THESIS

BETWEEN A ROCK AND A HARD PLACE; THE CHEMISTRY, BIOLOGY, AND LABILITY OF GLACIAL MELTWATERS IN THE AMERICAN WEST

Submitted by

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ABSTRACT

BETWEEN A ROCK AND A HARD PLACE; THE CHEMISTRY, BIOLOGY, AND LABILITY OF GLACIAL MELTWATERS IN THE AMERICAN WEST

Glaciers and rock glaciers supply water and nutrients to headwater mountain lakes and streams across all regions of the American West. The resulting changes in volume, timing, and chemistry of meltwater discharged by these features appears to be having significant effects on the adjacent alpine headwater ecosystems they feed. Whereas both glaciers and rock glaciers are sources of seasonal meltwater, sediment, and solutes to headwater ecosystems, differences in meltwater characteristics between glacial types, and its affect on biological productivity, is poorly documented.

Here we present a comparative study of the metal, nutrient, and microbial characteristics of glacial and rock glacial influence on headwater ecosystems in three mountain ranges of the contiguous U.S.: the Cascade Mountains, Rocky Mountains, and Sierra Nevada. Several meltwater characteristics (water temperature, conductivity, pH, heavy metals, nutrients, complexity of dissolved organic matter (DOM), and bacterial richness and diversity) differed significantly between glacier and rock glacier meltwaters, while other characteristics (Ca^{2+}, Fe^{3+}, SiO_2 concentrations, reactive nitrogen, and microbial processing of DOM) showed distinct charcteristics between mountain ranges regardless of meltwater source. Some characteristics were affected both by glacier type and mountain range (e.g. temperature, ammonium (NH_4^+) and

nitrate (NO_3^-) concentrations, bacterial diversity). Glaciers and rock glaciers had similar carbon concentrations, but differed in the structural composition of their DOM.

Incubations of DOM from glaciers and rock glaciers with a common subalpine bacterial assemblage were conducted to examine how observed differences in meltwater chemistry controlled bacterial productivity and metabolism. DOM pools from glaciers and rock glaciers were similar in size and chemical diversity, but differed in the chemical compounds they contained. Glacier meltwaters had higher proportions of bioavailable compounds compared with rock glaciers. A smaller portion of DOM from rock glaciers was bioavailable, but both glacial types are enriching alpine headwaters with bioavailable DOM that can support heterotrophic production. Due to the high numbers of rock glaciers and the accelerating loss of low latitude glaciers, the results presented here suggest that rock glacier meltwaters may be representative of what future biogeochemical inputs will be in currently ice-glaciated watersheds.

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1. INTRODUCTION

1.1 PRIMER IN GLACIAL BIOGEOCHEMISTRY

Glaciers and rock glaciers are melting worldwide from climate change, mobilizing ice-locked organic matter, minerals, and nutrients. The release of these meltwater constituents has implications for downstream chemical cycling and heterotrophic activity [Milner et al. 2009; Singer et al. 2012]. Headwater alpine ecosystems fed by glacial features have higher nutrient concentrations in meltwater streams than headwaters fed only by perennial snow [Baron et al. 2009; Hood et al. 2009; Saros et al. 2010]. Dissolved organic matter (DOM) from glaciers has been shown to be more labile and able to support greater amounts of downstream biological activity than DOM from more allochthonous, or terrestrial sources [Barker et al. 2006; Hood et al. 2009; Singer et al. 2012]. Taken together, these results suggest that glaciers are impacting their local ecosystems with potential to alter fundamental ecological aspects in important headwater ecosystems.

Glaciers may also be a source of pollutants to alpine headwaters. Atmospheric pollutants are able to travel great distances and collect in alpine ecosystems [Blais et al. 2001; Baron et al. 2009; Hood et al. 2012]. The melting of glacial ice can concentrate chemical constituents to concentrations high enough to have ecological impact. Glacier meltwaters have concentrations of the pollutants hexacholorocyclohexane (HCH) and dichlorodiphenyltrichloroethane (DDT) that were an order of magnitude higher than meltwaters fed only by snow [Bizzotto et al. 2009]. These products of human pollution

are likely retained at high concentrations in alpine ice due to their low volatility at low temperatures, as well as limited absorption due to limited contact with soils [Slemmons et al. 2013]. Little work has examined the geochemistry of rock glacier meltwaters, however early studies have shown metals in rock glacier-fed streams have been shown to be high enough to cause mutations in stream biology [Theis et al. 2013; Illyashuk et al. 2014].

The alpine regions of the American West have many more rock glaciers than ice glaciers. Rock glaciers may differ from ice glaciers in how they impact biogeochemical processes [Ives 1940; Millar and Westfall 2008]. Rock glaciers, which are frozen, heterogeneous masses of ice and rock, move through plastic deformation. As periglacial features, rock glaciers often represent the lowest altitudinal reaches of alpine permafrost [Gruber and Haeberli 2012]. Most active rock glaciers face in northeasterly direction, occupying former Pleistocene-age ice glacier cirques [Janke 2007; Millar et al. 2013]. Unlike ice glaciers, frost weathering of the surrounding headwall supplies rock debris to the rock glacier surface, which preserves the internal ice core [Janke 2007]. Ice loss in permafrost features is often orders of magnitude slower than rates of ice loss from glaciers, and quantities of permafrost feature water usually exceed glacial ice in alpine environments [Woo 2012]. This difference in melting rates between feature types could have an affect on chemical inputs to headwater ecosystems.

Some glaciers are becoming rock glaciers under warming. As ice glaciers continue to thaw, continued ablation and melt of ice can result in the formation of a rock glacier [Outcalt and Benedict 1965; White 1971; Krainer and Mostler 2000]. By comparing differences between currently active glaciers and rock glaciers, we can apply

a space-for-time substitution to examine potential consequences for glacial-fed headwater ecosystems under warming alpine climate scenarios. As a first identification of what differences in rock glacier and glacier meltwaters will mean for ecosystems, it is important to make basic physical, chemical, and biological comparisons to understand the breadth and scope of potential consequences during this increasingly common geomorphological transition.

Previous research has focused on the hydrology and geomorphology of rock glacier melt [Ives 1940; Janke 2007; Janke and Frauenfelder 2008; Krainer and Mostler 2000; White 1971], but rock glacier shrinkage will also result in changes in the thermal regime, weathering products, and changes in nutrients and DOM, all of which have the potential to alter fundamental biogeochemical and ecosystem processes. Debris-covered glaciers, which are very similar in their geomorphology to rock glaciers, take up larger amounts of CO_2 per area and suppress melting rates compared to ice glaciers. This is due to the weathering processes within the debris on their surface [Franzetti et al. 2013; Wang et al. 2014]. Rock glaciers may act in a similar manner and act as a scrubber for atmospheric CO_2. Although there has been little work to date on the effects of rock glacier melt on the ecology of local ecosystems, there is some evidence that rock glacier melt affects local populations and ecosystem processes. Elevated sulfate and metal concentrations from the outflow of rock glaciers were reported to cause changes and mutations in chironomids and other invertebrates near the outflow of rock glaciers [Ilyashuk et al. 2014; Thies et al. 2013].

Substantial chemical cycling occurs subglacially through microbially mediated processes [Boyd et al. 2011, Ansari et al. 2013]. In the streams fed by glacial meltwater,

it has been suggested that glacial recession is homogenizing *in-situ* microbial populations [Wilhelm et al. 2013]. To the author's knowledge no research has examined the microbial communities in the outflow of rock glaciers, however it has been suggested that there is a positive relationship between the amount of sediment in the subglacial environment and the size and diversity of the microbial population present [Sharp et al. 1999]. The sediment-rich intra-rock glacial and sub-rock glacial environment may support more abundant and diverse microbial communities than ice glaciers with similar physical and chemical parameters. Differences among glacier and rock glacier microbial communities may drive differences in microbial transformations of organic matter between glaciers and rock glaciers.

1.2 DISSOLVED ORGANIC MATTER LABILITY IN GLACIATED HEADWATERS

Organic matter currently has an operational but not molecular definition for lability. Little is known about the control molecular structure places on bioavailability in freshwater ecosystems. Much of this is due to the heterogeneity of DOM in natural systems. The percent protein within DOM has been shown to one of the greatest contributors to bioavailability, with increasing protein being positively correlated with DOM bioavailability [Fellman et al. 2010]. Studies show humic compounds once thought to be recalcitrant are actually bioavailable [Wetzel 2003; Mann et al. 2012]. Novel techniques, including gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), Fourier transform infrared-mass spectrometry (FTIR-MS), and nuclear magnetic resonance imagery (NMR), are allowing rigorous examination of metabolic byproducts and are placing better molecular

parameters on DOM lability [Bowen and Northen 2010]. Differences in bioavailability between glaciers and rock glaciers are unknown.

Ancient DOM from ice glaciers is bioavailable [Singer et al. 2012], and able to support secondary productivity in adjacent ecosystems [Hood et al. 2009]. DOM from ice glaciers is enriched with proteinaceous compounds created *in situ* [Barker et al. 2006]. Little is known about DOM composition and lability from rock glaciers, but the greater inputs of plant and soil-like organic compounds from the surface of the rock glaciers could reduce the lability of rock glacier DOM compared to that of glaciers. Alpine ecosystems can show strong carbon and nutrient limitations [Bernasconi et al. 2011; Singer et al. 2012], and the composition and lability of DOM from glaciers can play a critical role in ecosystem function and downstream activity [Fellman et al. 2010]. The composition of DOM can also have nonchemical effects on ecological activity, as DOM can control the amount of photo bleaching occurring in alpine lakes and can control the depth of the photic zone [Foreman et al. 2013; Slemmons et al. 2013]. DOM could also act as a metal complexing agent, with ecological implications for systems fed by rock glaciers due to high metal concentrations in their outflow [Williams 2006; Ilyashuk et al. 2014].

1.3 RESEARCH QUESTIONS AND HYPOTHESES

This examination of background literature on lead to the principal unanswered questions that I addressed during my thesis research:

1. Is there a difference in the biogeochemistry of meltwaters from glaciers and rock glaciers in the western United States?

2. Is there a difference in the bioavailability of DOM in meltwaters from glaciers and rock glaciers?

I expanded the idea framed by Slemmons et al. (2013) and developed a conceptual model of the putative ecological effects of glacial and rock glacier meltwaters. In this model (Figure 1), glacial type (glacier or rock glacier) controls both the physical and chemical parameters of the outflow. These in turn control the microbial activity occurring both subglacially and in the adjacent ecosystem.

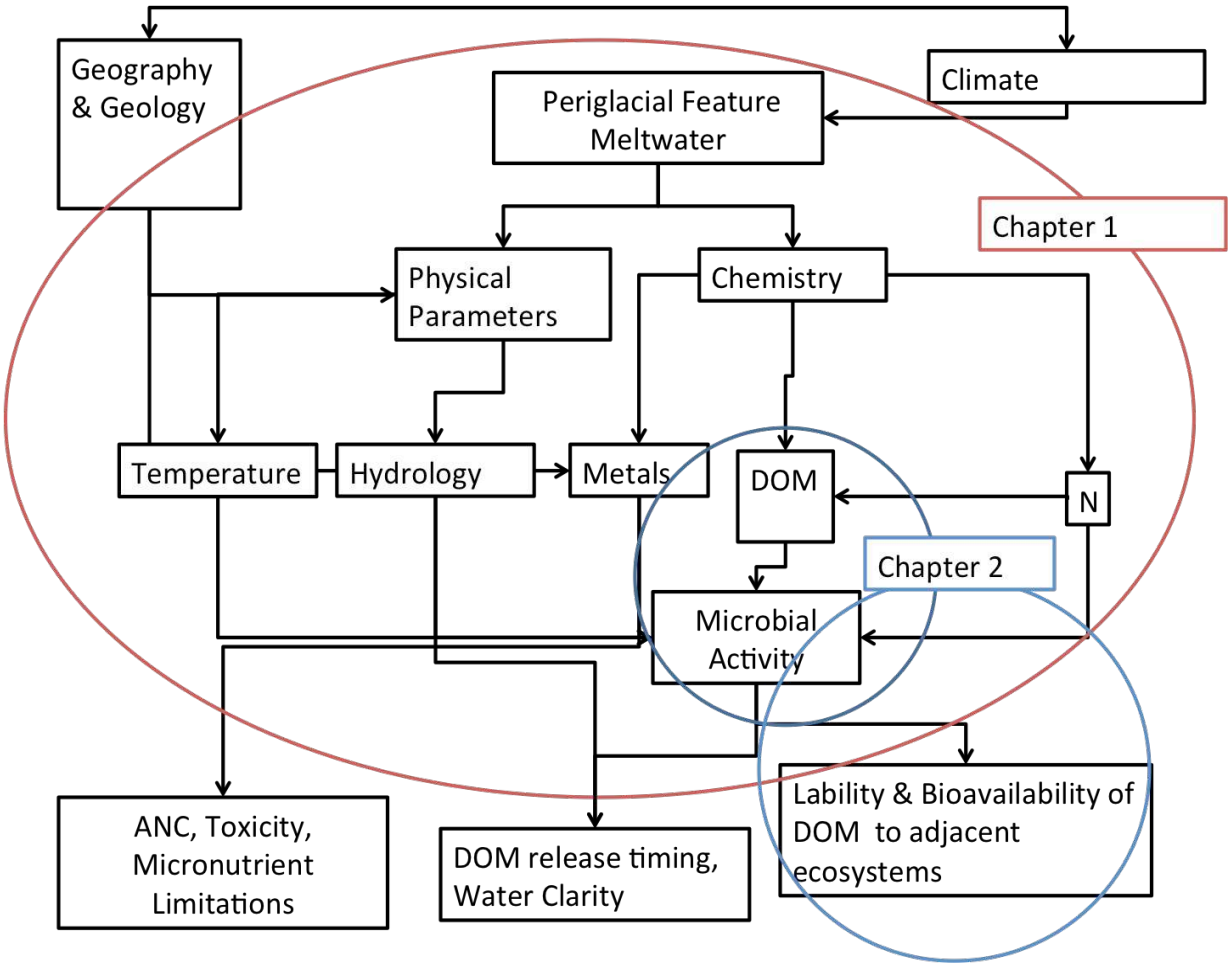


Figure 1: Conceptual model of ecosystem implications for glacial melt. The red circle is representative of survey work described in Chapter 1. research, while the blue circles are representative of Chapter 2 research.

HYPOTHESIS 1: Differences in glacier type will result in differences in chemical (e.g. metal concentrations), biological (e.g. 16S sequencing), and DOM composition diversity (e.g. fluorescence and molecular mass-spectrometry) in the feature outflow.

HYPOTHESIS 2: Differences in DOM composition between glacier types will result in differences in DOM availability between glacier types.

1.4 FIELD SURVEYS

To address whether biogeochemical differences exist between glaciers and rock glaciers (Hypothesis 1), I conducted a biogeochemical survey of glacier and rock glacier meltwater streams drawn from three geographically distinct alpine regions of the American West (the volcanoes of the Cascade Range of Washington, Oregon and northern California, the Rocky Mountains of Colorado and Wyoming, and the Sierra Nevada of southern California). I selected my sample sites to be representative of mountain ranges with different geologies and climates, and of both types of glaciers. I collected samples from 9 sites in 2012, 27 sites in 2013, and 40 sites in 2014 at the outflow of ice glacier and rock glacier features. In all three years, samples were collected in the late summer (August-September) to allow for the greatest contribution of ice melt and the least amount of seasonal snowmelt. The 2012 field survey was conducted on the Front Range of Colorado, sampling paired ice glaciers, rock glaciers, and snow-fed reference streams in three distinct watersheds: Loch Vale, the Rawah Wilderness, and the North Fork of the Big Thompson region of Rocky Mountain National Park (Table 1). In 2013 the survey was expanded to include additional sites (Table 2), including the Arapaho ice and rock glaciers northwest of the town of Boulder, CO, as well as 3 sites within Sierra Nevada of California, 11 sites within Cascades of Oregon,

and 5 sites in the Tetons of Northwestern Wyoming. The survey work was completed in 2014, with 6 new sites in the Cascades, 14 new sites in the Rockies, and 6 new sites in the Sierra Nevada. In total, 25 unique glaciers and 24 unique rock glaciers were sampled during the summers of 2012, 2013, and 2014. Water and sediment samples at the terminus outflow of each glacier and rock glacier were collected according to standard methods (http://www.nrel.colostate.edu/projects/lvws/pages/accesstodata/fieldlabmethods.html), and were analyzed for a suite of chemical (e.g. metal concentrations), biological (bacterial DNA sequencing), and physical (e.g. Temperature) measures.

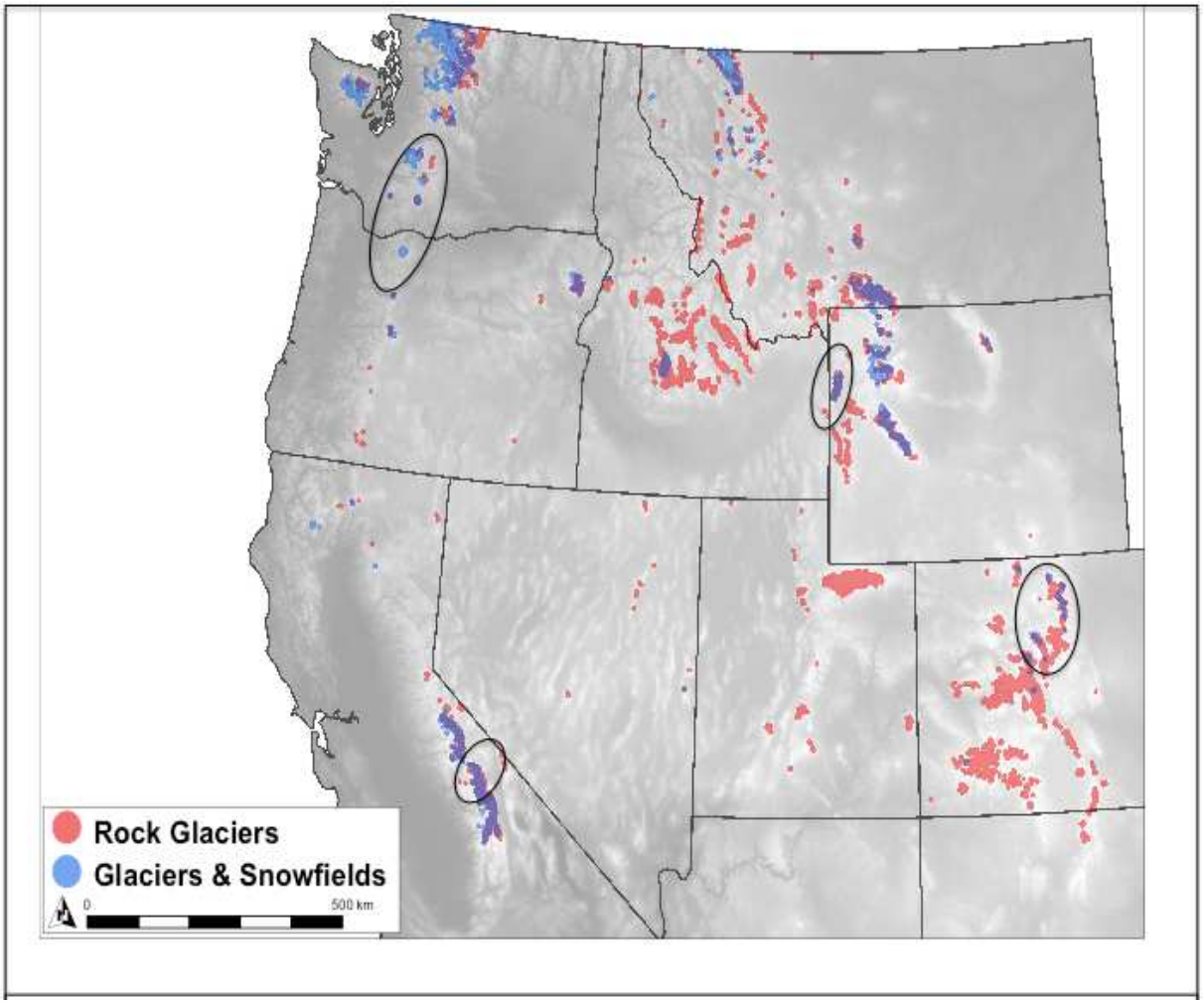


Figure 2: Distribution of glaciers and rock glaciers within the Western United States, with circles over areas of study sites.

Table 1: 2012 Survey Sites

SITE	RANGE	FEATURE TYPE	UTM_E	UTM_N	ELEV. (M)
Andrews Glacier	ROCKY	Glacier	442225	4459895	3505
Husted Lake Inflow	ROCKY	Snow-Fed	448107	4484571	3383
Island Rock Glacier	ROCKY	Rock Glacier	420215	4497859	3274
Loomis Lake Inflow	ROCKY	Snow-Fed	440746	4465463	3115
Louise Rock Glacier	ROCKY	Rock Glacier	447334	4484418	3398
Rawah Glacier	ROCKY	Glacier	419023	4502552	3312
Rowe Glacier	ROCKY	Glacier	445270	4482002	4007
Taylor Rock Glacier	ROCKY	Rock Glacier	443037	4458749	3327
Twin Lake Inflow	ROCKY	Snow-Fed	420422	4499690	3349

Table 2: 2013 Survey Sites

SITE	RANGE	FEATURE TYPE	UTM-E	UTM-N	ELEV. (M)
McCall Glacier	CASCADE	Glacier	-121.4505	46.519019	2056
South Cascade Glacier 1	CASCADE	Glacier	-121.05492	48.362333	1829
Adams Glacier 1	CASCADE	Glacier	-121.52437	46.22534	2260
Adams Glacier 2	CASCADE	Glacier	-120.40696	48.250517	2165
South Cascade Glacier 2	CASCADE	Glacier	-121.05492	48.362333	1829
Goat Rocks	CASCADE	Rock Glacier	-121.4535	46.539033	1994
Adams Rock Glacier 1	CASCADE	Rock Glacier	-121.55267	46.22709	1913
Adams Rock Glacier 2	CASCADE	Rock Glacier	-120.41256	48.256543	2042
North Cascades Rock Glacier 1	CASCADE	Rock Glacier	-120.41369	48.290884	2180
North Cascades Rock Glacier 2	CASCADE	Rock Glacier	-120.4130	48.2912	2179
North Cascades Rock Glacier 3	CASCADE	Rock Glacier	-121.5243	46.22534	2260
Arapaho Glacier	ROCKY	Glacier	-105.38166	40.0147	3496
Andrews Glacier	ROCKY	Glacier	-105.4088	40.17254	3410
Rawah Glacier	ROCKY	Glacier	-105.57286	40.401268	3312
Arapaho Rock Glacier	ROCKY	Rock Glacier	-105.3873	40.01374	3694
Louise Rock Glacier	ROCKY	Rock Glacier	-105.37304	40.30526	3371
Taylor Rock Glacier	ROCKY	Rock Glacier	-105.40133	40.164005	3327
Island Rock Glacier	ROCKY	Rock Glacier	-105.56336	40.374071	3274
Middle Palisade Glacier	SIERRA	Glacier	-118.45839	37.076582	3527
North Palisade Glacier	SIERRA	Glacier	-118.50649	37.111465	3602
Agassiz Rock Glacier	SIERRA	Rock Glacier	-118.51940	37.122169	3613
Teton Glacier	TETON	Glacier	-110.47383	43.44457	3162
Middle Teton Glacier	TETON	Glacier	-110.80264	43.73233	3266
Paintbrush Rock Glacier 1	TETON	Rock Glacier	-110.48214	43.46988	2996
Paintbrush Rock Glacier 2	TETON	Rock Glacier	-110.47844	43.47008	2860

Table 3: 2014 Site Surveys

SITE	RANGE	FEATURE TYPE	UTM_N	UTM_E	ELEV. (M)
ADAMS GLACIER	CASCADES	G	-121.524371	46.225340	2257
DILLER GLACIER	CASCADES	G	-121.763392	44.140898	2274
ELIOT GLACIER	CASCADES	G	-121.660903	45.394917	1891
LAVA GLACIER	CASCADES	G	-121.491400	46.232268	2400
PROUTY GLACIER	CASCADES	G	-121.758203	44.112986	2438
ADAMS ROCK GLACIER	CASCADES	RG	-121.552670	46.227090	1910
DILLER ROCK GLACIER	CASCADES	RG	-121.765737	44.145730	2321
PROUTY ROCK GLACIER	CASCADES	RG	-121.750503	44.106983	2442
ANDREWS GLACIER	ROCKIES	G	-105.680639	40.288370	3467
ARAPAHO GLACIER	ROCKIES	G	-105.646351	40.023378	3738
CONTINENTAL GLACIER	ROCKIES	G	-109.691389	43.000833	3450
ISABELLE GLACIER	ROCKIES	G	-105.640994	40.063373	3634
PECK GLACIER	ROCKIES	G	-105.663810	40.068332	3458
POWELL GLACIER	ROCKIES	G	-106.338675	39.762535	3819
ROWE GLACIER	ROCKIES	G	-105.645890	40.487127	3999
ST. VRAIN MAIN LOBE	ROCKIES	G	-105.667730	40.163962	3702
GORE GLACIER	ROCKIES	G	-106.332046	39.752469	3495
ARAPAHO ROCK GLACIER	ROCKIES	RG	-105.637699	40.022482	3581
CONFUSION ROCK GLACIER	ROCKIES	RG	-106.182873	39.445576	3562
DUCK LAKE ROCK GLACIER	ROCKIES	RG	-106.331853	39.759668	3706
GIBRALTAR ROCK GLACIER	ROCKIES	RG	-105.654799	40.155336	3463
LOUISE ROCK GLACIER	ROCKIES	RG	-105.625321	40.508941	3418
NAVAJO ROCK GLACIER	ROCKIES	RG	-105.636092	40.061200	3492
PECK ROCK GLACIER	ROCKIES	RG	-105.664310	40.071642	3271
POWELL ROCK GLACIER	ROCKIES	RG	-106.339080	39.764031	3770
ST. VRAIN EAST LOBE	ROCKIES	RG	-105.659327	40.162104	3549
TAYLOR ROCK GLACIER	ROCKIES	RG	-105.671197	40.275568	3417
BOLAM GLACIER	SIERRA	G	-122.204342	41.428681	3097
CONNESS GLACIER EAST	SIERRA	G	-119.313354	37.968609	3525
CONNESS GLACIER WEST	SIERRA	G	-119.318549	37.971285	3491
GOETHE GLACIER	SIERRA	G	-118.707668	37.210199	3667
BOLAM ROCK GLACIER	SIERRA	RG	-122.209437	41.429724	3006
GOETHE ROCK GLACIER	SIERRA	RG	-118.714092	37.220051	3596
MIDDLE PALISADE ROCK GLACIER	SIERRA	RG	-118.449419	37.084854	3342
NORTH LAKE ROCK GLACIER	SIERRA	RG	-118.620354	37.230261	2830

1.5 LABORATORY INCUBATIONS OF GLACIER AND ROCK GLACIER DOM

I addressed the differences in the bioavailability of carbon in the outflow of glaciers and rock glaciers using microbial assays (Hypothesis 2). Bioavailability of DOM was examined by measuring metabolic respiration (dissolved oxygen levels) in bottle

bioassays, of the same carbon concentration, using a common mixed microbial community. Bottle bioassay incubations were repeated for eight sites from the Rocky Mountains in Colorado. Four glaciers and four rock glaciers were sampled. The use of an established microbial community in the incubation, which is independent of the site, along with standardized carbon concentrations, worked as an analytical tool. It allowed us to assess lability independently of any differences that may have existed in the endemic microbial community at each site. It also controlled for any biological home-field advantage that may exist between site-specific DOM and microbial community. Removal of this variable allowed for a direct comparison of organic matter community composition and carbon bioavailability between glacial types.

1.6 CHAPTER DESCRIPTION

The two chapters of my research for the completion of my masters were independent, but closely related in the applicability of their results. Chapter 2, in press in the *Journal of Geophysical Research: Biogeoscience*, examines differences in the chemistry and bacterial communities present between glaciers and rock glaciers across the American West. We found differences in the temperature, chemistry, and biology of glaciers and rock glaciers. Some biogeochemical attributes we controlled by glacier type and others were more controlled by geographical and geological attributes. Chapter 3 builds on the results of chapter 2 by examining differences in the lability of dissolved organic matter pools between glaciers and rock glaciers on the Front Range of Colorado. We found differences in the lability of DOM between glacier types, and were able to attribute these to specific chemical compounds through the use of high-resolution gas chromatography mess spectrometry before and after microbial

incubation. Following the two chapters of the research for my masters is a section devoted to the implications of my work, and suggestions for the future directions research on the biogeochemistry of alpine glaciers and rock glaciers should take.

2. THE DIFFERING BIOGEOCHEMICAL AND MICROBIAL SIGNATURES OF GLACIERS AND ROCK GLACIERS

2.1 INTRODUCTION

Across the American West alpine glaciers and rock glaciers are contracting due to rising air temperatures [Diaz and Escheid 2007; McCabe and Fountain 2013]. The resulting changes in volume, timing, and chemistry of meltwater discharged by these features appears to be having significant effects on the adjacent alpine headwater ecosystems they feed [Battarbee et al. 2009; Bogdal et al. 2009]. For example, glacial derived dissolved organic matter (DOM) from ice can be an important source of chemical energy to headwater ecosystems that in some cases fuels heterotrophic respiration much further downstream [Hood et al. 2009, 2015; Singer et al. 2012]. In addition, it is clear that both glaciers and rock glaciers influence hydrographs and water temperatures of alpine streams [Fountain and Tangborn 1985*;* Cable et al. 2011; Dunnette et al. 2014; Millar et al. 2013]. The loss of these important ice features is homogenizing downstream temperature gradients, altering stream microbial community structure [Wilhelm et al. 2013]. Whereas both glaciers and rock glaciers are sources of seasonal meltwater, sediment, and solutes to headwater ecosystems [Baron et al. 2009; Saros et al. 2010; Singer et al. 2012; Thies et al. 2007], the differences between meltwater characteristics of each glacier type are poorly documented.

Alpine ice glaciers (hereafter simply identified as “glaciers”) are discriminated from rock glaciers primarily on the basis of surface appearance and estimated rock

content contained within the feature. Glaciers have surfaces of snow and ice and contain relatively low concentrations of rock debris; whereas rock glaciers have surfaces composed of rock debris whose internal structure may be composed of either rock debris with void spaces between the rocks filled with ice [Haeberli 1985] or bulk ice, like a glacier, mantled with a veneer (~> m thick) rock debris [Potter 1972]. This latter form is known as a debris-covered glacier. It is not possible to easily distinguish between a debris-covered glacier and a rock glacier [Clark et al. 1994] therefore here we refer to both as “rock glaciers”. Across the American West rock glaciers are far more common both in number and in geographic range than glaciers (Figure 1). There are approximately 8300 glaciers and perennial snowfields in the United States, of which about 2000 are considered to be glaciers [Fountain et al. 2007]. In comparison the continental United States contains more than 10,000 identified rock glaciers [A. Fountain per. comm.]. Glaciers, however, have received far more attention than rock glaciers, largely due to their ease of visual identification both in the field and remotely.

The geomorphological characteristics between glaciers and rock glaciers are likely to strongly influence their meltwater characteristics [Mattson 2000; Williams et al. 2006]. For example, the continuous talus surface of rock glaciers thermally insulates internal ice (reducing melt) and provides a vapor pressure gradient barrier to sublimation [Janke 2007]. Consequently, daily runoff from rock glaciers is not flashy compared to glaciers. As such, rock glaciers have slower recession rates than glaciers, with the potential to affect headwater biogeochemistry further into the future than glaciers [Millar and Westfall 2013; Woo 2012]. Given the much greater fraction of rock within rock glaciers compared to glaciers, far more mineral surface area is in contact

with ice and undergoing active chemical weathering [Illyashuk et al. 2014]. Relative to glaciers, these greater rock glacier meltwater solute concentrations can more readily alter community assemblages of primary producers [Ilyashuk et al. 2014; Thies et al. 2013]. Nutrient release can also be higher from rock glaciers than glaciers [Williams et al. 2007]. Additionally, rock glaciers can change the characteristics and biological processing of carbon compounds entering alpine watersheds [Williams et al. 2006].

Here we compare physical, chemical, and microbiological characteristics between glacier and rock glacier meltwaters collected from three mountain ranges of the American West. We asked whether meltwater chemistry and microbiology differed between glaciers and rock glaciers. We also asked if there were characteristic differences in glacier and rock glacier meltwater among mountain ranges.

2.2 METHODS

We conducted a survey of glacier and rock glacier meltwater streams drawn from three geographically distinct alpine regions of the American West (the volcanoes of the Cascade Range of Washington, Oregon and northern California, the Rocky Mountains of Colorado and Wyoming, and the Sierra Nevada of southern California) (Figure 3). We selected our sample sites to be representative of mountain ranges with different geologies and climates, and of both types of glaciers. In total, 25 glaciers and 24 rock glaciers were sampled, during the summers of 2012, 2013, and 2014 (Figure 4).

2.2.1 REGIONAL FEATURE DESCRIPTIONS

Cascade Mountain features were characterized by relatively low mean elevations (2563 ± 503 m) and low mean slopes (23.8° ± 5.4°), and were predominantly underlain by volcanic geology. Rocky Mountain features sampled were characterized by relatively

high mean elevations (3678 ± 223 m) on steep mean slopes (34.4° ± 7.5°), and underlain by both plutonic and metamorphic geology. Sierra Nevada features sampled were characterized by relatively high mean elevations (3679 ± 193 m) on steep mean slopes (30.9° ± 3.8°), and were predominantly underlain by granite. Detailed topographic characteristics, including contributing drainage area, aspect and relief, for each alpine region sampled are provided as Supplemental Information.

The three mountain ranges have different climates. Climatic data were drawn from PRISM modeled 1981–2010 mean atmospheric conditions [PRISM Climate Group, 2015]. Cascade Mountain sites have relatively higher mean annual precipitation (2675 ± 588 mm, ≈ 58% as snow) and mean annual air temperatures (−0.2 ± 2.1 °C). Rocky Mountain sites are relatively drier and colder, with mean annual precipitation of 1237 ± 331 mm (≈ 49% as snow) and mean annual air temperatures of −2.2 ± 1.1 °C. Sierra Nevada sites are also dry and cold, with mean annual precipitation of 1092 ± 229 mm (≈ 57% ± 22% as snow) and mean annual air temperatures of −0.5 ± 1.2 °C. Wet atmospheric deposition data, taken from the National Atmospheric Deposition Program show that Rocky Mountain sites receive greater inorganic reactive nitrogen (N) deposition than the other two regions, with the Colorado Front Range reporting the greatest N deposition of approximately 3.0 kg N ha^{-1} yr^{-1} (Table 1) [NADP 2015 http://nadp.isws.illinois.edu/data/].

2.2.2 GLACIER AND ROCK GLACIER DESCRIPTIONS

We visited the 25 glacier and 24 rock glaciers more than once, so in all we collected 37 glacier meltwater samples (Cascade Mountains n=12, Rocky Mountains n=20, Sierra Nevada = 5) and 33 rock glacier meltwater samples (Cascade Mountains

n=9, Rocky Mountains n=20, Sierra Nevada n=4). Glaciers and rock glaciers were selected based on proximity to each other, forming pairs within a similar geographic setting. PRISM 1981-2010 model output suggested glaciers sampled were quite comparable (Table 4) [PRISM Climate Group 2015]. Metamorphic geology underlays 29% of our sites, plutonic geology 49% of our sites and volcanic geology 22% of our sites (Supplemental Information).

2.2.3 SAMPLE COLLECTION METHODS

Samples were collected from outflow streams as close to the glacier or rock glacier terminus as possible. This ranged from immediately below the ice to up to 10 meters away. Each sample was collected in late summer (August–September, 2012–2014) to capture the greatest contribution of ice melt and least amount of seasonal snowmelt. Meltwater temperature and specific conductance were measured *in situ* with a hand-held probe (Thermo Scientific Orion 3-Star). Water and stream sediment samples from terminus outflow of each glacier or rock glacier feature were collected according to standard methods (http://www.nrel.colostate.edu/projects/lvws/pages/accesstodata/fieldlabmethods.html). Samples for pH, reactive nitrogen (NH_4^+ and NO_3^-), metal cation concentrations, and SiO_2 were collected in acid-washed Nalgene® HDPE plastic bottles, after rinsing three times with sample water. Samples collected for carbon and DOM measurement and total dissolved nitrogen (TDN) were collected in glass borosilicate bottles, sterilized in a muffle furnace (900 °C for 6 hours). Sediment samples collected for microbial analyses were collected in sterilized 60 mL HDPE plastic centrifuge tubes *in situ*, and then subsampled into 5 mL cryotubes within 6 hours of collection.

Samples for reactive nitrogen, pH, and metals were filtered (0.2 μm Millipore filter) within 24 hours of collection. Samples for carbon chemistry and TDN were filtered (Whatman GF/F) then acidified to ≈ pH 3 within 24 hours of collection. Samples collected for fluorescence analysis were not acidified. Immediately after being subsampled, cryotube samples for microbial community analysis were flash-frozen in liquid nitrogen to preserve the integrity of the nucleic acids.

2.2.4 LABORATORY ANALYSIS

We measured pH with a Radiometer Copenhagen TTT85 Titrator. Metals and other ions derived from weathering were measured using inductively coupled plasma optical emission spectrometry (ICP-OES) at the Environmental Sciences Research Laboratory (ESRL) at University of California, Riverside. Dissolved silica (SiO_2), ammonium (NH_4^+), nitrate (NO_3^-), total inorganic nitrogen (TIN), total dissolved nitrogen (TDN), and dissolved organic carbon (DOC) were analyzed using standard methods at the EcoCore facility at Colorado State University. Fluorescence and UV scans were completed for estimates of humification index (HIX), specific ultraviolet absorption at 254 nm (SUVA254), fluorescence index (FI), and freshness index (β:α). Humification Index (HIX) serves as an indicator of the humicity of organic matter [Zsolnay et al. 1999], and SUVA254 as an indicator of aromaticity [Weishaar et al. 2003]. Combined, HIX and SUVA 254 values allow us to estimate DOM complexity. Fluorescence Index (FI) is an indicator of proteineitity [McKnight et al. 2001; Cory and McKnight 2005], and indicative of the level of microbial processing in DOM. Freshness index (β:α) is an indicator of freshness of organic matter [Parlanti et al. 2000]. Fluorescence samples were analyzed on a Horiba Scientific Aqualog.

3.2.5 MICROBIAL ANALYSIS

Samples for microbial community analysis were collected from sediments fed by meltwaters at the terminus of the glacier and rock glacier for 23 sites in 2012 and 2013. PCR amplification was performed for each DNA sample in triplicate and pooled. To facilitate multiplexed sequencing, barcoded primers with Illumina adapters and linkers were used to amplify the V4 region of bacterial 16S rRNA genes [Caporaso et al. 2011; Caporaso et al. 2012]. PCR reactions were performed with KAPA2G Fast HotStart ReadyMix (KapaBiosystems, Wilmington, MA, USA). Negative controls were included to test for contamination. Amplicon concentrations were measured with a PicoGreen dsDNA assay (Life Technologies, Grand Island, NY, USA). The amplicons were cleaned with the UltraClean PCR Clean-Up Kit (MoBio Laboratories Inc., Carlsbad, CA), and sequenced on an Illumina MiSeq platform at Michigan State University. Sequences were demultiplexed, and forward and reverse 16S rRNA gene reads were merged.

3.2.6 DATA ANALYSIS

Data were analyzed using the R programming language, with the t.test and lmfit functions with parameters set for non-parametric Welch-Satterthwaite test and ANOVA test, respectively. Plot function and ggplot2 package were used for figures. Humification Index (HIX) was calculated as cumulative area under 435–480 nm emission at 254 nm excitation divided by cumulative area under 300–345 nm at 254 nm excitation. Specific ultra violet absorption at 254 nm (SUVA254) was calculated as UV absorbance at 254 nm divided by measured DOC concentration (mg L^{-1}). Fluorescence Index (FI) was calculated as emission at 470 nm divided by emission at 520 nm, both at 370 nm excitation. Freshness Index (β:α) was calculated as intensity of emission at 380 nm and

310 nm excitation divided by maximum intensity of emission between 420–435nm at 310 nm excitation. Microbial 16S sequences were analyzed using the Mothur program [June 2015; Kozich et al. 2013]. Sequences were unified, made unique, aligned, filtered, removed of chimeras, filtered, and assigned Operational Taxonomic Units (OTUs) using the MiSeq SOP [June, 2015; Kozich et al. 2013]. Bacterial taxa were assigned to OTUs using the Silva Comprehensive Ribosomal RNA Database (www.arb-silva.de). Samples were not rarefied. Alpha and beta diversity were estimated through rarefaction plots created in R.

2.3 RESULTS

2.3.1 DIFFERENCES IN GLACIER TYPE

Water samples from glaciers and rock glaciers differed significantly in physical and chemical characteristics. Across all three mountain ranges, rock glacier meltwaters had higher temperatures, pH, and conductivity than glacier meltwaters (Figure 5a-c). Rock glacier meltwaters were also enriched in a range of weathering products including SiO_2, Ca^{2+}, K^{+}, Mg^{2+}, and Sr^{2+}, but depleted in Fe^{3}, and Mn^{2+} relative to glaciers (Table 5). In addition, NO_3^{-} concentrations, TIN, and TDN, were significantly higher in meltwater samples from rock glaciers than glaciers. However, NH_4^{+} concentrations were more enriched in glacier meltwaters than rock glacier meltwaters (Figure 5d-f).

We evaluated differences in organic chemistry characteristics of the meltwaters. We found no significant difference in DOC concentrations between glacier and rock glacier meltwaters but clear differences in composition of fluorescing dissolved organic matter (FDOM) between glacier types (Table 6). Humification index (HIX) was twice as high, on average, in the meltwaters from rock glaciers than glaciers, consistent with

more complex, humic-like carbon being released from rock glaciers (Table 6). However, there was no clear difference in fluorescence index (FI) or freshness index (β:α) between glacier meltwater types (Table 6). Average FI for all samples combined (1.6 ± 0.15) suggested that most DOM from both glacier meltwater types was of microbial rather than terrestrial plant origin.

Evaluation of the 16S sequences showed clear differences in the bacterial communities between glacier and rock glacier stream sediments. The microbial communities sampled from rock glacier stream sediments had higher α-diversity (within sample diversity) compared to samples derived from glacial stream sediments (Figure 6a). Rock glacier stream sediments also had higher richness in microbial communities, with a total of 4,408 more unique operational taxonomic units (OTUs) unique to all rock glacier stream sediments than those found in all glacier stream sediment communities (Figure 6b). Whereas there were a considerable number of shared OTUs (7673) between glacial stream sediment types, there were also a large number of OTUs that were unique to each glacial stream sediment type with variability between sites as large as variability between glacier and rock glacier stream sediments.

The most common bacterial taxa present in both glacier and rock glacier sites were also the most abundant taxa within each sample. The most abundant genus, seen in all samples, was the psychrophile, *Polaromonas sp.* Also present in all samples were the nitrite-oxidizers *Nitrospira* sp. and the psychrophiles *Hymenobacter sp*., *Deinococcus sp.* and *Sulfuricurvum sp. Sulfuricurvum*, a sulfur-oxidizer previously found in glacial-fed meltwaters of the European Alps was also present in our glacier stream sediments, but not rock glacier stream sediments [Wilhelm et al. 2014]. Rock glacier

stream sediments had many more unique and identifiable genera compared to glacier stream sediments, including many genera that are noted to be tolerant of warmer temperatures and common to soil microbial communities, including *Anaerolineacea sp., Bryobacter sp.*, *Gemmatimonas sp.*, *Planctomycetaceae sp.*, *Sphingomodales sp.* and *Terrabacter sp..* Identifiable genera associated with rock glaciers were also more diverse than those associated with glaciers, while many of the OTUs endemic to the glacier sites did not have identified species within the Silva reference database.

2.3.2 REGIONAL DIFFERENCES

Beyond the difference in characteristics between glacier types our analyses identified characteristics that appeared to be primarily influenced by geography. Meltwaters from Rocky Mountain rock glaciers were warmer than rock glacier meltwaters from the Sierra Nevada or Cascade Mountains (Figure 5a). Conductivities were higher in the Cascade Mountains compared to the other mountain ranges, though the greatest difference in conductivity between glacier meltwaters (11 μS cm^{-1}) and rock glacier meltwaters (37 μS cm^{-1}) was found in Rocky Mountain sites. Differences in metals varied with mountain range and appeared to be related to parent material and bedrock geology (Table 5). Rocky Mountain glacier and rock glacier meltwaters had higher NO_3^- concentrations (1.17 ± 1.03 mg L^{-1}) than Cascade Mountain or Sierra Nevada features (0.16 ± 0.19 mg L^{-1} and 0.61 ± 0.51 mg L^{-1}, respectively) (Figure 5e). Similarly, NH_4^+ concentrations were higher in the Rocky Mountain glacier sites (0.16 ± 0.07 mg L^{-1}) than both other mountain ranges. As stated above, there was no significant difference in DOC concentrations between mountain ranges, however the fluorescence results suggested more DOM of microbial origin in the meltwaters of the Cascade

Mountains and Sierra Nevada compared to the Rocky Mountains (Table 6). The Cascade Mountains had a higher mean β:α ratio than both the Sierra Nevada and Rocky Mountains, indicative of “fresher” or less processed carbon being released from the glaciers and rock glaciers of the Cascade Mountains. SUVA254 was lower in the Rocky Mountains than Sierra Nevada and Cascade Mountain sites; meaning carbon from glacier and rock glacier effluent in the Rocky Mountains has lower aromaticity than that from the Cascade Mountains and Sierra Nevada (Table 6). The humification index (HIX) was nearly three times higher in rock glacier meltwaters of the Cascade Mountains and the Rocky Mountains than ice glacier meltwaters, suggestive of higher humicity and allochthonous sources of DOM in rock glacier effluent in these two mountain ranges.

We also found pronounced differences in microbial communities among mountain ranges. The microbial communities sampled in the Rocky Mountains had the highest α-diversity of any region (Figure 6a), with microbial community α-diversity being the lowest in the Sierra Nevada. Differences in microbial community α-diversity were significant for rock glacier samples in both the Sierra Nevada and Cascade Mountains while differences were more variable for microbial communities sampled in from the Rocky Mountains (Figure 6a). The Rocky Mountains also had the greatest richness in sediments fed by meltwaters, with 12,906 OTUs in total, 6643 of which were unique to the range (Figure 6c). The Sierra Nevada was the least diverse, with 1354 OTUs, only 113 (8%) of which were unique. Sierra Nevada sites shared very few OTUs with each of the other ranges individually, with only 30 OTUs shared between the Sierra Nevada and the Cascade Mountains, and 113 OTUs shared with only the Rocky Mountains. The

lower richness of the Sierra Nevada sites may partly be due to the smaller number of samples collected for the Sierra Nevada compared to the Cascade Mountains or Rocky Mountains, though individual site richness was much lower for each of the Sierra Nevada samples compared to all other individual samples from the other two mountain ranges (Figure 6a). The Cascade Mountains were intermediary in their microbial diversity, 9291 total OTUs, 3182 (34%) of which were unique. The Cascade Mountains also shared over 50% of their OTU diversity (5061 OTUs) with the Rocky Mountains (Figure 6c).

The most abundant bacterial taxa present in all ranges were the same taxa that were common between feature types, including *Gemmatimonas sp., Hymenobacter sp., Intrasporangiaceae sp.* and *Polaromonas sp.* Many unclassifiable gammaproteobacteria were shared by only the Cascade Mountains and the Rocky Mountains. *Flavobacterium* were exclusive to the Cascade Mountains, along with many *Acidthiobacillus* taxa, known for their metal oxidizing life strategies and tolerance of low pH environments. *Burkholderiales sp.* and *Terrabacter sp.*, and *Thiobacillus sp.* were the most abundant microbes exclusive to the Rocky Mountains. Nearly all the abundant taxa exclusive to the Sierra Nevada were unclassified.

2.4 DISCUSSION

Glaciers and rock glaciers sit at the interface of atmospheric and terrestrial environments [Slemmons et al. 2013]. They integrate atmospherically deposited chemicals and weathering products, process reactive compounds through biotic and abiotic pathways, and then release the altered solutes to alpine headwaters. Our results suggest that glacier type dictates both concentration of the weathering products

released and the complexity of organic matter exported via meltwaters (Table 5,6), while geographic region dictates the rock type that is weathered (and thus kind of weathering products released), the rate and intensity of weathering, and the compounds that are atmospherically deposited (Figure 5b, Table 4,5). The result is that some characteristics (e.g. temperature, weathering products, complexity of DOM) appear to be driven primarily by glacier type (i.e. rock or ice glacier) while other characteristics (e.g. NH_4^+, NO_3^-, microbial processing of DOM) appear to be more influenced by geographic characteristics.

Our survey suggests that specific characteristics of each mountain range control the amount of weathering products delivered to headwater ecosystems. For example, we found diminished differences between the weathering products of glacier and rock glacier meltwaters in the Cascade Mountains relative to the Sierra Nevada and Rocky Mountains. In contrast to the continental glaciers of the Rocky Mountains and Sierra Nevada, glaciers of the Cascade Mountains are maritime glaciers. As such, they sit at lower elevations, receive greater amounts of precipitation, and are volumetrically larger than other alpine ice features in the continental United States (Table 4, Supplemental Information). Glaciers in the Cascade Mountains are likely to have much higher subglacial mechanical and chemical weathering rates than other glaciers of the American West because of more persistent precipitation throughputs. Enhanced microbial respiration due to increased delivery of redox pairs in the zone of basal melting would increase CO_2 concentrations in the water, further increasing mineral dissolution through the production of carbonic acid [Montross 2013]. The effects of this increased carbonic acid production would be further exaggerated in the Cascade

Mountains, as the basaltic mineral complexes of the parent material are more readily weathered than the granitic bedrock of the Sierra Nevada and Rocky Mountains thus less likely to have pronounced differences in meltwater chemistry between glacier types.

Similarly, our results show that N concentrations in both glacial and rock glacial meltwaters appear to reflect regional atmospheric N deposition. The Rocky Mountains had NO_3^- and NH_4^+ concentrations twice as high in both glacier types relative to meltwaters from the other mountain ranges (Figure 5a-b). This is consistent with elevated N concentrations previously observed in surface waters of Rocky Mountain watersheds fed by glaciers [Baron et al. 2009; Saros et al. 2010; Williams et al. 2007](Supplemental Information). The Colorado Front Range, in particular, is a hotspot of N deposition due to the combination of wind patterns and concentrated human settlement and agricultural activity directly to the east [Baron et al. 2000].

Glaciers in the western United States act as delayed source of reactive N and other pollutants, effectively increasing the lag time between anthropogenic stressors (atmospheric deposition) and impact on the ecosystem. Therefore, even with recent reductions of anthropogenic N pollution, there may be a delayed response in the reduction of N concentrations and ecosystem recovery in alpine headwaters [Mast et al. 2014]. Whether the reactive N seen in meltwaters is of recent atmospheric origin prior to *in situ* biological processing remains unknown. However, distillation through evaporation and sublimation on the glacial surface could concentrate atmospherically sourced compounds to enhance microbial activity during base flow conditions or “hot moments” [Battin et al. 2004], periods when hydrological connectivity and temperature are at

optimal levels for biological processing of N and organic matter. It also appears that reactive N in the Rocky Mountains is not entering the production of organic matter within glaciers, as our results show lower fluorescence indices values of glaciers and rock glaciers in the Rocky Mountain sites compared to the Cascade Mountains and Sierra Nevada (Table 6). These lower values suggest lower N concentrations in the DOM of glacier meltwaters. This is consistent with less tight cycling of organic nitrogen, and may be further evidence of an N threshold being reached in the Rockies [Baron et al. 2000], as nitrogen is not being as tightly assimilated into biological DOM. This same phenomena of increasing temporal lag between atmospheric inputs and release to headwaters has been noted in other glaciated ranges including the Kenai, Chugach and Coast Mountains of Southeast Alaska (organic matter) [Hood et al. 2009], and Swiss Alps (pesticides) [Schmid et al. 2010].

Previous research has shown small glaciers contribute a disproportionate amount of DOM for their size, and fuel heterotrophic metabolism at great distances downstream [Hood et al. 2015]. The DOM values we observed for glaciers and rock glaciers were low, but similar to concentrations reported from large maritime glaciers [Hood et al. 2009]. Differences in the structure of organic matter released from glaciers and rock glaciers, as seen in our study (Table 6), could cause differences in alpine ecosystem activity through preferential lability of compounds specific to a glacial type. Previous research on glacial DOM from Southeast Alaska suggests glacier DOM is highly labile and fuels bacterial metabolism in neighboring waters [Hood et al. 2009], but the lability of rock glacier DOM remains unknown. Our results show rock glaciers had higher humification, or complexity, of organic matter than glaciers. This suggests that

rock glacial DOM is likely less labile than that of glaciers for two principal reasons. First, there is likely quantitatively more (and more diverse) biological activity occurring within the pore spaces, and stream sediments of the rock glacier, producing a broader range of more complex and recalcitrant DOM compounds than glaciers. Second, meltwaters of rock glaciers have greater amounts of complex organic compounds compared to glaciers due to leaching materials percolating through the rock-ice matrix and into meltwaters (Table 6). Both result in production of more complex metabolites in rock glacier meltwaters compared to glacier meltwaters. These hypotheses are consistent with our analyses of the bacterial communities associated with each glacial type, as we saw higher microbial diversity and DOM complexity in rock glacier stream sediments compared to glacier stream sediments (Table 6, Figure 6). Further research should use more descriptive methods of organic matter characterization (e.g. mass spectrometry), along with direct evaluation of DOM lability to evaluate differences in lability of DOM and biological processing between meltwaters of different glacier types.

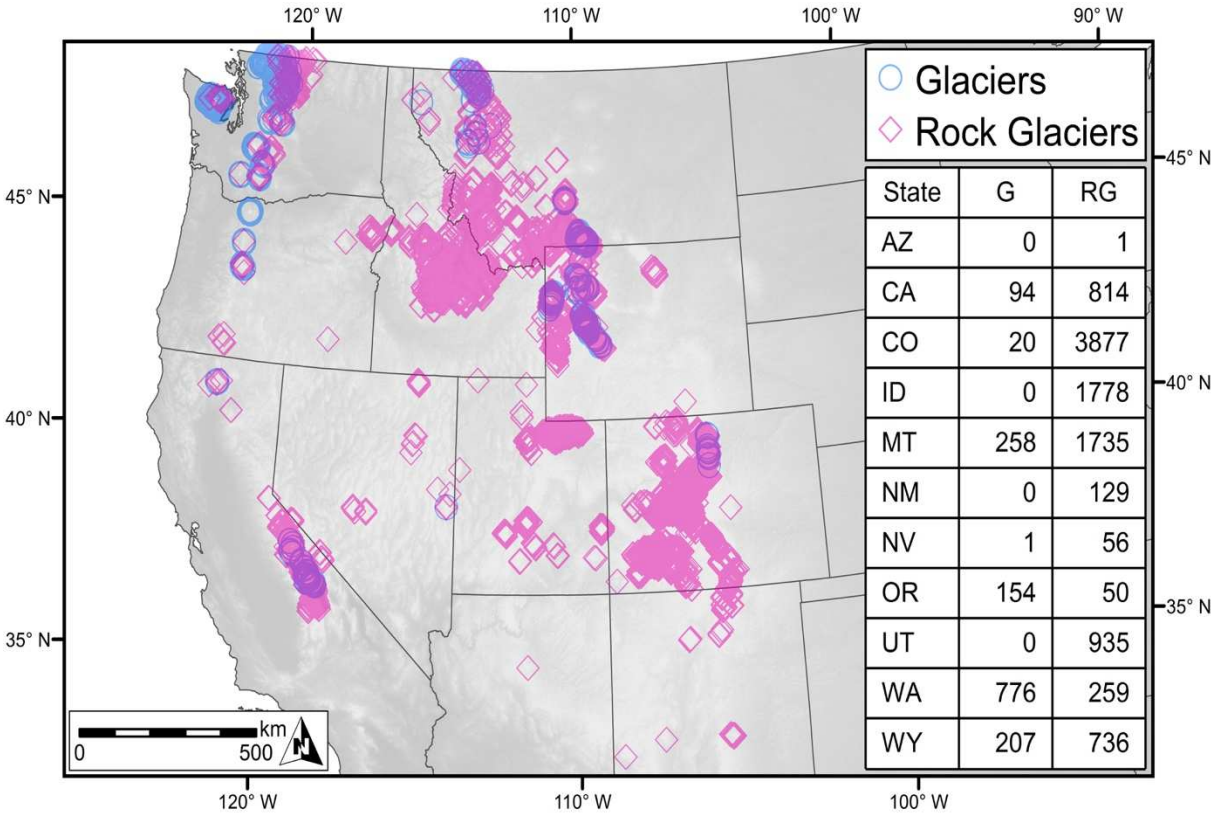
In this study the sediment-rich rock glacial environment supported more abundant and diverse microbial communities than those of glaciers (Figure 6a-c), This is consistent with a known positive relationship between size and diversity of the microbial population present and amount of sediment in the subglacial environment [Sharp et al. 1999]. Significantly, warmer temperatures in rock glacier effluent compared to that glaciers also likely reduced selective pressure for psychrophiles, and supported a more rich and diverse bacterial community (Figure 5, 6a-c). Taxa only found in rock glaciers also had more bacterial species in common with known soil microbes indicating more commonly and cosmopolitan microbial community. Biological diversity between

glaciers and rock glaciers at higher trophic levels should be examined, as low temperatures and increased sediment loads have been correlated with lower diversity of invertebrates in meltwater fed streams [Milner et al. 2009]. Subglacial environments are biologically active [Simon et al. 2009; Wilhelm et al. 2013, 2014], and our work shows that alpine glaciers and rock glaciers in the American West contribute biologically significant additions to alpine ecosystems. The commonality of *Polaromonas sp.* between all sites in our study, as well as cyrospheric ecosystems globally, suggests the *Polaramonas sp.* is common to many cold environments [Darcy et al. 2011; Margesin et al. 2012; Wilhelm et al. 2014]. However, with abundant unclassified taxa exclusive to meltwater fed glacial sediments, glaciers may represent areas of diversity and biological processing not shared by rock glaciers. This is supported by other studies that showed rare taxa in exclusively glacially fed streams to be disproportionately active [Wilhelm et al. 2014]. These unique microbial communities may be lost with the ongoing retreat of alpine glacial ice driven by climate change and may prove a ripe ground for discovery of novel bacterial taxa and unique metabolic pathways.

Over the coming century the differences in headwater characteristics between glaciers and rock glaciers will become more similar along with the glaciers themselves [Clarke et al. 2015; Radic et al. 2014]. Rock glaciers are predicted to linger longer than alpine glaciers, but eventually even they will likely be lost**.** Continued ablation of ice can turn some glaciers into rock glaciers [Outcalt and Benedict 1965; White 1971; Krainer and Mostler 2000]. For these cases, we can apply a space-for-time substitution by comparing differences between glaciers and rock glaciers within each range. This substitution allows for examination of potential future scenarios for presently glacial-fed

headwater ecosystems experiencing warming alpine climates. During the current stage of global glacial recession, the higher geochemical and microbial contributions rock glaciers compared to glaciers suggest that rock glaciers will have a pronounced impact on the biogeochemical processes of many alpine headwaters.

The results presented here combined with previous research suggest that rock glacier meltwaters may be representative of what future biogeochemical inputs will be in currently ice-glaciated watersheds. With increasing air temperatures, the elevated biogeochemical and microbial characteristics of rock glaciers compared to glaciers will likely dominate meltwaters that reach sensitive headwater ecosystems. Further, some glaciers are likely to become more rock glacier-like in the biogeochemistry of their meltwaters and increase the biogeochemical signal of rock glaciers on the alpine headwaters they feed. Our results suggest that both feature specific and range specific biogeochemical characteristic may place bottom up controls on ecosystem function. Understanding which biogeochemical characteristics will be a function of glacier type and which will be driven by region allows for better implementation of management strategies to protect and adapt to these changing headwater ecosystems.



State	G	RG
AZ	0	1
CA	94	814
CO	20	3877
ID	0	1778
MT	258	1735
NM	0	129
NV	1	56
OR	154	50
UT	0	935
WA	776	259
WY	207	736

Figure 3: Glacier and Rock Glacier Distribution Map. Locations of contiguous US glaciers and perennial ice features drawn from the Randolph Glacier Inventory and rock glaciers drawn from the Fountain Rock Glacier Inventory. Approximately 1500 glacial and perennial ice features are identified, yet >90% of them are clustered in just four states. Conversely, over 10,000 rock glaciers are identified and distributed across a broader geographic range.

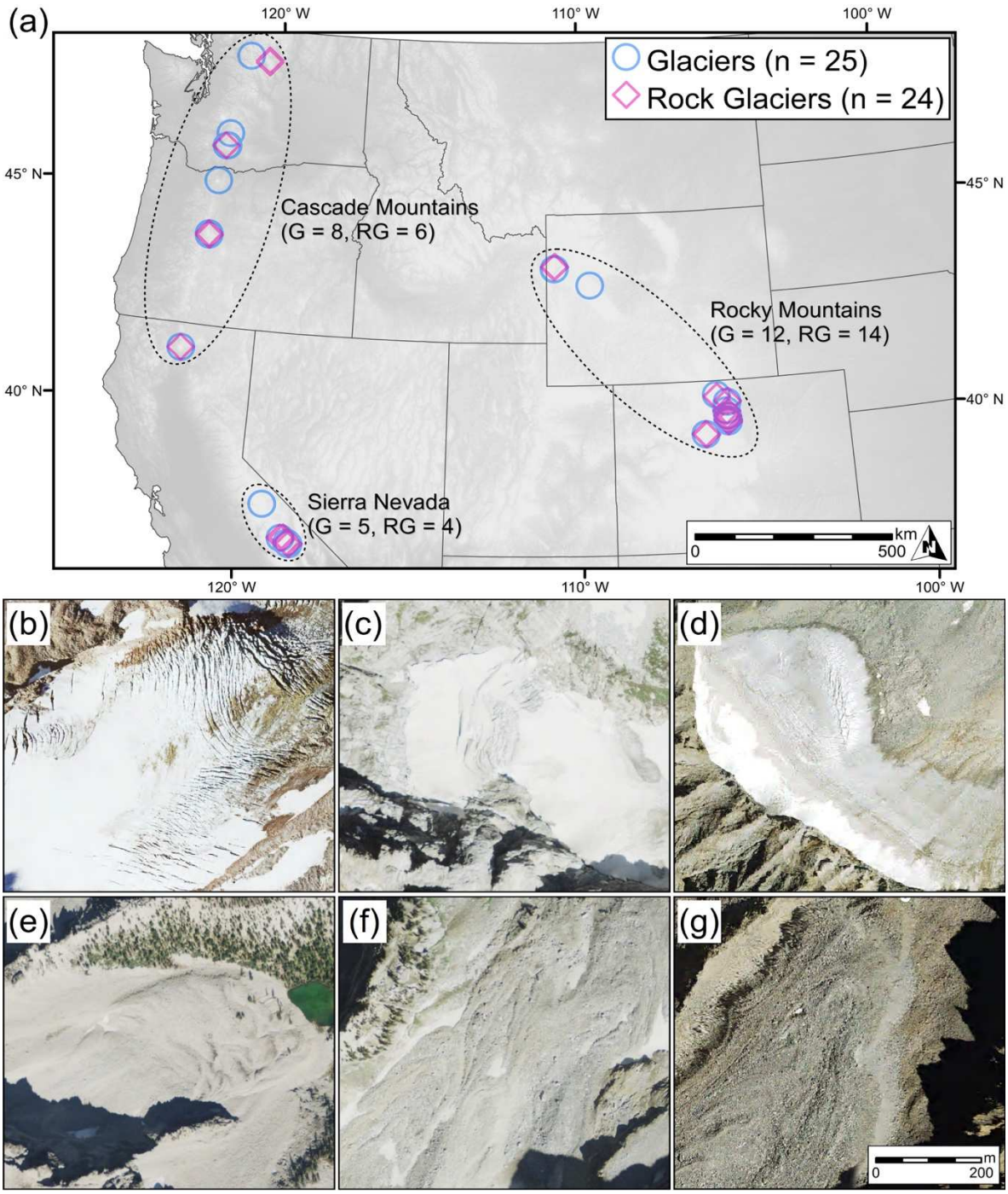


Figure 4: Sample Site Location Map with Examples. Sample site locations (a) and examples of representative features from each of the three mountain ranges (b-g). Eliot Glacier (b) and North Cascade Rock Glacier (e) are Cascade Mountain sites, Teton Glacier (c) and Paintbrush Rock Glacier 3 are Rocky Mountain sites, and Middle Palisade Glacier (d) and Agassiz Rock Glacier (g) are Sierra Nevada sites.

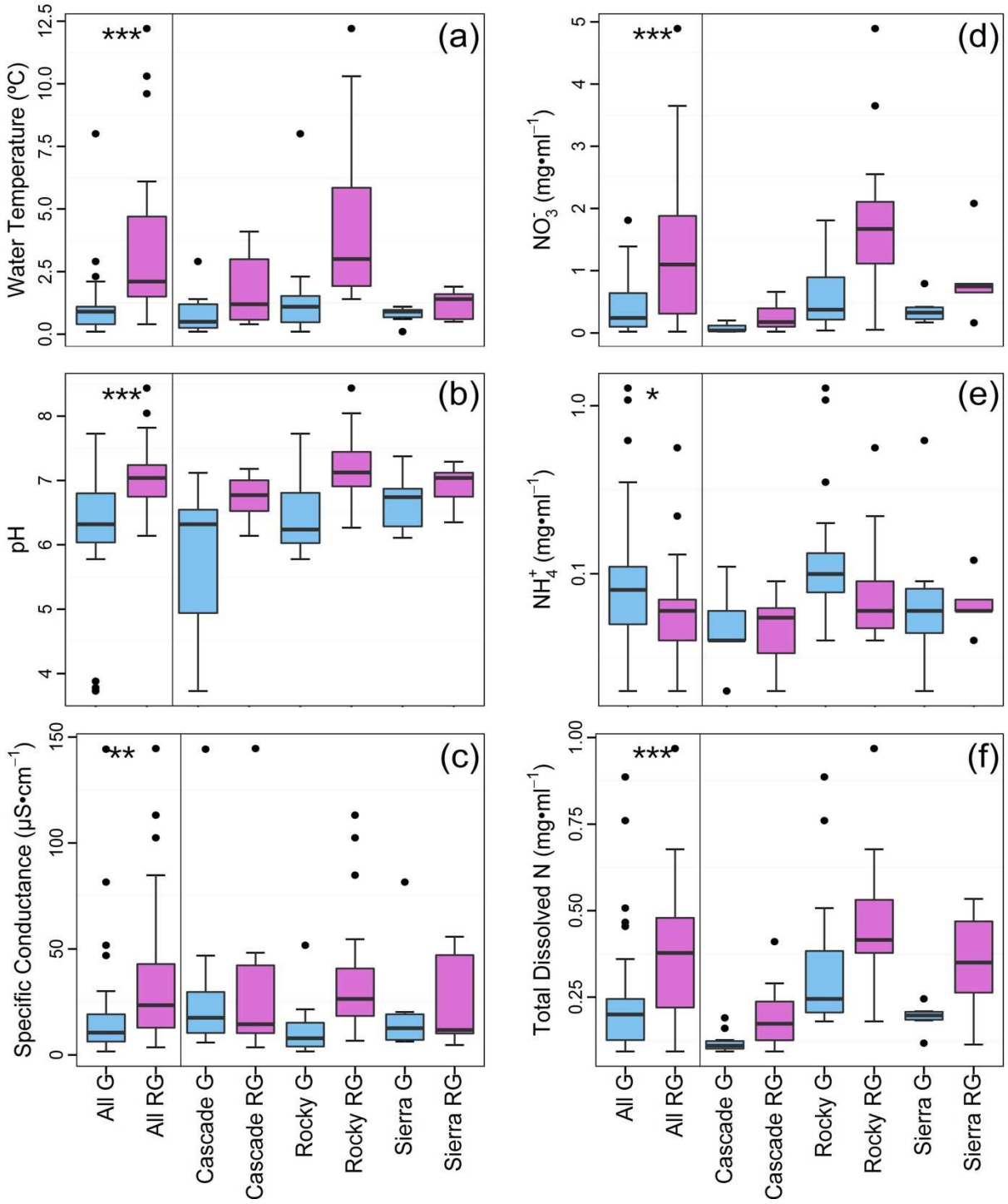


Figure 5: Physical and chemical measurements for glaciers and rock glaciers by mountain range. Glaciers are blue boxes, rock glaciers are pink boxes. Boxes represent upper and lower quartiles, whiskers indicate range of measurement, points indicate outliers, and bold bars indicate sample mean. * Indicates significance at $p<0.05$, ** at $p<0.01$, and *** at $p<0.001$ using Welch-Satterthwaite T-Test for nonparametric sample sets.

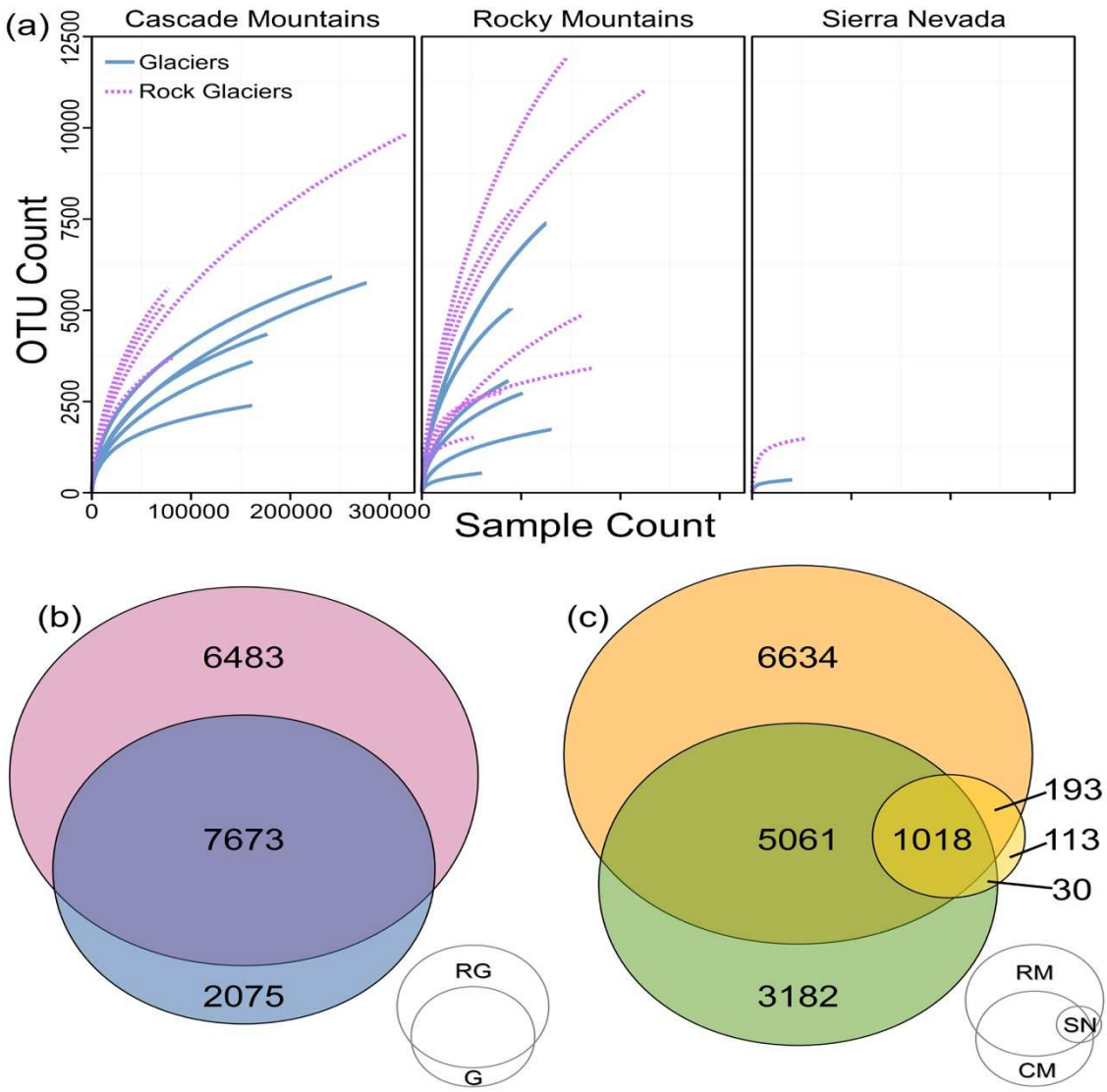


Figure 6: a. Rarefaction curves as an estimate of α-diversity for microbial communities sampled at the base of glaciers and rock glaciers in each of the surveyed mountain ranges. For each range, individual rock glaciers had higher microbial α- diversity than ice glaciers. Rock glaciers also had greater overall microbial richness (overall number of OTUs) at the measured sampling depth of each sample. Venn Diagrams showing overlap in membership between microbial communities sampled from b) glaciers and rock glaciers (labeled G and RG), and c) among Mountain Ranges (Cascade Mountains = CM, Rocky Mountains = RM, and Sierra Nevada = SN). All numbers are representative of Operational Taxonomic Units (OTUs) that are novel to their respective feature or area, or are common between overlapping spheres. Rock Glaciers had a greater number of unique OTUs, however there were a large number of cosmopolitan OTUs between feature types. The Rockies had the greatest number of OTUs, and shared the most OTUs with the Cascades. The Sierra Nevada had the fewest OTUs, and the majority were shared between all three mountain ranges.

Table 4: Site description, precipitation, and atmospherically deposited N for all sites sampled in our 2012-2014 survey.

Site	Mountain Range	Sample Coordinates	Sample Elevation (m)	Contributing Drainage Area (km^2)	Air Temperature (°C)	Precipitation (mm)	Precipitation As Snow (%)	Wet NO_3^- Deposition ($kg \cdot ha^{-1}$)	Wet NH_4^+ Deposition ($kg \cdot ha^{-1}$)
Individual Glaciers									
Adams Glacier	Cascade	46.225340°,-121.524370°	2257	2.29	-3.5	2547	77	5.64	5.22
Bolam Glacier	Cascade	41.428681°,-122.204342°	3100	1.03	-3.8	2208	83	4.29	2.57
Diller Glacier	Cascade	44.140898°,-121.763392°	2274	0.40	1.2	3098	43	3.30	4.06
Eliot Glacier	Cascade	45.394917°,-121.660903°	1890	2.70	-0.2	3652	67	8.70	8.33
Lava Glacier	Cascade	46.232268°,-121.491400°	2399	0.41	-1.2	2613	69	5.30	4.90
McCall Glacier	Cascade	46.519019°,-121.450510°	2053	0.25	0.9	2090	59	4.49	4.17
Prouty Glacier	Cascade	44.112986°,-121.758203°	2436	0.42	0.9	3432	31	3.29	4.03
South Cascade Glacier	Cascade	48.362333°,-121.054929°	1826	2.12	1.3	2791	57	6.23	4.73
Andrews Glacier	Rocky	40.288579°,-105.680264°	3462	0.32	-2.2	1183	48	10.30	5.71
Arapaho Glacier	Rocky	40.023378°,-105.646351°	3737	0.24	-3.5	1134	48	9.32	4.69
Continental Glacier	Rocky	43.341513°,-109.689746°	3682	1.96	-4.9	1045	54	5.46	3.50
Gore Glacier	Rocky	39.752469°,-106.332046°	3496	0.22	-1.7	883	45	6.88	3.83
Isabelle Glacier	Rocky	40.063373°,-105.640994°	3634	0.36	-2.7	1185	48	9.13	4.62
Middle Teton Glacier	Rocky	43.732330°,-110.802640°	3271	0.63	-3.1	2430	61	10.07	6.44
Peck Glacier	Rocky	40.068332°,-105.663810°	3461	0.19	-1.2	1129	45	8.99	4.57
Powell Glacier	Rocky	39.762535°,-106.338675°	3817	0.03	-2.9	911	44	6.86	3.82
Rawah Glacier	Rocky	40.670189°,-105.957956°	3499	0.19	-2.1	1144	47	8.32	4.71
Rowe Glacier	Rocky	40.487127°,-105.645890°	3999	0.02	-4.0	1282	62	7.49	3.94
Saint Vrain Glacier	Rocky	40.162104°,-105.659327°	3551	0.05	-2.4	1196	45	9.15	4.81
Teton Glacier	Rocky	43.740928°,-110.790954°	3206	0.48	-3.2	2473	61	10.28	6.59
East Conness Glacier	Sierra	37.968609°,-119.313354°	3527	0.06	0.1	1266	55	3.42	2.02
Goethe Glacier	Sierra	37.210199°,-118.707668°	3667	0.14	-1.2	1099	67	3.26	2.03
Middle Palisade Glacier	Sierra	37.076582°,-118.458395°	3518	0.58	-1.4	1212	67	3.25	1.98
North Palisade Glacier	Sierra	37.111465°,-118.506498°	3603	1.49	-1.9	1217	67	3.32	2.04
West Conness Glacier	Sierra	37.971285°,-119.318549°	3492	0.31	0.2	1266	55	3.44	2.04
Individual Rock Glaciers									
Adams Rock Glacier	Cascade	46.227090°,-121.552670°	1910	0.11	2.2	2915	43	5.95	5.52
Bolam Rock Glacier	Cascade	41.429724°,-122.209437°	3011	0.46	-1.5	2118	73	4.33	2.59
Diller Rock Glacier	Cascade	44.145730°,-121.765737°	2320	0.53	0.7	3157	42	3.38	4.18
North Cascades Rock Glacier One	Cascade	48.250517°,-120.406968°	2164	0.08	0.8	1439	62	3.32	2.57
North Cascades Rock Glacier Three	Cascade	48.290884°,-120.413696°	2182	0.30	0.2	1427	62	3.29	2.54
Prouty Rock Glacier	Cascade	44.106983°,-121.750503°	2443	0.60	0.8	3513	42	3.36	4.11
Arapaho Rock Glacier	Rocky	40.022482°,-105.637699°	3583	0.47	-3.0	1151	49	9.26	4.65
Confusion Rock Glacier	Rocky	39.749054°,-106.307559°	3558	0.04	-0.6	831	33	6.84	3.80
Duck Lake Rock Glacier	Rocky	39.759668°,-106.331853°	3702	0.11	-2.6	904	44	6.88	3.83
Gibraltar Rock Glacier	Rocky	40.155336°,-105.654799°	3463	0.03	-2.0	1170	45	8.54	4.46
Ilans Rock Glacier	Rocky	40.627544°,-105.943468°	3396	0.18	-1.1	1188	48	8.48	4.76
Louise Rock Glacier	Rocky	40.508941°,-105.625321°	3419	0.37	-1.4	1125	48	7.06	3.73
Navajo Rock Glacier	Rocky	40.061200°,-105.636092°	3496	0.15	-2.0	1200	49	9.46	4.77
Paintbrush Rock Glacier One	Rocky	43.783379°,-110.803140°	2974	0.09	-0.6	1630	57	9.43	6.03
Paintbrush Rock Glacier Three	Rocky	43.790463°,-110.778199°	2720	1.29	0.0	1668	57	7.79	4.97
Paintbrush Rock Glacier Two	Rocky	43.783451°,-110.797469°	2866	0.13	-0.3	1635	47	9.53	6.10
Peck Rock Glacier	Rocky	40.071642°,-105.664310°	3272	0.11	-0.6	1126	35	8.93	4.54
Powell Rock Glacier	Rocky	39.764031°,-106.339080°	3769	0.09	-2.8	915	44	6.85	3.81
Saint Vrain Rock Glacier	Rocky	40.163962°,-105.667730°	3704	0.30	-2.7	1196	45	8.91	4.66
Taylor Rock Glacier	Rocky	40.276985°,-105.669918°	3318	0.66	-2.0	1213	48	10.69	5.91
Agassiz Rock Glacier	Sierra	37.123760°,-118.519432°	3578	1.02	-1.4	1053	66	2.94	1.80
Goethe Rock Glacier	Sierra	37.220051°,-118.714092°	3596	0.28	-0.4	1104	67	3.26	2.03
Middle Palisade Rock Glacier	Sierra	37.084854°,-118.449419°	3342	1.63	-0.6	1090	67	2.99	1.81
North Lake Rock Glacier	Sierra	37.230261°,-118.620354°	2830	0.41	2.1	519	0	1.80	1.11
Mountain Range Summaries									
Cascade Mountain Glaciers	Cascade	45.302055°,-121.613506°	2279(374)	1.203(0.94)	-0.54(1.94)	2804(520)	61(16)	7.79(4.97)	1.5(0.7)
Cascade Mountain Rock Glaciers	Cascade	45.408488°,-121.349835°	2338(342)	0.347(0.202)	0.52(1.06)	2428(819)	54(12)	9.53(6.1)	1.14(0.45)
Rocky Mountain Glaciers	Rocky	41.007738°,-106.987388°	3568(213)	0.391(0.505)	-2.84(0.96)	1333(512)	51(7)	8.93(4.54)	2.62(0.62)
Rocky Mountain Rock Glaciers	Rocky	40.894153°,-106.918331°	3374(309)	0.287(0.33)	-1.54(0.99)	1211(255)	46(6)	6.85(3.81)	2.6(0.5)
Sierra Nevada Glaciers	Sierra	37.467628°,-118.860893°	3561(65)	0.514(0.517)	-0.85(0.86)	1212(61)	62(6)	8.91(4.66)	1.83(0.1)
Sierra Nevada Rock Glaciers	Sierra	37.164731°,-118.575824°	3336(309)	0.836(0.535)	-0.06(1.3)	941(245)	50(29)	10.69(5.91)	1.47(0.3)
All Glaciers	All	41.259140°,-115.820595°	3154(254)	0.68(0.77)	-1.7(1.7)	1779(842)	56(12)	6.41(2.54)	2.11(0.77)
All Rock Glaciers	All	41.155790°,-115.614663°	3109(546)	0.39(0.40)	-0.8(1.4)	1470(729)	49(15)	6.39(2.71)	2.05(0.81)

Table 5: Metal concentrations (mg L^{-1}) for glaciers and rock glaciers for the different ranges in the study. Standard deviations are in parenthesis. Bold values indicate statistically significant difference between paired sites at $p<0.05$ using Welch-Satterthwaite T-Test for nonparametric samples. Detection limit was 0.01 mg L^{-1} for SiO_2 and 0.001 mg L^{-1} for all other metals.

Sample Group	Al		Ca		Fe		K		Mg		Mn		SiO_2		Sr	
All Feature Summaries																
All Glaciers	**0.88**	**(0.73)**	1.80	(3.12)	**0.19**	**(0.36)**	0.22	(0.24)	0.22	(0.33)	0.01	(0.02)	1.37	(2.21)	0.01	(0.01)
All Rock Glaciers	0.72	(0.74)	**3.55**	**(3.05)**	0.03	(0.05)	0.44	(0.61)	**0.69**	**(1.13)**	0.00	(0.00)	**3.48**	**(4.39)**	**0.01**	**(0.01)**
Mountain Range Summaries																
Cascade Mountain Glaciers	0.73	(0.58)	3.15	(4.76)	**0.17**	**(0.13)**	0.24	(0.30)	0.26	(0.28)	0.02	(0.02)	2.47	(3.19)	0.01	(0.01)
Cascade Mountain Rock Glaciers	0.05	(0.01)	1.78	(1.53)	0.03	(0.00)	0.24	(0.03)	0.15	(0.10)	0.00	(0.00)	6.23	(6.40)	0.01	(0.00)
All Cascade Mountain Features	0.65	(0.59)	2.54	(3.66)	0.13	(0.13)	0.24	(0.23)	0.21	(0.22)	0.01	(0.02)	4.16	(5.13)	0.01	(0.01)
Rocky Mountain Glaciers	0.79	(0.70)	1.22	(1.81)	0.07	(0.13)	0.21	(0.23)	0.24	(0.42)	0.01	(0.00)	0.61	(0.56)	0.01	(0.01)
Rocky Mountain Rock Glaciers	0.59	(0.68)	**4.54**	**(2.95)**	0.03	(0.05)	0.53	(0.79)	**1.10**	**(1.43)**	0.00	(0.00)	**2.45**	**(2.95)**	0.02	(0.01)
All Rocky Mountain Features	0.67	(0.67)	3.00	(2.97)	0.04	(0.09)	0.40	(0.74)	0.70	(1.15)	0.00	(0.00)	1.55	(2.32)	0.01	(0.01)
Sierra Nevada Glaciers	1.45	(1.09)	0.77	(0.39)	**0.54**	**(0.74)**	0.18	(0.17)	0.11	(0.15)	0.01	(0.20)	1.76	(2.77)	0.01	(0.00)
Sierra Nevada Rock Glaciers	1.13	(0.83)	3.45	(4.27)	0.05	(0.04)	0.53	(0.03)	0.30	(0.33)	0.00	(0.00)	2.70	(3.57)	0.01	(0.01)
All Sierra Nevada Features	1.30	(0.87)	1.99	(3.05)	0.37	(0.63)	0.34	(0.40)	0.20	(0.25)	0.01	(0.02)	2.19	(3.03)	0.01	(0.01)

Table 6: Dissolved Organic Carbon (DOC) in mg L^{-1} and Fluorescing Dissolved Organic Matter (FDOM) Indices for glaciers and ranges within the study. Bold values indicate statistical significant difference between paired sites, with indicating * at $p<0.05$, ** at $p<0.01$, and *** at $p<0.001$.

Sample Group	DOC (mg•L^{-1})		Fluorescence Index		Freshness Index		Humification Index		SUVA254	
All Feature Summaries										
All Glaciers	**0.74**	**(0.49)**	1.60	(0.14)	0.85	(0.27)	0.62	(0.56)	2.27	(1.60)
All Rock Glaciers	0.82	(0.59)	1.60	(0.15)	0.85	(0.23)	**1.38**	**(1.87)***	2.00	(1.48)
Mountain Range Summaries										
Cascade Mountain Glaciers	0.46	(0.44)	1.66	(0.20)	1.09	(0.38)	0.47	(0.53)	3.73	(1.75)
Cascade Mountain Rock Glaciers	0.96	(0.92)	1.69	(0.19)	0.96	(0.29)	**1.32**	**(0.61)***	2.30	(1.92)
All Cascade Mountain Features	0.69	(0.72)	1.68	(0.19)	**1.03**	**(0.34)** **	0.86	(1.79)	3.09	(1.92)
Rocky Mountain Glaciers	0.92	(0.48)	1.55	(0.07)	0.74	(0.10)	0.77	(0.61)	1.34	(0.73)
Rocky Mountain Rock Glaciers	0.88	(0.41)	1.56	(0.12)	0.80	(0.22)	**1.64**	**(1.67)***	1.50	(0.98)
All Rocky Mountain Features	0.90	(0.44)	**1.55**	**(0.10)****	0.77	(0.17)	1.21	(1.33)	**1.42**	**(0.86)****
Sierra Nevada Glaciers	0.54	(0.43)	1.64	(0.09)	0.74	(0.07)	0.41	(0.38)	2.51	(1.39)
Sierra Nevada Rock Glaciers	0.31	(0.13)	1.63	(0.10)	0.85	(0.11)	0.44	(0.64)	3.46	(1.41)
All Sierra Nevada Features	0.43	(0.33)	1.63	(0.09)	0.79	(0.10)	**0.43**	**(0.48)****	2.94	(1.41)

3. DIFFERENCES IN BIOAVILABILITY AND CHEMICAL CHARACTERISTICS OF DISOLVED ORGANIC MATTER BIOAVAILABILITY BETWEEN GLACIER TYPES

3.1 INTRODUCTION

Meltwaters from mountain glaciers are the largest annual flux of carbon released from melting ice globally [Hood et al. 2015], yet the bioavailability of this carbon to alpine headwater ecosystems in the United States is unknown. Inland waters were once thought to act as a simple aqueduct with the ocean acting as the final repository, with little processing of organic matter, especially in headwaters, occurring on the path to the sea. It is now known carbon cycling in inland waters is the result of complex interactions among atmospheric-aquatic and terrestrial-aquatic interfaces [Jaffe et al. 2008; McCallister and Del Giorgio 2012; Stubbins et al. 2012; Mackay et al. 2013; Mosher et al. 2015]. Each interface has biotic and abiotic interactions, and leaves a signature on the aquatic carbon pool. Headwater streams typically represent a large portion of the total terrestrial-aquatic interface of an ecosystem [Wallin et al. 2015]. Glaciers and rock glaciers meltwaters feed some of the headwater streams within the western United States [Fountain and Tangborn 1985*;* Cable et al. 2011; Dunnette et al. 2014]. Glaciers bridge the atmospheric-terrestrial and terrestrial-aquatic interfaces, integrating atmospherically deposited chemicals and weathering products, processing reactive compounds through biological and inorganic pathways, and then releasing the altered solutes to alpine headwaters [Williams et al. 2007; Dubnick et al. 2010; Fellman et al. 2010; Stibal et al. 2010; Vermilyea et al. 2012]. Glaciers in greater mountain ranges of

Alaska and Europe are a known source of bioavailable dissolved organic matter (BDOM) [Singer et al. 2012; Hood et al. 2009; Fellman et al. 2015], but BDOM from the small ice glaciers in the American West is unknown. Rock glaciers (with similar C concentrations but different DOM pool structures compared to ice glaciers) are an order magnitude more abundant than ice glaciers in the United States and are more resistant to warming temperatures than ice glaciers [Fegel et al. in press].

Glacially derived DOM can be an important source of chemical energy to headwater ecosystems, with the potential to fuel heterotrophic respiration and metabolism much further downstream [Hood et al. 2009; Singer et al. 2012; Fellman et al. 2015]. DOM processing within glaciers is similar to lakes, whereby DOM is structured by *in situ* microbial activity in the sub-glacial environment and within cryoconite holes [Williams et al. 2007; Dubnick et al. 2010; Fellman et al. 2010; Stibal et al. 2010]. The portion of DOM available for biological processing is known as bioavailable dissolved organic matter (BDOM). While it is known organic matter from glaciers and rock glaciers in the western United States is released to headwaters [Fegel et al. in review], how much of this is available for biological (aka BDOM) processing remains unknown. Previous research has examined glacial BDOM in large glaciers in the Gulf of Alaska and in the European Alps, and found glacial DOM to be an important driver in ecosystem productivity [Hood et al. 2009; Singer et al. 2012, Fellman et al. 2015]. Whether this phenomenon occurs in the alpine regions of the United States remains unknown, as little is known about BDOM released from mountain ice glaciers in the contiguous US with even less being known about rock glacier effluent to influence headwater ecosystems.

There are more than 10,000 rock glaciers throughout the mountain ranges of the American West, nearly fives times the number of ice glaciers [Janke 2007; Millar and Westfall 2008; Fegel et al. in review; Johnson et al. in review]. Release of DOM from rock glaciers has the potential to be a widespread influence on headwater ecosystems as an important source of BDOM [Fegel et al. in review]. Furthermore, rock glaciers are likely to be retained in alpine ecosystems much longer than ice glaciers due to their slower melting rates. As ice glaciers are lost, rock glacier meltwaters may be representative of future DOM inputs to headwater ecosystems under climate warming [Outcalt and Benedict 1965; White 1971; Krainer and Mostler 2000]. Previous research has shown that carbon concentrations in ice glaciers and rock glaciers in the United States are similar [Fegel et al. in review], and comparable to carbon concentrations of ice glaciers worldwide [Hood et al. 2015]. However there appear to be consistent differences in the structure of DOM between ice glaciers and rock glaciers, suggestive of differences in bioavailability of DOM among each feature.

DOM pools in natural systems are complex, and consist of as many as 10,000 individual organic compounds [Hedges et al. 2000; Kim et al. 2006; Hockaday et al. 2009], with many more that have likely not been detected. Difficulties in experimentally connecting bioavailability to molecular diversity of the total DOM pool are partly due to this high level of heterogeneity [Derenne and Tu 2014]. The affects of individual compound bioavailabilities may not be representative of total DOM pool bioavailability. DOM pools with multiple compounds of high bioavailability may assert positive feedbacks on the bioavailability of the total DOM pool [Guenet et al. 2010]. Certain metabolites within DOM pools may also not be bioavailable individually, but may act as

cofactor metabolites that allow for other compounds to increase in bioavailability [Hilker 2014]. The relationship of total DOM pool diversity to functionality remains unknown. Total DOM pool diversity, or chemodiversity, could be explored using techniques and indices currently applied to biological community diversity [Hilker 2014]. Understanding how chemodiversity affects bioavailability will allow for better comprehension of DOM pool bioavailability as a whole.

The molecular controls on bioavailability and chemodiversity of total DOM pools from both types of glaciers are currently unknown. Molecular structure of DOM in aquatic ecosystems exerts a large control on bioavailability than in terrestrial ecosystems, where environmental and biological factors like reactive mineral surfaces, soil redox state, and presence of degraders may control bioavailability [Schmidt et al. 2011; Kellerman et al. 2014]. Most of the previous work describing molecular structure controls on the bioavailability of aquatic DOM has been limited to bulk quantification and broad functional group classification [Sleighter et al. 2014; Berggren and del Giorgio 2015; Mosher et al. 2015; Wallin et al. 2015]. Previous characterizations of DOM from ice glaciers and rock glaciers used bulk composition techniques [Barker et al. 2006; Williams et al. 2007; Singer et al. 2012; Fegel et al. in review] for analysis and thus may not completely describe the chemical composition total DOM pool, as only a small portion of DOM is identified with the techniques previously used [Stubbins et al. 2014], thus the molecular controls on bioavailability of total DOM pools between glacier types is currently unknown. The complexity of DOM pools in natural systems only adds to the uncertainty of DOM bioavailability between the two glacier types, however recent advancements are allowing for more descript methods of identifying bioavailability

based on molecular structure. Exact chemical identities for bioavailable compounds within DOM pools of natural systems that are metabolized have remain elusive, however recent advancements in environmental metabolomics now allow for specific compound identification within natural systems [Bundy et al. 2009; Bowen and Northen 2010].

We asked whether glacier meltwater bioavailability in the mountain ranges of the United States was similar to the pattern observed in other glaciated ecosystems and whether differences in DOM structure and diversity between ice glaciers and rock glaciers control biological productivity and metabolism. Here we present the results of laboratory incubations of DOM at the same carbon concentration from meltwaters from ice glaciers and rock glaciers. Incubations were paired with non-targeted metabolomics analysis of DOM via gas chromatography mass spectrometry (GC-MS) before and after incubation to determine differences in the specific chemical compounds metabolized (both catabolically and anabolically) by microbial processing. Our use of a standardized mixed microbial community between incubations of differing DOM pools allowed for direct analysis of differences in the efficiency and dynamics of microbial metabolism driven by total DOM pool structure.

3.2 METHODS

3.2.1 SITE DESCRIPTION

Paired ice glaciers and rock glaciers from four watersheds on the Front Range of Northern Colorado were selected based on their individual size (>0.5km^2) and the proximity of ice glacier to rock glacier within the watershed, forming pairs of feature with similar geographic parameters (Figure 7). Collection occurred in the late summer to

capture the greatest contribution of ice melt and minimize annual snowmelt contribution. Arapaho Glacier (-105.646351, 40.023378) and Arapaho Rock Glacier (-105.637699, 40.022482), located in the 4th of July Wilderness west of Boulder, CO were sampled on September 14th & 15th, 2014. Isabelle Glacier (-105.640994, 40.063373) and Navajo Rock Glacier (-105.636092, 40.061200) in the St. Vrain Wilderness, CO were sampled on September 5th, 2014. Peck Glacier (-105.663810, 40.068332) and Peck Rock Glacier (-105.664310, 40.071642) on the western side of Rocky Mountain National Park (RMNP), CO were sampled on September 7th, 2014**.** Andrews Glacier (-105.680639, 40.288370) and Taylor Rock Glacier (-105.671197, 40.275568) in the Loch Vale Watershed, RMNP, CO were sampled on September 19th and 20th, 2014**.** Full site descriptions can be found in Fegel et al. (in review).

3.2.2 FIELD EXTRACTION OF DOM

Samples from meltwaters at the terminus of each glacial feature were collected for DOM extraction. Sample meltwaters were collected in the early morning (0500-1000) to minimize diurnal variability in ice melt from solar radiation. DOM was extracted in the field from 20 L of meltwater at the terminus of each feature using the protocol established by Dittmar et al. 2008, omitting the salt extraction step (Supplemental Information). Collected meltwaters were passed through pre-combusted (450° C, 5hr) Whatman GF/F filters (GE Whatman, Pittsburg, PA, USA). Water samples were acidified to ~ pH 2 with 32% HCl acid. Bond Elut PPL carbon extraction cartridges (Agilent, Santa Clara, CA, USA) were condition with ~5mL HPLC-Grade methanol. The cartridge was filtered on a vacuum hand pump, with 2.5L of filtered meltwater pulled through each conditioned cartridge [Dittmar et al. 2008]. Vacuum pressure was never

allowed to exceed 15 mmHg. Cartridges were kept under vacuum 5 minutes after the last of the meltwater was pulled through, in order to dry. The vacuum line was left in each empty collection bottle in order to minimize contamination. Each cartridge was then eluted with 10mL HPLC- grade methanol per cartridge into a cleaned, combusted, and pre-weighed 120mL borosilicate bottle. The eluent from all of the cartridges for each sample was combined and collected into the same pre-tared borosilicate glass bottle.

3.2.3 PREPARATION FOR METABOLOMIC ANALYSIS

Running clean N_2 gas over the open samples evaporated methanol and the DOM quantity was determined by comparing pre-tared and post evaporation bottle weights (Supplemental Information). Samples were prepped for metabolomic analysis by redilution of 2 mg of concentrated DOM back into fresh HPLC-grade methanol. Post incubation DOM samples were collected for each sample separately, filtered through 0.2 μm Millipore filters (EMD Millipore, Billerica, MA, USA) to remove accumulated biomass, freeze-dried, and redissolved into HPLC-grade methanol.

3.2.4 METABOLOMICS

Metabolomics is a method of evaluating the molecular structure and functionality of DOM using mass spectrometry techniques. Metabolomics is an inherently ecological technique for assessing chemical functionality within natural systems, however very few environmental studies have used metabolomic approaches to address the bioavailability of DOM for microbial processing, with the exception being Logue et al. 2015. Both pre and post incubation DOM samples were run for gas chromatography-mass spectroscopy (GC-MS) at the Proteomics and Metabolomics Facility at Colorado State

University. Pre incubation samples were run during the same sample run on the same instrument to ensure instrument relativity. Extracted samples were resuspended in 50 µL of pyridine containing 50 mg mL^{-1} of methoxyamine hydrochloride, incubated at 60ºC for 45 min, sonicated for 10 min, and incubated for an additional 45 min at 60ºC. 50 µL of N-methyl-N-trimethylsilyltrifluoroacetamide with 1% trimethylchlorosilane (MSTFA + 1% TMCS, Thermo Scientific, Waltham, MA, USA) was added and samples were incubated at 60 ºC for 30 min, centrifuged at 3000xg for 5 min, cooled to room temperature, and 80 µL of the supernatant was transferred to a 150 µL glass insert in a GC-MS autosampler vial. Metabolites were detected using a Trace GC Ultra coupled to a Thermo ISQ mass spectrometer (Thermo Scientific, Waltham, MA, USA). Samples were injected in a 1:10 split ratio twice in discrete randomized blocks. Separation occurred using a 30 m TG-5MS column (0.25 mm i.d., 0.25 µm film thickness, Thermo Scientific, Waltham, MA, USA) with a 1.2 mL min^{-1} helium gas flow rate, and the program consisted of 80ºC for 30 sec, a ramp of 15ºC per min to 330ºC, and an 8 min hold. Masses between 50-650 m/z were scanned at 5 scans sec^{-1} after electron impact ionization [Broeckling et al. 2014].

3.2.5 DOM CONSUMPTION EXPERIMENTS

Concentrated DOM samples from each of the eight study sites were incubated *in vitro* at the same carbon concentrations, with unfiltered water from the Loch, Loch Vale watershed, RMNP, CO, USA, a nearby subalpine lake [Baron et al. 1992]. Exposing different DOM samples to a common mixed microbial community allowed us to examination differences in the rate and quantity of DOM respired during the incubation. Before the initiation of the experiment, lake water was stored at 4.5°C for 24 months in

order to decrease bioavailable carbon. After this period, 2 L of aged lake water was filtered through a pre-combusted (450° C, 5hr) 1.0µm Whatman GF/F filter (GE Whatman, Pittsburg, PA, USA) to remove the majority of protists and metazoans capable of consuming bacteria. 3mL of this filtered-aged lake water was aliquoted, preserved at 2% Formalin (37% Formaldehyde), and set aside for enumeration. 2 L of filtered-aged lake water was placed in the 15°C incubator in an uncapped, nontransparent Erlenmeyer flask to equilibrate. Next, a second aliquot of filtered-aged lake water was taken for pre-incubation DOC/TN analysis. Incubations were standardized to the same carbon concentration (4 mg L^{-1}) using temperature equilibrated MilliQ water, and TOC was measured using standard methods at the EcoCore facility at Colorado State University (http://www.nrel.colostate.edu/projects/lvws/pages/accesstodata/fieldlabmethods.html). Samples were poured into biological oxygen demand (BOD) bottles and individual incubations were inoculated with the same volume of microbial culture (Supplemental Information). Each ice glacier and rock glacier site was incubated in two individually created replicates. Two control incubations of exclusively the mixed microbial lake water culture and MilliQ water were created. A single BOD bottle containing only MilliQ was created to serve as a blank.

Respiration through the incubation was measured as dissolved oxygen (DO) consumption. DO was measured at 1-minute intervals, during the 10-week incubation using an Oxy-4 fiber-optic dissolved oxygen probe (PreSens, Regensburg, Germany). The incubation was terminated before the samples become hypoxic (< 4 mg L^{-1} dissolved oxygen), and all measurements with amplitude less than 20000 amps were

removed because of the potential affect of an improper connection between optic cables and the optode can have on DO value measured. Remaining incubated samples were filtered through a 0.2 μm Millipore polycarbonate filters (EMD Millipore, Billerica, MA, USA) to remove biomass. Post incubation carbon concentrations were measured using standard methods (http://www.nrel.colostate.edu/projects/lvws/pages/accesstodata/fieldlabmethods.html) (Supplemental Information). Each 3mL aliquot of post incubation sample was preserved at 2% overall concentration of Formalin (37% Formaldehyde), and standard Acridine Orange DNA staining methods were used to measure bacterial cell counts [Hobbie et al. 1977]. Samples were prepared in near dark conditions. All prepared stains were filtered through 0.2 μm Millipore polycarbonate filters (EMD Millipore, Billerica, MA, USA) to remove particulates. Preserved cell count samples were filtered through black 0.2 μm Whatman polycarbonate filters (GE Whatman, Pittsburg, PA, USA) at 178mm Hg. Black filters were used to maximize visual difference between fluorescing cells and the black background filter. Vacuum pressure was removed and a 0.1 μg mL^{-1} solution of the DNA stain 3,6-tetramethyl diaminoacridine (Acridine Orange, AO) was added to the filter paper of each sample and allowed to sit for 5 min. Each filter paper was then placed on a glass slide with low-fluorescing immersion oil. A cover slip was added and cells were counted.

Bacterial cells were counted on a Olympus Vanox AHBT3 (Center Valley, PA, USA) connected to a DC USH 200MB lamp (Ushio, Cypress, CA, USA) using 1250x magnification with an oil immersion lens. Low fluorescing oil was used for the slide-lens connection. The microscope filters were set to the DM505 mirror, with a 20BP545

exciter filter and a 180515F barrier filter, resulting in an excitation wavelength of 490nm. Bacterial cells were counted for each field of view of a gridded ocular, for 30 views, or 300 cells, whichever came first.

3.2.6 DATA ANALYSIS

GC-MS data was analyzed using principal component analysis (PCA) in R. For each sample, raw data files were converted to .cdf format, and a matrix of molecular features as defined by retention time and ion mass (m/z) was generated using XCMS package in R for feature detection and alignment. Raw peak areas were normalized to total ion signal, outlier features were detected based on total signal and PC1 of PCA, and the mean area of the chromatographic peak was calculated among replicate injections (n=2). Features were grouped based on an in-house clustering tool, RAMClustR, which groups features into spectra based coelution and covariance across the full dataset, whereby spectra are used to determine the identity of observed compounds in the experiment [Broeckling et al. 2014]. Compounds were annotated based on spectral matching to in-house, NISTv12, Golm, Metlin, and Massbank metabolite databases. The peak areas for each feature in a spectrum were condensed via the weighted mean of all features in a spectrum into a single value for each compound. The use of metabolite databases, each having been experimentally verified, allows for the development of metrics to confirm a high level of certainty that the proper compound assignment has been given to each feature cluster (Supplemental Information). However, because databases serve as a proxy for compound identification, and known compound standards for each of the 2000 identified peaks were not run placed within our instrument, a small amount of uncertainty still exists in

compound identity. Therefore, compounds identified by cluster features within our study are better denominated as candidate compounds. Cluster features assigned candidate compounds in our study had high similarity (>90%) to feature clusters from known standard compounds within the databases used (Supplemental Information).

Analysis of variance was conducted on each compound using the aov function in R, and p-values were adjusted for false positives using the Bonferroni-Hochberg method in the p.adjust function in R. Post-incubation samples were corrected for ionizing intensities of the added water culture by subtracting the peak intensities of the exclusively microbial culture for each chemical candidate from each post incubation sample. PCA was conducted on mean-centered and Pareto variance-scaled data using the pcaMethods package in R. These PCA components were used to identify differences in compounds present between ice glaciers and rock glaciers, as well as between pre and post incubation samples. C:N ratios were calculated using the values from the standardized pre incubation DOC/TDN measurements.

Molecular rank was calculated by ordering candidate compounds by their normalized ion intensity. Molecular rank was calculated for pre and post incubation sample averages. Chemodiversity was calculated using the Shannon-Wiener diversity index [Shannon and Weaver 1948]**,** and treating each unique compound identified though GC-MS as a ‘species’**.** We did this for DOM composition both before and after incubation and estimated changes in chemodiversity through microbial metabolism. The Shannon Wiener index was chosen for the measurement of chemodiversity because of it’s ability to show differences in diversity from set of samples with large differences in the number of compounds present. Previous work has used the Chao1 index to

measure chemodiversity [Kellerman et al. 2014], however Chao1 is better suited for identifying whether the entire chemodiversity of the sample was identified, not necessarily measuring the actual chemodiversity of the data, thus Chao1 was not applicable for our research questions.

Oxygen consumption was averaged for each glacier type. Confidence intervals were calculated at α=0.05. A third order polynomial was used to smooth data (R^2>0.999) and 95% confidence intervals were plotted in the programming language R. Berner's Multi-G model was used to model carbon pool bioavailability [Berner 1980; Guillemette and del Giorgio 2011], modeled through the SAS. Dissolved oxygen curves generated from the incubation were fit to the equation:

Equation 1: Two-Pool Decay Model Equation

$$Y = B_1^{kt} + B_0$$

Where Y is the total carbon pool, B_1 is the bioavailable carbon pool, k is the decay rate constant of the bioavailable pool, t is time, and B_0 is the recalcitrant carbon pool. We then used a least square means to test for statistical differences in carbon pool sizes (i.e. B_1 and B_0) between ice glaciers and rock glaciers. C consumed was calculated as the difference in pre and post incubation DOC values. Respiratory Quotient (RQ) was calculated using Equation 2:

Equation 2: Respiratory Quotient

$$RQ = \frac{C\ Consumed\ (mg\ L^{-1})}{O_2\ Consumed\ (mg\ L^{-1})}$$

As an additional estimate of DOM quality, bacterial growth efficiency (BGE) was calculated to examine how carbon was cycled within each incubation. BGE is the amount of microbial biomass produced per unit of C assimilated, and is calculated using Equation 3 [del Giorgio and Cole 1998]:

Equation 3: Bacterial Growth Efficiency (BGE)

$$BGE = \frac{Bacterial\ Production\ Rate\ (mol\ C\ L^{-1}\ Hr^{-1})}{Bacterial\ Carbon\ Assimilation\ Rate\ (mol\ C\ L^{-1}\ Hr^{-1})}$$

Thus BGE, is a ratio of carbon respired to total carbon demand for the mixed bacterial community. Bacterial production rate was measured as the amount of carbon created as bacterial biomass (20 femtograms C per bacterial cell) per hour. Bacterial carbon assimilation rate was equivalent to total carbon consumed throughout the incubation, measured in each filtered post incubation sample using standard TOC/TDN methods (http://www.nrel.colostate.edu/projects/lvws/pages/accesstodata/fieldlabmethods.html).

3.3 RESULTS

3.3.1 ANALYSIS OF ICE GLACIER VS. ROCK GLACIER DOM COMPOSITION

DOC concentrations in the meltwaters of ice glaciers and rock glaciers were low and not statistically different (G=0.92 ± 0.48 mg L^{-1}, RG= 0.88 ± 0.48 mg L^{-1}) (Supplemental Information). C:N ratios were not different between ice glaciers and rock glaciers (G = 2.35 ± 0.62, RG = 1.85 ± 0.83) before incubation (Table 7). Chemodiversity was high before incubation, but not significantly different for ice glaciers and rock glaciers (G = 2.83 ± 0.11, RG = 2.80 ± 0.02) (Table 7). Metabolomic analysis (GC-MS) indicated over 2000 DOM compounds consisting of a sum total of14571 mass spectral features within the meltwaters of the four ice glaciers and four rock glaciers of our study. Each DOM compound ranged between 3-170 individual mass spectral features that were clustered together to form a compound identity. 328 compounds were annotated with candidate compounds, while 1705 compounds were unable to be annotated in the current metabolite libraries (Supplemental Information). Most candidate compounds were simple sugars, sugar acids, amino groups, and nucleic acids. The top 25 compounds present by molecular rank within the DOM pool before incubation were shared by ice glaciers and rock glaciers, and included mostly organic acids (Figure 8. Panel B.).

PCA analysis suggested 33 compounds as driving differences in the DOM pools between glacier types (Figure 9. Panels A, B), only a few of which were able to be assigned known candidate compounds in the most current GC-MS libraries (Figure 9. Panel B, supplemental information). Ice glaciers meltwaters were enriched in the simple sugar maltose and the amino acid glutamate compared to meltwaters of rock glaciers

(Figure 9. Panel B). Rock glacier meltwaters were enriched in organic acids compared to ice glacier meltwaters.

3.3.2 INCUBATIONS

Microbes cultured with DOM from ice glacier meltwaters consumed more oxygen and consumed oxygen more rapidly than microbes incubating with rock glacier DOM (Figure 10). Carbon consumption was similar between glacier types, but respiratory quotients (RQ) for ice glaciers were slightly lower than rock glaciers (G = 0.56 ± 0.14, RG = 0.69 ± 0.11) (Table 7). Bacterial Growth Efficiency (BGE) was higher in incubations of DOM from glaciers compared to rock glaciers (G = 0.263 ± 0.134, RG = 0.157 ± 0.159) (Table 1). Differences in BGE between glacier types would have been even larger, however Peck Rock Glacier had unusually high BGE (0.387) and drove most of the increase, as well as the variability in the average BGE of rock glaciers (Table 7). The results of RQ and BGE combined indicate that quickly consumed bioavailable DOM was more tightly recycled, and stored as biomass in ice glacier meltwater DOM incubations than in rock glacier meltwater DOM incubations.

According to the multi-G decomposition model Ice glaciers had a larger portion of BDOM, bioavailable carbon (B_1), in their measured DOM pool compared to rock glaciers (Table 8). This equated to an average of 58.829 ± 9.73% of the DOM pool being BDOM for ice glaciers, and an average of 37.34 ± 10.23% of the DOM pool being BDOM for rock glaciers. Inversely, rock glaciers had a larger portion of DOM that was less bioavailable (B_0). The two glacier types had no difference in the decay constant, k.

3.3.3 ANALYSES OF DOM AFTER INCUBATION

The DOM pools of ice glaciers and rock glaciers were homogenized by microbial metabolism throughout the duration of our incubation (Figure 9. Panel C.). Microbial metabolism also rarified the DOM pool, meaning a handful of very abundant compounds were produced and many compounds of middle molecular rank decreased in intensity (Figure 8. Panel A.). Ice glaciers and rock glaciers shared the most abundant chemical compounds by molecular rank before and after incubation (Supplemental Information). Molecular ranks were reorganized by the incubation, resulting in different compounds having the highest intensities (through GC-MS analysis) post incubation (Figure 8. Panel B). Many of the organic acids and sugars present before incubation were consumed. The incubation resulted in increases in the peak intensity of many amino acids that we present at lower intensities in DOM pools pre-incubation. Glacier type controlled the direction of change in chemodiversity of the DOM pool throughout the incubation. Ice glacier meltwater DOM chemodiversity significantly increased through microbial metabolism, while rock glacier DOM chemodiversity significantly decreased (Table 7).

3.4 DISCUSSION

Our results demonstrate that the chemically complex DOM released from ice glaciers and rock glaciers in the United States is capable of stimulating bacterial productivity. Chemodiversity was similar between glacier types, however the structure of individual compounds present within each glaciers' DOM pool resulted in changes in the to quality of the DOM for microbial metabolism. Ice glacier DOM incubations appeared to process bioavailable DOM quickly, then recycle processed metabolites and produce more

biomass. Rock glacier incubations appeared to use more of the bioavailable DOM pool for catabolic activity. Regardless of different starting materials, our common mixed microbial culture homogenized DOM pools and produced similar candidate compound metabolites. Organic acids representative of microbial metabolism replaced many of the abundant sugars that were preferentially consumed in DOM pools of both ice glaciers and rock glaciers. Our work highlights the growing need for better metabolite database development and exemplifies the possibilities of GC-MS approaches for ecological metabolomics.

The results from our study of glaciers in Colorado show similar patterns to those seen in the Arctic and the European Alps, where bioavailable carbon from glaciers is capable of supporting microbial production [Hood et al. 2009; Singer et al. 2012; Fellman et al. 2015]. Carbon concentrations in ice glacial and rock glacial meltwaters of our study were low, but similar to those observed other glaciers globally [Dubnick et al. 2010; Stubbins 2012; Singer et al. 2012; Hood et al. 2015]. Despite the low C concentrations seen in our study, glacier DOM is disproportionately bioavailable compared to DOM in streams leached from terrestrial sources [Volk et al. 1997; Kim et al. 2006]. BDOM values for ice glaciers in our study were high (~50%), a comparable value to those seen in the European Alps (58%), and those seen in the Gulf of Alaska (23-66%) [Hood et al. 2009; Singer et al. 2012]. This functional characteristic now appears to be common to glaciated ecosystems globally.

Our study expands our comprehension of glacial DOM bioavailability by examination of DOM from rock glaciers. Though complete mapping of rock glaciers has only been completed for the contiguous United States and portions of South America,

early results suggest rock glaciers may be prolifically more abundant than ice glaciers in headwater ecosystems [Falaschi et al. 2015; Rangecroft et al. 2015; Johnson et al. in review; Fegel et al. in press]. Rock glacier DOM may contribute to ecosystem productivity for much longer than ice glaciers due to the slower recession of rock glaciers compared to ice glaciers [Woo 2012]. At similar carbon concentrations and with slightly smaller portions of BDOM (~37%), as observed in our study, rock glaciers may play just as critical of a role in biological metabolism as ice glaciers. Furthermore current glacial carbon modeling neglects the contribution of rock glacial carbon [Hood et al. 2015].

Bacterial growth efficiency (BGE) in our study was directly related to glacial type, with ice glacier DOM being a more nutritious source for bacterial production than DOM from rock glaciers. Our values for both ice glaciers and rock glaciers are similar to values observed in freshwater lakes and streams, where 15%-30% of DOM consumed is used for cellular production of biomass [del Giorgio and Cole 1998]. Microbes in our study incubated with DOM rock glaciers preferentially respired more of the DOM consumed compared to ice glaciers. Relative proportions of CO_2 respired versus C sequestered as biomass in glaciated headwater may be higher in rock glacier fed streams compared to ice glaciers. With a change to more rock glacier dominated biogeochemistry in headwaters [Fegel et al. in press], DOM released from rock glaciers may contribute a higher CO_2 flux to the atmosphere compared to ice glaciers.

Complexity of organic matter released from glaciers is as high as DOM complexity in other freshwater systems [Dubnick et al. 2010; Fellman et al. 2010; Singer et al. 2012]. The results of our study have allowed better parameters to be placed on

how complexity of this organic matter may place controls on productivity. Though chemodiversity was very similar between glacier types in our study, it was altered by microbial metabolism, resulting in inverse changes in chemodiversity between glacier types. Application of chemodiversity techniques to chemical ecology questions thus may be most useful when applied to functional instead of observational studies. The application of biodiversity techniques common to community-level studies, may serve useful for understanding how chemical heterogeneity affects ecosystem function [i.e. Hilker 2014; Kellerman et al. 2014]. Care must be taken in the application of biodiversity techniques to chemistry, as the diversity of chemistry is often orders of magnitude higher than biological diversity, and differences in compound concentrations within a single sample may span orders of magnitude. Analytical techniques used to measure may also bias how chemodiversity will be quantified, thus special care must be taken as chemodiversity methods are developed.

Similar to the history of DNA sequencing techniques, technological advancements paired with ecological application are allowing for better controls to be placed on chemical functionality based on molecular characterization. Ecological metabolomic techniques are moving chemical ecology from bulk classification methods to more descript quantification. Furthermore, metabolomics allows for functional assessment based on candidate compound characterizations. Our work exemplifies this transition, as bulk classification of DOM from glaciers has been shown to have low humic-protein ratios [Lafreniere and Sharp 2004; Williams et al. 2007; Dubnick et al. 2010; Fellman et al. 2010] Our work has expanded this bulk classification framework to assign descript molecular characterizations to the humic and protein components.

Metabolomics has allowed us to take this a step further and examine how this low humicity characterization is altered by metabolism. Thus, our work removes strictly operational definitions from bioavailable compounds in DOM and places descript molecular definitions on lability. Metabolomic approaches using mass spectrometry, like those in our study and a few others [Logue et al. 2015], offer a novel method of linking chemistry to ecological function.

Our results show clear differences in bioavailability due to chemical differences between glacier types, however some compounds present within the total DOM pool may not be fully expressed by our metabolomic analysis. Many metabolites in our study could not be confidently assigned candidate compounds. Some of these unassigned metabolites may be common to natural systems, but have yet to be verified with standards within the databases we used. Known metabolites are often a small portion of data obtained through mass spectrometry (<10%), with much of the data reflecting unknown metabolites or those yet to be verified with standards [Jansson et al. 2009]. Previous studies using the same techniques may have encountered similar difficulties, resulting in confidence using bulk compositional measurements instead of specific metabolite identities [Logue et al. 2015]. The high number of compounds unable to be annotated with candidate assignments in our study, likely reflects the infancy of metabolite databases for ecological application, and exposes the need for more ecological metabolite standards to be added to the current metabolite databases.

We propose that DOM released from ice glaciers is enriched in bioavailable compounds compared to rock glaciers for two reasons. First, DOM inputs to the ice within rock glaciers before melting are likely more complex than input to ice glaciers.

This is due to the ability of the rock glacier surface to act as a host to the growth of terrestrial plants including mosses, lichens, and organisms as complex as evergreens [Wahrhaftig 1959; Burga et al. 2004]. Less bioavailable compounds from these organisms percolate through the rock glacier unaltered and are released into meltwaters, where they are a less bioavailable source of DOM. Second, DOM in ice glaciers is likely more readily locked into the ice matrix than in rock glaciers, whereas rock glacier DOM may be in contact with open pore space, sediments, and liquid water. DOM within ice glaciers may be completely locked in ice and unavailable for microbial processing. The subglacial environment is often anoxic [Tranter et al. 2005], leaving compounds in an energetically rich state until released from ice melt. This allows for microbial metabolism of bioavailable compounds once unlocked from the ice. This microbial metabolism occurs before DOM is released from the rock glacier into adjacent streams and lakes, and is what was measured by our study.

Increases in the bioavailability of DOM entering the alpine from both glacier types in the United States could promote increased ecosystem productivity through a bottom up control on the aquatic food web, as is seen in glaciated systems at higher latitudes [Fellman et al. 2015]. The timing of DOM consumption through microbial metabolism was altered by differences in the DOM pool present before incubation between ice glaciers and rock glaciers. The commonality of many candidate compounds identified in our metabolic analysis of DOM expands the application of our results for the prediction of DOM bioavailability beyond glaciated ecosystems. The DOM inputs to alpine lakes and streams from glacial melt will be increasingly dominated by rock glacier-like DOM inputs, as rock glaciers will likely contribute to alpine hydrology for longer than ice

glaciers. This contribution will be paired with a decrease in the bioavailability of DOM and potential increases in the amount of CO_2 respired to the atmosphere.

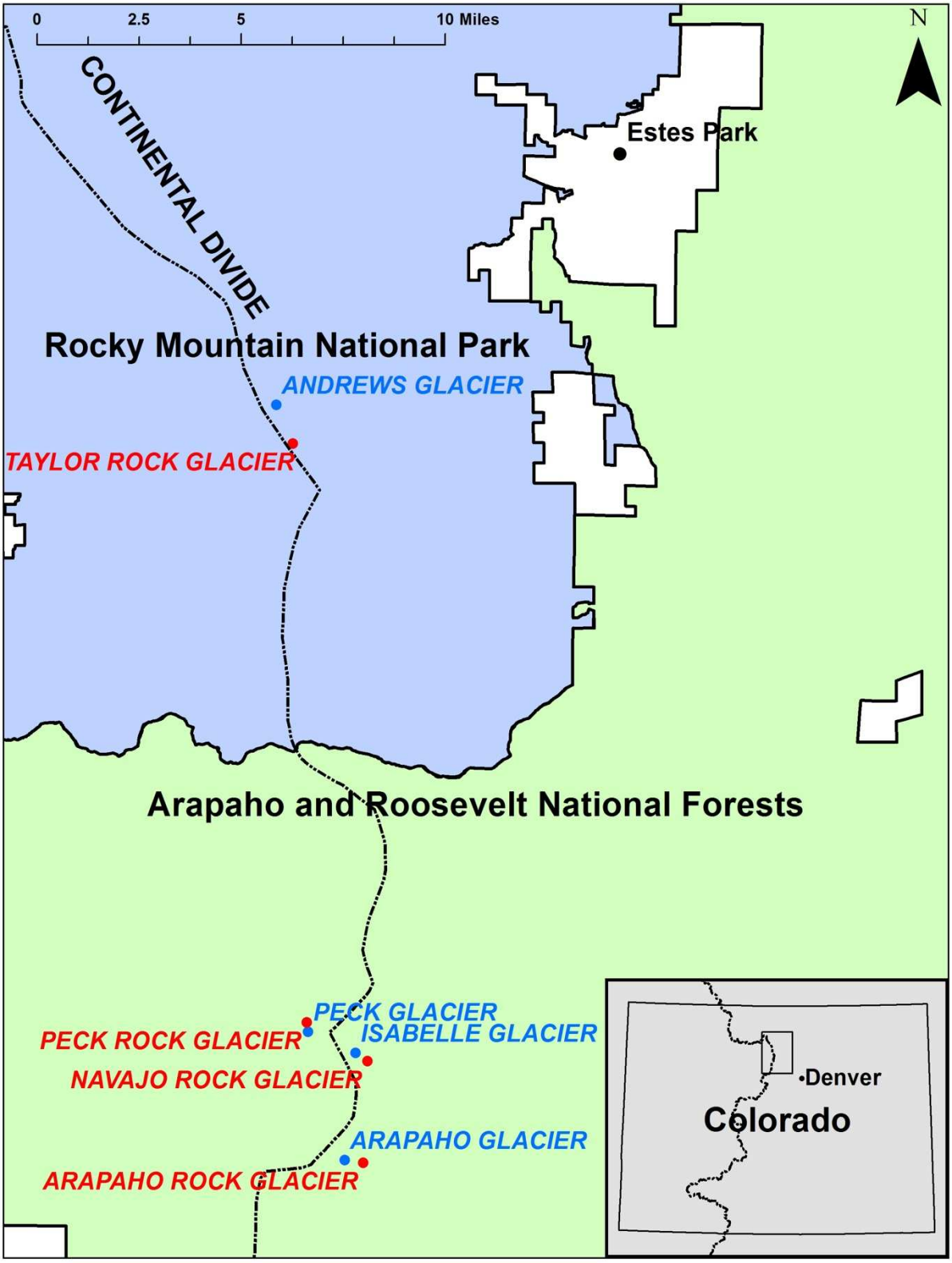
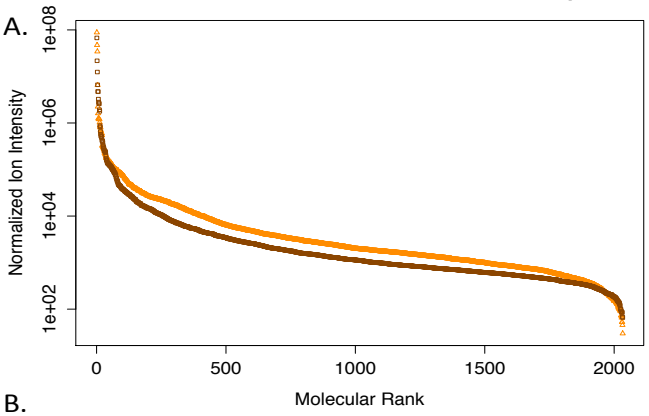


Figure 7. Site map for DOM bioavailability study

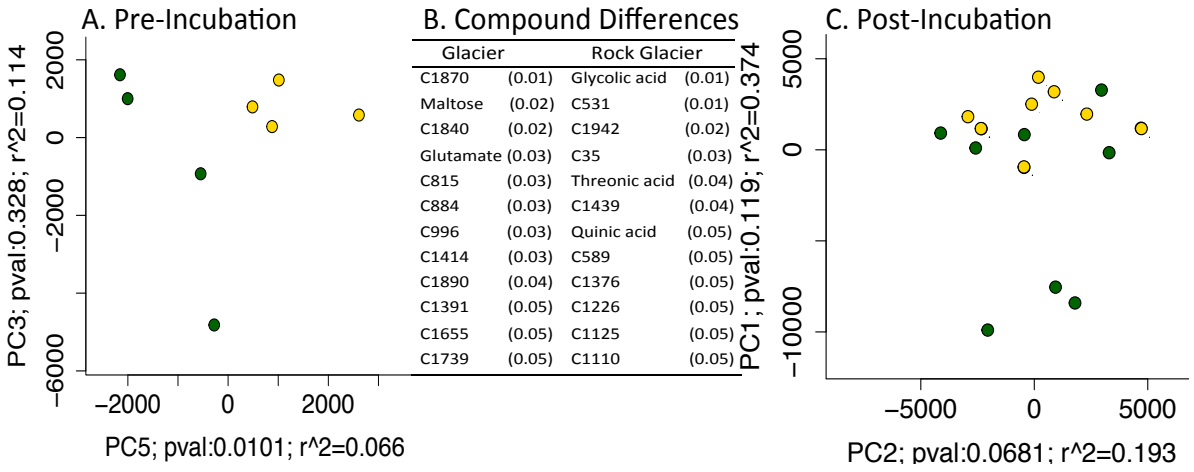
A.



B.

Pre Incubation	Post Incubation
Succinic Acid	Glycerol
Phthalic acid, octyl 2-propylpentyl ester	Benzenecarbothioic acid, 2,4,6-triethyl-, S-(2-phenylethyl) ester
Terephthalic acid, octyl 2,4,4-trimethylpentyl ester	Pyroglutamate
Succinic acid, 3-chlorophenyl 4-methoxybenzyl ester	2-Phenoxathiinamine-10,10-dioxide
Pentadecan-1-ol, n- (1TMS)	Serine
Hydroxylamine (3TMS)	1-Ethoxy-2-propanol
Homosalate, TMS derivative	Palmitic acid
p-Cyanophenyl 4'-heptyl-4-biphenylcarboxylate	Sucrose
Scopoletin, TBDMS derivative	Beta-alanine
Diiodoacetylene.1	Phenol, 2,4,6-tri-tert-butyl-
Palmitic acid	Aspartate
Diiodoacetylene	Boric Acid
Phosphoric acid	Stearic acid
Methanone, (2-amino-5-chlorophenyl)(2-fluorophenyl)-	Norleucine
Stearic acid	Homoserine
2-Thiophenecarboxylic acid, phenyl ester	Glycine
Phenylethylmalonamide, 2TMS derivative	Pentadecan-1-ol, n-
3-Methyl-3-(N-methyl-2-pyrrolyl)-1,2-diphenylcyclopropene	Pinitol, D-
Dibenz[d,f]cycloheptane, 5-amino-1,2,3,8-tetramethoxy-	l-Valine, N-methoxycarbonyl-, isohexyl ester
Furan-2-carboxylic acid [2-(2,2,6,6-tetramethyl-piperidin-4-yl)-ethyl]-amide	3,5-Di-tert-butyl-4-hydroxybenzoic acid
1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purine-8-carbaldehyde-oxime	1H-Benzimidazole, 1-ethyl-
p-Cyanophenyl p-(2-butoxyethoxy)benzoate	Phosphoric acid
Succinic acid, 2-fluorophenyl 2,4-dimethylpent-3-yl ester	Tyrosine
Homoserine	Sarcosine

Figure 8. Molecular distribution of GC-MS detected compounds A. Distribution of compounds by ion intensity before (Orange) and after (Brown). Many of the midrange compounds, most of which were unidentifiable in NIST, and GOELM libraries, present before incubation were metabolized. B. List of the top 25 compounds present before and after incubation. Though the top 25 compounds were present in similar normalized intensities before and after incubation, the compounds present were altered through metabolism.



Glacier		Rock Glacier	
C1870	(0.01)	Glycolic acid	(0.01)
Maltose	(0.02)	C531	(0.01)
C1840	(0.02)	C1942	(0.02)
Glutamate	(0.03)	C35	(0.03)
C815	(0.03)	Threonic acid	(0.04)
C884	(0.03)	C1439	(0.04)
C996	(0.03)	Quinic acid	(0.05)
C1414	(0.03)	C589	(0.05)
C1890	(0.04)	C1376	(0.05)
C1391	(0.05)	C1226	(0.05)
C1655	(0.05)	C1125	(0.05)
C1739	(0.05)	C1110	(0.05)

Figure 9. PCA Analysis of GC-MS compounds in glaciers (yellow) and rock glaciers (green) before incubation (Panel A.) and after incubation (Panel C.) with sub-alpine lacustrine microbes for 10 weeks. Clear differences in DOM compounds were present between glaciers and rock glaciers before incubation (Panel B.), however these differences were removed by microbial metabolism through the incubation period. Many compounds that were different between glacier types were unable to be annotated in current metabolite databases, however glaciers were enriched in maltose and glutamate compared to rock glaciers. Rock glaciers were enriched in organic acids. P-values for compounds that were different between glacier types are given in parentheses (Panel B.)

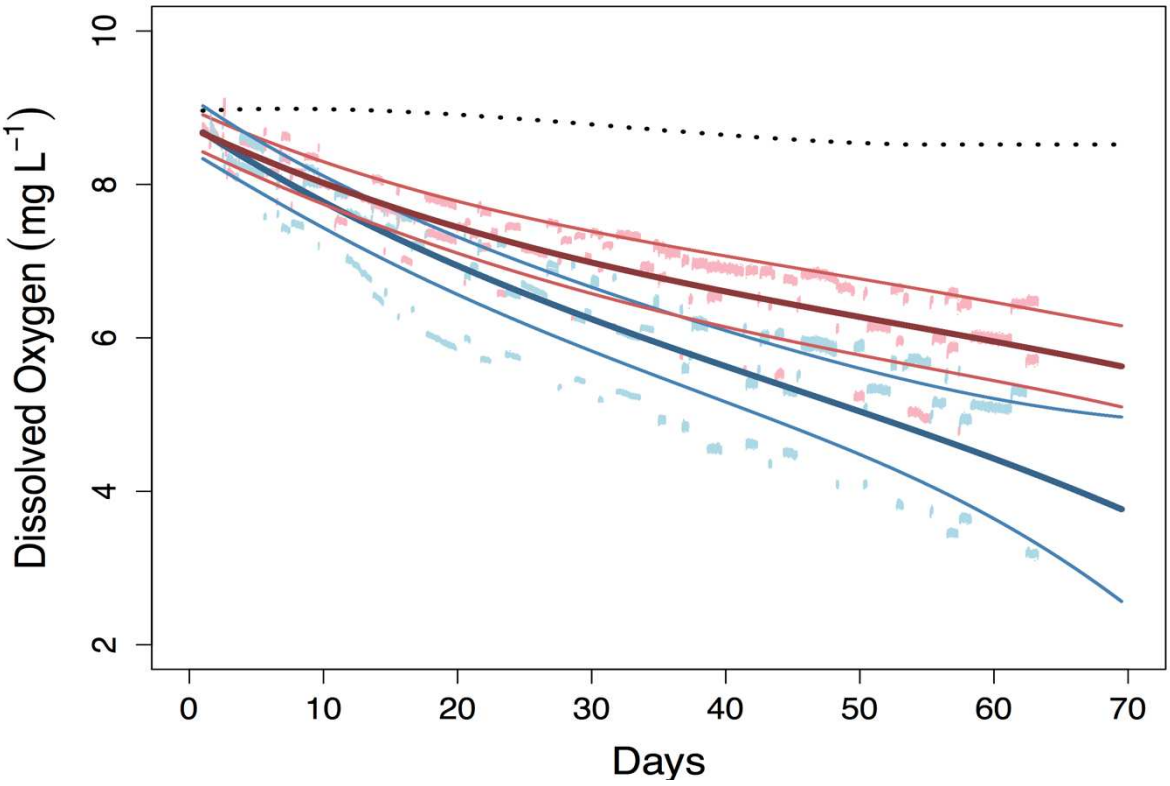


Figure 10. Results from our laboratory incubation of DOM from four different glaciated watersheds on the Front Range of Colorado. Here, values are averaged for each of the four glaciers (blue) and rock glaciers (red), and smoothed using a third order polynomial regression function (R^2=0.999). 95% Confidence intervals are shown in light blue for glaciers and in pink for rock glaciers. Glaciers were significantly faster in their consumption of oxygen throughout the incubation.

Table 7. Characteristics of DOM from each of the four glaciers and rock glaciers within the study. C:N is the ratio of Carbon to Nitrogen for pre incubation DOM. O2 and C consumed within the incubation are in mg L^{-1}. RQ is the respiratory quotient, calculated as C consumed/O2 consumed. BGE is the bacterial growth efficiency, calculated as Bacterial Production Rate/Carbon Consumption Rate (del Giorgio et al. 1998). SW is the Shannon Wiener Diversity Index, and is a measure of both richness and evenness of chemodiversity within the DOM pool identified through GC-MS. Loss in SW is the change is SW between pre and post incubation samples. C:N showed no relationship to RQ, *BGE, or SW. RQ values were low, and suggestive of continuous cycling of DOM metabolites throughout the incubation. SW was higher in pre incubation glaciers than rock glaciers, and there was an increase in SW in all glaciers while most rock glaciers experienced a decrease in SW throughout the experiment.

Site	Type	C:N	O2 Consumed (mg/L)	C Consumed (mg/L)	RQ	BGE	Shannon-Weiner	Loss in SW
ISABG	G	1.65	6.034	2.503	0.415	0.368	2.922	0.189
PG	G	2.02	3.946	1.891	0.479	0.388	2.892	0.876
ANDG	G	3.00	4.105	2.636	0.642	0.125	2.678	0.130
ARAPG	G	2.73	3.965	2.836	0.715	0.171	2.842	0.519
PRG	RG	2.63	3.524	2.088	0.593	0.387	2.796	0.535
NAVRG	RG	1.48	2.807	1.711	0.609	0.034	2.832	-0.315
ARAPRG	RG	2.45	4.120	3.004	0.729	0.136	2.786	-1.095
TRG	RG	0.86	2.694	2.246	0.834	0.073	2.799	-0.009
AVG G		2.35 (0.62)	4.51 (1.02)	2.47 (0.41)	0.56 (0.14)	0.263 (0.13)	2.83 (0.11)	0.43
AVG RG		1.85 (0.83)	3.29 (0.67)	2.26 (0.54)	0.69 (0.11)	0.157 (0.16)	2.80 (0.02)	-0.22

Table 8. Results from carbon decay model where BO represents the C (mg L^{-1}) in recalcitrant pool of DOM, B1 mg L^{-1} is the bioavailable pool of C (mg L^{-1}) and K is the decay constant in C (mg L^{-1} $Hour^{-1}$). Percent BDOM (Biologically Available DOM) results from the Multi G model show glaciers have a larger percentage of carbon within their total DOM pool compared to rock glaciers.

Site	Type	B_0	B_1	Percent BDOM	Percent Recalcitrant	k
ANDG	G	3.203	5.593	48.225	51.775	0.001
ARAPRG	G	3.718	5.205	47.738	52.262	0.001
ISABG	G	1.348	7.027	60.897	39.103	0.001
PG	G	3.781	5.237	46.055	53.945	0.001
ARAPRG	RG	5.320	3.804	32.054	67.946	0.002
NAVRG	RG	5.636	3.422	29.888	70.112	0.001
PRG	RG	3.310	5.241	45.740	54.260	0.001
TAYRG	RG	4.694	3.827	36.590	63.410	0.001
Glacier	G	3.377 (1.086)	6.350 (1.062)	52.829 (9.731)	47.171 (7.026)	0.001 (0.000)
Rock Glacier	RG	4.740 (1.255)	4.074 (1.121)	37.176 (10.23)	62.824 (6.842)	0.001 (0.000)

4. IMPLICATIONS OF RESEARCH

My research examines what increasing temperatures will mean for glaciated alpine ecosystem biogeochemistry. With increasing air temperatures and eventual loss of glacier ice, the elevated biogeochemical and microbial characteristics of rock glaciers compared to glaciers will likely dominate meltwaters reaching sensitive headwater ecosystems. Further, some glaciers are likely to become more rock glacier-like in their morphology. Changes in morphology will be paired with changes in biogeochemistry of meltwaters, and an increase the biogeochemical signal of rock glaciers on the alpine headwaters they feed. For some meltwater biogeochemical constituents this change in glacier type will control meltwater chemistry, while other meltwater biogeochemical attributes will be independent of glacier type, and driven primarily by geographical setting. Our results suggest that both feature specific and mountain range specific biogeochemical characteristics may place bottom up controls on ecosystem function. Understanding which biogeochemical characteristics will be a function of glacier type and which will be driven by region allows for better implementation of management strategies to protect and adapt to these changing headwater ecosystems.

The results of my work show that DOM from both glacier and rock glacier meltwaters is bioavailable and capable promoting biological metabolism. This glacial characteristic now appears to be common to glacial meltwaters globally [Hood et. al 2009; Singer et al. 2012; Fellman et al. 2015]. My work expands this understanding to rock glaciers, which are not currently included in glacier carbon models. Our results

show that even though DOM from rock glaciers is bioavailable, it represents a less nutritious source of DOM for microbial metabolism than ice glaciers, thus more carbon is respired from microbes consuming rock glacial DOM compared to ice glaciers where carbon is more readily recycled and sequestered as biomass. As rock glaciers melt they may be a larger source of CO_2 to the atmosphere than ice glaciers.

Ecosystem function for small alpine glaciers and rock glaciers within the United States may be limited to directly adjacent headwaters. However in many other glaciated alpine regions of the world, glacier and rock glacier meltwater biogeochemistry is affecting both natural ecosystems and human livelihood. Both glacier types act like water towers for chemicals, releasing stored pollutants trapped in glacial and rock glacial ice [Blais et al. 2001]. Some of these pollutants originate from before modern clean air and water policies were implemented. Sulfides from Roman-era smelting are melting out of rock glacial ice in Europe in concentrations so high that the meltwater is nonpotable and mutating biology within streams [Theis et al. 2013]. Glacial meltwaters have also been shown to have an order of magnitude higher concentration of chlorinated pollutants [Bizzotto et al. 2009]. Similar patterns are likely to be seen within glacier meltwaters in areas of acid mine drainage within South America, where low temperatures and limited soil contact within glacial meltwaters likely allows for contaminates to persist in glacial ice [Slemmons et al. 2013]. Our results show that here in the United States changes in glacier meltwater biogeochemistry may not be detrimental to ecosystem function and anthropogenic use. However the physical and chemical byproducts of human activity are leaving their imprint on glacial ice in the United States even at great distance [Baron et al. 2009; Saros et al. 2010]. Particularly

in the United States, land disturbance and human induced draught have increased to amount of nutrient containing dust reaching alpine ecosystems, and therefore within glacial ice [Painter et al. 2007].

Increases in dust on snow and ice events will alter the timing of glacial melt and the biogeochemistry of meltwaters. Much of the dust on snow will come from land use disturbance at great distance from the ice [Painter et al. 2007; Rhoades et al. 2010]. Soot will become an increasing driver in changes in glacial ice albedo with increases in drought and resulting forest fires. Soot deposition will have the compounded effect of supplying carbon to the alpine ecosystem. This organic carbon could combine with DOM in glacial meltwaters and form harmful disinfection byproducts in areas where glacial meltwater is treated for human consumption [Smith et al. 2011]. Dust on ice will also affect the functionality of glaciers as a source of hydroelectric power due to changes in the timing runoff rate and the inability of hydroelectric systems to utilize increases in runoff when necessary. A 1% decrease in hydrologic flow during times when run-off from when ice can be used results in a 3% decrease in power created [Laghari 2015]. Changes in the timing of ice melt from dust deposition will dramatically effect regions like Pakistan, which receives 37% of their power from hydroelectric dams fed by alpine glacial melt.

DOM released from glacial and rock glacial meltwaters is likely to have both physical and chemical affects on headwater ecosystems. Alpine ecosystems are often carbon limited [Bernasconi et al. 2011], and the carbon released as DOM from glaciers can support heterotrophic metabolism [Hood et al 2009; Singer 2012; Fellman et al. 2015]. Our results show that though DOM concentrations are low in glaciers in the

United States, DOM is labile enough character to promote metabolism. The concentrations of DOM within glacial ice are likely to increase with continued ablation due to the known distillation affect of glacial melt [Blais et al. 1998]. Therefore with continued contraction of glacial ice, the concentration of labile DOM released to alpine lakes and streams may become increasingly significant.

Alpine lakes and streams are known to experience strong photo bleaching, which limits the photosynthetic productivity occurring within the lake [Hylander et al. 2011]. UV radiation also limits bacterial growth efficiency for microbes living on glacial ice surfaces, explaining the large amount of carbon that is respired instead of accumulated as biomass [Foreman et al. 2013]. DOM is known to inhibit light attenuation. DOM, along with glacial flour, can act as a sunscreen, and may decrease the depth of the photosynthetic zone and block harmful UV radiation. DOM in glacial systems may thus be acting like a mechanical primer for the sequestration of carbon by photosynthetic activity within alpine lakes. Through both chemical and mechanical pathways, the release of DOM from glacial and rock glacial ice may be controlling ecosystem productivity from the bottom up.

5. FUTURE RESEARCH DIRECTIONS

Our work measured the outflow of glaciers and rock glaciers within the late season to minimize the inputs of annual snowmelt to the samples collected. Assumptions are made using this method, however no feasible alternative exists. Glaciers can be cored, but coring is nearly impossible for rock glaciers. Therefore direct measurement of chemical constituents locked in rock glacial ice, but before melt, is unobtainable. Future research could verify samples collected from rock glacier outflows are in fact exclusively ice meltwaters through the use of radiometric dating of meltwaters paired with estimated ages for ice from the geologic literature.

When separated geographically, regional differences in atmospheric deposition also seem to control the amount of nitrogen melted out from glaciers. Both rock glaciers and ice glaciers in Colorado have elevated nitrogen values compared to other mountain ranges and are located in alpine ecosystems well documented to have high nitrogen deposition [Baron 1992]. With a transition to NH_4^+ as the dominant species of reactive nitrogen to alpine ecosystems of the Front Range, understanding how atmospheric nitrogen is processed and exported from glacial and periglacial features may prove difficult. Sourcing reactive nitrogen previously stored in glaciers versus nitrogen recently deposited will likely be discriminated through modern stable isotopic techniques like $\Delta^{17}O\text{-}NO_3$.

Alpine ecosystems have been shown to be sensitive to atmospherically deposited acids [Psenner and Schmidt 1992]. With elevated pH values and metal

concentrations, our results imply that the acid neutralizing capacity (ANC) of an alpine ecosystem may be related to the type of glacial meltwaters at their inputs, with waters being fed by rock glaciers having higher acid neutralizing capacity than ice glacier fed meltwaters. Particularly in alpine ecosystems affected by increased anthropogenic deposition of acids (NO_x, SO_4), glacier meltwaters may mediate the consequences of human pollution to the alpine. Further research measuring the ANC of meltwaters from glaciers and rock glaciers in the American West would be useful for understanding what loss of glacial ice will mean for future alpine ecosystems affected by human pollution.

Glaciers in the American West are more susceptible to inter- and intra- annual climate variability than any other type of glacial feature globally due to their high elevation, small size, and patterns of snow deposition. Outside of maritime glaciers within the Coastal Ranges of Oregon and Washington and glaciers within the Gulf of Alaska, very little of the affect of glacial nutrients, DOM, and physical characteristics are likely felt by ecosystems not directly adjacent to the ice. This is likely not a globally common phenomenon. Meltwaters of alpine glaciers and rock glaciers within the Tien Shan of China and the Andes of South America may have biogeochemical consequences for ecosystem function much further away. Ecosystem function extends into use as an anthropogenic resource in these areas. Ironically the biogeochemistry from glaciers in these regions is may be more negatively disturbed from human activity than any other glacial region on the planet. Future research on the biogeochemistry of glacier and rock glacier melt should focus on these areas. As with many other affects of climate change, the brunt of the blow will be felt by those that are the least capable of mitigating the consequences. Most of the nomadic and herding communities that are

the most directly affected by the biogeochemistry of glacial meltwaters will be those that suffer the consequences of elevated pollutant, metal, and nutrient concentrations within glaciated meltwaters.

Carbon from mountain glaciers represents 63% of the global carbon flux from melting ice [Hood et al. 2015]. In my research I measured the structure and concentration of this carbon. Further research should quantify the carbon flux from mountain glaciers and rock glaciers using both carbon concentration measurements and discharge data. This may prove difficult to do at the scale necessary for global budgeting. Difficulties will be further compounded with the inclusion of rock glacier carbon flux measurements. Rock glaciers are an order of magnitude greater in their abundance, but also much more difficult to identify in the field. Outflow paths of meltwater from both glacier types may also become partially or fully subterranean before the feature terminus. Therefore the most logical method for calculating carbon flux from mountain glaciers and rock glaciers will be studies pairing satellite imagery, aerial photography, and ground penetrating radar as techniques to measure the volume of water present as ice within glaciers and rock glaciers. Combining these results with carbon concentration measurements from my study, and the work of others will allow for better parameters to be placed on glacier and rock glacier carbon flux measurements [Singer et al. 2012; Williams 2007].

My research showed differences in the bioavailability of organic matter between glaciers and rock glaciers to lacustrine microbes. Respiratory quotients (RQ) were slightly different between the two glacier types as well. However these RQ values were calculated as total carbon consumed throughout the incubation, measured as pre and

post incubation DOC. Further research should used paired O_2 and CO_2 sensors within the incubation to explore real-time RQ dynamics. The ability to measure continuous changes in RQ would allow for the pinpointing of when the first labile DOM compounds are consumed, as well as when metabolites from the original labile pool are recycled for a secondary metabolic activity. Running incubations in greater duplication would allow for specific incubations to be terminated when DOM pools of different labilities have been consumed. Metabolites could be examined using GC-MS techniques at that time, allowing for a better understanding of the complex time-series dynamics DOM plays in aquatic ecosystems. Furthermore, DNA sequencing paired with greater duplication in the incubation would allow for the examination of functionality of specific microbial genera on known DOM compounds.

My results from GC-MS annotation of DOM compounds identified many chemical structures that were significantly different between glaciers and rock glaciers, as well as compounds common to both glacial types that were unable to be identified in the current GC-MS databases. Many of these non-annotated compounds are potentially more complex and polarizing than then gas chromatography column is able to detect. Further research should use alternative techniques like liquid chromatography mass spectrometry (LC-MS) to examine and potentially chemically identify these more complex metabolites.

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7. SUPPLEMENTAL MATERIALS

7.1 DETAILED SITE AND CLIMATE DATA FOR THE DIFFERING BIOGEOCHEMICAL AND MICROBIAL SIGNATURES OF GLACIERS AND ROCK GLACIERS.

Journal of Geophysical Research Biogeochemistry

Supporting Information for

The Differing Biogeochemical and Microbial Signatures of Glaciers and Rock Glaciers

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Table S1. Available at:

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

Additional Supporting Information (File uploaded separately)

Caption for Table S1

Introduction

This supporting information table provides detailed geographic, geologic and atmospheric site characteristics for contributing drainage areas (i.e. watersheds) immediately upslope of each sample collection point. Contributing drainage areas (CDAs) were delineated from the 1-arc second (30 meter) resolution USGS National Elevation Dataset digital elevation model using Spatial Analyst tools in ArcGis 10.3. These CDAs represent the geographic area where all surface runoff generated will flow past the sample collection point. Water samples collected are expected to integrate biogeochemical signals from the entire upslope CDA in some measure, though in virtually every case samples were collected immediately below the glacier or rock glacier terminus and overwhelmingly comprised of glacier or rock glacier meltwater. All CDA characteristics are area weighted values calculated using Zonal Statistics tools in ArcMap 10.3 and derived from official published data sources commonly used in

landscape ecology. All geospatial datasets were reprojected to the USGS Albers equal area projection prior to analysis.

Table S1. Detailed geographic, geologic and atmospheric site characteristics based on contributing drainage areas (i.e. watersheds) immediately upslope of meltwater sample collection points.

Detailed Column Descriptions:

Feature Name: Common or working name of the glacier or rock glacier sampled.

Feature Type: Type of glacial feature sampled, either glacier or rock glacier.

Mountain Range: Mountain range of feature sampled, Cascade Mountains, Rocky Mountains or Sierra Nevada.

Sample Longitude: Longitude of sample collection point, given in decimal degrees based on WGS84 geodetic datum and USGS Albers equal area projection.

Sample Latitude: Latitude of sample collection point, given in decimal degrees based on WGS84 geodetic datum and USGS Albers equal area projection.

Sample Elevation (m): Elevation of sample collection point coordinates derived from the 1-arc second (30 meter) resolution USGS National Elevation Dataset, given in meters.

Contributing Drainage Area (CDA) (m^2): Area of the contributing drainage area upslope of the sample collection point, given in square meters.

CDA Elevation Maximum (m): Contributing drainage area maximum elevation derived from the 1-arc second (30 meter) resolution USGS National Elevation Dataset, given in meters.

CDA Elevation Mean (m): Contributing drainage area area-weighted mean elevation derived from the 1-arc second (30 meter) resolution USGS National Elevation Dataset, given in meters.

CDA Slope (°): Contributing drainage area area-weighted slope derived from the 1-arc second (30 meter) resolution USGS National Elevation Dataset, given in degrees.

CDA Eastness: Contributing drainage area area-weighted eastness derived from the 1-arc second (30 meter) resolution USGS National Elevation Dataset. Eastness is an ecologically relevant measure of area aspect and varies from 1 (perfectly east facing slope) to -1 (perfectly west facing slope).

CDA Northness: Contributing drainage area area-weighted northness derived from the 1-arc second (30 meter) resolution USGS National Elevation Dataset. Northness is an ecologically relevant measure of area aspect and varies from 1 (perfectly north facing slope) to -1 (perfectly south facing slope).

CDA Primary Geology: Contributing drainage area primary geology class derived from the USGS Geologic Map of North American.

CDA Secondary Geology: Contributing drainage area primary geology type derived from the USGS Geologic Map of North American.

CDA Geology Age: Contributing drainage area geology age derived from the USGS Geologic Map of North American.

CDA Temp Max Winter (°C): Contributing drainage area area-weighted average maximum monthly temperature for Dec-Jan-Feb derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in degrees C.

CDA Temp Max Spring (°C): Contributing drainage area area-weighted average maximum monthly temperature for Mar-Apr-May derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in degrees C.

CDA Temp Max Summer (°C): Contributing drainage area area-weighted average maximum monthly temperature for Jun-Jul-Aug derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in degrees C.

CDA Temp Max Fall (°C): Contributing drainage area area-weighted average maximum monthly temperature for Sep-Oct-Nov derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in degrees C.

CDA Temp Max Annual (°C): Contributing drainage area area-weighted average maximum monthly temperature for all 12 months derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in degrees C.

CDA Temp Mean Winter (°C): Contributing drainage area area-weighted average mean monthly temperature for Dec-Jan-Feb derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in degrees C.

CDA Temp Mean Spring (°C): Contributing drainage area area-weighted average mean monthly temperature for Mar-Apr-May derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in degrees C.

CDA Temp Mean Summer (°C): Contributing drainage area area-weighted average mean monthly temperature for Jun-Jul-Aug derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in degrees C.

CDA Temp Mean Fall (°C): Contributing drainage area area-weighted average mean monthly temperature for Sep-Oct-Nov derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in degrees C.

CDA Temp Mean Annual (°C): Contributing drainage area area-weighted average mean monthly temperature for all 12 months derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in degrees C.

CDA Temp Min Winter (°C): Contributing drainage area area-weighted average minimum monthly temperature for Dec-Jan-Feb derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in degrees C.

CDA Temp Min Spring (°C): Contributing drainage area area-weighted average minimum monthly temperature for Mar-Apr-May derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in degrees C.

CDA Temp Min Summer (°C): Contributing drainage area area-weighted average minimum monthly temperature for Jun-Jul-Aug derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in degrees C.

CDA Temp Min Fall (°C): Contributing drainage area area-weighted average minimum monthly temperature for Sep-Oct-Nov derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in degrees C.

CDA Temp Min Annual (°C): Contributing drainage area area-weighted average minimum monthly temperature for all 12 months derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in degrees C.

CDA Precip Winter (mm): Contributing drainage area area-weighted cumulative precipitation for Dec-Jan-Feb derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in millimeters.

CDA Precip Spring (mm): Contributing drainage area area-weighted cumulative precipitation for Mar-Apr-May derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in millimeters.

CDA Precip Summer (mm): Contributing drainage area area-weighted cumulative precipitation for Jun-Jul-Aug derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in millimeters.

CDA Precip Fall (mm): Contributing drainage area area-weighted cumulative precipitation for Sep-Oct-Nov derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in millimeters.

CDA Precip Annual (mm): Contributing drainage area area-weighted cumulative precipitation for all 12 months derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in millimeters.

CDA Precip Snow Percent Winter: Contributing drainage area area-weighted fraction of cumulative precipitation for Dec-Jan-Feb likely falling as snow (calculated as cumulative precipitation in months with mean air temperatures < 0°C divide by cumulative precipitation for the time period) derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in percent.

CDA Precip Snow Percent Spring: Contributing drainage area area-weighted fraction of cumulative precipitation for Mar-Apr-May likely falling as snow (calculated as cumulative precipitation in months with mean air temperatures < 0°C divide by cumulative precipitation for the time period) derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in percent.

CDA Precip Snow Percent Summer: Contributing drainage area area-weighted fraction of cumulative precipitation for Jun-Jul-Aug likely falling as snow (calculated as cumulative precipitation in months with mean air temperatures < 0°C divide by cumulative precipitation for the time period) derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in percent.

CDA Precip Snow Percent Fall: Contributing drainage area area-weighted fraction of cumulative precipitation for Sep-Oct-Nov likely falling as snow (calculated as cumulative precipitation in months with mean air temperatures < 0°C divide by cumulative precipitation for the time period) derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in percent.

CDA Precip Snow Percent Annual: Contributing drainage area area-weighted fraction of cumulative precipitation for all 12 months likely falling as snow (calculated as cumulative precipitation in months with mean air temperatures < 0°C divide by cumulative precipitation for the time period) derived from the 800 meter resolution PRISM Climate Group 30-Year Normals (1981-2010), given in percent.

CDA Wet H^+ Deposition Annual (kg*ha^-1): Contributing drainage area area-weighted average annual hydronium wet deposition for 2003-2013 derived from National Atmospheric Deposition Program annual gradient maps, given in kilograms per hectare.

CDA Wet $NH4^+$ Deposition Annual (kg*ha^-1): Contributing drainage area area-weighted average annual ammonium wet deposition for 2003-2013 derived from National Atmospheric Deposition Program annual gradient maps, given in kilograms per hectare.

CDA Wet $NO3^-$ Deposition Annual (kg*ha^-1): Contributing drainage area area-weighted average annual nitrate wet deposition for 2003-2013 derived from National Atmospheric Deposition Program annual gradient maps, given in kilograms per hectare.

CDA Wet $SO4^{2-}$ Deposition Annual (kg*ha^-1): Contributing drainage area area-weighted average annual sulfate wet deposition for 2003-2013 derived from National Atmospheric Deposition Program annual gradient maps, given in kilograms per hectare.

7.2 SUPPLEMENTARY INFORMATION FOR CHAPTER 2

Metabolite data from the experiment is publically available and can be found at:

https://www.nrel.colostate.edu/projects/lvws/data.html

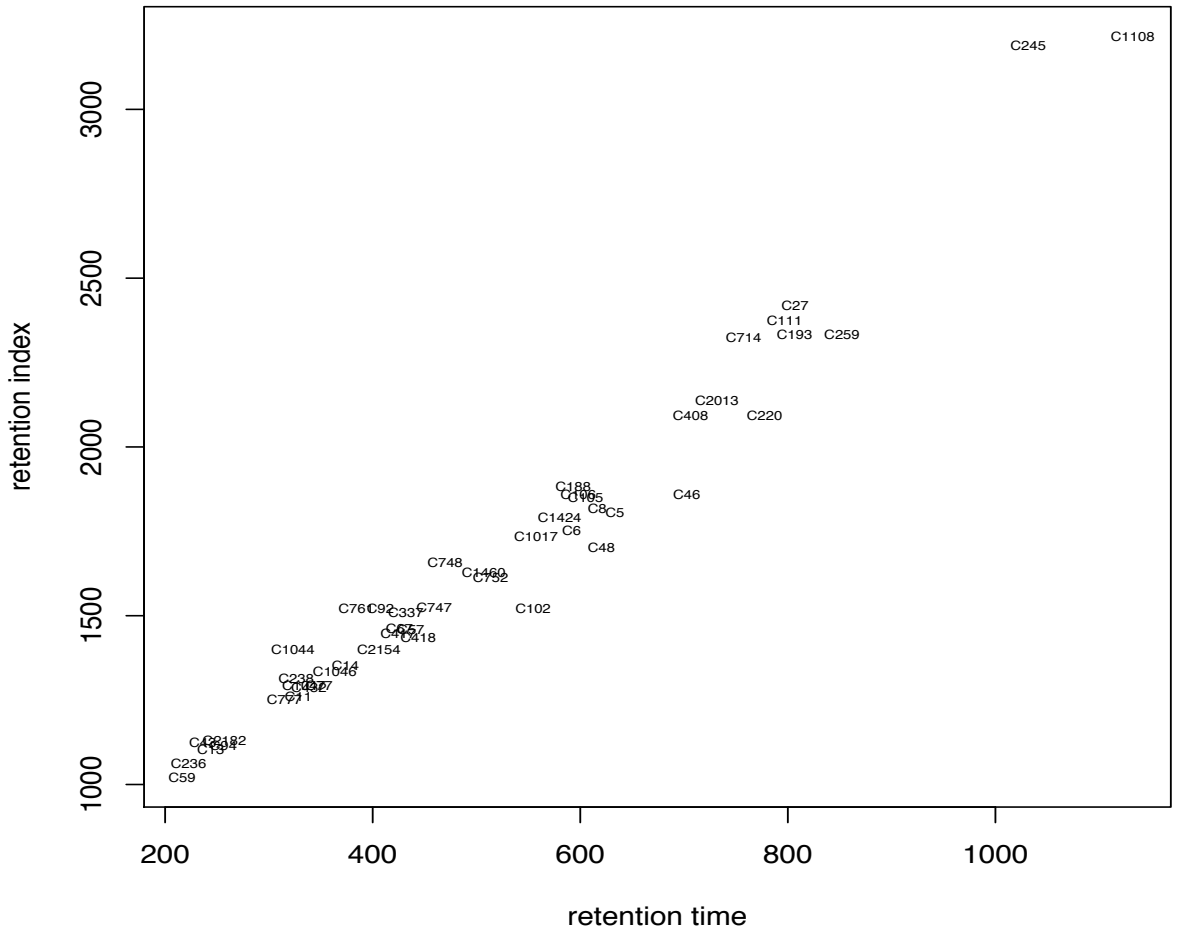

Figure 11. Golm database compound retention index vs. candidate compound retention time for GC-MS data. Retention index is based on the GOLM standards while the retention time is the independent of candidate compound assignment. Therefore a linear relationship between retention index and retention time is practical measure of confidence in candidate compound assignment for cluster features identified within experimental samples.

Table 9. DOM amounts collected from concentrated glacier and rock glacier meltwaters.

Site	Bottle (g)	Total (g)	Sample (mg)	Water Volume (L)	Estimated DOC Value (mg/L)
Peck Glacier	96.9898	96.9983	8.5	18	0.47
Arapaho Glacier	96.9175	96.9290	11.4	15	7.83
Navajo Rock Glacier	97.989	98.0023	13.3	20	0.67
Arapaho Rock Glacier	94.8669	94.8705	3.6	15	0.24
Taylor Rock Glacier	96.7476	96.7542	6.6	20	0.33
Isabelle Glacier	97.7814	97.7865	5.1	18	0.28
Andrews Glacier	96.1427	96.1533	10.6	20	0.53
Peck Rock Glacier	96.1476	96.1593	11.7	19	0.62

Table 10: Chemical recipe for concentrated glacier and rock glacier DOM incubations

Site	Blank corrected C (mg/L)	target concentration (mg/L)	vol tea (mL)	vol milli-Q (mL)	total vol (mL)	water (mL)
Peck Glacier	57.38	4	4.88	4.16	70	60.96
Arapaho Glacier	40.90	4	6.85	2.19	70	60.96
Navajo Rock Glacier	69.29	4	4.04	5.00	70	60.96
Arapaho Rock Glacier	73.49	4	3.81	5.23	70	60.96
Taylor Rock Glacier	46.03	4	6.08	2.95	70	60.96
Isabelle Glacier	30.99	4	9.04	0.00	70	60.96
Andrews Glacier	59.19	4	4.73	4.31	70	60.96
Peck Rock Glacier	73.60	4	3.80	5.23	70	60.96

7.2.1 DISSOLVED OXYGEN CALIBRATION STANDARD PREPARATION METHODS

To create the 100% DO Standard, a flask will be filled with DI water and will be allowed to equilibrate in the incubator for several days. A 0% DO Calibration Standard will also be created. 50mL of 0.5 M HNO_3 solution will be created by adding 1.59 mL of 69.6% HNO_3 to a 50 mL volumetric flask, then filling to line with deionized water. 10 mL of cobalt nitrate solution will be created by adding 0.05 g cobalt nitrate ($Co(NO_3)_2*H_2O$)

to 10 mL volumetric flask, then filling to the top with the 0.5 M HNO_3 solution. Finally, the standard will be created by adding 50µL of cobalt nitrate solution to 100 mL volumetric flask and then adding Add 1.0 g of sodium sulfite (Na_2SO_3). Finally, the 100mL flask will be filled with DI water. This calibration standard will be placed in the incubator and allowed to equilibrate for the same period of time as the 100% DO standard. See calculations below.

Calculations

0.5 M HNO_3 solution

$$\frac{0.5\ mol\ HNO_3}{0.5\ L\ H_2O} = \frac{15.7\ M\ HNO_3}{x\ L\ H_2O}$$

$$x = 0.00159L\ or\ 1.59\ mL\ HNO_3$$

Cobalt nitrate solutions (required 1000 mg Co/1000 mL 0.5 M HNO_3)

$$\frac{1000\ mg}{1000\ mL} = {}^{1\ mg\ Co}/_{1\ mL\ HNO3}$$