate change and atmospheric nitrogen deposition.

In recent decades, increasing trends and high inter-annual variability of alpine stream NO_3^- concentrations have been observed in the US and Canadian Rocky Mountains as well as the Swiss and Italian Alps (Williams et al. 1997; Watmough et al. 2004). Historical data from the Loch Vale watershed in Rocky Mountain National Park, CO, revealed that annual stream NO_3^- concentration trends increased >50% from the 1990s to 2006 commensurate with warmer summer temperatures, but not with changes in atmospheric deposition patterns (http://nadp.sws.uiuc.edu/sites/siteinfo.asp?id=CO98&net=NTN, Baron et al. 2009). The coincidence of changes in stream NO_3^- with temperature suggests that global change may be affecting alpine water chemistry.

Elevated NO_3^- concentrations in terrestrial and aquatic systems influence alpine ecosystem health and services. Nitrate is a soluble form of reactive nitrogen. Its availability is often limiting for the growth of plants and microbes. Nitrate enrichment in oligotrophic alpine waters can result in increased primary productivity and altered aquatic biodiversity. It may also result in eutrophication (Campbell et al. 2000; Sickman

et al. 2003; Wolfe et al. 2003). Water quality is important not only for ecosystem health, but because alpine catchments serve as fresh water reservoirs for many metropolitan areas around the world (Barnett et al. 2005). Nitrogen enrichment also threatens the integrity of national park and wilderness areas managed as protected natural resources.

Elevated stream NO_3^- and its potential consequences for alpine ecosystems have motivated scientists to explore the mechanisms controlling high-elevation NO_3^- concentrations and how they are influenced by global change. It is possible that high stream NO_3^- concentrations in the alpine are the direct result of atmospheric inputs. However, patterns in atmospheric nitrogen deposition do not necessarily align with changes in stream chemistry (Baron et al. 2009).

Glacier melt has also been proposed as the primary source of increased stream NO_3^- (Saros et al. 2010). Depositional NO_3^- can become concentrated in glacial ice due to evaporation. Increasing temperatures accelerate deglaciation, which can release NO_3^- from the ice, or expose old soil organic matter with nitrogen. The release of glacier-entrained NO_3^- and thaw of old soil organic matter may contribute to the stream chemistry trends observed commensurate with climate warming.

Changes in temperature, moisture, and nutrient availability are important controls of microbial growth and activity, which suggests that global change may also be linked to stream NO_3^- concentrations via shifts in microbial nutrient processing. Nitrification, a two-step microbial pathway in which NH_4^+ is oxidized to NO_3^-, is known to be an important nitrogen cycling process that directly influences NO_3^- availability (Amberger & Schmidt 1987). Nitrification rates are a function of temperature, moisture conditions and NH_4^+ availability. Ammonium availability is the primary process control in many soil

environments (Schlesinger 1997). Alpine catchments receiving atmospheric NH_4^+ deposition are likely to have relatively high nitrification potential. Increasing summer temperatures coupled with abundant atmospheric nitrogen deposition may stimulate microbial nitrification, and this, too, may contribute to increases in alpine NO_3^- concentrations.

These observations motivated the following study, in which the potential effects of global change on the dynamics of alpine microbial communities and activity, including carbon and NO_3^- processing, were investigated. Carbon processing was studied in order to learn more about fundamental microbial dynamics in the alpine. I asked how temperature and moisture conditions controlled microbial biomass growth and respiration rates, as well as how these qualities varied between diverse alpine soil environments. Nitrate processing was studied to determine the potential for microbial nitrification to contribute to changing stream NO_3^- concentration trends commensurate with warming temperatures. I asked what the important controls were of net and gross rates of nitrification, as well as whether or not nitrifier abundance was a significant predictor of nitrification rates.

Because alpine ecosystems are extreme environments some have previously hypothesized that alpine soils were largely abiotic and devoid of significant microbially-mediated biogeochemical cycling (Williams & Melack 1991). However microbial processes are now regarded as critical drivers of alpine nutrient cycling and ecosystem dynamics (Lipson et al. 1999; Brankatschk et al. 2011). A more complete understanding of microbial dynamics in remote high-elevation ecosystems will inform our ability to forecast and mitigate consequences of anthropogenic drives of change in the alpine.

2. THE EFFECTS OF TEMPERATURE AND MOISTURE ON MICROBIAL CARBON PROCESSING ACROSS A GRADIENT OF SOIL DEVELOPMENT IN AN ALPINE ECOSYSTEM

2.1 INTRODUCTION

Accelerating rates of glacier melt have been observed in alpine regions worldwide since the mid-1980s (Paul 2004). Deglaciation incrementally exposes sediments to primary succession and creates gradients of soil development that range from barren newly-exposed terrain to older densely-vegetated meadows and forests.

At all stages of development, alpine soils are extreme environments characterized by exposure to high winds and solar radiation, cold temperatures and a short growing season (Ley et al. 2004). Because of these harsh conditions it was hypothesized by some that alpine soils were largely abiotic and devoid of significant microbially-mediated biogeochemical cycling (Williams & Melack 1991). However microbial processes are now recognized as critical drivers of alpine nutrient cycling and ecosystem dynamics (Lipson et al. 1999; Brankatschk et al. 2011).

The importance of microorganisms as drivers of ecosystem function extends to newly-deglaciated sediment. The earliest stages of soil formation in the alpine are facilitated by microbial activity (Kohls et al. 1994; Nemergut et al. 2007; Schmidt et al.

2008). Microbial communities quickly colonize newly-exposed soils (Tscherko 2003). Microbial carbon and nitrogen fixation and biogeochemical processing supply nutrients to these recently-deglaciated sediments and promote their development.

Because of the harsh environmental conditions, these "young" soils may remain unvegetated for many years, and therefore do not receive significant inputs of organic matter from plant litter or root exudates, unless plant matter is blown in from adjacent alpine tundra (Sigler & Zeyer 2004).

Despite their critical role in soil development and ecosystem function, little is understood about what controls the relative size, structure, activity or behavior of alpine microbial communities among variable alpine soil environments (Ley et al. 2004; King et al. 2008; Schmidt et al. 2008). The chemical, physical and biological properties of deglaciated alpine soils vary widely (Ohtonen et al. 1999; Ley et al. 2004). Some studies have identified similar processes controlling microbial communities across levels of succession, while others have found that fundamentally different dynamics are operating (Ohtonen et al. 1999; Schmidt et al. 2008).

Alpine catchments that are currently deglaciating afford a unique opportunity to study the maturation of microbial communities, Microbial community physiology reflects the evolutionary history of a community as a result of the environmental characteristics under which it became established. However, the first communities to colonize truly "fresh" or recently-exposed soils do not demonstrate these historical effects; instead their physiology should reflect current conditions more accurately than other communities (Paul & Clark 1996).

We studied alpine soils along a developmental gradient in order to compare how their respective microbial communities were controlled by changing temperature and moisture conditions. Three alpine soils from the Loch Vale watershed in Rocky Mountain National Park, CO were characterized for field microclimate conditions, pH, texture, nutrient pool sizes and microbial biomass concentrations. Soils were incubated under a range of temperature and soil moisture conditions, after which we measured rates of net nitrogen mineralization, changes in microbial biomass, the relative abundance of bacteria and archaea, and rates of respiration. We tested these hypotheses:

(1) Microbial abundance and activity increase from outwash to talus to meadow

(2) Microbial abundance and activity show a positive response to increasing temperature and moisture availability.

(3) The physical environment across soil types is similar enough that microbial community size, structure and activity will respond in similar fashion to temperature and soil moisture treatments.

2.2 METHODS AND MATERIALS

2.2.1 Site Description

The soils used in this study were sampled from the alpine/subalpine Loch Vale watershed in Rocky Mountain National Park, CO ($40^o17'17''$; $105^o39'43''$) (Fig. 1). Glaciers in Loch Vale have been retreating since the little Ice Age, and ablation rates may have recently increased in response to warming (Diaz & Eischeid 2007).

Continuous ecological research and monitoring of Loch Vale began in 1981 (Baron

1992). The climate is high-mountain continental with a mean annual temperature of

1.4^oC and with 103.4 cm of mean annual precipitation

(http://www.nrel.colostate.edu/projects/lvws/data.html).

Loch Vale receives direct inputs of NO_3^- and NH_4^+ via atmospheric nitrogen

deposition and is considered a nitrogen-saturated catchment based on high NO_3^-

concentrations in surface water and increased nitrogen losses from the watershed

(Baron et al. 2000; Burns 2004). The Loch Vale Watershed National Atmospheric

Deposition Program (NADP) site (CO98) has recorded average rates of total wet annual

nitrogen deposition of >7.5 kg N/ha yr^{-1} since 2000

(http://nadp.sws.uiuc.edu/sites/siteinfo.asp?id=CO98).
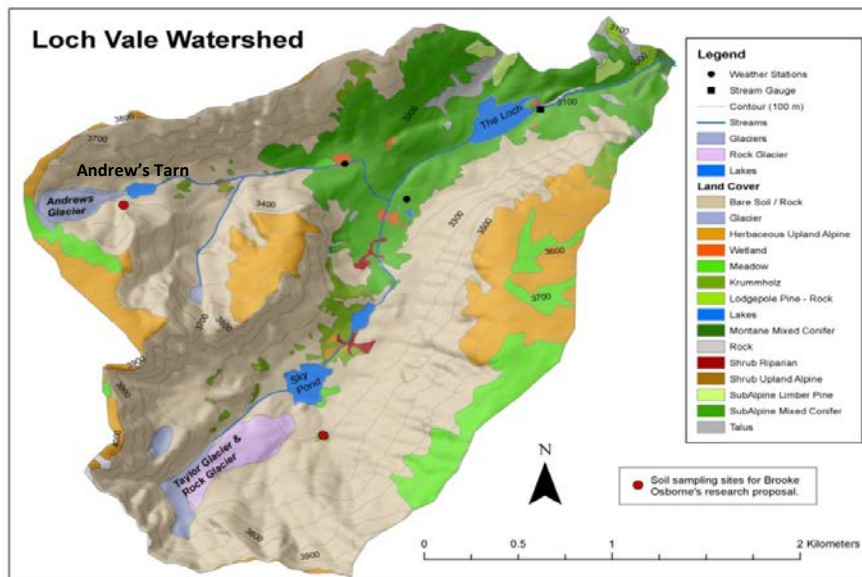
Figure 1, Annotated map of the Loch Vale watershed, Rocky Mountain National Park, CO, USA

2.2.2 Soil collection sites

Soils were collected above the watershed's two high-elevation headwater lakes: Andrews Tarn and Sky Pond (Fig. 1). Collection sites represented soils at different levels of development, from newly-exposed sediments to vegetated meadow soils common to alpine watersheds. The least-developed soil we sampled was outwash sediment below the shallow surface of Andrew's Tarn, which is located at the base of Andrew's Glacier. The intermediate soil was collected from pockets in a sparsely-vegetated talus field west of Andrew's Tarn and Andrew's Glacier. Densely-vegetated meadow soil was collected from a sedge meadow to the southeast of Sky Pond. These soils are identified as "outwash," "talus" and "meadow" throughout the study.

2.2.3 Soil Collection and Characterization

Field temperature and moisture conditions in the outwash, talus and meadow were measured to characterize each soil and design appropriate treatment conditions for laboratory incubation experiments. Soil temperature was recorded in the talus field and meadow every 30 minutes from July 14, 2010 to August 10, 2010 by two Thermochron iButton™ temperature sensors implanted in each soil. Outwash temperature data was not collected because outwash sediment was inaccessible when the iButtons were installed.

Five samples of each soil were collected on August 10, 2010 using trowels, covered with plastic bags. Samples were stored on ice in double quart-sized plastic bags during their transport to the Natural Resource Ecology Laboratory at Colorado State University. Upon arrival, the contents of the five quart-sized bags from each

sample site were pooled, sieved to 4 mm, homogenized by hand and stored at -80°C until subsequent analyses.

Water-holding capacity (WHC) and gravimetric water content (GWC) were measured at the time of collection. The subsamples used to evaluate WHC and GWC were never frozen in the laboratory. To determine WHC three 5g subsamples were placed into individual plastic funnels lined with Whatman no. 41 filter papers. Each sample was then submerged in water. The experiment was contained in a cooler that was sealed to prevent evaporation. The funnels containing each saturated sample were elevated, allowing the soils to drain overnight through the filters. Water-holding capacity was calculated as the volume of water retained in each sample divided by the total weight of the saturated soil. The GWC of each soil was quantified by subtracting the oven-dry weight (105°C) of subsamples from field wet weight. Field % WHC was calculated for each soil by dividing field water content (soil wet weight minus dry weight) by WHC, expressed as percentage.

Parameters measured in each sieved soil included pH, C:N, and dissolved inorganic nitrogen (DIN). A VWR Model 8000 pH meter was used to determine pH in 1:1 soil/deionized water solutions. Carbon and nitrogen content were quantified using a LECO Tru-SPEC elemental analyzer (Leco Corp., St. Joseph, MI) in soil subsamples that were dried at 105°C for 24 hours and ground on a ball mill. The DIN concentrations, estimated as the sum of NH_4^+-N and NO_3^--N concentrations, were quantified in 1:5 grams of dry soil/mL 2M potassium chloride (KCl) extracts. After shaking for one hour on an orbital shaker, the extracts were gravity filtered through Whatman no. 41 filter

papers and analyzed on an Alpkem Flow Solution IV Automated wet chemistry system (O.I. Analytical, College Station, TX).

Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) as well as soil microbial biomass carbon and nitrogen (MBC and MBN, respectively) concentrations were determined using a chloroform slurry-extraction technique (Fierer et al. 2003). Each soil sample was divided into two 10 g subsamples, and each subsample was extracted in a mason jar with 0.5 M K_2SO_4. One subsample from each set was also treated with 0.5 mL of chloroform at the same time that K_2SO_4 was introduced. The slurries were shaken for 4 hours on an orbital shaker and then vacuum-filtered through glass fiber filters. The extracts were analyzed for carbon and nitrogen using a continuous flow autoanalyzer (Schimadzu, Columbia, TN). Flushed MBC and MBN were calculated as the difference between carbon and nitrogen concentrations detected in control samples and the chloroform-treated samples. The carbon and nitrogen concentrations measured in the non-chloroform treated control samples were interpreted as soil DOC and DON.

2.2.4 Laboratory incubation and rates of respiration

Outwash, talus and meadow subsamples of ~35 g were incubated at three different temperatures and with two moisture treatments in a full factorial design for 45 days. Soil respiration and changes in microbial biomass as well as relative bacterial and archaeal abundance were monitored during the incubation.

The selected temperature (4^oC, 12^oC and 25^oC) and moisture treatments were based on field observations and long-term Loch Vale Watershed climate data

(http://www.nrel.colostate.edu/projects/lvws/data.html). Treatments were selected to simulate microclimates similar to those each soil experienced in the field, as well as reasonable deviations in temperature and moisture. Moisture treatments were substrate-specific, and ranged from 25% to 100% WHC (Fig. 2). Subsamples of outwash, talus and meadow were prepared for incubation by being air-dried to 20% WHC and re-wetted to the appropriate moisture levels. Wetted substrates were transferred in triplicate to 0.24 L Mason jars equipped with septa.

% Water Holding Capacity				
	25	50	75	100
2.5	TM	TO	M	O
10	TM	TO	M	O
25	TM	TO	M	O

Figure 2, Incubation temperature and moisture treatments

A 7-day pre-incubation was carried out before the start of the long-term incubation in which soil CO_2 concentrations in the headspace of the each Mason jar were measured daily to confirm that microbial activity had stabilized. Respiration was also measured throughout the 45-day incubation. This was achieved using an infrared gas analyzer (IRGA). When CO_2 levels approached 3% jars were flushed with breathing air for 15 minutes. Rates of MBC-specific respiration were calculated by dividing total respiration rates by the concentrations of MBC quantified in each sample. This form of

standardized respiration informs us about the relative activity of the microbial community present as well as how efficiently carbon is being used by that community.

2.2.5 Rates of net nitrogen mineralization

Net rates of nitrogen mineralization integrate several processes that affect the soil DIN pool, including mineralization, nitrification, denitrification, and nitrogen immobilization. Net nitrogen mineralization was calculated by subtracting the KCl-extractable NO_3^- and NH_4^+ present in untreated, homogenized outwash, talus and meadow samples from the NO_3^- and NH_4^+ measured in all treated soils at the completion of the long-term incubation.

2.2.6 DNA extraction & quantification

Shifts in the relative abundance of bacteria and archaea were assessed by comparing ratios of bacterial and archaeal 16S gene abundance at the start of the incubation (immediately following the 7-day pre-incubation) and at the end of 45 days. The bacterial and archaeal 16S genes are universal, and therefore inform us about the total abundance of bacteria and archaea in each soil.

At both time points, total genomic DNA was isolated from 0.25 g soil subsamples using a PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) according to the manufacturer's protocol. Extracts were quantified or DNA concentrations on a multifunction Tecan Infinite M200 plate reader using a Quant-IT dsDNA High-Sensitivity Assay Kit (Invitrogen) using the instructions provided. DNA extracts were stored at -20°C for approximately one month prior to analysis. This yielded results expressed as ng DNA/µL extract.

2.2.7 Quantitative PCR assays

Bacterial and archaeal 16S gene abundance was quantified in the DNA extracts using quantitative real-time PCR (qPCR) performed on a MyIQ Single-Color Real-Time PCR Detection System (Bio-Rad). All reactions were loaded into clear 96-well plates in triplicate to confirm reproducibility of the method. Every plate contained unknowns, a seven-point standard curve, and a non-template control. Reactions totaled 25 µL and contained 12.5 µL of ABsolute SYBR Green QPCR mix (Thermo Scientific) and 0.25 µL of bovine serum albumin. Standard curves were comprised of serially diluted (10^1-10^7 copies/µL) cloned amplicons (30ng/µL stock solution) for each gene of interest. Bacterial 16S genes were quantified in 1ng of DNA extract using 1.25 µL of the forward and reverse primers EUB338 (5'-ACTCCTACGGGAGGCAGCAG-3') and Eub518 (5'-ATTACCGCGGCTGCTGG-3') (Fierer et al. 2005). Cycling conditions for bacterial 16S were 15 min at 95^oC, 35 cycles of 94^oC for 15 s, 30 s at 53^oC, and 30 s at 72^oC. Archaeal 16S was quantified using 10ng of DNA extract and 0.75µL of Arch967f (5'-AATTGGCGGGGGAGCAC-3') and ARCH1060r (5'-GGCCATGCACCWCCTCTC-3') (Cadillo-Quiroz et al. 2006). Temperature cycles included 15 min at 95^oC, 35 cycles of 94^oC for 15 s, 30 s at 56^oC, and 30 s at 72^oC. All primers were concentrated at 10µM. Every qPCR run was concluded with a melting curve to assess the occurrence of primer dimer. Amplification efficiencies ranged from 94 - 99% and R^2 values of all qPCR runs were >0.9.

2.2.8 Statistical Analysis

The effects of temperature and moisture on microbial biomass and rates of respiration were analyzed using multiple regressions. Temperature and moisture were treated as categorical variables. All residuals were power transformed to achieve normality using the Box-Cox method. Significance was accepted at a level of probability (P) of <0.05. Statistical analyses were performed with SAS JMP 9.0 (SAS Institute Inc., Cary, North Carolina).

2.3 RESULTS

2.3.1 Soil parameters

Soil temperature data recorded the month prior to sample collection showed that talus soil experienced higher temperatures and greater diurnal temperature variability than meadow soil (Fig. 3). In talus, soil temperature averaged 11.8^{o}C but ranged from a low of 4.9^{o}C to 25.8^{o}C. Meadow soils fluctuated from 1.4^{o}C to 8°C and averaged 5.5^{o}C. Outwash temperatures were not logged. However, because outwash soil was submerged in Andrew's Tarn at the base of Andrew's Glacier we assumed that it fluctuated little from 1°C. Unpublished iButton data collected from Sky Pond outwash sediment during the 2011 growing season supported this assumption (E. Hall, pers. commun.).
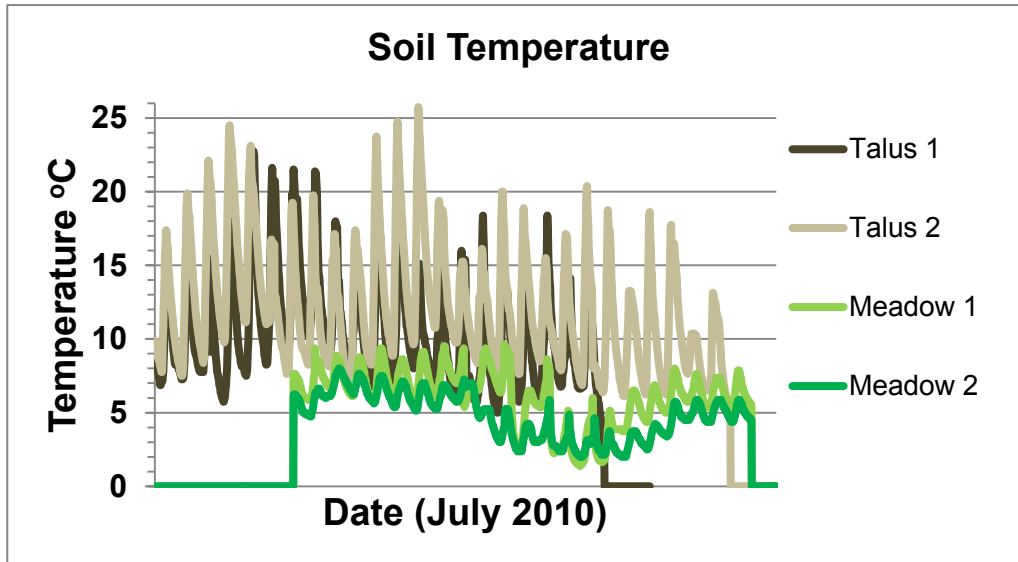
Figure 3, Soil temperature data

Outwash was under water at the time of collection. Talus and meadow were at 88% and 85% WHC. There was a 0.6 cm rain event on August 10, 2010, the morning of soil sample collection.

Clay content was 12% in outwash soil, while talus and meadow contained 20% and 25% clay respectively. pH in outwash, talus and meadow soils was 5.9, 5.0 and 5.2, respectively (Table 1). Dissolved organic carbon and nitrogen availability increased along the gradient of soil development, being lowest in outwash, intermediate in talus and highest in meadow soil (Table 1). Dissolved organic carbon increased from outwash (0.36 mg/kg) to talus (1.34 mg/kg) and from talus to meadow (16.62 mg/kg). Dissolved organic nitrogen levels were comparable in outwash and talus (0.26 and 0.35 mg/kg), but increased by an order of magnitude in meadow soil to 3.19 mg/kg (Table 1).

The largest DIN concentration was 24.2 mg/kg, observed in meadow soil. Outwash contained 17.49 mg/kg and talus held 15.68 mg/kg (Table 1). In outwash, both total carbon and total nitrogen content were below detection limit. Talus C:N was 8.65.

Meadow contained a higher ratio than talus with a ratio of 12.86 (Table 1). Microbial

biomass carbon and MBN concentrations increased with from outwash to talus to

meadow soil (Table 1).

Table 1, Average soil parameters at the time of collection: BDL, below detection limit; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; DIN, dissolved inorganic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen

	% clay	pH	DOC (mg/kg)	DON (mg/kg)	DIN (mg/kg)	C:N	MBC (mg/kg)	MBN (mg/kg)	MBC:N
Outwash	12.2	5.8	0.36	0.26	17.5	BDL	0.05	BDL	-
Talus	20.0	5	1.34	0.35	15.7	8.65	0.42	0.07	6.0
Meadow	25.0	5.2	16.6	3.19	24.2	12.9	8.69	0.56	15.5

Microbial biomass

The total and relative abundance of MBC and MBN changed in each soil over the

course of 45-day incubation. In outwash, microbial growth occurred under all treatment

conditions. Talus soils incubated under dried conditions behaved differently than talus

soils incubated with higher soil moisture (Table 2). Those under drier conditions (25%

WHC) lost MBC along with the coldest, wettest sample (incubated at 4^oC and 50%

WHC), while MBC increased in all other talus treatments. Microbial biomass carbon in

meadow soil incubated at 4^oC and 75% WHC increased more than any other soil.

However, all other meadow soils experienced decreases in MBC (Table 2).

Concentrations of MBN increased in outwash samples but decreased in talus. Wet

meadow samples experienced the greatest MBN increases of all by an order of

magnitude, while those at 25% WHC were relatively small or negative (Table 2). The

change in MBC:MBN during the incubation was positive in all outwash and talus

samples, indicating a relative increase in MBC over MBN. The ratio of MBC to MBN decreased in all meadow soils (Fig. 4).

The effects of temperature and moisture treatments on microbial biomass concentrations were variable among soils. Levels of MBC in outwash were not explained by incubation conditions. However, shifts in MBC:MBN were maximized at $10^{o}C$ in outwash (Table 3). Increasing moisture increased MBC and MBC:MBN in talus soil (Table 3). In meadow soil, 86% of the variation observed in MBC was described by temperature and moisture, but temperature and moisture treatment conditions were not significant predictors of MBC:MBN (Table 3).

Table 2, Average rates of change in MBC and MBN: MBC, microbial biomass carbon; MBN, microbial biomass nitrogen

		Outwash		Talus		Meadow	
% WHC		50%	100%	50%	100%	50%	100%
Δ MBC (µg/kg/d)	$4^{o}C$	2.28	2.38	-3.92	-1.29	-63.10	192.80
	$12^{o}C$	2.08	2.44	-1.64	1.80	-68.70	-18.77
	$25^{o}C$	2.50	3.24	-3.75	3.53	-86.10	-1.45
Δ MBN (µg/kg/d)	$4^{o}C$	1.95	1.82	-0.54	-0.65	3.18	27.99
	$12^{o}C$	1.06	1.34	-0.11	-0.24	1.65	16.05
	$25^{o}C$	1.03	1.51	-0.60	-0.78	-2.66	11.71

2.3.2 Respiration

Average rates of respiration were highest in meadow samples. Talus and outwash soil respired at similar rates throughout the incubation (Table 4). However respiration standardized for each soil's initial concentration of microbial biomass carbon (representing "MBC-specific activity") presented the opposite trend (Table 4). Rates standardized for MBC were used to compare the relative activity of the MBC present
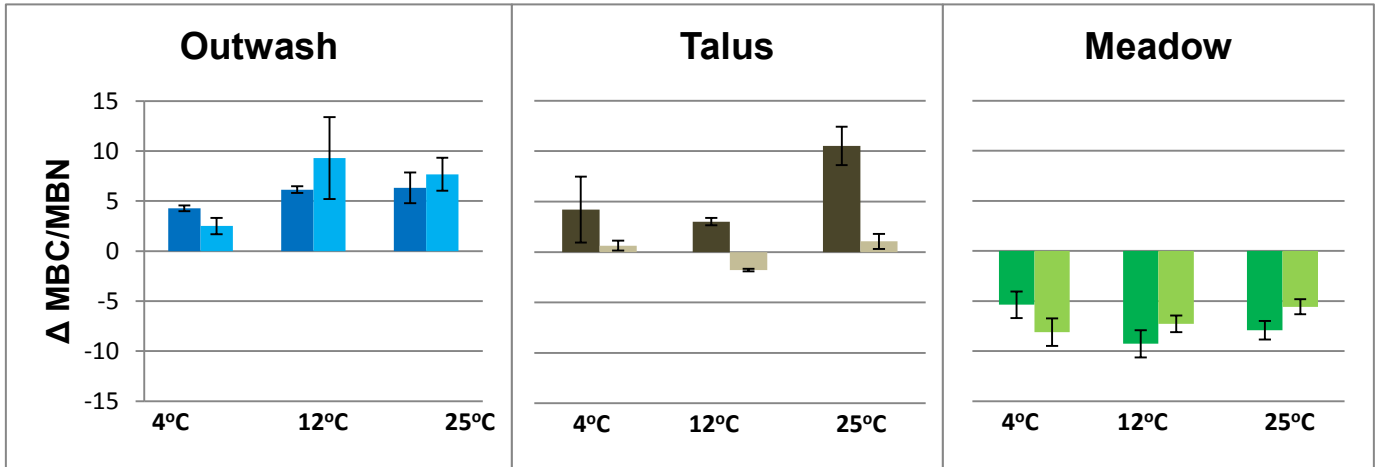
Figure 4, Changes in MBC:MBN with laboratory temperature and moisture treatments over the 45 day incubation: MBC, Microbial biomass carbon; MBN, Microbial biomass nitrogen.

among soils. Outwash showed the highest rates of MBC-specific respiration, followed

by talus and meadow soil (Table 4).

Total and specific respiration rates were controlled by temperature and moisture

conditions. Both increased with increasing temperature and moisture, explaining ~99%

of variability (Table 3). Soil respiration was positively correlated with MBC overall.

However, in individual soil analyses MBC abundance was not a control of respiration

(Table 3).

2.3.3 Net nitrogen mineralization

Net nitrogen mineralization is the rate of net change in the DIN pool. Positive

nitrogen mineralization rates were observed only in meadow soil only. Samples wetted

to 25% WHC averaged 0.9 mg N/kg, while samples treated with 75% WHC averaged

2.6 mg N/kg. The negative rates measured in talus and outwash signified net nitrogen

immobilization. Talus soils had the highest rates of net nitrogen immobilization,

Table 3, Standardized regression coefficients of multiple regression analyses for change in microbial biomass carbon (MBC) (µg/kg/d), respiration (µg/kg/d), and MBC-specific respiration (µg/kg/d): Multiple regression results were deemed significant at P<0.05 and highly significant (*) at P<0.001. Temperature and moisture were analyzed as categorical variables; their coefficients are relative to 4°C and 50% WHC.

	Δ MBC (µg/kg/d)			Respiration (µg/kg/d)			Respiration/MBC (µg/kg/d)		
	Outwash	Talus	Meadow	Outwash	Talus	Meadow	Outwash	Talus	Meadow
Temperature (°C)	-	-	-	[25°C]: 0.77*	[25°C]: 0.55*	[25°C]: 0.71*	[25°C]: 0.77*	[10°C]: -0.09 [25°C]: 0.57*	[25°C]: 0.64*
Moisture (%WHC)	-	[25%]: -0.66	[25%]: -0.74	[100%]:0.56*	[25%]: -0.84*	[25%]: -0.67*	[100%]: 0.56*	[25%]: -0.81*	[25%]: -0.68*
ΔMBC (µg/kg/d)	na	Na	na	-	-	-	na	na	na
	-	-	T[12°C] x M[25%]:0.31	T[12°C] x M[100%]:0.1 T[25°C] x M[100%]: 0.08	T[25°C] x M[25%]: -0.08	T[12°C] x M[25%]: -0.16	T[12°C] x M[100%]: 0.1	T[25°C] x M [25%]: -0.23*	T[12°C] x M[25%]:-0.13 T[25°C] x M[25%] -0.14
Model R²	-	0.43	0.84	0.97	0.99	0.98	0.99	>0.99	>0.99

Table 4, Average rates of respiration and biomass-specific respiration, in µg/g/d

		Outwash		Talus		Meadow	
% WHC		50%	100%	50%	100%	50%	100%
Respiration (µg/g/d)	$4^{o}C$	0.15	1.06	0.05	0.92	0.3	3.93
	$12^{o}C$	1.17	3.65	0.11	2.46	1.61	17.19
	$25^{o}C$	2.73	5.40	0.51	6.43	9.28	29.25
Respiration/MBC (µg/g/d)	$4^{o}C$	3.17	22.58	0.12	2.19	0.03	0.45
	$12^{o}C$	25.01	77.79	0.27	5.84	0.19	1.98
	$25^{o}C$	58.09	115.01	1.20	15.27	1.07	3.37

ranging from 0.08 to 0.22 mg N/kg. Outwash immobilized nitrogen at an average rate of 0.1 mg/kg.

2.3.4 Bacterial:Archaeal 16S

The relative abundance of bacterial and archaeal 16S was modified in all soils during the incubation. This suggests that a change in microbial community structure occurred over time in each soil. Meadow soil ratios increased the most, followed by outwash. In talus, however, bacterial:archaeal 16S decreased (Fig. 5).

2.4 DISCUSSION

This investigation was carried out in order to determine how microbial communities at different stages of development respond to changes in temperature and moisture conditions. We found that many parameters of microbial community growth and behavior were influenced by temperature and moisture, but that they did not always
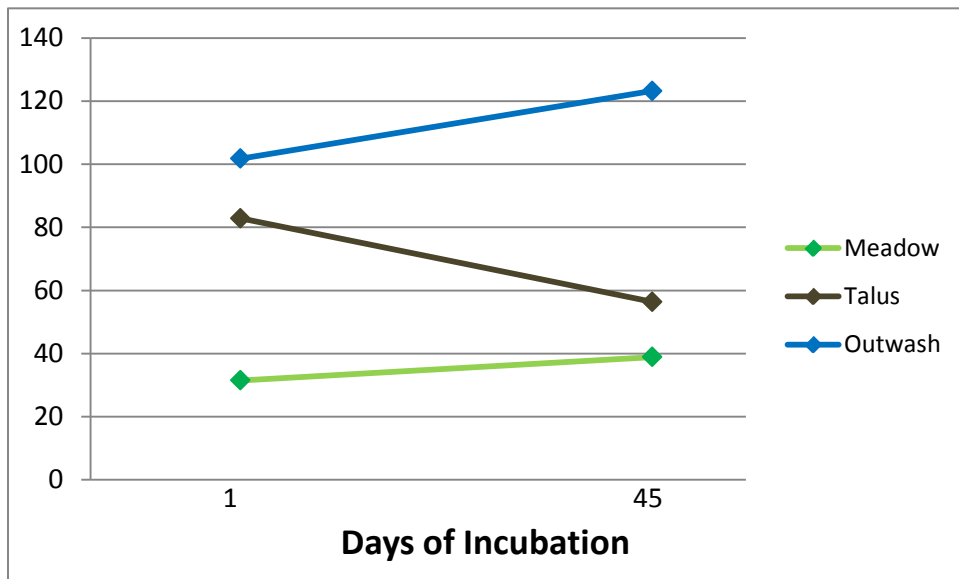
Figure 5, Changes in bacteria:archaea over a 45-day incubation for all temperature and moisture treatments

respond positively as we hypothesized. Treatment effects were varied among soils. This suggests that the microbial communities were operating under distinct mechanisms of response.

2.4.1 Gradient characterization

The trends in DOC, DON, C:N, % clay, and MBC observed in outwash, talus and meadow soil parameters were similar to those documented along gradients of development in other alpine sites and regions. Consistent with previous studies, we observed large incremental increases in DOC, DON, clay content, and C:N as well as microbial community size and activity with alpine soil succession across short distances (Ohtonen et al. 1999; Ley et al. 2004; Brankatschk et al. 2011). The increasing MBC trend with relatively development (from outwash to talus to meadow) that we observed

supported our first hypothesis that microbial abundance would increase with soil development. In Loch Vale, many of these parameters increased by orders of magnitude between outwash, talus and meadow (Table 1, Table 2, Fig. 1). Developing microbial communities in alpine soils are capable of adapting and optimizing the resources that are available.

2.4.2 Microbial biomass carbon and respiration

The concentrations of MBC in Loch Vale outwash, talus and meadow soils were low compared to other late-summer alpine soils. The Loch Vale meadow contained MBC ~1/7 the size of the lowest concentrations measured in other vegetated alpine sites along the Colorado Front Range, while talus concentrations were lower but more similar to those in other talus soils (Lipson et al. 1999; Ley et al. 2001, 2004). We have found no prior studies that quantified glacial outwash MBC. For context, other extreme environments like Antarctic glacial moraines and the Arctic polar desert average 0.06 and 0.15 mg/kg MBC, while in Loch Vale soils MBC which ranged from 0.00005 to 0.009 mg/kg (Jones et al. 2000; Tscherko et al. 2003) (Table 1). Low microbial biomass in all three of these very different soils suggests that there may be a universally limiting control in the Loch Vale Watershed.

During the course of the incubation, MBC levels remained lower than those observed in other alpine sites and regions (Lipson et al. 1999; Ley et al. 2001, 2004). However, rates of Loch Vale soil respiration were within the range of rates measured in other alpine soils, both in the field and during laboratory experiments (Ohtonen et al.

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1999; Ley et al. 2004). This indicates that the microbial communities were small, but responded with a relatively high level of activity under incubation conditions.

2.4.3 Treatment effects on microbial community size, activity and structure

The positive response of respiration to warmer temperatures and the lack of temperature-sensitivity observed in rates of MBC growth indicate that metabolic activity was limited by temperature but microbial biomass was not. The increase in respiration with warmer temperatures supported our first hypothesis.

The positive response of microbial community size and activity to increased moisture that we observed has been documented in the alpine by others and supported our first hypothesis (King et al. 2008). Inadequate soil moisture limits substrate diffusion and reduces microbial mobility (Paul & Clark 1996). The importance of moisture in regulating soil biological activity suggests that water availability was a limiting factor in talus and meadow soils. This cannot be said of outwash soil because it was submerged at the time of collection. However the most microbial biomass and highest rates of respiration were observed at the highest moisture treatment of 100% WHC. Talus and meadow were wetted to >80% WHC at the time of collection. However, talus and alpine meadows along the Colorado Front Range are generally dry during the summer months (JJA) (Sickman et al. 2003; Ley et al. 2004; King et al. 2008). It is possible that the rain event on the morning of sample collection resulted in temporarily exaggerated soil water content that was not representative of summer meadow and talus conditions. If that was the case, the increased MBC and respiration in response to moisture indicates that late-summer meadow and talus were limited by moisture.

Temperature and moisture treatments not only influenced the abundance and activity of microorganisms, but also affected microbial community structure. Changes in the relative abundance of bacterial and archaeal 16S over the duration of the 45-day incubation indicate changes occurring in community structure at the molecular level. The shifts observed in MBC:MBN indicate that microbial biomass content varied during the incubation (Paul & Clark 1996).

Of the parameters measured during the incubation, including change in MBC, net N mineralization, bacteria:archaea, and MBC:MBN, only soil respiration showed similar trends in response to temperature and moisture controls by all three soils. Microbial community size and structure responded to incubation treatment conditions as well, but varied by soil. Changes in MBC were not correlated with respiration. These observations refute our third hypothesis, that the microbial communities of each soil would have fundamentally similar responses to treatment controls. It appears that outwash, talus and meadow soils had divergent mechanisms of community adjustment to changes in microclimate. However, the relative levels of activity within those communities respond predictably to changing microclimate conditions.

2.4.4 Outwash microbial communities

Although outwash was so recently exposed and contained the lowest nutrient and MBC concentrations at the time of collection, it experienced the greatest increase in MBC and the fastest rates of MBC-specific respiration (Table 2, Table 4). Outwash also had negative rates of net nitrogen mineralization, suggesting nitrogen immobilization and community growth. These strong growth responses to warming treatments

suggested that microclimate conditions were limiting in outwash soil. This was not

surprising because outwash soil likely experienced constant temperatures of $\sim 1^{o}C$ in the

field and was saturated with water. High rates of MBC-specific respiration suggested

that carbon use was relatively inefficient in the outwash, and that carbon was not

limiting the growth of microbial biomass (Ohtonen et al. 1999). In the early successional

stages, when temperature limitations are lifted, microbial communities are often unable

to incorporate substrates efficiently but instead respire rapidly (Ohtonen et al. 1999;

Price & Sowers 2004). A decrease in resource use efficiency in exchange for maximum

growth is a commonly-observed trade-off within microbial communities (Lipson et al.

2006).

2.4.5 Meadow microbial communities

Microbial communities inhabiting the meadow site, which was the most

developed of the three soils studied, used energy more efficiently than communities

inhabiting talus or outwash, as evidenced by a low standardized respiration rate (Table

4). This decrease in MBC-specific respiration with development has been observed

elsewhere in the western US (Ohtonen et al. 1999). Interestingly, meadow soil samples

were the only ones in which MBC:MBN decreased during the incubation, yet they

gained more MBN than the other soils. This suggests that nitrogen was not a limiting

resource, which is expected from a nitrogen saturated catchment like Loch Vale. Others

have also observed late-stage microbial communities unable to reach high rates of

standardized respiration (Ohtonen et al. 1999). Instead, they remained in an energy-

saving state accumulating carbon into biomass, as we observed in Loch Vale meadow samples.

2.4.6 Implications

There were significant step-wise increases in microbial biomass and activity with increasing soil succession and nutrient availability from outwash to talus to meadow soils, which suggests that alpine microbial communities are able to mature from barren soils exposed after deglaciation and acclimate to their environment and the nutrients available (Wallenstein & Hall 2011). But the changes in community structure and variable responses to temperature and moisture observed in microbial community size and behavior indicate that as they are adapting, the soils operate under fundamentally different modes of microbial community function (Sigler & Zeyer 2004). The results imply that predicting the effects of global change on alpine microbial processes may require careful consideration of the variable levels of soil succession represented.

3. ENVIRONMENTAL DRIVERS OF NITRIFICATION IN ALPINE MICROBIAL ECOSYSTEMS

3.1 INTRODUCTION

Human-caused climate change and increasing rates of atmospheric nitrogen deposition are destabilizing environmental trends characteristic of the past 10,000 years (Rockström et al. 2009). In many parts of the world, these disturbances are responsible for modifying air temperature and precipitation patterns and distributing unprecedented levels of reactive nitrogen (Matson et al. 2002; Galloway et al. 2004; IPCC 2007).

High-elevation ecosystems demonstrate acute vulnerability to the effects of atmospheric nitrogen deposition due to their characteristically thin soils, sparse vegetation and limited nutrient availability (Burns 2004). Previously nitrogen limited alpine systems of the US and Europe have shifted to state of nitrogen saturation as a result of atmospheric deposition since the post-WWII increase in human population and synthetically-available fertilizers (Williams et al. 1996; Burns 2004; Rogora & Mosello 2007).

In addition to atmospheric nitrogen deposition, the influence of climate change is now being observed in alpine regions worldwide (IPCC 2007). Record high annual temperatures have been observed in both the western US and the European Alps since

the year 2000 (Diaz & Eischeid 2007; Cannone et al. 2008). Climate warming in mountains alters precipitation patterns and, as highlighted in this study, shrinks alpine glaciers (IPCC 2007; Meier et al. 2007).

The response of ecosystems to changes in important biogeochemical controls like temperature, moisture, and nutrient availability are driven by microbial processes (Hu et al. 1999; Morgan 2002; Horz et al. 2004; Falkowski et al. 2008). Not only are microbial processes critical drivers of ecosystem function, but microbial communities may be among the first organisms to adapt to changing environmental conditions due to short generation times (Finlay et al. 1997). Despite early and consequential responses to global change, interactions between microbial communities and climate change and atmospheric deposition are not well understood (Panikov 1999; Zak et al. 2000; Asner et al. 2001; L et al. 2001; Matson et al. 2002).

Alpine ecosystems being altered by climate change and atmospheric nitrogen deposition offer a unique opportunity for studying microbial processes. The alpine is an extreme and heterogeneous environment, characterized by exposure to high winds and solar radiation, a short growing season, and complex hydrologic cycling processes (Ley et al. 2004). Deglaciated alpine systems are also host to soils along a wide gradient of development, from newly-exposed sediments to densely vegetated soils due to glacier melt (Campbell et al. 2000). It has been proposed that microbial communities adapted to harsh environments like the alpine exist at the limits of their tolerance and therefore are sensitive to even small changes in controls of community development and activity, like temperature, moisture, and nutrient availability (Williams et al. 1998; Ley et al. 2004).

Regardless of the harsh environmental conditions and changing biogeochemical controls, microbially-mediated nitrogen cycling is important in the alpine (Fig. 6). Isotopic signatures of surface water in alpine regions of the eastern and western US indicate that >50% of lake NO_3^- is transformed by microbes, and specifically nitrified, prior to being flushed or leached from soil (Amberger & Schmidt 1987; Campbell et al. 2002a; Sickman et al. 2003).
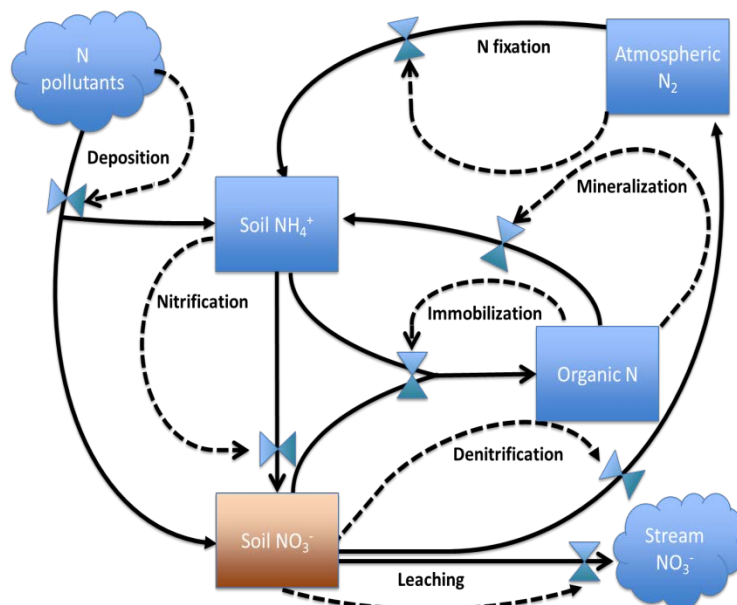
Figure 6, Nitrogen cycling processes and pools in alpine ecosystems affecting NO_3^- availability

Nitrification involves ammonium (NH_4^+) oxidation to NO_3^- by way of nitrite (NO_2^-). Ammonia-oxidizing bacteria and archaea, called nitrifiers, carry out the first and rate limiting-step of nitrification (Leininger et al. 2006). The production of NO_3^- regulates soil inorganic nitrogen concentrations and ecosystem nitrogen losses (Barnard 2005). The availability of inorganic nitrogen affects carbon cycling and the productivity,

development and diversity of plant and microbial communities (Oren et al. 2001; van der

Heijden et al. 2008). Nitrification is a good model for the study of microbial processes

affected by global change because nitrifying microorganisms are ubiquitous and their

activity is influenced by environmental changes (Zak et al. 2000; Kowalchuk & Stephen

2001).

Ammonium availability is the primary control of nitrification (Schlesinger 1997).

Alpine ecosystems are naturally nutrient-poor environments (Ley et al. 2004). The direct

addition of NH_4^+ to soils and surface water as atmospheric nitrogen deposition alters

alpine nutrient dynamics. The Loch Vale Watershed National Atmospheric Deposition

Program (NADP) site in Rocky Mountain National Park, CO (CO98) has recorded

average rates of annual wet NH_4^+ deposition between 1.5 and 2 kg N/ha yr^{-1} and >7.5

kg N/ha yr^{-1} of total wet atmospheric nitrogen deposition since 2000

(http://nadp.sws.uiuc.edu/sites/siteinfo.asp?id=CO98&net=NTN).

Evidence from one high-elevation region suggests that nitrification rates in

systems with high NH_4^+ availability are responding to changes in temperature and

moisture in ways that are detectable and relevant at the ecosystem scale (Rogora et al.

2008). Annual stream NO_3^- concentration trends in Loch Vale increased >50% from the

1990s to 2006 commensurate with warmer summer temperatures (Baron et al. 2009).

Similar changes in NO_3^- availability have been recorded in the Swiss and Italian Alps

and northern Rocky Mountains, including Yellowstone and Glacier National Parks

(Rogora 2007; Saros et al. 2010).Studying the effects of changing environmental

conditions on nitrification in diverse soils from a nitrogen saturated alpine catchment will

increase our understanding of nitrogen cycling in extreme environments as well as the

biogeochemical controls and process dynamics of nitrification under the influence of global change.

The objective of this investigation was to determine how temperature and moisture affect NO_3^- production in nitrogen rich alpine soils with a range of physical, chemical and biological properties. To achieve this, we measured nitrification rates and nitrifier abundance in alpine soils along a gradient of development from the Loch Vale Watershed incubated under a range of temperatures and soil moisture levels. We predicted that field soils would contain high concentrations of inorganic nitrogen. Under NH_4^+ saturated conditions we posed the following hypotheses:

(1) *Nitrification rates would increase with temperature, moisture and nitrifier abundance.*

(2) *Rates of NO_3^- production would increase with increasing amount of soil carbon.*

3.2 MATERIALS AND METHODS

3.2.1 Site Description

The Loch Vale Watershed is a remote alpine/subalpine catchment located east of the continental divide in Rocky Mountain National Park ($40^o17'17''$; $105^o39'43''$) (Fig. 1). It has been the location of long-term ecological research and monitoring since the early 1980s. The catchment faces northeast and covers 660 ha ranging in elevation from 3,110 to 4,009 m above sea level. The Loch Vale Watershed contains two sub-basins, each with high-elevation headwater lakes: Andrews Tarn in the north and Sky Pond to

the south (Fig. 1). Mean annual precipitation is 103.41 cm, with 20.5 cm falling during the summer months (JJA). Mean annual temperature is 1.4^{o}C, while summer temperatures average 11.6^{o}C (http://www.nrel.colostate.edu/projects/lvws/data.html). Parent material is biotite gneiss and schist (Baron 1992).

3.2.2 Soil collection sites

Three alpine soils were collected from the Loch Vale Watershed. The selected sites represented stages of soil succession. Newly exposed sandy outwash sediment was sampled at the base of Andrew's Glacier under the surface of Andrew's Tarn. The "intermediate soil" came from pockets in a poorly vegetated talus field west of Andrew's Tarn. Meadow soil was collected from a wet sedge meadow to the southeast of Sky Pond (Fig. 1). These soils are identified as "outwash", "talus", and "meadow" respectively.

3.2.3 Substrate Collection & Parameterization

Field temperature conditions were measured to characterize each soil environment and to provide boundary temperature and moisture conditions for subsequent laboratory incubations. Thermochron iButton™ temperature sensors buried in the talus and meadow soils in duplicate recorded temperatures every 30 minutes for one month prior to collection, from July 14, 2010 to August 10, 2010). Outwash was inaccessible under Andrew's Glacier at the time of iButton installment; its temperature was therefore not recorded.

On August 10, 2010 trowels wrapped in plastic were used to fill quart-sized bags with five samples of each soil of ~1kg each. The samples were double-bagged and transported on ice to the Natural Resource Ecology Laboratory at Colorado State University. The contents of the five quart-sized bag from each sample site were pooled and sieved to 4 mm, homogenized and stored at -80^oC until used for the experiments reported below.

Water-holding capacity (WHC) and gravimetric water content (GWC) were measured for each soil in triplicate and used to asses field moisture conditions. To determine WHC three 5g subsamples were placed into individual plastic funnels lined with Whatman no. 41 filter papers. Each sample was then submerged in water. The experiment was contained in a cooler that was sealed to prevent evaporation. The funnels containing each saturated sample were elevated, allowing the soils to drain overnight through the filters. Water-holding capacity was calculated as the volume of water retained in each sample divided by the total weight of the saturated soil. The GWC of each soil was quantified by subtracting the oven-dry weight (105^oC) of subsamples from field wet weight. Field % WHC was calculated for each soil by dividing field water content (soil wet weight minus dry weight) by WHC, expressed as percentage.

Parameters measured in each sieved soil included pH, C:N, and dissolved inorganic nitrogen (DIN). A VWR Model 8000 pH meter was used to determine pH in 1:1 soil/deionized water solutions. Carbon and nitrogen content were quantified using a LECO Tru-SPEC elemental analyzer (Leco Corp., St. Joseph, MI) in soil subsamples that were dried at 105^oC for 24 hours and ground on a ball mill. The DIN concentrations,

estimated as the sum of NH_4^+-N and NO_3^--N concentrations, were quantified in 1:5 grams of dry soil/mL 2M potassium chloride (KCl) extracts. After shaking for one hour on an orbital shaker, the extracts were gravity filtered through Whatman no. 41 filter papers and analyzed on an Alpkem Flow Solution IV Automated wet chemistry system (O.I. Analytical, College Station, TX).

Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) as well as soil microbial biomass carbon and nitrogen (MBC and MBN, respectively) concentrations were determined using a chloroform slurry-extraction technique (Fierer et al. 2003). Each soil sample was divided into two 10 g subsamples, and each subsample was extracted in a mason jar with 0.5 M K_2SO_4. One subsample from each set was also treated with 0.5 mL of chloroform at the same time that K_2SO_4 was introduced. The slurries were shaken for 4 hours on an orbital shaker and then vacuum-filtered through glass fiber filters. The extracts were analyzed for carbon and nitrogen using a continuous flow autoanalyzer (Schimadzu, Columbia, TN). Flushed MBC and MBN were calculated as the difference between carbon and nitrogen concentrations detected in control samples and the chloroform-treated samples. The carbon and nitrogen concentrations measured in the non-chloroform treated control samples were interpreted as soil DOC and DON.

3.2.4 Laboratory incubations and rates of respiration

To determine the effects of changing environmental conditions on alpine soil nitrifier and nitrification dynamics, a long-term (45 days) and a short-term (24 hours) laboratory incubation were carried out in which soil samples, in triplicate, received three

temperature and two moisture treatments in a full factorial design. Nitrifier abundance and net rates of nitrification under treatment conditions were determined in all soils during the 45 day incubation. The 24 hour incubation was conducted to measure gross rates of nitrification in talus and outwash soils under the treatment conditions.

The incubation treatments represented reasonable temperature and moisture fluctuations based on measured field conditions. The three incubation temperatures selected were 4^oC, 12^oC and 25^oC. Moisture treatments were soil-specific, but ranged from a low of 25% to 100% WHC (Fig. 2). For both short- and long-term incubations soils were air dried at room temperature to 20% WHC and re-wetted to the appropriate moisture levels. Wetted substrates were incubated in triplicate in 0.24 L acid-washed Mason jars equipped with septa.

Prior to each incubation soils underwent a 7-day pre-incubation under the same temperature and moisture conditions in which soil CO_2 concentrations in the headspace of the each Mason jar were measured daily to determine when microbial activity had stabilized. Respiration was also measured throughout the 45-day incubation. This was achieved using an infrared gas analyzer (IRGA). When CO_2 levels approached 3% jars were flushed with breathing air for 15 minutes. Rates of MBC-specific respiration were calculated by dividing total respiration rates by the concentrations of MBC quantified in each sample.

3.2.5 DNA extraction & quantification

Nitrification can be carried out by bacteria and archaea. Nitrifying bacteria and archaea possess unique genes that code for this ability. The presence of these genes

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does not represent the abundance of microorganisms that are actively nitrifying; only the abundance of microorganism that is capable of nitrifying.

Changes in nitrifier abundance were determined by comparing bacterial and archaeal amoA gene abundance at the start of the long-term incubation (immediately following the 7-day pre-incubation) and at the end of 45 days. At both time points total genomic DNA was isolated from 0.25 g subsamples using a PowerSoil DNA Isolation Kit, following the manufacturer's protocol (MoBio Laboratories, Carlsbad, CA). Extracts were quantified on a multifunction Tecan Infinite M200 plate reader using a Quant-IT dsDNA High-Sensitivity Assay Kit (Invitrogen) according to the instructions provided. DNA extracts were stored at $-20^{o}C$ for approximately one month prior to analysis. This yielded results expressed as ng of DNA/μL of extract.

3.2.6 Quantitative PCR assays

Bacterial (AOB) and archaeal (AOA) nitrifier abundance was quantified within DNA extracts using quantitative real-time PCR (qPCR) performed on a MyIQ Single-Color Real-Time PCR Detection System (Bio-Rad). Reactions were loaded into clear 96-well plates. Each plate included unknowns, a seven-point standard curve, and a non-template control. All reactions were run in triplicate to confirm reproducibility of the method. Standard curves were comprised of serially diluted (10^1-10^7 copies/μL) cloned amplicons (30ng/μL stock solution) for each gene of interest. Reactions totaled 25μL and contained 12.5μL of ABsolute SYBR Green QPCR mix (Thermo Scientific) and 0.25μL bovine serum albumin (BSA). All primers were concentrated at 10μM. Bacterial amoA was quantified using 10 ng of extracted DNA as template and 1.25 μL of forward

and reverse primers amoA-1F (5'-GGGGTTTCTACTGGTGGT-3') and amoA-2R (5'-CCCCTCKGSAAAGCCTTCTTC-3') (Rotthauwe et al. 1997). Archaeal amoA was quantified using 10 ng of DNA extract and 0.75µL of the archaeal amoA primer set Arch-amoAF – Arch-amoAR (5'-STAATGGTCTGGCTTAGACG-3' and 5'-GCGGCCATCCATCTGTATGT-3') (Francis et al. 2005). Cycling conditions for both bacterial and archaeal amoA quantification reactions were 15 min at 95°C, 35 cycles of 94°C for 30 s, 30 s at 55°C, and 30 s at 72°C. All qPCR runs were concluded with a melting curve to assess whether primer dimer had occurred. Amplification efficiencies ranged from 93-99.8% and R^2 values were >0.9.

3.2.7 Net Nitrification

Net nitrification is defined as the net change in the soil NO$_3^-$ pool resulting from multiple nitrogen cycling processes, including nitrification, denitrification, and nitrogen immobilization (Fig. 6). Net nitrification was calculated by subtracting the extractable NO$_3^-$ present in untreated, homogenized outwash, talus, and meadow samples from the extractable NO$_3^-$ measured in all treated soils at the completion of the long-term incubation.

3.2.8 Gross nitrogen transformation rates

Gross nitrification and NO$_3^-$ consumption rates in talus and outwash soils were measured during the 24 hour incubation by using the ^{15}N pool dilution technique described by Kirkham and Bartholomew (Kirkham & Bartholomew 1954). Gross NO$_3^-$ consumption rates represented the combined effect of NO$_3^-$ immobilization by

microorganisms and denitrification (Fig. 6). Prior to the start of the 24-hour incubation soils underwent a 7-day pre-incubation period identical to the long-term incubation.

Immediately following the pre-incubation, background NO_3^- concentrations were quantified in subsamples representing each soil and treatment regime using the same method described above. Background NO_3^- concentrations were used to calculate the quantity of label needed to raise background levels by the appropriate percentages. The percentages to which background NO_3^- levels were raised with $^{15}NO_3^-$-N were selected to be minimal, so as not to greatly alter NO_3^- availability, but sufficient for subsequent analyses. Microbial biomass data from the long-term incubation were used to adjust these percentages, based on predictions of how rapidly NO_3^- was likely to turn over. Talus samples at 4^oC and 25% or 50% WHC were raised to 130% and 80% of background levels respectively, while all other samples were raised by 30%.

Soils were transferred into double gas-permeable polyethylene plastic bags. Five atom % ^{15}N-enriched KNO_3 dissolved in deionized water to 100 and 150 ppm was added drop-wise to each bag of soil and manually homogenized until the desired percent background levels were achieved. This increased soil water content by 0.26% to 0.96%.

Within ten minutes of ^{15}N addition, half of the soil in each bag was removed using sterile spoons and transferred into beakers where they were extracted in 2M KCl at a ratio of approximately 1:2 in order to assess extraction efficiency. The remaining soil was incubated for an additional 24 hours before being extracted in the same manner. After incubation, the soil extracts' NO_3^- concentrations were tested to confirm the

successful addition of $K^{15}NO_3^-$ and to identify samples with nitrogen levels too low for subsequent analyses.

Following Stark and Hart (1996), KCl extracts from both time points were diffused onto acidified filter disks and analyzed for $^{15}NO_3^-$ levels (Stark & Hart 1996). Half of the total extract volume from each sample was added to a specimen cup. KCl extracts with low NO_3^- concentrations were supplemented with KNO_3^- (0.037 atom %) as needed so that each sample contained at least 30 µg of NO_3^--N, which was required for accurate ^{15}N analysis. Additional 2M KCl was also added to bring the total volume of each specimen cup up to 53mL. Subsamples of these solutions were run on the AlpKem autoanalyzer to verify that extractable N levels ranged from 30 to 80 µg for subsequent analysis.

Acid traps were created by acidifying two Whatman quartz fiber filter disks with 10 µL of 2M $KHSO_4$ and sealing them in polytetrafluoroethylene (PTFE). Traps were suspended from specimen cup lids using stainless steel wire to prevent contamination.

In the first 6-day diffusion, 0.3 g of magnesium oxide (MgO) and an acid trap were introduced to the specimen cups, which were immediately closed and sealed with Parafilm. This removed NH_4^+ from the extract solutions. At the end of 6 days, new acid traps, 0.3 g of MgO and 0.5 g of Devarda's alloy were added. Cups were then sealed for an additional 6 days, during which time NO_3^- was converted to NH_4^+ and diffused into the specimen cup head spaces as NH_3 to be trapped on the acidified quartz filter disks.

At the end of the second diffusion, acid traps were removed from the PTFE tape using clean forceps and pierced with 2'' pieces of stainless steel wire. Wires were set in Styrofoam blocks and dried in desiccators over concentration H_2SO_4 for 24 hours.

The ^{15}N levels of the nitrogen trapped on the dried filter disks were determined using a VG Isochrom continuous flow IRMS (Isoprime Inc., Manchester, UK) connected to a Carlo Erba NA 1500 elemental analyzer at the EcoCore analytical service facility, Colorado State University, Colorado, USA. Gross NO_3^- transformation rates were calculated using the equations from Kirkham and Bartholomew (1954). Data from any samples containing <0.45 atom % ^{15}N were removed from the gross nitrification and gross NO_3^- consumption data sets because such low atom percentages indicated methodological error had occurred. All negative production and consumption values were replaced with zeros.

3.2.9 Statistical Analysis

The effects of temperature, moisture and soil properties on net and gross nitrification and changes in AOB abundance were analyzed using multiple regressions. Temperature and moisture were treated as categorical variables. Residuals were power transformed using the Box-Cox method to achieve normality. Significance was accepted at a level of probability (P) of <0.05. Statistical analyses were performed with SAS JMP 9.0 (SAS Institute Inc., Cary, North Carolina).

3.3 RESULTS

3.3.1 Soil parameters

IButton temperature sensor data indicated that talus soil experienced higher and more variable temperatures than meadow soil during the month prior to sample

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collection. Talus soil temperatures spanned 4.9°C - 25.8°C, with an average of 11.8°C,

while meadow soils fluctuated from 1.4°C to 8°C and averaged 5.5°C (Fig. 3). Outwash

temperature was not recorded for this study. However, because the soil was submerged

in Andrew's Tarn at the base of Andrew's Glacier we assumed that it fluctuated little

from 1°C. Unpublished iButton data collected in Sky Pond outwash sediment during the

2011 growing season supported this assumption (E. Hall, pers. commun.).

Outwash was wetted to 100% WHC at the time of collection. Talus and meadow

were wetted to 88% and 85% WHC. There was 0.6 cm of rain the morning of soil

collection on August 10, 2010.

Clay content was ~12% in outwash, while talus and meadow contained 20% and

25% clay. pH in outwash, talus, and meadow soils was 5.8, 5.0 and 5.2, respectively

(Table 5). Dissolved organic carbon and nitrogen concentrations increased with soil

development. They were highest in the organic meadow (DOC: 16.62, DON: 3.19

mg/kg), intermediate in talus (DOC: 1.34, DON: 0.35 mg/kg), and lowest in outwash

soils (DOC: 0.36, DON: 0.26 mg/kg) (Table 5). Total soil C:N also increased from

outwash to talus to meadow. Both carbon and nitrogen content were below detection

limit in outwash samples. The C:N of talus was 8.65, which increased to 12.86 in

meadow soil (Table 5).

Dissolved inorganic nitrogen greatly exceeded DON concentrations in all soils.

Meadow soil contained the largest DIN pool (the sum of NO_3^- and NH_4^+ concentrations)

at 24.2 mg/kg. Outwash and talus had DIN concentrations of 17.49 mg/kg and 15.68

mg/kg respectively (Table 5). In contrast, the DON concentrations were 0.26 mg/kg in

outwash, 0.35 mg/kg in talus, and 3.19 mg/kg in meadow. Ammonium exceeded NO_3^- in

all soils. The NH_4^+:NO_3^- ratios were 19 in outwash, 13 in meadow, and 7 in talus soil (Table 5).

Microbial biomass carbon varied by an order of magnitude among soils, from 0.047 mg/kg in outwash to 0.421 mg/kg in talus and 8.690 mg/kg in meadow (Table 5).

Table 5, Average soil parameters at the time of collection: DOC, dissolved organic carbon; DON, dissolved organic nitrogen; MBC, microbial biomass carbon; BDL, below detection limit

	% clay	pH	DOC (mg/kg)	DON (mg/kg)	C: N	NO_3^- (mg/kg)	NH_4^+ (mg/kg)	MBC (mg/kg)
Outwash	12.16	5.8	0.36	0.26	BDL	0.87	16.62	0.047
Talus	20.00	5.0	1.34	0.35	8.65	2.00	13.69	0.421
Meadow	25.00	5.2	16.62	3.19	12.86	1.72	22.48	8.690

3.3.2 AmoA gene abundance

Nitrifier communities were dominated by bacteria. At the start of the long-term incubation, AOB copy numbers exceeded AOA in all soils and treatments by 2-4 orders of magnitude. At the end of the long-term incubation, AOA abundance dropped to near or below detection limit.

Temperature and moisture treatment conditions predicted 82% of the variability in bacterial nitrifier abundance in outwash and 74% in meadow soil (Table 6). Nitrifier gene abundance decreased with increasing temperature. In outwash soil, greater soil moisture (100% WHC) decreased the concentration of nitrifiers, while moisture had the opposite effect in meadow samples. The effects of temperature and moisture were co-dependent. In talus soil nitrifier abundance did not respond to temperature or moisture,

but decreased with increasing DOC availability, which predicted 71% of variability (Table 7).

3.3.3 Net nitrification

Net nitrification was observed in all samples with the exception of talus soil incubated at 12°C and 25°C wetted to 50% WHC. The negative rates measured in these talus samples were -0.0004 and -0.013 mg/kg soil d^{-1} (Table 6). Net nitrification observed in meadow samples responded strongly to the moisture treatment. At 75% WHC rates of nitrification were an order of magnitude higher in meadow soils than average rates in outwash (0.11 mg/kg soil d^{-1}). Net nitrification in meadow samples at 25% WHC was comparable to or slightly lower than mean outwash rates (Table 6). Rates of net nitrification in talus soil responded the least to temperature and moisture treatments (Table 6).

In outwash, NO_3^- production was greatest at 12°C and 100% WHC was tightly correlated with DOC and DON concentrations (Table 7). Net nitrification decreased with increasing DOC availability, and decreased with increasing DON. Bacterial nitrifier abundance at the start of the long-term incubation also increased outwash net nitrification (Table 7). Together these variables predicted >99% of the variability observed. In talus, net nitrification decreased with increasing temperature and moisture (Table 7). In meadow soils, >99% of net nitrification variability was explained by increasing rates at higher temperatures and 75% WHC (Table 7).

3.3.4 Gross nitrification

Similar rates of gross nitrification were measured in outwash and talus soils. In outwash, rates ranged from 0.44 to 1.98 mg/kg d^{-1}, while meadow rates were higher and spanned 2.03 mg/kg d^{-1} to 12.00 mg/kg d^{-1}. Maximum gross nitrification occurred at 12^oC in outwash and increased with increasing DON availability (Table 7). In the talus samples, gross nitrification was not directly influenced by temperature. Variability in gross rates of talus nitrification was predicted by nitrifier abundance at the start of the long-term incubation and the availability of DOC as influenced by temperature and moisture. Nitrate production increased with increasing moisture and decreased DOC (Table 7).

3.3.5 Gross NO_3^- consumption

Rates of NO_3^- consumption were less than NO_3^- production in all treatments (Table 6). Gross NO_3^- consumption rates in talus and outwash were not explained by temperature, moisture, DOC, DON or nitrifier abundance (Table 7). More NO_3^- was consumed in "wet" talus soil (50% WHC) than in "wet" outwash samples (100% WHC) (Table 6).

3.4 DISCUSSION

The effects of temperature and moisture on microbial NO_3^- processing were studied in order to better understand what controls NO_3^- production in the alpine. This research was carried out in part to contribute to our understanding of whether or not there could be a biological link between global change and stream NO_3^- concentrations.

Table 6, Average rates of net nitrification, gross nitrification, gross NO_3^- consumption, and net N mineralization in mg/kg/d

		Outwash		Talus		Meadow	
% WHC		50%	100%	25%	50%	25%	75%
Net Nitrification	4°C	0.001	0.138	0.009	0.046	0.001	0.138
mg/kg/d	12°C	0.029	0.254	0.005	0.000	0.138	0.254
	25°C	0.064	0.165	0.002	-0.013	0.254	0.001
Gross Nitrification	4°C	0.442	1.241	0.116	1.275	-	-
mg/kg/d	12°C	1.984	0.520	0.203	2.039	-	-
	25°C	0.854	0.684	-	1.752	-	-
Gross NO_3^- Consumption	4°C	0.135	0.901	0.070	0.920	-	-
mg/kg/d	12°C	0.443	0.039	0.315	1.934	-	-
	25°C	0.569	0.000	-	0.218	-	-
Net N Mineralization	4°C	-0.059	-0.091	-0.080	-0.138	0.001	0.138
mg/kg/d	12°C	-0.144	-0.018	-0.124	-0.216	0.138	0.254
	25°C	-0.154	-0.114	-0.077	-0.215	0.254	0.001

Nitrate is produced by all three soils under a wide range of microclimate conditions. Variation in rates of net nitrification was explained largely by nitrifier abundance, DOC concentrations, and temperature and moisture. However the effect of these controls was depended on soil type. Data suggested that the effects may have also been the result of the microclimate conditions, namely cold, warmer, and highly variable or more homeostatic, under which the respective microbial communities developed.

3.4.1 Nitrogen saturation

The inorganic nitrogen concentrations in outwash, talus and meadow at the time of collection were uncharacteristically high for alpine soils in the western US (Williams et al. 1995; Osborne n.d.) This observation was supported by DIN concentrations measured in alpine soils from Grand Teton National Park in northwest Wyoming and the Niwot Ridge Long Term Ecological Research (LTER) site, located 50 km south of the

Table 7, Standardized regression coefficients of multiple regression analyses for net nitrification rates (mg/kg/d), gross nitrification rates (mg/kg/d), and changes in AOB abundance (#copies/ng DNA/d): Multiple regression results were deemed significant at $P<0.05$ and highly significant (*) at $P<0.001$. Temperature and moisture were analyzed as categorical variables; their coefficients are relative to $4^{\circ}C$ and 50% WHC.

	Net Nitrification (mg/kg/d)			Gross Nitrification (mg/kg/d)		AOB abundance (# copies/ng DNA/d)		
	Outwash	Talus	Meadow	Outwash	Talus	Outwash	Talus	Meadow
Temperature ($^{\circ}$C)	[12°C]: 0.28	[25 $^{\circ}$C]: -0.65	[12 $^{\circ}$C]: 0.12 [25 $^{\circ}$C]: 0.34*	[25 $^{\circ}$C]: -1.85	-	[12 $^{\circ}$C]: 0.35 [25 $^{\circ}$C]: -0.83*	-	[25 $^{\circ}$C]: -0.52
Moisture (%WHC)	[100%]: 0.46	[25%]: -0.17	[25%]: -0.83*	-	-	[100%]: -0.33	-	[25%]: 0.46
DOC (mg/kg/d)	-0.53	-	-	-	-	-	-0.85*	-
DON (mg/kg/d)	0.52	-	-	-1.9	-	-	-	-
AOB (# copies/ng DNA)	0.79	-	-	-	1.13	-	-	-
	T[12 $^{\circ}$C] x M[100%]: 0.17 M[100%] x DON: 0.61* M[100%] x AOB: -0.73	T[25 $^{\circ}$C] x M[25%]: 0.52	T[12 $^{\circ}$C] x M[25%]: -0.14* T[25 $^{\circ}$C] x M[25%]: -0.27*	M[100%] x DOC: -0.53 T[25 $^{\circ}$C] x DON: -1.26 T[25 $^{\circ}$C] x AOB: 2.01	T[12 $^{\circ}$C] x DOC: 2.93 M[50%] x DOC: 2.56	T[25 $^{\circ}$C] x M[25%]: 0.31 T[25 $^{\circ}$C] x M[100%]: -0.5	-	T[25 $^{\circ}$C] x M[25%]: 0.52
Model R^2	>0.99	0.83	>0.99	0.94	0.98	0.82	0.72	0.74

Loch Vale watershed in Colorado's Front Range. Data collected from these regions

included >120 different vegetated and unvegetated alpine and subalpine sites that were

sampled over five different growing seasons. They reported KCl-extractable NH_4^+ levels

ranging from 0.51 mg/kg to a high of 6.56 mg/kg soil and NO_3^- concentrations from

below detection limits to 1.96 mg N/kg (Williams et al. 1997; Bieber et al. 1998; Hood et

al. 2003; Darrouzet-Nardi & Bowman 2011; Osborne, unpublished data). Extractable

NH_4^+ concentrations measured in outwash, talus and meadow were more than twice as

high as the highest concentration observed in Niwot Ridge or the Grand Tetons (Table

5). In Loch Vale soils, NH_4^+ to NO_3^- ratios were also comparatively high. Average

$NH_4^+:NO_3^-$ in Niwot Ridge soils ranged between ~4.5 and 6.0, while outwash and

meadow ratios in Loch Vale were 19.1 and 13.1 respectively.

3.4.2 AOB dominance

Bacterial AmoA gene concentrations were greater than archaeal AmoA in all

soils, suggesting that bacterial nitrifiers outnumbered archaeal nitrifiers. A growing body

of literature highlights archaeal nitrifiers as being ubiquitous in the environment and

dominant in a wide range of both aquatic and terrestrial ecosystems (Leininger et al.

2006; Nicol & Schleper 2006; Prosser & Nicol 2008). However, exceptions exist and

have been used to study the distinct niches of AOA and AOB. Two prominent

observations have emerged. Archaeal nitrifiers are adapted to low-pH and low nutrient

environments, while bacterial nitrifiers generally succeed at higher pH levels and have

an advantage under nitrogen-fertilized conditions (Nicol et al. 2008; Erguder et al. 2009;

Martens-Habbena et al. 2009; Di et al. 2010; Schleper 2010). Because alpine regions

are broadly characterized as low-nutrient extreme environments (even those experiencing high rates of atmospheric deposition) we might have expected greater AOA abundance. It is possible that the low AOA levels detected were the result of primer bias. However, our data were not the first to show AOB dominance in alpine soils. Bacterial nitrifier abundance was ~80 times higher than archaeal nitrifier abundance along an alpine glacier forefield chronosequence in the Swiss Alps (Brankatschk et al. 2011). Like Loch Vale, the soils from this chronosequence contained high DIN concentrations as a result of atmospheric deposition. The most developed soils in the Swiss Alps study reached 12.8 g NH_4-N/kg soil. It is possible that alpine AOB growth occurred in response to nitrogen fertilization by atmospheric deposition. Studies highlighting the advantage of AOB in fertilized soils have been conducted primarily in agricultural soils or grasslands with DIN 1-2 orders of magnitude higher than those observed in Loch Vale (Hallin et al. 2009; Wang et al. 2009; Di et al. 2010). However, due to the relatively low abundance of DOC and MBC, these relatively low-nutrient alpine soils were considered nitrogen saturated and carbon starved.

3.4.3 Controls of nitrification

As hypothesized (1), temperature and moisture were dominant predictors of nitrification. Together they explained >65% of the variation observed in net nitrification overall.

Excess NO_3^- was produced in all three soils, as evidenced by the positive rates of net nitrification in all but two talus treatments and the higher rates of gross nitrification than NO_3^- consumption (Table 6). Temperature, moisture, DOC and DON availability,

and AOB abundance explained >97% of the variation in net nitrification. However the effects of these controls were dependent on soil type, as described below.

Net rates of nitrification were greatest under higher moisture conditions, supporting our hypothesis . This positive response to moisture was not surprising because, although nitrification is an aerobic pathway, soil moisture supports substrate diffusion and microbial metabolism (Linn & Doran 1984; Stark & Firestone 1995). Nitrification is an oxidation pathway. However relatively high rates of net nitrification were observed in outwash soil samples wetted to 100% WHC (Table 6). Because outwash was coarsely textured water pooled in the bottom of the jar and the soil samples did not go fully anaerobic (Bollmann & Conrad 1998).

The influence of temperature on net nitrification was not always positive as hypothesized. Instead it varied by soil. In outwash, maximum net nitrification and gross nitrification occurred at the intermediate temperature treatment: 12°C. Nitrate production in talus decreased with increasing temperature, while production in meadow soil increased. Net nitrification often increases with temperature to an optimum that is above maximum ambient conditions due to greater metabolic activity (Paul & Clark 1996; Stark 1996). We saw this trend clearly in meadow soil. Net nitrification in talus showed the opposite trend.

Gross nitrification rates in talus were not temperature sensitive. This indicated that the observed net nitrification trend resulted from increased NO_3^- consumption, by immobilization or denitrification, under warmer temperature conditions (Fig. 6). Microbial biomass nitrogen concentrations decreased in talus over the duration of the incubation, which suggested that nitrogen immobilization was negative and that the relationship

between temperature and net nitrification was likely the result of increased denitrification.

The lack of temperature control on nitrification rates in talus and different optimal temperatures observed in meadow and outwash suggested that microbial communities were adapted to the local temperature regimes under which they developed. High temperature variability, as observed in talus soil, has been associated with the presence of thermally tolerant temperature generalists and/or more diverse microbial communities (Wallenstein & Hall 2011). Stable temperature regimes, like those recorded in outwash and meadow, are thought to select for temperature specialists that operate most efficiently within a narrower range of temperatures (Waldrop & Firestone 2006; Wallenstein & Hall 2011). Specialist communities respond to changing conditions more strongly than generalists that evolve under dynamic conditions (Elena & Lenski 2003). The lower temperature of maximum outwash nitrification ($12^{o}C$) relative to meadow ($25^{o}C$) indicated the adaptation of their respective microbial communities to temperatures more similar to those they experienced in the field (Fig. 3). The lack of temperature dependence expressed in talus soil suggested the presence of microbial generalists.

The same temperature responses observed in rates of net nitrification were also true for changes in AOB abundance. Temperature was an important predictor of AOB abundance in outwash and meadow soil. Nitrifier abundance was higher at $12^{o}C$ in outwash and $25^{o}C$ in meadow. However AOB did not respond to temperature in talus samples (Table 7).

Bacterial nitrifier abundance at the start of the long-term incubation explained 33% of net nitrification in meadow soil. Nitrifier abundance was not a significant predictor of net nitrification in talus, but it was a predictor of gross nitrification rates. Net as well as gross nitrification in outwash increased with AOB. Functional gene presence often does not align with process rates, and community structure and function are instead linked by other chemical or physical controls (Brankatschk et al. 2011). However in these soils AOB abundance was a positive predictor of nitrification rates and supported our first hypothesis.

3.4.4 Soil gradient

Net nitrification rates did not support our second hypothesis that NO_3^- production would increase with soil development. Instead the highest rates of net NO_3^- production were observed in outwash and meadow, with the lowest rates of net NO3 production in talus. Unlike meadow and talus, outwash nitrification may have been driven in part by a lack of available carbon. Outwash soil not only had the lowest DOC concentration, but also the lowest DOC/DON ratio (Table 5). The negative relationship between DOC and rates of nitrification has been observed in many systems (Hart et al. 1994; Cookson et al. 2007; Montaño et al. 2007). Autotrophic nitrifiers have a competitive advantage under low carbon availability, as observed in outwash soil, because the immobilization of nitrogen by heterotrophic microbes is reduced and NH_4^+ is available to nitrifiers as their energy source (Verhagen et al. 1993; Booth et al. 2005).

Despite limited vegetation and soil development, alpine field studies have identified talus as a potentially important source of stream water NO_3^- in the alpine

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(Campbell et al. 2000, 2002b; Burns 2004; Ley et al. 2004). However NO_3^- production in talus was the lowest of the three soils we studied with laboratory assays (Table 6). The field observations were likely the result of direct hydrologic connections between steep talus slopes and surface water (Campbell et al. 2000; Burns 2004). The limited NO_3^- produced in talus may leach directly into stream water instead of being recycled as in more-developed vegetated soils or in Mason jars (Bieber et al. 1998).

The chemical and physical characteristics of meadow soil were the most divergent in comparison to outwash and talus. Meadow soil contained the most DOC, DON, and MBC by an order of magnitude (Table 5). The C/N ratio as 12.86 was four times higher than that of talus. Meadow soils also had the largest overall DIN pool. Nitrifiers were most abundant in meadow. Rates of net nitrification in meadow samples were strongly dependent on temperature. The high rates that might be expected from such a comparatively well-developed soil were observed only under 75% WHC. This may have been the result of the meadow's more developed microbial community. Soils that are warmed without sufficient moisture for optimal microbial metabolism sometimes respond with significant reduction in microbial activity (Burns 2004). Decreased NO_3^- concentrations have been observed in dry alpine meadows of the Niwot Ridge LTER (Bowman et al. 1993; Fisk et al. 2010). But increased moisture in alpine meadows has been seen to shift microbial community dominance to favor species that increase rates of nitrogen mineralization and nitrification, and ultimately lead to more NO_3^- leaching (Bowman & Steltzer 1998; Steltzer & Bowman 1998).

3.4.5 Implications

Net NO_3^- production can occur in a broad range of alpine soils under NH_4^+ saturated conditions, and we found unexpectedly high productivity in glacial outwash. Temperature and moisture were important controls of NO_3^-, but their influence varied. Temperatures greater than field conditions resulted in increased NO_3^- production in outwash and meadow soils. Drier moisture conditions decreased nitrification. The diverse nitrifier communities and controls of nitrification represented in outwash, talus and meadow suggest that alpine microbial communities could ultimately adapt to changing temperature and moisture regimes resulting from global change, however NO_3^- production trends would likely be altered.

4. CONCLUSIONS

By characterizing outwash, talus and meadow soils we observed that deglaciation can result in diverse soil environments within relatively close proximity. Many of the parameters of soil physical, chemical and biological properties that were measured varied by over an order of magnitude and showed an accumulation of nutrients and microbial biomass with development. Temperature conditions were also variable. Different microclimate conditions likely influence and interact with the process of soil development, creating an added level of complexity and resulting in greater alpine soil diversity. This cautions against making generalizations when thinking about soil environments in alpine ecosystems.

The variable responses of microbial community size, structure and behavior to changes in temperature and moisture conditions that we measured in Loch Vale soils imply that that at different stages of succession alpine microorganisms operate under individual modes of community function. In addition to their relative levels of soil succession, this observation may in part be the result of the unusual microclimate conditions under which communities become established. Microbial community physiology is a reflection of that community's evolutionary history and therefore the environmental conditions to which it has been exposed through time. More recently established communities, like those in outwash and talus soils, have relatively shorter

histories and therefore reflect contemporary conditions more accurately (Paul & Clark 1996). This is one example of how variable microclimate conditions may be shaping the diverse microbial communities.

The net production of NO_3^- by all three soils under the wide range of experimental conditions applied during the incubations suggests that there may be a biologically-mediated source of NO_3^- in Loch Vale and that it responds to changes in temperature and moisture. The distinct responses of nitrification in each soil to temperature treatments supports the concept that the range of microclimates represented play an important role in microbial community development and activity. Nitrate processing during the laboratory incubations also suggest that nitrifier abundance may be a reasonable metric for rates of NO_3^- production.

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