Linking the chemistry and reactivity of dissolved organic matter from low-latitude glaciers and rock glaciers

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Abstract

As glaciers in the western United States thaw in response to warming, they release dissolved organic matter (DOM) to alpine lakes and streams. Biological availability of DOM from small mountain glaciers is unknown. Differences in DOM bioavailability between glacier types like rock and ice glaciers remains undefined, yet rock glaciers outnumber ice glacier approximately ten to one at low latitudes. To assess which components of aquatic DOM are most reactive and the potential for glacial DOM from low latitude glaciers to subsidize heterotrophy in alpine headwaters we evaluated reactivity and molecular composition of DOM from ice glaciers and rock glaciers from four paired catchments (each with a glacier and rock glacier at their headwaters). Biological reactivity was linked to molecular composition by evaluating the chemical characteristics of each DOM pool pre- and post-incubation using common microbial community laboratory assays paired with untargeted mass spectrometry-based metabolomics. Glacier and rock glacier DOM was similar in concentration and chemodiversity, but differed in composition. When incubated with a common microbial community, DOM from ice glacier meltwaters contained a higher proportion of bioavailable DOM (BDOM), and resulted in greater bacterial growth efficiency. Differences in DOM reactivity between glacier types was determined by differences in the relative abundance of only a few dozen compounds. Though BDOM was lower in rock glaciers, because rock glaciers are more abundant and are expected to have greater longevity, we propose that both glacial types will be important sources of bioavailable DOM to alpine headwaters over the coming years to decades.

Introduction

Glaciers bridge the atmospheric-terrestrial-aquatic interface, integrating atmospherically deposited chemicals with weathering products and releasing reactive solutes to adjacent surface waters (Williams et al. 2007; Dubnick et al. 2010; Fellman et al. 2010; Stibal et al. 2010; Stubbins et al. 2012). Between continental and mountain glaciers, mountain glaciers release the largest flux of carbon from melting ice annually (Hood et al. 2015). Dissolved organic matter (DOM) from these large mountain glaciers of the European Alps and Alaska has been shown to stimulate heterotrophic respiration (Hood et al. 2009; Singer et al. 2012). Glaciers and rock glaciers, each with distinct geophysical attributes, are common in many of the alpine headwaters of the western U.S. (Fegel et al. 2016). Glaciers are massive ice bodies that form and persist in areas where annual snow accumulation is greater than annual snow ablation at decadal or longer time spans. Rock glaciers are flowing bodies of permafrost, composed of coarse talus and granular regolith both bound and lubricated by interstitial ice (Berthling, 2011). In the western United States, rock glaciers are an order of magnitude more abundant than ice glaciers and are more resistant to warming temperatures than ice glaciers (Fegel et al. 2016). Yet little is known about the quantity or quality of DOM being released from smaller glaciers or rock glaciers in mountain headwater ecosystems or their potential to affect low-latitude alpine ecosystems.

The chemical composition of DOM from natural aquatic systems is complex (Hedges et al. 2000; Kim et al. 2006; Hockaday et al. 2009), and glacier and rock glacier meltwaters are not likely to be an exception. Previously, assessment of the bioavailability of DOM relied on bioassays that measure the rate and amount of DOM consumed over time (e.g. Amon and Benner 1996; del Giorgio and Cole 1998; Guillemette and del Giorgio 2011). While some coarse characterizations of molecular composition (Benner 2002; Berggren and del Giorgio 2015) or the

lability of individual compounds (del Giorgio and Cole 1998) have previously been applied to characterize bioavailable DOM (BDOM), only recently has the research community begun to apply high-resolution analytical chemistry to assess the molecular characteristics of environmental DOM (e.g. Kellerman et al. 2014, 2015; Andrilli et al. 2015). Whereas no single method can identify the entire spectrum of compounds present in an environmental DOM pool (Derenne and Tu 2014), mass spectrometry allows for molecular identification of thousands of specific DOM compounds, simultaneously yielding a more specific and broader metric for DOM analysis compared to spectroscopic techniques such as fluorescence or ultraviolet absorption.

Simultaneous identification and quantification of total BDOM is not an easy task. Difficulties in experimentally connecting molecular characterization of DOM pools to their bioavailability are partly due to the highly diverse constituent compounds that compose natural DOM (Derenne and Tu 2014). The effects of individual compounds identified to be bioavailable (e.g. amino acids in glacial meltwaters (Fegel et al. 2016) may not be representative of the total BDOM pool. It is also possible that DOM with a high diversity of labile compounds may have positive feedbacks on the bioavailability of the total DOM pool by asserting priming effects on the total pool (Guenet et al. 2010). In addition, certain metabolites within DOM pools may not be bioavailable individually, but may act as cofactor metabolites that allow for a mutualistic increase in bioavailability [Hilker 2014]. Thus, the relationship between DOM pool characteristics and lability remains largely unknown in most natural systems. However, some patterns are beginning to emerge, like the consistently high lability of proteinaceous DOM that has been found in glacial, estuarine, and marine environments [Andrilli et al. 2015]. Defining these fundamental relationships between chemical composition of DOM and lability has the

potential to provide a better understanding for how DOM pools contribute to heterotrophy across a broad spectrum of aquatic ecosystems.

There are defining characteristics in the origin of DOM that differentiate glaciers from rock glaciers. DOM derived from ice glaciers is structured by in situ microbial activity and can be an important source of chemical energy to headwater ecosystems (Hood et al. 2009; Singer et al. 2012; Fellman et al. 2015; Fegel et al. 2016). DOM derived from rock glaciers is a conglomeration of carbon compounds percolated through from vegetation growing on the rock glacier surface and microbial processing within the rock glacier itself (Wahrhaftig and Cox, 1959; Williams et al. 2007; Fegel et al. 2016). Previous research has shown that DOM released from glaciers and rock glaciers in the western United States differ in their optical properties (Fegel et al. 2016). Whether or not these differences in the optical properties of DOM between glacial types translates to differences in their reactivity or proportion of BDOM remains unknown.

To address this, we asked whether differences in the composition of DOM between ice glaciers and rock glaciers affected differences in microbial metabolism and whether DOM chemistry in ice and rock glacier meltwater in the western United States is similar to what has been reported for other glacial meltwaters. Here we present the results of laboratory incubations of DOM from ice glacier and rock glacier meltwaters with a common microbial community. Incubations were bookended (i.e. analyzed before and after incubation) with non-targeted metabolomic analysis of DOM via gas chromatography mass spectrometry (GC-MS) to determine differences in the specific chemical compounds metabolized by microbial processing. By exposing DOM from different sources to a common microbial community we were able to

assess the lability of each DOM source and analyze which differences in the molecular composition of DOM affected its lability and carbon use efficiency.

Methods

Site Description

Paired ice glaciers and rock glaciers within four watersheds on the Front Range of Northern Colorado were selected based on their size (>0.5km^2) and the proximity to each other within the watershed, forming pairs of features with similar geographic parameters (Figure 1). We collected samples of glacier meltwater in the late summer to capture the greatest contribution of ice melt and minimize annual snowmelt contribution. We sampled the four pairs in September 2014. Sample sites were: Isabelle Glacier (-105.640994,40.063373) and Navajo Rock Glacier (-105.636092,40.061200), Arapaho Glacier (-105.646351,40.023378) and Arapaho Rock Glacier (-105.637699, 40.022482), Peck Glacier (-105.663810,40.068332) and Peck Rock Glacier (-105.664310,40.071642) in the Indian Peaks Wilderness west of Boulder, CO; and Andrews Glacier (-105.680639,40.288370) and Taylor Rock Glacier (-105.671197,40.275568) in the Loch Vale Watershed in Rocky Mountain National Park (Figure 1a). A complete site description for each site is given in Fegel et al. (2016).

Field extraction of DOM

At each glacier, meltwaters were collected in the early to mid-morning (0500-1000) to minimize diurnal variability in ice melt from solar radiation. DOM was extracted from 20 L of meltwater collected at the terminus of each feature in the field using a slightly modified protocol established by Dittmar et al. (2008, Supplemental Information). Briefly, meltwater samples were passed through pre-combusted (450° C, 5hr) Whatman GF/F filters (GE Whatman, Pittsburg, PA, USA), acidified to ~ pH 2 with 32% HCl, and concentrated using Bond Elut PPL carbon

extraction cartridges (Agilent, Santa Clara, CA, USA). Concentrated DOM was eluted in the field with 10mL HPLC grade methanol per cartridge into cleaned, combusted, and pre-weighed 120mL borosilicate bottles.

Metabolite Analyses

Once in the laboratory, pre-incubation DOM samples were dried under a clean N_2 stream and weighed (http://www.nrel.colostate.edu/projects/lvws/data.html). Each sample (n=8) was prepared for metabolomic analysis by dissolving the dried OM into fresh HPLC-grade methanol to a final concentration of 2 mg ml^{-1}.

After the microbial bioassays (in which all samples were run in duplicate), post-incubation DOM samples were collected separately from each individual microcosm (n=16). Water from each microcosm was filtered through pre-leached 0.2 μm Millipore filters (EMD Millipore, Billerica, MA, USA) to remove microbial biomass, freeze-dried, and the total remaining DOM was weighed and re-dissolved into HPLC-grade methanol (http://www.nrel.colostate.edu/projects/lvws/data.html). In order to increase the volatility of molecules analyzed through GC-MS, samples were derivatized with trimethylsilane (TMS) using standard protocols (Supplemental Materials) (Pierce, 1968).

Metabolomics

Both pre- and post- incubation OM samples were analyzed with inline gas chromatography-mass spectroscopy (GC-MS) at the Proteomics and Metabolomics Facility at Colorado State University. 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

158 min^{-1} helium gas flow rate, and the program consisted of 80°C for 30 sec, a ramp of 15°C per
159 min to 330°C, and an 8 min hold. Masses between 50-650 m/z were scanned at 5 scans sec^{-1} after
160 electron impact ionization (Broeckling et al. 2014).

161 **DOM lability experiments**

162 Concentrated DOM samples from each of the eight study sites were diluted to 4 mg L^{-1} C and
163 incubated *in vitro* with a natural microbial community collected from The Loch, a small sub-
164 alpine lake in Rocky Mountain National Park, CO, USA, (-105.6455, 40.2976). Unfiltered lake
165 water collected from The Loch was aged for 2 years at 5 °C in order to remove the majority of
166 the bioavailable carbon. At the start of the experiment DOM concentration of the lake water was
167 0.7 mg L^{-1} C. At the initiation of the incubations 2 L of aged lake water was filtered through a
168 pre-combusted (450° C, 5hr) Whatman GF/C filter (1.2µm nominal pore size) (GE Whatman,
169 Pittsburg, PA, USA) to remove bacterial grazers (e.g. protists and metazoans). Three aliquots of
170 filtered-aged lake water was preserved with 2% formalin (37% formaldehyde), and set aside for
171 enumeration of bacteria at the initiation of the experiment (i.e. t=0), and a second set of three
172 aliquots (7mL) was used for initial DOC/TN analysis on a Shimadzu TOC-VWS analyzer
173 (Shimadzu Corp., Kyoto, Japan). To create normalized concentrations (4 mg L^{-1} C), in each
174 incubation bottle we added 60.96 mL of unfiltered Loch water, between 3.80 and 9.06mL of
175 concentrated DOC solution (depending on the initial concentration), and filled to 70mL total
176 volume with DI; resulting in standardized concentrations of 4 mg L^{-1} C in each incubation bottle.
177 During the experiment, microcosms that received DOM from ice glacier and rock glacier sites
178 were incubated alongside control incubations that contained lake water and the common
179 microbial community but with no added DOM (i.e. experimental control). In addition, an

180 analytical blank of MilliQ water was incubated to correct for any instrumental drift that occurred
181 during the experiment.
182 All microcosms were incubated simultaneously at 15 °C for 10 weeks. To calculate microbial
183 respiration, we measured changes in dissolved oxygen (DO) at 1-minute intervals in each
184 microcosm using an Oxy-4 fiber-optic dissolved oxygen probe (PreSens, Regensburg, Germany).
185 The incubation was terminated when the fastest metabolizing microcosm approached 4mg L^{-1}
186 DO to avoid hypoxia and the potential for anaerobic metabolism. All measurements with
187 amplitude less than 20000 amps were removed because of the potential for inaccurate readings of
188 DO. Absolute values from the raw fiber optic measurements were corrected for analytical drift
189 by subtraction of changes in signal from the MilliQ water analytical control over the course of
190 the experiment.
191 **Bacterial Cell Counts and Bulk Chemistry**
192 From each microcosm we collected a 2 mL aliquot post-incubation and preserved it with 2%
193 formalin (final concentration) to assess changes in cell abundance during the course of the
194 incubation. Aliquots were filtered onto 0.2 μm Millipore polycarbonate filters (EMD Millipore,
195 Billerica, MA, USA) and stained with Acridine Orange (Hobbie et al. 1977, Supplemental
196 Information) for enumeration. Post-incubation we assessed TOC and TDN
197 (http://www.nrel.colostate.edu/projects/lvws/data.html) for each microcosm. To ensure that
198 filtration did not contribute significant additional carbon to the dissolved carbon pool, a subset of
199 blank Millipore filters flushed with MilliQ was analyzed for TOC and TN. All filtration of
200 microcosm water for chemical analysis was conducted on pre-leached Millipore filters that
201 contributed insignificant amounts of carbon to the filtrate
202 (http://www.nrel.colostate.edu/projects/lvws/data.html).

Data Analysis

Metabolite Analysis

Compound annotation was prioritized based on order of normalized intensity and statistical difference between glacier types. Putative identification (candidate compounds based on physicochemical characteristics and spectral similarity with spectral libraries (Sumner et al. 2007), was made using spectral matching to in-house, NISTv12, Golm, Metlin, and Massbank metabolite databases. Clustered features assigned candidate compounds in our study had high similarity (>90%) to spectra from known standard compounds within the databases used (Supplemental Information). Molecular rank was calculated by ordering candidate compounds by their normalized ion intensity for pre- and post- incubation sample averages for all features. Chemical diversity was calculated using the Shannon-Wiener diversity index (Shannon 1948) by treating each unique chemical compound identified though GC-MS as a 'species'. This was done for DOM composition both before and after incubation in order to estimate changes in chemical diversity through microbial metabolism.

Analysis of DOM Reactivity

Oxygen consumption rates were fit to a Berner-Multi-G two-pool decay model to estimate the size of the labile pool (BDOM) and the recalcitrant pool. Oxygen consumption was averaged for each glacier type and confidence intervals were calculated at $\alpha=0.05$. Data were smoothed using a third order polynomial ($R^2>0.999$) and 95% confidence intervals were plotted. Berner's Multi-G model was used to model carbon pool bioavailability (Berner 1980; Guillemette and del Giorgio 2011), using SAS. Dissolved oxygen curves generated from the incubation were fit to the 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 used a least square means to test for statistical differences between ice glaciers and rock glaciers in the size of the B_1 and B_0 pools. Total C consumed was calculated as the difference in pre- and post-incubation DOC values.

To address the metabolic quality of consumed carbon we calculated Respiratory Quotient (RQ) as the carbon consumed (in mg L^{-1}) divided by the oxygen (as O_2) consumed (in mg L^{-1}).

Bacterial growth efficiency (BGE) was calculated to examine how efficiently each carbon source was incorporated into bacterial biomass. To calculate BGE we divided bacterial production (BP) by the sum of bacterial respiration and bacterial production (BR + BP)(del Giorgio and Cole 1998). Thus, BGE is a ratio of carbon incorporated into biomass relative to total carbon consumed for the bacterial community. BP was measured as change in cell number over time and converted to units C using an estimate of 20 fg C per bacterial cell (Borsheim and Bratbak 1987) as a conversion. Carbon consumption rate was measured as carbon consumed during the course of the incubation by measuring DOC content in each filtered pre- and post-incubation sample using a TOC/TDN analyzer.

Statistical Analyses

The DOM composition for each treatment (glaciers vs. rock glaciers) was compared with ANOVA for 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. Post-incubation samples were corrected for compounds added by the lake water by subtracting the peak intensities for each chemical candidate within the lake water (i.e. experimental control) from each post incubation sample.

We identified differences in compounds present in each sample using PCA conducted on mean-centered and pareto variance-scaled data using the pcaMethods package in R. We analyzed GC-MS spectra using principal component analysis (PCA) in R (R Core Team, 2014). For each sample, raw data files were converted to .cdf format, and a matrix of molecular features as defined by retention time and mass to charge ratio (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). We grouped all spectral features based on an in-house clustering tool, RAMClustR, which uses spectra based coelution and covariance across the full dataset groups features (Broeckling et al. 2014). We used the t-test configured for non-parametric Welch-Satterthwaite test to compare differences in RQ, BGE, chemodiversity, and loss of chemodiversity between glacier types. Our use of a Welch-Satterthwaite test allowed for comparison of samples of unequal variance and distribution.

Results

Ice glacier and rock glacier DOM composition

Neither the chemical diversity (mean=2.82), nor the C:N ratio (mean=2.1) differed between glaciers and rock glaciers (Table 1). In addition, the molecular composition was similar in each glacier type for the top 25 most abundant molecules. Combined, the glacier and rock glacier DOM molecular composition consisted of 2033 compounds. Each individual compound consisted of 3 to170 individual mass spectral features, for a total of 14,571 unique mass spectral features in the complete dataset. Of the 2033 compounds, 328 were chosen for annotation based either on high ion intensity (n=278) or significantly different relative abundances between glacier and rock glacier sources (n=33). Annotated compounds consisted primarily of simple sugars, amino acids and other organic acids (http://www.nrel.colostate.edu/projects/lvws/data.html).

There were significant differences between glacier types in the molecular composition of less abundant compounds (Table 2), although the C:N ratio, chemical diversity, and compounds with greatest ion intensity were similar. PCA analysis of the complete dataset suggested 33 key compounds, ordinated on axes three and five of our PCA analysis (Figure 2A), were responsible for differences in the ice and rock glacier DOM pools (Table 2). Of those 33 compounds, 5 were assigned putative structures (Table 2, also see http://www.nrel.colostate.edu/projects/lvws/data.html) with ice glacier meltwaters significantly enriched in the simple sugar maltose and the amino acid glutamate, and rock glacier meltwaters enriched in the primary organic acids, glycolate, threonate and quinate (Table 2). The other 28 compounds represented components of the DOM pool that differentiated DOM composition from each glacier type, but were not present in the databases we employed and had no known structural analogs.

Incubations

The bacterial respiration rates of DOM from each glacier type were not significantly different, as estimated by the decay constant k (Table 3). However, a significantly larger portion of ice glacier DOM was bioavailable, (i.e. BDOM, B_1 = 58.8 ± 9.7%) compared to rock glacier DOM (B_1= 37.3 ± 10.2%, $p < 0.01$, Table 3). Bacterial growth efficiency (BGE) was higher for microbial communities incubated with ice glacier DOM compared to rock glacier DOM (G = 0.26 ± 0.13, RG = 0.16 ± 0.16, Table 3). In general, more oxygen was consumed per mg organic carbon metabolized in incubations that contained glacial DOM compared to those that contained rock glacier DOM, even though the amount of carbon consumed between treatments was similar (Figure 4, Table 3). This resulted in a lower respiratory quotient (RQ) for glacial derived DOM compared to rock glacier derived DOM overall.

Post-incubation DOM analysis

Analysis of DOM after the incubation period allowed us to assess how microbial metabolism altered the composition of DOM. Incubation with a common microbial community both rarified and homogenized DOM between glacier types, resulting in fewer compounds with high ion intensities, and more compounds with low ion intensities (Figure 3). This reorganized the molecular rank-abundance curve, resulting in a different set of compounds with the highest relative abundance in the pre vs. post-incubation dataset (Table 4). However, similar to pre-incubation DOM composition, post-incubation ice glacier and rock glacier derived DOM shared the same abundant compounds (Figure 2B) (http://www.nrel.colostate.edu/projects/lvws/data.html). Interestingly, the chemical diversity of DOM between glacier types diverged during the course of the incubation. At the end of the incubation glacial DOM had significantly higher diversity and rock glacier DOM had

significantly lower diversity compared to pre-incubation DOM for the same glacial type (Table 1, Figure 2C). Combined with molecular rank abundance data (ordered on ion intensity), this change in chemical diversity resulted in an increase in the richness of glacier DOM pools by microbial metabolism and a decrease in richness in rock glacier DOM during the incubation. For both DOM sources, many of the organic acids and sugars present pre-incubation were consumed while amino acids that were present at lower intensities in pre-incubation DOM pools increased in relative abundance in post-incubation DOM pools. While there were differences in the chemical diversity of rock and ice glacier derived DOM post-incubation, PCA suggested were no differences in the overall molecular composition between glacier types (Figure 2B). This trend did not follow for all rock glaciers, as one replicate of Taylor Rock Glacier (lowest left point, Figure 2A & 2B) saw few changes in its principal components after the incubation. Interestingly, while no significant difference was seen in GC-MS compounds between glacier type post incubation (Figure 2B), both replicates of Peck Rock Glacier (lower rightmost points Figure 2B) showed separation along the y-axis (PC1) of their PCA, a separation that was not seen before microbial metabolism (Figure 2A). The reasons for this remain unclear, but may be due geographical separation (climatological differences) between Peck Rock Glacier and all other sites.

Discussion

Our results demonstrate that chemically complex DOM released from Rocky Mountain ice glaciers and rock glaciers stimulated bacterial respiration and productivity. Each DOM source had unique molecular characteristics that reflected their quality as an energy source for microbial metabolism. Glacier and rock glacier DOM shared many of the same organic compounds, but differences in the relative concentrations of just 33 compounds appeared to drive differences in

the bioavailability of DOM between glacier types. Enrichment in simple sugars of glacier DOM contributed to higher bioavailability of glacier DOM as measured by size of the BDOM pool and bacterial growth efficiency (BGE). The DOM from rock glaciers, was enriched in less bioavailable, primary organic acids. In spite of these differences, the bacteria homogenized DOM from both sources during the incubations, resulting in more similar DOM composition at the end of the incubation compared to the composition of the DOM before the incubation. In our experiment, the source of the DOM affected its composition and reactivity and interaction with the microbial community metabolism re-structured its chemistry.

Glaciers are a source of labile DOM

The results from our study of glaciers in Colorado showed similar patterns to previous studies of glacial DOM in the Arctic and the European Alps, where bioavailable carbon from glaciers also stimulated microbial production (Hood et al. 2009; Singer et al. 2012; Fellman et al. 2015). Carbon concentrations in meltwaters from ice and rock glaciers in our study were low (0.5- 1.5 mg C L^{-1}), but similar to the global average of 0.97 mg C L^{-1} that has been previously reported for glaciers (Dubnick et al. 2010; Stubbins 2012; Singer et al. 2012; Hood et al. 2015). The percentage of DOM that was bioavailable (BDOM) from ice glacier DOM in our study (~50%) was comparable to values of BDOM seen in DOM derived from glaciers in the European Alps (58%), and within the range of values reported from glaciers in the Alaskan Range (23-66%) (Hood et al. 2009; Singer et al. 2012). In all of these studies, including the results reported here, glaciers had higher BDOM concentrations than other surficial freshwaters (Volk et al. 1997). Previous reports on freshwater noted 16.5-34.5% of BDOM, which more closely resembled the amount of BDOM we report from rock glacier BDOM (37% on average).

We propose that ice glaciers have higher proportions of BDOM compared to rock glaciers for two reasons. First, rock glaciers host mosses, lichens, and vascular plants, including woody shrubs and trees (Wahrhaftig and Cox, 1959; Cannone and Gerdol 2003; Burga et al. 2004). Dissolved organic compounds released from these allochthonous phototrophs include a wide variety of complex organic acids and polymers that are potentially difficult for microbes in freshwaters to metabolize, resulting in a higher proportion of recalcitrant DOM within rock glaciers (Wetzel 1992, Rovira and Vallejo 2002). The potential for the development of protosoils within the rock glacier matrix may promote the presence of liquid pore water. DOM from rock glaciers may already be partially processed by microbes within this protosoil environment by the time it reaches the sub-rock-glacial surface water. This pre-processing may result in DOM with a level of lability more similar to what is delivered to inland surface waters and derived from the soil SOM matrix (Fellman et al. 2010). Conversely, BDOM in ice glaciers comes from autocthonous sources (i.e. microbial production and processing on the ice surface and within the ice interstitial space (Fellman et al. 2009) and atmospherically deposited aerosols (Hood et al. 2012), both of which appear to be more biologically available than terrestrial sources. Ice glacier BDOM may be preserved in the ice matrix and physically inaccessible to microbial degradation. In contrast, rock glacier DOM may be in contact with intraglacial sediments and liquid water, allowing for further processing through microbial metabolism. In addition, sub-glacial environments below ice glaciers are often anoxic (Tranter et al. 2005), allowing some compounds to remain in a chemically reduced state until released with ice melt. This would allow for rapid metabolism of these energetically-preferable, reduced compounds once unlocked from the ice and released to an oxic environment (Hood et al. 2009). Thus the production of DOM within each feature differs and pathways by which that DOM is delivered to the glacial

386 meltwater differ. These mechanisms would result in the differences in the proportion of BDOM
387 we observed in our bioassays of DOM lability.
388 The molecular structure of glacial DOM from large glaciers has been mechanistically linked to
389 its lability and shown to be more labile than marine or freshwater derived material due to the
390 presence of protein and amino-sugar components of microbial origin (Andrilli et al. 2015). This
391 is consistent with studies from other inland waters that show that higher DOM bioavailability
392 correlates with enrichment of amino acids and simple sugars (Lafreniere and Sharp 2004;
393 Williams et al. 2007; Dubnick et al. 2010). Taken together with the results presented here, we
394 suggest that DOM enriched in amino acids and simple sugars may be a key trait that defines
395 BDOM and be characteristic of glacial meltwaters worldwide.
396 **Glacier DOM Diversity**
397 The diversity of compounds in organic matter released from glaciers in this study was similar
398 to the DOM diversity found in other freshwater systems (Dubnick et al. 2010, Guillemette and
399 del Giorgio 2011, Kellerman et al. 2014). We found that chemical diversity was very similar
400 between ice glaciers and rock glaciers. However, the diversity of DOM between glacial types
401 was altered by microbial metabolism in different ways when incubated. Chemical diversity in
402 incubations with glacial DOM increased during the course of the incubation, whereas chemical
403 diversity decreased in incubations with rock glacier DOM. This suggests that when BDOM is a
404 smaller fraction of total DOM, microbial communities may use previously processed DOM to
405 fuel their own heterotrophic metabolism. This would reduce chemical diversity through the
406 consumption of primary metabolites (i.e. simple organic acids).
407 Our study expands the understanding of DOM complexity in inland waters by assessing the
408 glacial contribution of DOM to alpine headwaters and moving beyond broad functional groups

and compound classes identification to identification of specific compounds (Dubnick et al. 2010; Fellman et al. 2010; Singer et al. 2012, Fegel et al. 2016). By using a mass spectrometry based technique we were able to identify individual candidate compounds through metabolomics paired with total chemical pool complexity. Our results are more detailed, but consistent with previous analyses that used a fluorescence index (Fegel et al. 2016). Results from Fegel et al. (2016) showed higher fluorescence index (FI) values in ice glacier meltwaters compared to that of rock glaciers, indicating a higher presence of proteinaceous (i.e. nitrogen containing) compounds in ice glacier meltwaters. Here, using metabolomics, we identified higher proportions of specific amino acids within ice glacier meltwaters compared to rock glaciers that are likely the cause of the increased FI values observed (Fegel et al. 2016). Application of a community level analytical approach such as mass spectrometry-based metabolomics provides the opportunity to disentangle the critically important components of complex DOM pools for aquatic C cycling, however only a few studies have used metabolomic approaches to address the bioavailability of DOM as a heterotrophic subsidy (see Kujawinski 2011 for a review; Logue et al. 2015).

Whereas our results provide unambiguous evidence that differences in bioavailability are due to chemical differences between DOM that differ in origin from glacier types, some components of the DOM pool are likely not assessed by the GC-MS we employed. GC-MS measures molecules up to 1200 amu with electron impact ionization, leaving larger molecules unidentified. Further, some of the components important for glacial C processing could not be identified using the most current databases. While we were unable to determine exactly what those compounds were, we are able to say what they were not. Many of the unannotated compounds that were significantly different between glaciers and rock glaciers had m/z (mass to charge ratio) values

432 greater than 200 (Table 2), indicative of secondary metabolites with large molecular weights
433 relative to primary metabolites, most of which have mass to charge (m/z) values between 60 and
434 205. Products of primary metabolism, e.g. amino acids, organic acids, sugars and peptides are
435 well represented in mass spectrometry databases and therefore more easily identified with a GC-
436 MS approach. Less well represented are larger molecular weight products of secondary
437 metabolism such as terpenes, alkaloids, polyketides, aromatic structures, and products of mixed
438 biosynthesis. Known metabolites are often a small portion of data obtained through mass
439 spectrometry (<10%), with much of the data reflecting unknown metabolites or those yet to be
440 verified with standards (Jansson et al. 2009). Yet, the quality of mass spectrometry databases and
441 representation is rapidly improving, and this will likely be a critically important source of
442 information for understanding the relationship between molecular composition of DOM and its
443 lability in future research. The high proportion of compounds that could not be annotated with
444 candidate assignments in our study reflects the infancy of using mass spectrometry databases in
445 environmental applications. It exposes the need for more environmentally derived spectra and
446 standards from secondary metabolism to be added to the current metabolite databases. With the
447 expansion of metabolic databases, and the verification of environmentally-derived spectra to
448 known standard compounds, metabolomic techniques will provide the ability to directly assess
449 the functionality of in situ metabolic pathways from natural systems.

450 **Distribution of Glaciers and Rock Glaciers**

451 Complete mapping of rock glaciers has only been completed for the contiguous United States
452 and portions of South America, yet early results suggest rock glaciers may be exceptionally more
453 abundant than ice glaciers in headwater ecosystems (Falaschi et al. 2013; Rangecroft et al. 2015;
454 Fegel et al. 2016). In addition to their ubiquity, rock glacier derived 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 only ~15% less BDOM (~37% in rock glaciers compared to 52% in glacier derived DOM), as observed in our study, rock glaciers may play a role in heterotrophic metabolism of alpine headwaters. Current glacial carbon modeling neglects the contribution of rock glacial carbon (Hood et al. 2015), yet our results suggest that rock glaciers supply a significant source of organic matter for metabolism and will continue to do so for decades to come.

In conclusion, the work presented here suggests that as long as the cryosphere is present, DOM entering alpine streams and lakes from both glacier and rock glaciers is likely to continue to contribute to ecosystem heterotrophy during summer melt. We found clear differences in the proportion of BDOM and microbial growth efficiency between glacier and rock glaciers DOM. This suggests a higher potential for secondary production in systems with glacial headwaters compared to those with rock glacier headwaters. The applicability of metabolomics to the analysis of natural DOM provides the potential to expand the application of our results for the prediction of BDOM beyond glaciated ecosystems to a broad range of inland waters. At the same time our approach exposes the need for better metabolite database development for ecological metabolomic approaches. In the coming decades, the DOM inputs to alpine lakes and streams will increasingly be dominated by rock glacier-like DOM inputs, as ice glaciers are lost and rock glaciers continue to contribute to alpine hydrology due to their slower ablation rates. Despite differences in chemical compounds and BDOM pool sizes between glacier types, both glaciers and rock glaciers represent an important heterotrophic subsidy of organic carbon to alpine headwaters that will fuel ecosystems processes from the bottom up for years to come.

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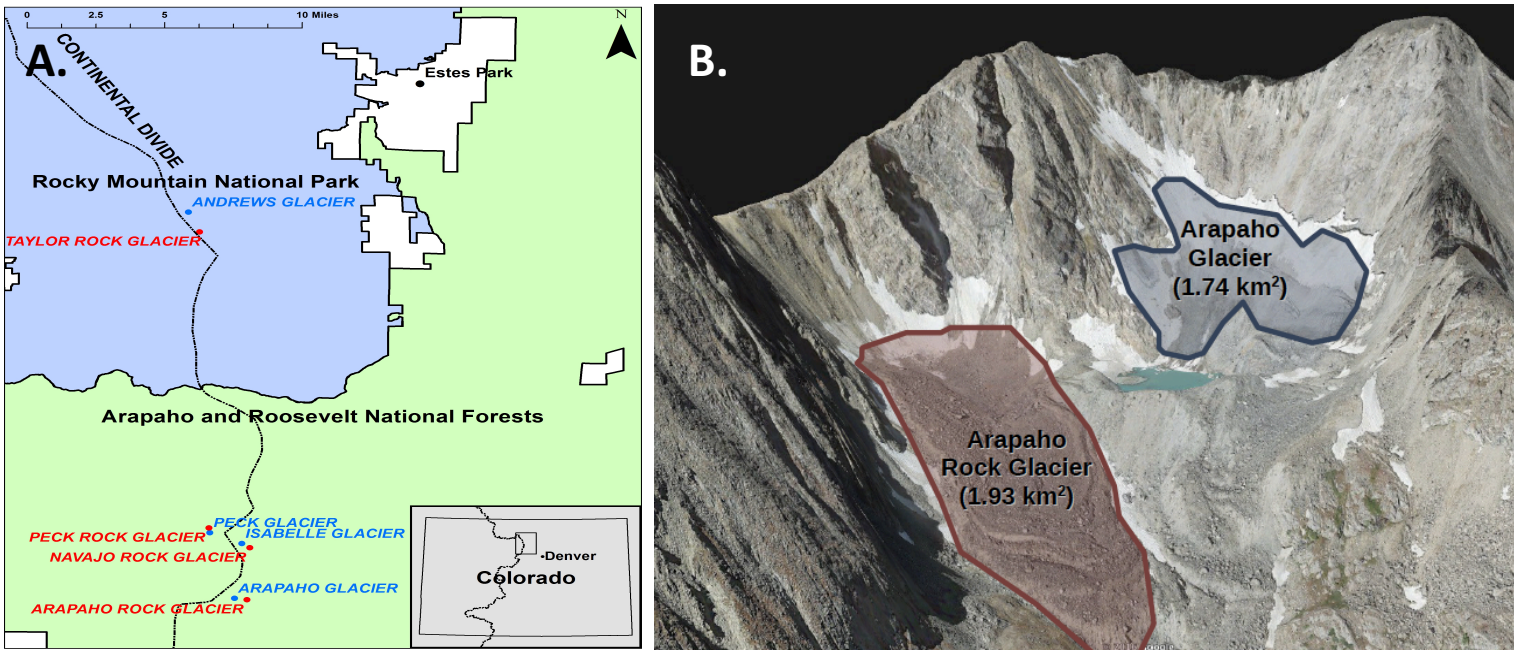
Figure Legends

Figure 1 A) Stars note the approximate location of each glacier feature that was sampled for this study within the state of Colorado. In total four pairs of glaciers and rock glaciers were sampled along the Front Range of Colorado. **(B) Isabelle Glacier and Navajo Rock Glacier** form a pair of a glacier and a rock glacier from the same watershed are shown here to illustrate the differences between the two types of features.

Figure 2 PCA GC-MS Analysis DOM compounds **A)** PCA plots showing separation along PC5 between glaciers (blue) and rock glaciers (red) before incubation and **B)** no significant difference between glacier types after incubation (p-value of 0.119 and 0.0681 for PC1 and PC2, respectively). **C)** The Shannon-Wiener Index (SW) for chemical diversity was similar between glaciers and rock glaciers before incubation, however microbial metabolism increased chemical diversity in glacier DOM and decreased chemical diversity in rock glacier DOM.

Figure 3 Molecular distribution of GC-MS identified compounds A) Distribution of compounds by ion intensity pre- (orange) and post-incubation (brown). Many of the compounds present before incubation that were of intermediate abundance were metabolized. The most abundant molecules were different between pre- and post- incubation metabolomics analysis (see Table 4).

Figure 4 Dissolved oxygen consumption curves from glaciated watersheds (n=8). Values were averaged for each of the glaciers (blue) and rock glaciers (red), and the analytical control (dotted black). All curves were smoothed using a third order polynomial regression function (R^2=0.999). Here we show 95% Confidence Intervals in light blue for glaciers and in pink for rock glaciers. Glaciers consumed a larger portion of available DOM during the course of the incubation.



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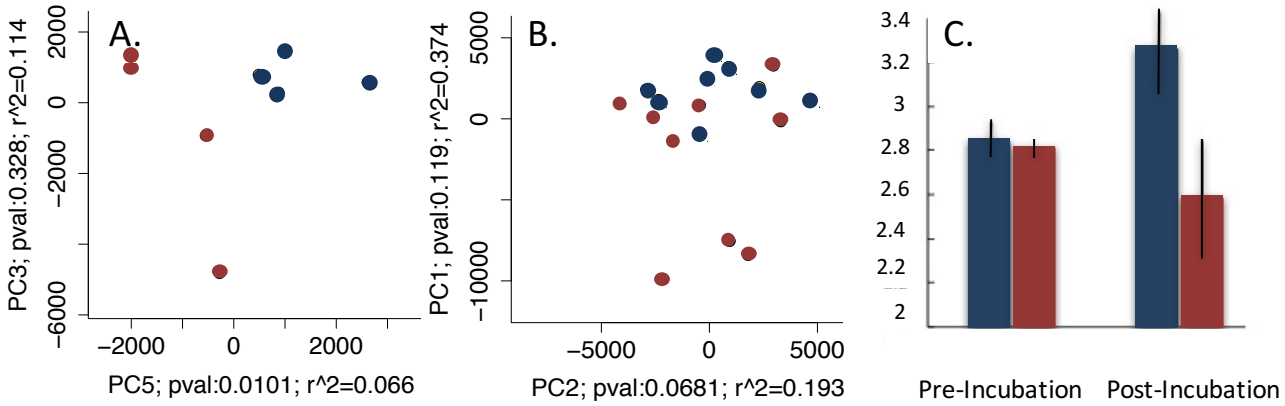

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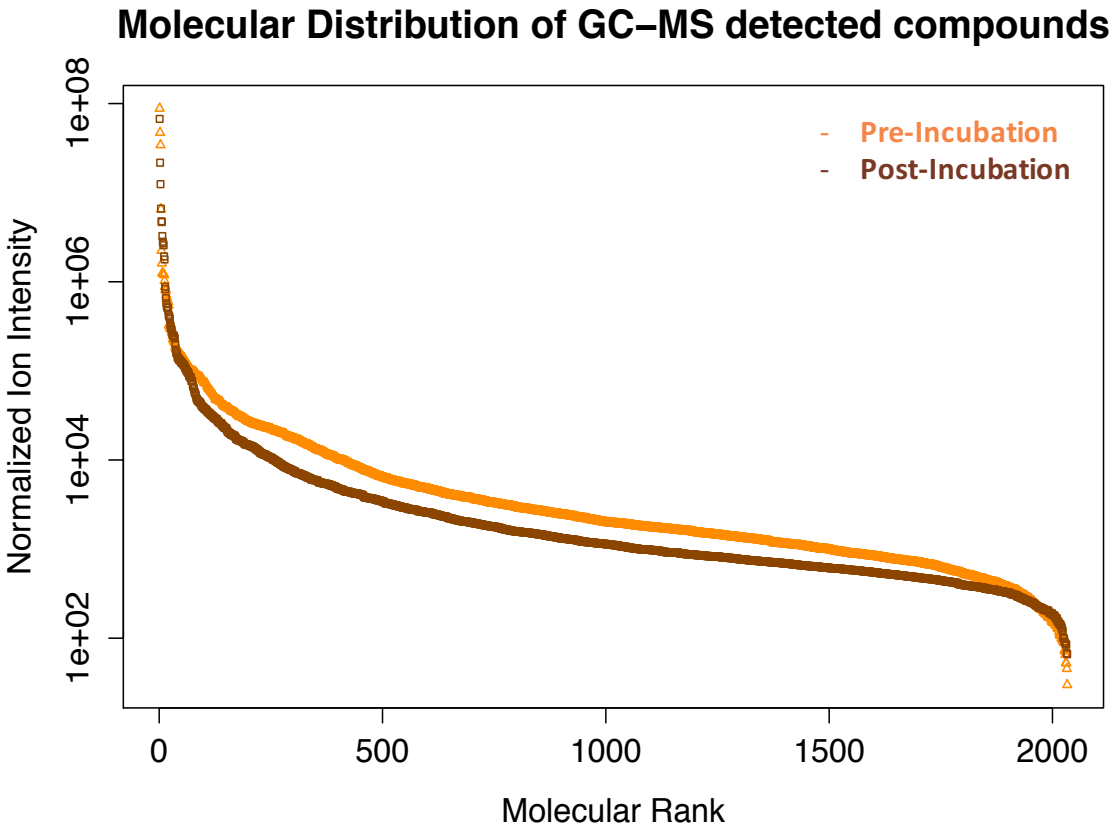

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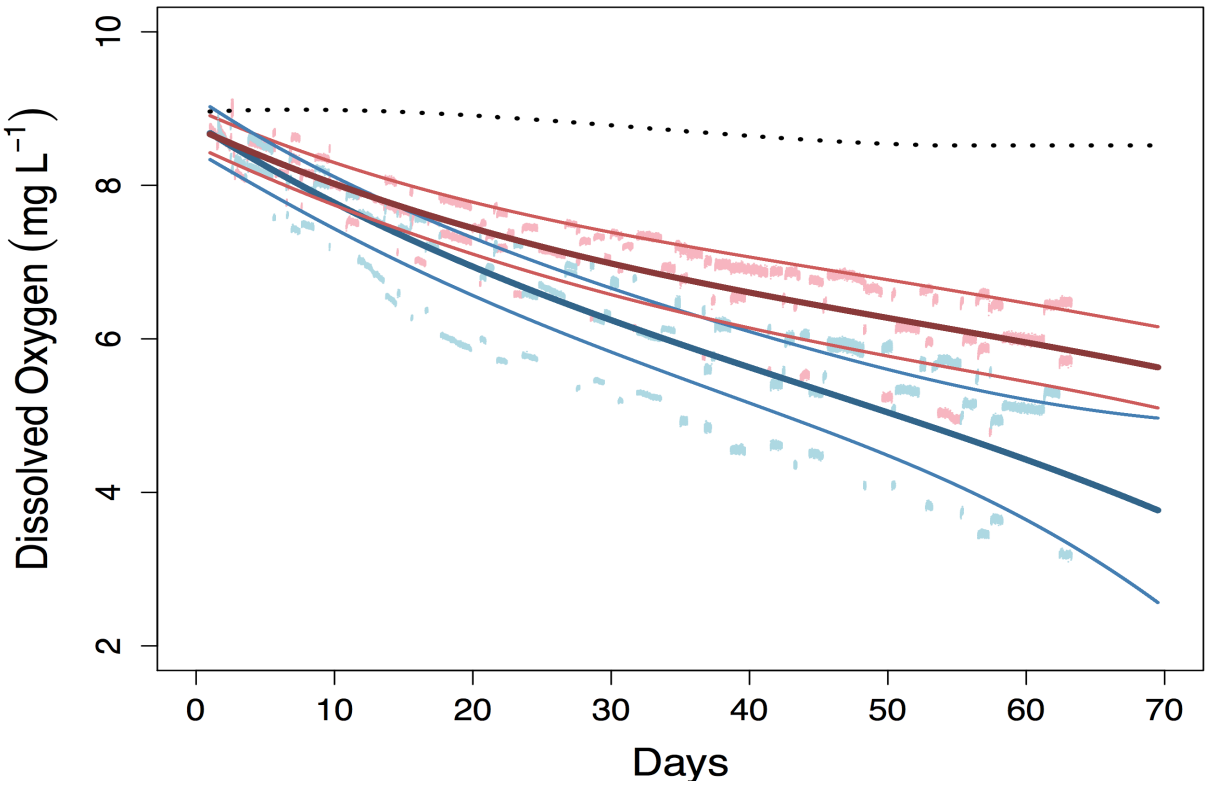

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Tables

Table 1 Characteristics of DOM and microbial metabolism fed DOM from glaciers and rock glaciers. C:N is the ratio of carbon to nitrogen for pre-incubation DOM. D.O (dissolved oxygen) and DOC (dissolved organic carbon) represent the change in concentration of each during the course of the incubation (mg L^{-1}). RQ = respiratory quotient, SW = Shannon Wiener Diversity Index, and ΔSW = change is SW of DOM before and after the incubation. Bold values represent significant differences between glaciers and rock glaciers ($p<0.05$), standard deviations of mean values are listed in parentheses.

Site	Type	C:N	D.O.	DOC	RQ	SW	ΔSW
Isabelle	G	1.65	6.03	2.50	0.42	2.92	0.19
Peck	G	2.02	3.95	1.89	0.48	2.89	0.88
Andrews	G	3.00	4.11	2.64	0.64	2.68	0.13
Arapaho	G	2.73	3.97	2.84	0.72	2.84	0.52
Peck	RG	2.63	3.52	2.09	0.59	2.80	0.54
Navajo	RG	1.48	2.81	1.71	0.61	2.83	-0.32
Arapaho	RG	2.45	4.12	3.00	0.73	2.79	-1.10
Taylor	RG	0.86	2.69	2.25	0.83	2.80	-0.01
Mean	G	2.35 (0.62)	4.51 (1.02)	2.47 (0.41)	0.56 (0.14)	2.83 (0.11)	**0.43**
Mean	RG	1.85 (0.83)	3.29 (0.67)	2.26 (0.54)	0.69 (0.11)	2.80 (0.02)	-0.22

Table 2 DOM compounds that defined the differences between glaciers and rock glaciers before incubation along PC5 from Figure 3A. Mass to charge ratios (m/z) ratios are the largest ion observed for each compound. Normalized intensities (N.I.), an indicator of compound concentration, are given for glaciers and rock glaciers. Bold values indicate which glacier type had a higher normalized intensity (N.I) on average.

DOM Peak	m/z	N.I. Glacier	N.I. Rock Glacier
Maltose	568.3	**4817**	2201
Glutamate	365.3	**5393**	2704
C996	94.2	**28959**	3589
C884	565.3	**14584**	1468
C815	419.1	**2486**	569
C1890	613.2	**805**	346
C1870	642	**1516**	1229
C1840	640.1	**384**	245
C1739	489.1	**2407**	1376
C1655	480.2	**17313**	9597
C1414	507.2	**550**	479
Threonic Acid	411	117	**2730**
Quinic Acid	409.2	2136	**7891**
Glycolic Acid	279.2	2920	**16696**
C589	649.5	1605	**1868**
C531	343.2	7446	**22845**
C35	348.2	1422	**2188**
C1942	535.1	846	**1062**
C1439	406.2	598	**861**
C1376	440.2	2623	**3610**
C1226	634.1	708	**1602**
C1125	468.1	3550	**7504**

Table 3 Results from carbon decay model where B_O represents the size of the recalcitrant pool of DOM (mg C L^{-1}), B_1 is the size of the bioavailable pool (mg C L^{-1}), k is the decay constant (mg C L^{-1} h^{-1}), and BGE = bacterial growth efficiency. Glaciers had a larger percentage of BDOM compared to rock glaciers and rock glaciers had a larger proportion of recalcitrant C compared to glaciers. BGE was also higher for glaciers than rock glaciers. Bold values represent significant differences between glaciers and rock glaciers ($p<0.05$) and standard deviations of mean values are listed in parentheses.

Site	Type	B_0	B_1	k	% BDOM	% Recalcitrant	BGE
Andrews	Glacier	3.20	5.59	0.001	48.23	51.77	0.37
Arapaho	Glacier	3.72	5.20	0.001	47.74	52.26	0.39
Isabelle	Glacier	1.35	7.03	0.001	60.90	39.10	0.39
Peck	Glacier	3.78	5.24	0.001	46.06	53.94	0.17
Arapaho	Rock Glacier	5.32	3.80	0.002	32.05	67.95	0.13
Navajo	Rock Glacier	5.64	3.42	0.001	29.89	70.11	0.03
Peck	Rock Glacier	3.31	5.24	0.001	45.74	54.26	0.14
Taylor	Rock Glacier	4.69	3.83	0.001	36.59	63.41	0.07
Mean G	Glacier	**3.38 (1.09)**	**6.35 (1.06)**	0.001 (0.000)	**52.83 (9.73)**	**47.17 (7.03)**	**0.263 (0.13)**
Mean RG	Rock Glacier	4.74 (1.26)	4.07 (1.12)	0.001 (0.000)	37.18 (10.23)	62.82 (6.84)	0.157 (0.16)

Table 4 The top 25 compounds present in pre- and post-incubation samples organized by normalized intensity. Glaciers and rock glaciers had the same top 25 compounds before and after incubation. Microbial metabolism altered compounds present, resulting in a different set of compounds post incubation.

Pre-Incubation	**Post-Incubation**
Succinic Acid	Glycerol
Octyl 2-propylpentyl ester-phthalic acid	2,4,6-triethyl-, S-(2-phenylethyl) ester-benzenecarbothioic acid
Octyl 2,4,4-trimethylpentyl ester-terephthalic acid	Pyroglutamate
3-chlorophenyl 4-methoxybenzyl ester-succinic acid	10-dioxide-2-phenoxathiinamine-10
pentadecan-1-ol	Serine
Hydroxylamine	1-Ethoxy-2-propanol
Homosalate	Palmitic Acid
p-cyanophenyl 4'-heptyl-4-biphenylcarboxylate	Sucrose
Scopoletin	Beta-alanine
Diiodoacetylene-1	2,4,6-tri-tert-butyl-phenol
Palmitic Acid	Aspartate
Diiodoacetylene	Boric Acid
Phosphoric Acid	Stearic Acid
(2-amino-5-chlorophenyl)(2-fluorophenyl)-methanone	Norleucine
Stearic Acid	Homoserine
phenyl ester-2-thiophenecarboxylic acid	Glycine
phenylethylmalonamide	n-pentadecan-1-ol
3-Methyl-3-(N-methyl-2-pyrrolyl)-1,2-diphenylcyclopropene	D-pinitol
5-amino-1,2,3,8-tetramethoxy-deibenz[d,f]cycloheptane	N-methoxycarbonyl-,isohexyl ester-l-valine
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	1-ethyl-1H-Benzimidazole
p-Cyanophenyl p-(2-butoxyethoxy)benzoate	Phosphoric acid
2-fluorophenyl 2,4-dimethylpent-3-yl ester-succinic acid	Tyrosine
Homoserine	Sarcosine
Pre-Incubation	Post-Incubation