LINKING THE CHEMISTRY AND REACTIVITY OF DISSOLVED ORGANIC MATTER FROM GLACIERS AND ROCK GLACIERS

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Abstract

As glaciers thaw in response to warming, they release dissolved organic matter (DOM) to alpine lakes and streams. Composition and reactivity of DOM released from alpine glaciers is poorly understood. Differences in DOM composition and bioavailability between glacier types like rock and ice glaciers remain undefined, and rock glaciers outnumber ice glaciers approximately ten to one in the United States. To assess the potential for glacial DOM to subsidize heterotrophy in alpine headwaters of the western United States we evaluated reactivity and molecular composition of DOM from eight glacial meltwaters. We assessed biological reactivity by incubating DOM samples with common bacterial community and evaluated the chemical characteristics of each sample's DOM with untargeted gas chromatography mass spectrometry-based metabolomics (GC-MS) before and after incubation. Prior to incubations, ice glacier and rock glacier DOM was similar in carbon concentration and chemical diversity, but had significant differences in DOM composition. Incubation with a common bacterial community showed that DOM from ice glacier meltwaters contained a higher proportion of bioavailable DOM (BDOM), and resulted in greater bacterial growth efficiency. After incubation with the common bacterial community, DOM composition from each source pool was statistically indistinguishable between glacier types. This study provides one approach of using MS-based metabolomics to qualitatively assess effects of DOM composition on differences in bioavailability of DOM pools. Our results suggest even though rock glaciers had significantly less BDOM than ice glaciers, both glacial types still have potential to be important sources of BDOM to alpine headwaters over coming decades.

Introduction

Glaciers integrate atmospherically deposited chemicals with weathering products and release 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). Alpine glaciers appear to be responsible for the release of a larger flux of carbon (0.58 +/- 0.07 Tg C) from melting ice annually when compared to continental glaciers (Hood et al. 2015). Dissolved organic matter (DOM) from large alpine glaciers of the European Alps and Alaska has been shown to be bioavailable and can thus support microbial heterotrophy (Hood et al. 2009; Singer et al. 2012). Ice glaciers (hereafter referred to as glaciers) and rock glaciers, each with distinct geophysical attributes, are also common in many of the alpine headwaters of the western U.S. (Fegel et al. 2016). Glaciers are perennial ice features that move 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 this report we use the word 'glacier' to refer to alpine glaciers largely composed of ice and snow and 'rock glacier' to refer to the permafrost feature described above.

In the western United States, rock glaciers are an order of magnitude more abundant than glaciers and are more resistant to warming air temperatures than glaciers, yet little is known about the quantity or quality of DOM being released from these smaller glaciers and rock glaciers in mountain headwater ecosystems (Woo et al. 2012; Fegel et al. 2016). Assessment of the bioavailability of aquatic DOM to date has largely 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). Bioassays present difficulties in assessing DOM

consumption because each assessment requires an independent incubation, creating the potential for confounding effects due to differences in the microbial communities among incubations. Broad molecular characterizations of DOM (Benner 2002; Berggren and del Giorgio 2015) have also previously been applied to estimate bioavailable DOM (BDOM), however often these analyses only address differences in lability of either coarse categories of organic molecules or individual model compounds rather than the DOM pool as a whole (e.g., del Giorgio and Cole 1998). Recently the research community has begun to explore high-resolution analytical chemistry to assess the molecular characteristics of environmental DOM (e.g., Kujawinski 2002; 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 (MS) defines structural characteristics of thousands of individual compounds in DOM. In general MS yields a more specific and broader metric for DOM analysis compared to spectroscopic techniques such as fluorescence or ultraviolet-visible (UV-Visible) absorption.

Understanding the capacity and constraints of the various MS-based analytical approaches is important for analysis and interpretation of the composition of BDOM. There are a variety of different MS-based platforms and separation modalities that can be used to characterize DOM. For instance, gas chromatography (GC) separations are based on compound volatility whereas liquid chromatography (LC) separations can be based on polarity, size, or ion exchange. Post-separation MS detection can involve many different ionization methods such as electron impact (EI), electrospray (ESI), atmospheric pressure chemical ionization (APCI) among others, and these ionization techniques can be paired with a range of mass analyzers from a unit resolution single quadrupole to ultrahigh resolution Fourier transform ion cyclotron resonance (FT-ICR). Each technique has advantages and disadvantages (Kind and Feihn 2010). For example, FT-

ICRMS can provide molecular formula assignments but cannot differentiate structural isomers, while EI-based methods provide diagnostic fragmentation patterns represented in searchable spectral libraries containing over half a million compounds (Halket et al. 2005). In this study we used compound derivatization followed by GC separation due to the ease of separation of small polar metabolites that we anticipated would be an important component of the BDOM pool. We chose EI ionization to maximize the number of compounds that would be identifiable by comparison of their fragment spectra within existing EI-MS databases.

There are a suite of challenges in trying to concurrently understand the amount and composition of bioavailable DOM. Difficulties in experimentally linking the molecular characterization of DOM pools to the proportion of BDOM are partly due to the highly diverse constituent compounds that compose natural DOM (Derenne and Tu 2014). The metabolic effects of individual compounds identified to be bioavailable, may not be representative of the total BDOM pool. It is also possible that interactions among compounds in a diverse DOM pool may alter the size of the BDOM pool. For example, labile compounds may have positive feedbacks on the bioavailability of the additional less available compounds i.e. priming effects (Guenet et al. 2010). These are some of the reasons that the relationship between DOM constituents and the BDOM pool remains largely unknown in most natural systems. However, some broad patterns are beginning to emerge, like the consistently high bioavailability of proteinaceous DOM that has been found in glacial, estuarine, and marine environments (Andrilli et al. 2015). Further defining the relationship between the composition of DOM and its lability has the potential to estimate reactivity of DOM in the absence of laboratory bioassays. Understanding how constituent molecules contribute to DOM reactivity would provide a better understanding for how DOM pools contribute to heterotrophy across a broad spectrum of aquatic

ecosystems and the potential to better understand residence times of different components of DOM in the environment.

We hypothesized that differences in the origin of DOM between glaciers and rock glaciers would result in differences in molecular composition of DOM between glacier types and thus differences in lability. DOM derived from glaciers originates from *in situ* microbial activity (phototrophy on the glacier surface, and heterotrophy in the glacier porewaters and subsurface) and atmospheric deposition of organic matter, and can be an important source of DOM and chemical energy to headwater ecosystems (Anesio et al. 2009; Hood et al. 2009; Singer et al. 2012; Stubbins et al. 2012; Fellman et al. 2015; Fegel et al. 2016). DOM derived from rock glaciers is a conglomeration of carbon compounds originating from multicellular vegetation growing on the rock glacier surface and microbial processing within the rock glacier itself (Wahrhaftig and Cox, 1959; Williams et al. 2007). Previous research has shown that DOM released from glaciers and rock glaciers in the western United States differ in their optical properties, with glaciers having higher amounts of protein-like DOM than rock glaciers, as identified through fluorescence spectroscopy (Fegel et al. 2016).

In this study, we assessed differences in the composition of DOM between glaciers and rock glaciers and asked which of those differences (if any) correspond to differences in the proportion of BDOM from each glacier type. We also asked whether the composition and reactivity of DOM in glacier and rock glacier meltwaters in the western United States is similar to what has been reported for other glacial meltwaters globally. Here we present the results of laboratory incubations with a common bacterial community of DOM from four pairs of glacier and rock glacier meltwaters (8 DOM sources in total) in the western United States. Incubations were analyzed before and after incubation with GC-MS and a non-targeted metabolomic workflow

was used to define differences in DOM from glaciers and rock glaciers. By exposing DOM from different sources to a common bacterial community we were able to assess differences in the lability of each DOM source and analyze differences in the molecular composition of DOM before and after its exposure to the bacterial community. In addition to measuring respiration in each microcosm, we measured change in bacterial cell numbers, allowing us to estimate the amount of carbon from each glacial source that was allocated to anabolic bacterial metabolism (i.e., bacterial production) relative to catabolic bacterial metabolism (i.e., bacterial respiration).

Methods

Site description

Paired 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 glacial features with similar geographic parameters (Figure 1). We collected samples of glacier meltwater in September 2014 to capture the greatest contribution of ice melt and minimize annual snowmelt contribution (Figure 1, Table 1). A complete description for each site is provided in Fegel et al. (2016).

Field extraction of DOM

Meltwaters were collected in the early to mid-morning (05:00-10:00) to minimize diurnal variability in ice melt among glaciers. DOM was concentrated 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). 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 (Bond Elut-PPL, 500 mg 6

mL Agilent, Santa Clara, CA, USA). PPL cartridges have been shown to have good retention for a variety of DOM molecules except for those with very high polarity (log D lower than approximately -3.4), where Log D is a measure of lipophilicity and likelihood for partitioning to octanol versus water. A low log D indicates low lipophilicity, greater polarity, and greater likelihood of partitioning to water (Raeke et al. 2016). PPL cartridges were first conditioned with ~5 mL methanol (MeOH). Cartridges were placed on a vacuum manifold, and 2.5 L of the 20 L total of filtered, acidified meltwater was eluted per syringe cartridge. Cartridges were left on the vacuum line for 5 minutes to dry, leaving the vacuum line in collection bottle to minimize contamination.

Concentrated DOM on each PPL cartridge was eluted in the laboratory with 10 mL HPLC grade MeOH per cartridge into cleaned, combusted, and pre-weighed 120 mL borosilicate bottles. Bottles were weighed after MeOH was evaporated to calculate the amount of DOM collected. All 8 syringes per sample (2.5 L meltwater per syringe, 20 L in total) were collected into the same borosilicate glass bottle. DOM was isolated from MeOH in each bottle by running clean N_2 gas over the open samples until all MeOH was evaporated. Samples were kept at room temperature until metabolomic analysis and incubation.

DOM lability experiments

Dried DOM extract samples from each of the eight sites were diluted to 4 mg L^{-1} C with MilliQ (>18Ω H2O) and incubated in vitro with a natural bacterial community collected from a small sub-alpine lake in Rocky Mountain National Park, CO, USA, The Loch (-105.6455, 40.2976). Unfiltered lake water collected from The Loch was aged for ~2 years at 5 °C to remove the majority of the BDOM. At the start of the experiment DOM concentration of the lake water was 0.7 mg L^{-1} C, with a pH of around 6.7. The goal of the bioassay was to determine

which portions of the glacial DOM pools could be consumed by a naturally occurring bacterial community. Community structure (i.e. relative abundance of constituent bacterial populations) in the aged lake water was undoubtedly different than when it was first sampled. However, aging the lake water allowed us to reduce the ambient DOC concentration and presumably remove the majority of labile compounds. At the same time it provided a common community of naturally occurring aquatic alpine bacteria that should not be preferentially disposed to rock glacier or glacier DOM as it was taken from a watershed containing both glacier types and at a similar distance from both. Prior to the incubations 2L of aged lake water was filtered through a pre-combusted (450° C, 5hr) Whatman GF/C filter (1.2 μm nominal pore size) to remove bacterial grazers (e.g. protists and metazoans). At the initiation of the experiment (t=0), three aliquots of filtered-aged lake water were preserved with 2% formalin (final concentration, from 37% formaldehyde) and set aside for enumeration of bacteria. A second set of three aliquots (7 mL) was used for initial DOC/TDN analysis on a Shimadzu TOC-VWS analyzer (Shimadzu Corp., Kyoto, Japan). To create normalized concentrations of DOM (4 mg L^{-1} C), in each incubation bottle we added 60.96 mL of > 1.2 μm filtered Loch water, between 3.80 and 9.06 mL of concentrated glacial DOM solution (depending on the initial concentration), and filled the remaining volume to 70 mL total volume with MilliQ Water (>18Ω H2O). The total MilliQ additions added to each microcosm ranged between 0 and 5.2 mL. This resulted in concentrations of 4 mg L^{-1} C in each incubation bottle for all DOM sources (Table S1). To account for respiration of the lake water DOM in the absence of glacier DOM additions, microcosms that received DOM from glacier and rock glacier sites were incubated alongside incubations that contained only GF/C filtered lake water, but with no added DOM (experimental control). In addition, to correct for any instrumental drift that occurred during the experiment a

microcosm of MilliQ water was also incubated alongside all other microcosms and monitored throughout the experiment (analytical control).

All microcosms were incubated simultaneously at 15 °C. To calculate microbial respiration, we measured changes in dissolved oxygen (DO) at 1-minute intervals in each microcosm using an Oxy-4 fiber-optic dissolved oxygen probe for at least six hours, every other day (PreSens, Precision Sensing GmbH, Regensburg, Germany). The incubation was terminated at 10 weeks, when the fastest metabolizing microcosm approached 4 mg L^{-1} DO to avoid hypoxia and the potential for anaerobic metabolism in anoxic microzones within each bottle. All measurements with amplitudes less than 20000 were removed because of the potential for inaccurate readings of DO, due to the minimum detection limit of the Oxy-4 probe (PreSens Oxy-4 mini Instruction Manual, Precision Sensing GmbH, 2011). Absolute values from the raw fiber optic measurements were corrected for analytical drift of the probe by subtraction of changes in signal from the MilliQ water analytical control over the course of the experiment.

Bacterial cell counts and bulk chemistry

From each microcosm we collected a 2 mL aliquot pre- (described above) and post-incubation and preserved it with 2% formalin (final concentration) to assess changes in cell abundance during the course of the incubation. Aliquots were filtered onto 0.2 μm Millipore polycarbonate filters and stained with Acridine Orange for enumeration, following the methods of Hobbie et al. (1977). We also assessed TOC and TDN pre- and post-incubation for each microcosm (Table S2). To ensure that filtration did not contribute significant additional carbon to the dissolved carbon pool, a subset of blank Millipore filters was flushed with MilliQ and analyzed for TOC and TDN (Table S2). All filtration of microcosm water for chemical analysis was conducted on pre-leached Millipore filters that contributed insignificant amounts of carbon to the filtrate

(Table S2). DOC/TDN values are accurate within +/-5% using a non-purgeable dissolved organic carbon method (EPA method 415.1, Shimadzu Corporation, Columbia, MD).

Metabolite analyses

Each sample was prepared for metabolomic analysis by dissolving the dried DOM into fresh HPLC-grade methanol to a final concentration of 2 mg mL^{-1} C. Percent recovery of DOM averaged 70.93% +/- 16.94% (Table S3).

After the bacterial bioassays (in which all samples were run in duplicate), post-incubation DOM samples were collected separately from each individual microcosm (n=16). All 70mL of water from each microcosm was filtered through pre-leached 0.2 μm Millipore filters, freeze-dried, and the total remaining DOM was also weighed and re-dissolved into HPLC-grade methanol to a final concentration of 2 mg mL^{-1} C for GC-MS analysis. To increase the volatility of molecules analyzed through GC-MS, all samples for metabolomics analysis were derivatized with trimethylsilane (TMS) using standard protocols (SI: Derivatization Process for GC-MS, Pierce 1968). The preparation and concentration of DOM may also include some soluble inorganic compounds, which were also detected by GC-MS analysis.

Metabolomics

Both pre- and post- incubation OM samples were analyzed with inline gas chromatography-mass spectrometry (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 30m 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 80°C for 30 sec, a ramp of 15°C per min to 330°C, 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). We confirmed that the most abundant compounds detected were consistent with retention on a PPL cartridge by calculating their log D at pH 2 (structure calculation at chemicalize.com, ChemAxon, data not shown).

Data analysis

Metabolite analysis

Compound annotation was prioritized based on order of normalized intensity (which correlates with abundance of a compound) and statistical difference between glacier types. Compound annotation (candidate compounds based on physiochemical 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, and represent TMS derivatized molecules. Clustered features that were annotated had high similarity (>90%) to spectra from known standard compounds within the databases used (Figure S1). Molecular rank was calculated by ordering DOM 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 DOM compound as a 'species'. This was done for DOM composition both before and after incubation to estimate changes in chemical diversity through microbial metabolism. Chemical superclasses for the most abundant annotated compounds were assigned using conversion of chemical name to InChI Code using the Chemical Translation Service (http://cts.fiehnlab.ucdavis.edu), and then the InChI Code was converted to compound superclass using ClassyFire (http://classyfire.wishartlab.com, Feunang et al. 2016). The compound superclasses in ChemOnt, ClassyFire's taxonomy, are the

second level of hierarchical classification. Compounds with the same superclass share generic structural features that describe their overall composition or shape.

Analysis of DOM reactivity

Oxygen consumption rates were fit to a Berner-Multi-G two-pool decay model to estimate the size of the bioavailable DOM pool (BDOM) and the recalcitrant DOM pool. Oxygen consumption was averaged for each glacier type and confidence intervals were calculated at α =0.05. We smoothed data using a third order polynomial (R^2>0.999) and plotted 95% confidence intervals. We then used Berner's Multi-G model to estimate BDOM pool size (Berner 1980; Guillemette and del Giorgio 2011), using SAS. Dissolved oxygen curves generated from the incubation were fit to the equation:

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

Where B_T is the total carbon pool converted from measured moles of oxygen to moles of carbon, with the assumption of a respiratory quotient (RQ) of 1. B_1 is the BDOM carbon pool, k is the decay rate constant of the BDOM pool, t is time, and B_0 is the recalcitrant carbon pool. We used a Wilcoxon signed rank test to compare statistical differences between glacier and rock glacier B_1 and B_0 pool sizes. Total C consumed was calculated as the difference in pre- and post-incubation measured DOC values.

BGE was calculated to examine how efficiently each carbon source was incorporated into bacterial biomass. To calculate BGE we divided bacterial production by the sum of bacterial respiration and bacterial production (del Giorgio and Cole 1998). Thus, BGE is a ratio of carbon incorporated into biomass relative to total carbon consumed by the bacterial community.

Bacterial production 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). Carbon consumption rate was measured as the difference between DOC concentration in each microcosm at the beginning and the end of each incubation.

Statistical analyses

For each sample, raw data GC-MS 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. We normalized raw peak areas to total ion signal in each sample to account for non-biological systematic instrument variation. The mean area of the chromatographic peak was calculated among replicate injections (n=2). Molecular feature comparisons are based on relative abundance or fold changes of specific compounds. We grouped all spectral features based on an in-house clustering tool, RAMClustR, which uses spectra based coelution and covariance across the full dataset to group features (Broeckling et al. 2014). Peak areas for each feature in a spectrum were condensed into a single value by adjusting the weighted mean of all features in the same spectrum for each compound. Differences in DOM composition for each compound between treatments (glaciers vs. rock glaciers), and pre/post incubation were compared with ANOVA using the aov function in the programming language R, and p-values were adjusted for false positives using the Bonferroni-Hochberg method in the p.adjust function (R Core Team, 2014). Common MS contaminants were removed from the dataset prior to further processing (Keller et al. 2008). We visualized differences in DOM composition by glacier type and pre/post incubation using PCA conducted on normalized, mean-centered and pareto-scaled data using the pcaMethods package (R Core Team, 2014). PCA analyses were completed separately for glacier types (pre-incubation) and for

the pre/post incubation comparison. P-values on the PCA axes (Figure 2) are the result of an ANOVA on the PC scores using the paired rock/ice glacier design with the aov function in R. We used the non-parametric Wilcoxon signed rank test for glacier pairs to compare differences in BGE, C:N, chemical diversity, and change in chemical diversity between glacier types as these variables did not meet the expectations of equal variance and normal distribution required by parametric tests.

Results

Glacier and rock glacier DOM

When all samples were included, the composition of glacier and rock glacier DOM consisted of 2,033 compounds (Table S5). Of those 2,033 compounds each individual compound consisted of 3 to 170 individual mass spectral features, for a total of 14,571 unique mass spectral features in the dataset. Of the total 2,033 compounds, 328 were chosen for annotation based either on high ion intensity (n=278) or significant difference in relative abundances between glacier and rock glacier sources as determined by ANOVA (n=33, Table 2). Prior to incubation, neither the chemical diversity or the C:N ratio of the DOM were significantly different between glaciers and rock glaciers (Table 3). In both glacial features we observed low C:N ratios of DOM (2.35 +/- 0.62 for glaciers, 1.85 +/- 0.83 for rock glaciers) which is consistent with the elevated inorganic nitrogen reported from glaciers in Colorado and other alpine ecosystems (Williams et al. 2006; Barnes et al. 2014; Fegel et al. 2016, Saros et al. 2010; Slemmons et al. 2013). The top 25 most abundant molecules were similar between glacier types prior to incubation, had intensities much higher than most other compounds within the DOM pools, and were primarily from chemical superclasses including benzenoids, phenylpropanoids and polyketides, and organic acids (Figure 3, Table 5, Table S5).

A complete PCA analysis of the 2033 compounds was expressed on 7 axes, with majority of variation (63.2%) along axes 1 and 2, but no categorical variables in our study correlated with separation along either axis 1 or 2. The 33 compounds that did differ significantly among glacier types (Table 2, Figure 2A) ordinated on axes 3 and 5 (explaining 26% of the variability of the entire GC-MS dataset, Table 2). Of the 33 compounds that differed significantly between glacier types (Table 2), we were able to assign putative structure to only five compounds. Of those 5 compounds, glacier meltwaters were 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 that differed significantly between glacier types were not present in the databases we employed and had no known structural analogs (the pseudomolecular ions, intensities, and retention times for each of the 28 unannotated compounds are reported in Tables 2 and S5). Before the incubation, only DOM from Taylor Rock Glacier had a noticeably different composition from the other glaciers and rock glaciers (Figure 2A), however it was not clear what was driving that difference.

Incubations

Respiration results from all incubations were fit to a two-pool decay model that allowed us to estimate the initial rate of respiration (*k*), the size of the BDOM pool (B_1), and recalcitrant DOM pool (B_2), from each DOM source. The initial bacterial respiration rates of DOM from each glacier type were not significantly different, as estimated by the decay constant *k* (Table 4). However, a significantly larger portion of DOM was bioavailable in glacier meltwater, (B_1 = 66.0 ± 10.6%) compared to rock glacier meltwater (B_1= 46.4 ± 8.9%, $p < 0.01$, Table 4). Bacterial growth efficiency was also greater for bacterial communities incubated with glacier DOM compared to rock glacier DOM (BGE_G = 0.26 ± 0.13, BGE_{RG} = 0.16 ± 0.16, Table 4). DOM was

also consumed in the lake water control during the incubation, as DOC concentration decreased from 0.7 mg L^{-1} C at the beginning of the experiment to 0.3 mg L^{-1} C at the end. This change in DOM was an order of magnitude less than the ~ 3 mg L^{-1} C that was consumed during the course of the incubations for the microcosms amended with rock glacier or glacier DOM and should not have impacted the difference in BDOM observed between glaciers and rock glaciers. This small change in DOM in the lake water controls is also consistent with the small changes in the observed cell counts in the lake water control incubations relative to cell changes in the microcosms that received glacier or rock glacier DOM, suggesting limited microbial activity in the lake water controls (Table S4).

Post-incubation DOM

Incubations with the common bacterial community altered the composition of the DOM from each glacier type, resulting in fewer compounds with high ion intensities (rarified), and more compounds with low ion intensities (homogenization, Figure 3). This restructured and reorganized the molecular rank-abundance curve (Figure 3), resulting in a different set of compounds with the highest relative abundance in the post vs. pre-incubation DOM pools (Table 5). However, similar to pre-incubation DOM composition, post-incubation glacier and rock glacier derived DOM still shared the same most abundant compounds (Table 5, Table S5). The rank abundance curve of the post-incubation samples was similar in structure (Figure 3) and composition (Table 5) to that of the alpine lake DOM pool that supported the common microbial community prior to inoculation (i.e., the DOM of the experimental control). Chemical diversity, as assessed by the Shannon-Wiener diversity index, of DOM between glacier types diverged during the course of the incubations. Post-incubation glacial DOM had significantly higher diversity (SW = 3.26) compared to pre-incubation glacial DOM (SW=2.83), whereas post-

incubation rock glacier DOM had significantly lower diversity (SW=2.58) compared to pre-incubation rock glacier DOM (SW=2.80, Table 3, Figure 2C). This divergence in chemical diversity between DOM from each glacial type was primarily due to an increase in the richness (number of compounds present) between pre- and post-incubation glacier DOM pools and a decrease in the evenness (relative abundance among different compounds) between the pre- and post-incubation rock glacier DOM. For both DOM sources incubation with a common microbial community generally decreased the relative abundance of compounds from the benzenoid and additional unannotated superclasses and increased the relative abundance of compounds from the organic acids and organic oxygen compound superclasses (Table 5). For instance, following incubation a benzenoid derivative of succinic acid decreased more than 7000 fold, and the organic acids serine, lactate, and aspartate each increased more than 900 fold (Table 5). While there were differences in the chemical diversity of rock glacier and glacier derived DOM post-incubation, a PCA no longer showed significant separation in the overall molecular composition between glacier types as it did prior to incubation (Figures 2A and B). Whereas no significant difference was observed for compounds between glacier types post incubation (Figure 2B), both microcosms with Peck Rock Glacier DOM post incubation (lower rightmost points Figure 2B) showed separation from all other glacier types along the y-axis (PC1) of the ordination.

Discussion

Our results demonstrate that chemically complex DOM released from both glaciers and rock glaciers supported bacterial respiration and bacterial secondary production. There were also clear differences in the size of the BDOM pools between glacier types, with glaciers having a higher proportion of BDOM than rock glaciers. At the time of sampling, glacier and rock glacier DOM shared many of the same compounds, but we did find significant differences in the relative

abundances of 33 compounds between glacier types. Incubation with a common bacterial community decreased the chemical diversity of rock glacier DOM but increased the chemical diversity of glacial DOM and resulted in more similar DOM composition between glacier types. In addition, incubation with a common microbial community resulted in a DOM pool similar to that of the aged alpine lake water, suggesting that microbial metabolism homogenized DOM to similar composition and structure, presumably reflective of the composite metabolism of the microbial community.

Glaciers are a source of BDOM

The proportion of BDOM (66%) from glacier meltwaters in Colorado was similar to what has been reported in previous studies of glacial DOM in southeast Alaska (23-66%), the European Alps (58%), and the Tibetan plateau (46-69%, Hood et al. 2009; Singer et al. 2012; Spencer et al. 2014; Fellman et al. 2015). Taken together glacier DOM around the world appears to be enriched in BDOM relative to what has been reported for other inland waters (4-34.5%) (Volk et al. 1997; Wiegner et al. 2006; Guillemette and del Giorgio 2011).

The mean amount of BDOM we estimated for rock glaciers (46%) was similarly high, and within the range reported from other North American glaciers. Carbon concentrations in meltwaters from glaciers and rock glaciers in our study were low (0.5- 1.5 mg C L^{-1}), but also similar to previously reported mean glacial meltwater DOM concentration of 0.97 mg C L^{-1} (Dubnick et al. 2010; Stubbins et al. 2012; Singer et al. 2012; Hood et al. 2015). The volume of ice within each feature and estimates of discharge would allow us to estimate the total pool size of BDOM within glaciers and rock glaciers and an annual amount of BDOM released from each. Unfortunately, quantification of internal ice volume of rock glaciers is currently not possible. In addition, even with the best available technologies, the numerous shallow meltwater flow paths

from both glacier types makes estimating discharge exceedingly difficult. Therefore, we focus our discussion on the reported differences between both the reactivity and composition of DOM that we sampled from each glacier type.

We suggest that differences in both the origin and delivery of DOM between each glacier type in part explains why we saw higher proportions of BDOM in glaciers relative to rock glaciers. 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). DOM released from these multi-cellular phototrophs include a wide variety of complex organic acids and polymers that are related to the secondary growth of the phototroph and are potentially difficult to metabolize (Wetzel 1992; Rovira and Vallejo 2002). Conversely, BDOM in glaciers is more likely to come from microbial production; either on the ice surface, within the meltwater channels, or below the glacier (Fellman et al. 2009), and from atmospherically deposited aerosols (Stubbins et al. 2012). Organic molecules from microbial biomass or those small enough to be volatile should be more biologically available than plant derived organic matter, especially those compounds associated with the secondary metabolism of plants (Sun et al. 1997; Raymond and Bauer 2001; Berggren et al. 2009). The molecular structure of glacial DOM assessed by ultra high-resolution MS has been shown to be more labile than marine or freshwater derived material due to the presence of protein and amino-sugar components of microbial origin (Andrilli et al. 2015). This is consistent with studies from other inland waters that show that higher BDOM correlates with enrichment of amino acids and simple sugars (Lafreniere and Sharp 2004; Williams et al. 2007; Dubnick et al. 2010). For the few compounds that could be annotated in this study, maltose, a simple sugar, and glutamate, an amino acid were more abundant in glacier meltwater relative to rock glacier meltwater. Conversely organic acids were more abundant in

rock glacier meltwater relative to glacier meltwater. However, the main difference in the composition of glacier and rock glacier DOM was largely driven by compounds with unknown molecular structures of relatively large molecular weight that are not represented in compound databases. Analysis of the DOM pool with GC-MS most likely did not capture the full suite of compounds responsible for the observed differences in BDOM, as discussed below.

In addition to the origin of DOM in each glacier feature, the pathway the DOM takes to the meltwater outlet also likely differs between glacial features. DOM from rock glaciers may be partially processed by microbes as it travels through the rock glacier's protosoil. 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 a terrestrial soil matrix (Fellman et al. 2010). In comparison, glacier BDOM may be preserved in the ice matrix and physically inaccessible to microbial degradation before it is released in glacier meltwater. Thus, the origin of DOM within each glacier type and the pathways through which DOM arrives at outflow are different. These differences in origin and delivery between glacier types are consistent with the differences in the proportion of BDOM between each glacier type that we found. Differences in origin and delivery between glacier types are also consistent with the more similar values between rock glacier BDOM and other surface freshwaters compared to glacier BDOM and other surface waters.

DOM changes during the Incubation

Before incubation with a common microbial community, we found that chemical diversity was very similar between glacier and rock glacier DOM (Table 3) and similar to the chemical diversity of DOM found in other freshwater ecosystems (Dubnick et al. 2010; Guillemette and del Giorgio 2011; Kellerman et al. 2014). However, alteration of the DOM during incubation with a common microbial community differed by glacial type. We suggest that a balance

between a) the introduction of new metabolites from the common microbial pool relative to b) the loss of BDOM may have affected how the diversity of DOM was altered during the incubation. For both glacial types incubation with a common bacterial community most likely homogenized the DOM pool by eliminating the most labile compounds and adding microbial metabolites through exudates and cellular lysis. As discussed above rock glacier DOM likely contained more processed DOM (i.e., DOM that had been exposed to diverse microbial communities) and less bioavailable DOM (i.e., secondary metabolites from plants) relative to glacier DOM. Because rock glacier DOM had been more thoroughly exposed to a microbial community before it was collected (relative to glacier DOM), many of the microbial metabolites contributed by the common microbial community were likely already present in rock glacier DOM. Thus, changes in rock glacier DOM composition during the incubation was likely due to loss of secondary metabolites and "more processed" components of the DOM pool. This is consistent with the lower bacterial growth efficiency we observed in the rock glacier relative to the glacier DOM incubations. However, the "less-processed" glacial DOM may have increased in diversity due to a disproportionate increase in novel microbial metabolites (relative to the rock glacier DOM) introduced by the common microbial community during the incubation.

The shift in the DOM pool for both glacial types towards compounds provided by microbial metabolism is supported by the presence of low molecular weight compounds, often considered to be highly labile. For example, serine, an amino acid, increased in molecular rank from 1099 to 6, and the sugar sucrose increased in molecular rank from 65 to 5 (Table 5) between the pre-incubation and post-incubation DOM pools, respectively. We expect that microorganisms would rapidly consume these compounds to levels below detection, however, rather than being leftover after the incubation (i.e., recalcitrant compounds inaccessible to microbes), it is more likely that

these compounds are an example the increasing dominance of microbial biomass in the DOM pool (i.e., compounds originating as microbial metabolites) during microbial processing of DOM. This shift in DOM composition towards DOM of microbial origin during the course of the incubation is further supported by an increased similarity of the DOM structure (Figure 3) and composition (Table 5) between the post-incubation DOM pool for both glacier types and the alpine lake water control (i.e., experimental control). It is also consistent with the increasing realization that many environmental DOM pools are more likely composed of compounds resulting from microbial metabolism rather than recalcitrant plant metabolites (e.g., Cotrufo et al 2014). Without a labeled starting material (e.g. an isotopically labeled DOM pool) it is not possible to definitively determine the relative contribution of turnover from microbial metabolism to the post-DOM pool size (and even with that approach it is a difficult task), however this mechanism provides an important and parsimonious explanation of the composition of post-incubation DOM and the presence of compounds thought to be labile in the post-incubation DOM pool.

Analysis of composition and lability of DOM

This study expands the understanding of DOM complexity in inland waters by assessing the glacial contribution of DOM to alpine headwaters and moving beyond broad functional groups and compound classes descriptors to identification of specific compounds, albeit very few (Dubnick et al. 2010; Fellman et al. 2010; Singer et al. 2012; Fegel et al. 2016). However, by using a MS-based technique we were able to identify molecular properties of individual candidate compounds as well as examine a broader swath of chemical diversity from each DOM pool. This approach provides chemical detail at both the individual compound and compositional pool levels. Application of an 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 (e.g., see Kujawinski et al. 2011 for a review; Logue et al. 2015).

Although MS based analysis of DOM has important advantages it also comes with a series of challenges and constraints. One challenge comes from the selective DOM extraction using PPL cartridges, which often do not retain very polar compounds (Raeke et al. 2016). However, given the low concentration of DOM (typically > 2 mg C per L) in the glacial meltwaters, a concentrating procedure was necessary to perform the GC-MS analyses. Whereas our results provide evidence that differences in bioavailability were concurrent with chemical differences between DOM that differ in origin from glacier types, some components of the DOM pool were not assessed by the GC-MS method we employed. Our GC-MS method scanned for molecules up to 650 *m/z*, leaving larger molecules undetected. Further, many of the components of glacial DOM pools (e.g., the majority of the 33 compounds that differed between features) could not be identified using the current databases (NISTv12, Golm, Metlin, and Massbank). Whereas we were unable to determine what those compounds were, we can say what they were not. Among the compounds that were significantly different between glaciers and rock glaciers, the annotated compounds were all primary metabolites with a pseduomolecular ion *m/z* less than 500. However, the majority (70%) of the unannotated compounds had a pseduomolecular *ion m/z* greater than 500, suggesting they may be products of secondary metabolism rather than primary metabolism based on their larger molecular size (Table 2). Primary metabolites are considered those metabolically produced compounds that are essential for survival, and are also common to most organisms. Secondary metabolites are generally not required for survival, but are organism

or group specific, and may confer some competitive advantage for the organisms that produces them. Secondary metabolites also tend to be more structurally complex than primary metabolites (Luckner 1984). Products of primary metabolism, e.g. amino acids, organic acids, sugars, and peptides are well represented in mass spectrometry databases and therefore more easily identified with a GC-MS approach. Less well represented are larger molecular weight products of secondary metabolism such as terpenes, alkaloids, polyketides, aromatic structures, and products of mixed biosynthesis. 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 (Bowen and Northen 2010). Yet, the quality of mass spectrometry databases and representation is rapidly improving, and this will likely be a critically important source of information for understanding the relationship between molecular composition of DOM and its lability in future research.

The high proportion of compounds that could not be annotated with candidate assignments in our study reflects the infancy of ecosystem metabolomics (Raes and Bork 2008). It exposes the need for more environmentally derived spectra and standards from secondary metabolism to be added to metabolite databases. With the expansion of metabolic databases, and the verification of environmentally-derived spectra to known standard compounds, metabolomic techniques have the potential to provide the ability to more directly assess the reactivity of DOM pools from a range of aquatic ecosystems.

Conclusion

Glaciers and rock glaciers in the Colorado Rockies have similar DOM content to other glaciers worldwide and have the capability to support heterotrophic metabolism with high

(relative to other inland waters) percentages of BDOM in their meltwaters. Our study expands on previous work to characterize the molecular structure of DOM in glacial meltwaters by our novel application of metabolomic techniques to glacial meltwater. Whereas we determined that 33 chemical compounds differed among glacier types using GC-MS, our results likely only represent a fraction of the chemical compounds controlling the bioavailability of total DOM pools and it is unknown if these compounds would be universally diagnostic for differences in other paired sets of glacier and rock glaciers or other similar sources of DOM. Other chemical characterization techniques suited towards identifying larger molecules such as liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS) may provide additional insight into which compounds drive bioavailability differences between complex DOM pools. In this study, only 5 of the 33 compounds that were found to be significantly different between glacier types could be annotated. However, as ecological metabolite libraries expand, repeated assessments of diverse DOM pools with untargeted MS approaches have the potential to link molecular structure with the biological lability among a broad range of aquatic ecosystems.

Rock glaciers are far more abundant than glaciers in some alpine headwater ecosystems (Falaschi et al. 2013; Rangecroft et al. 2015; Fegel et al. 2016). Current glacial carbon modeling neglects the contribution of rock glacial carbon (Hood et al. 2015), yet our results suggest that rock glaciers supply a consistent source of BDOM for heterotrophic metabolism in headwater ecosystems. Rock glacier derived DOM may contribute to ecosystem productivity for much longer than glaciers due to the slower recession of rock glaciers compared to glaciers (Woo 2012; Millar and Westfall 2010). At similar carbon concentrations and with only ~20% less BDOM (~46% in rock glaciers compared to ~66% in glacier derived DOM), rock glaciers may

play just as large if not larger role in heterotrophic metabolism in alpine headwaters as glaciers now and in the near future.

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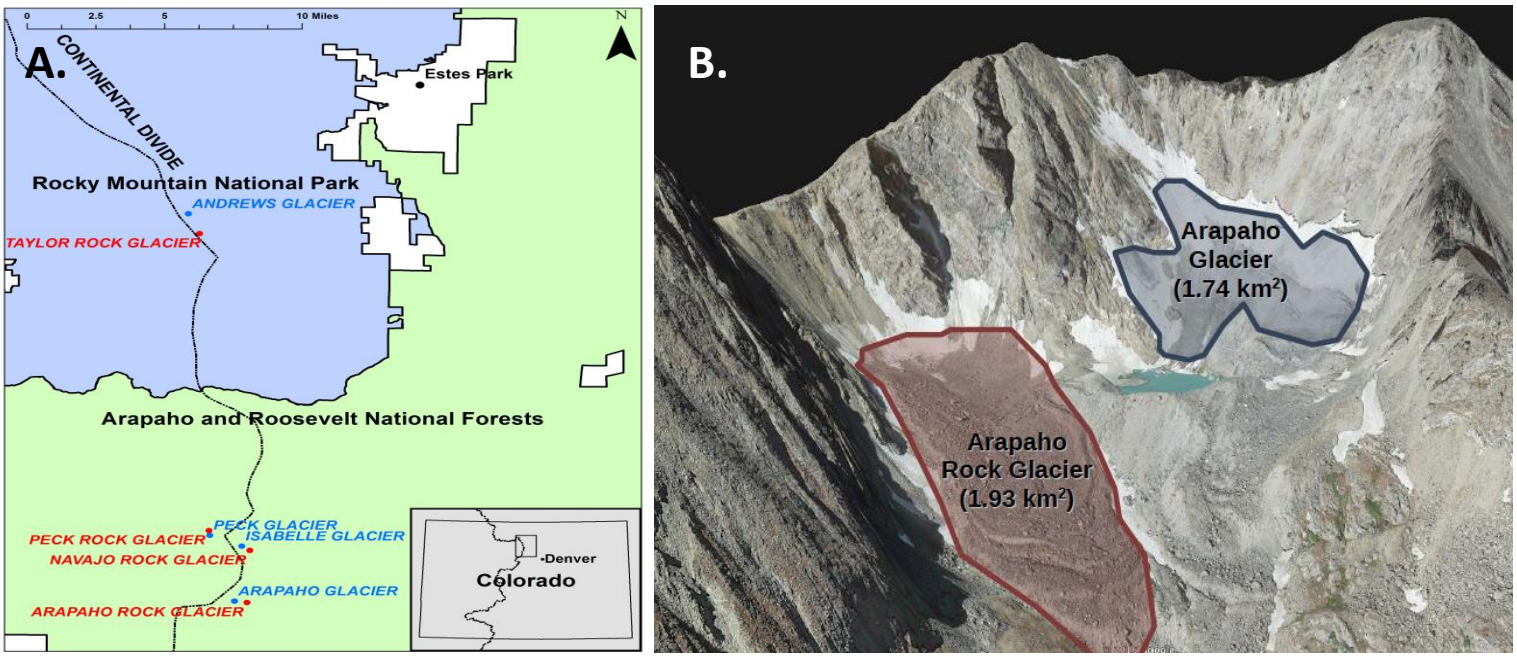
Figure 1 Site Map A) Stars note the approximate location of each glacier feature that was sampled for this study within the state of Colorado. In total four pairs of glaciers and rock glaciers were sampled along the Front Range of Colorado. **(B) Isabelle Glacier and Navajo Rock Glacier** form a pair 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 analysis by glacier type for DOM compounds identified using GC-MS 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. Statistical analysis of differences between glacier types can be found in Table 3.

Figure 3 Molecular distribution of GC-MS identified compounds Distribution of compounds by ion intensity pre- (orange) and post-incubation (red/brown) and the experimental control (green). 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 5).

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 BDOM during the incubation.



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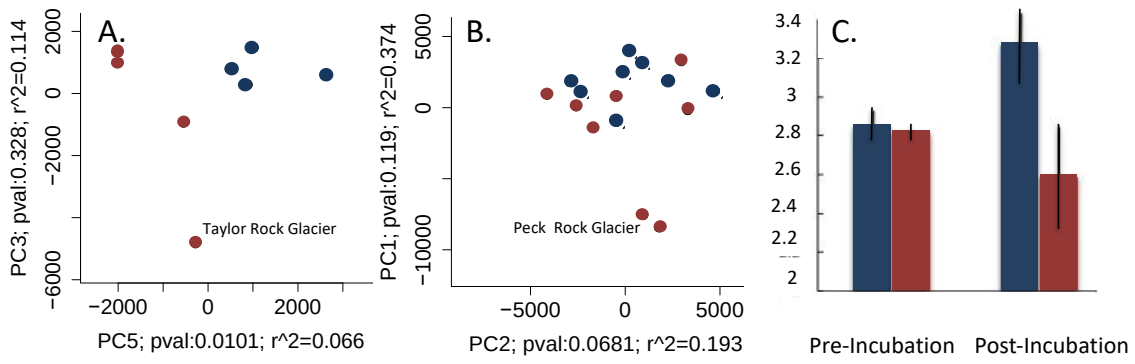

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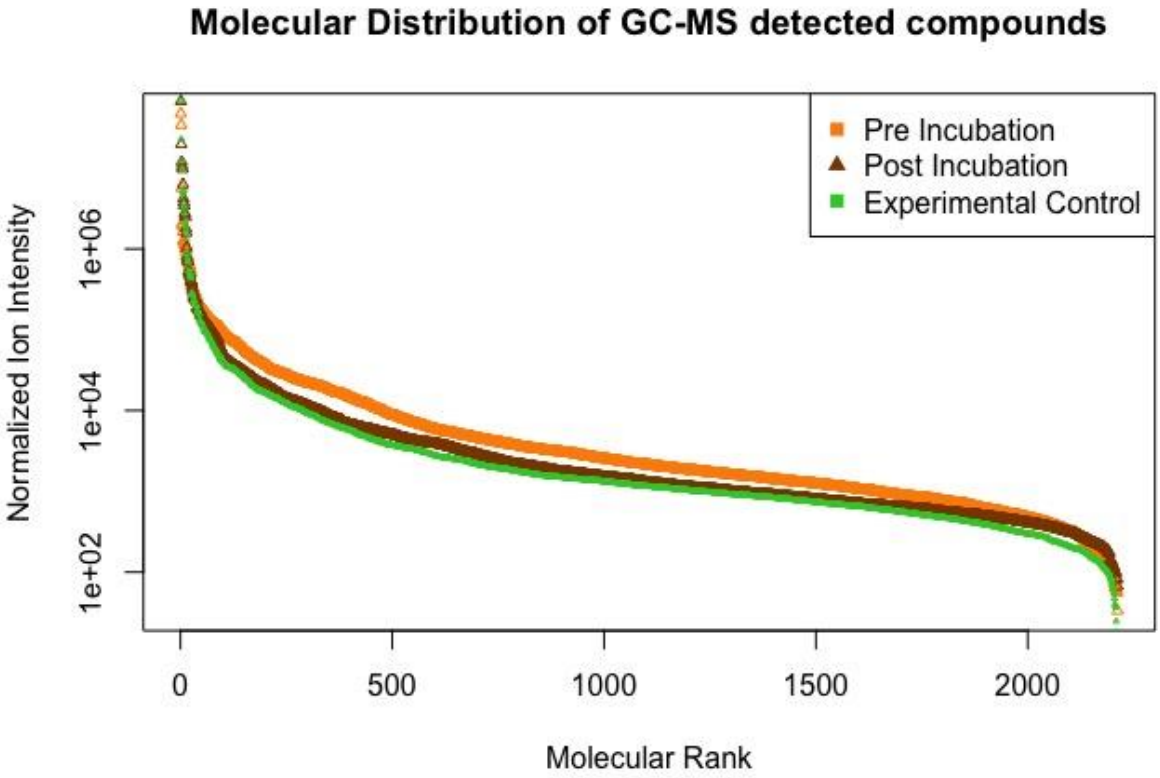

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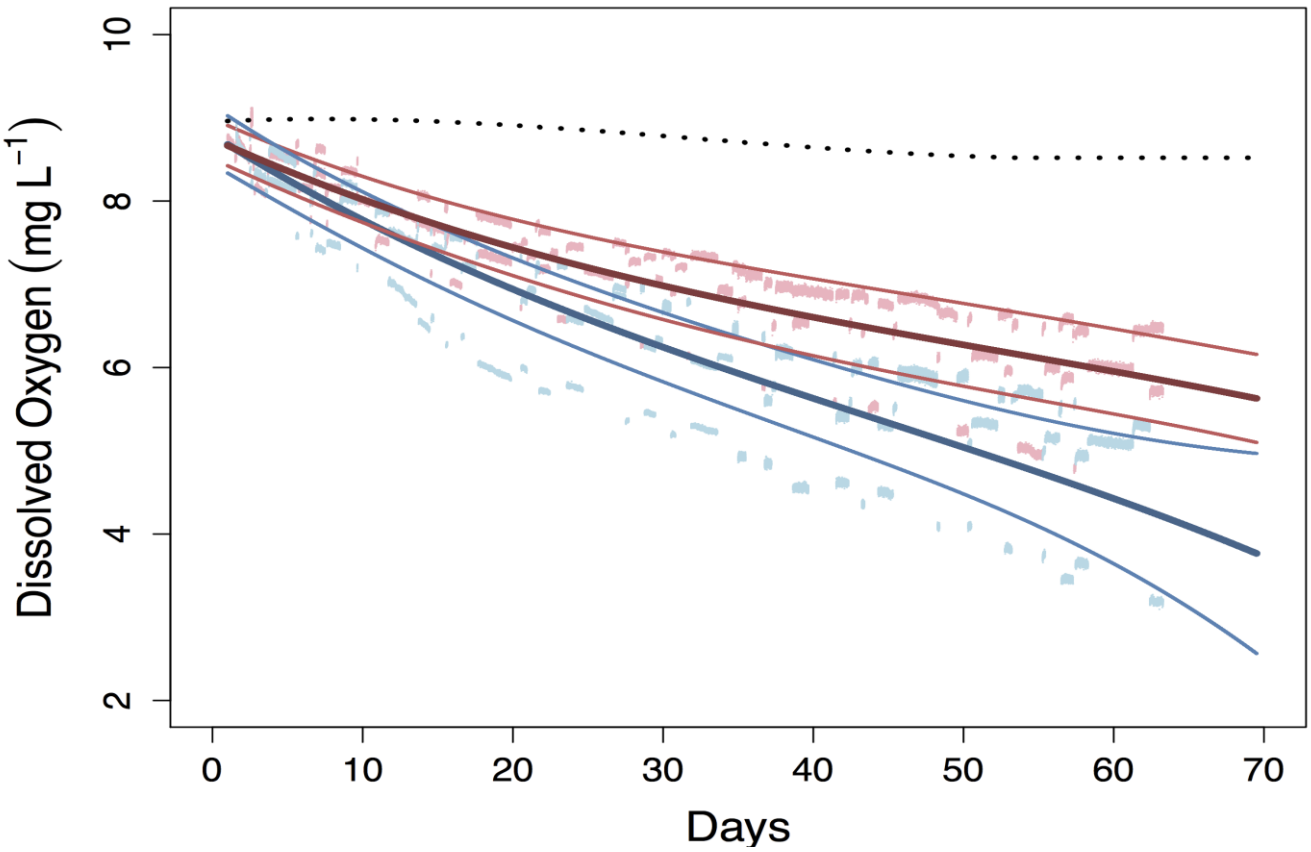

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Tables

Table 1 Site description for the four glaciers and rock glaciers sampled within our study**.** Italicized names represent non-official names of features in study. Coordinates are given in Universal Transverse Mercator North (UTM_N) and Universal Transverse Mercator East (UTM_E).

SITE	***FEATURE TYPE***	***UTM_N***	***UTM_E***	***ELEVATION (M)***
Andrews Glacier	Glacier	-105.681	40.288	3467
Taylor Rock Glacier	Rock Glacier	-105.671	40.276	3417
Arapaho Glacier	Glacier	-105.646	40.023	3738
Arapaho Rock Glacier	Rock Glacier	-105.638	40.022	3581
Isabelle Glacier	Glacier	-105.641	40.063	3634
Navajo Rock Glacier	Rock Glacier	-105.636	40.061	3492
Peck Glacier	Glacier	-105.664	40.068	3458
Peck Rock Glacier	Rock Glacier	-105.664	40.072	3271

Table 2 DOM compounds that defined the differences between glaciers and rock glaciers before incubation, as identified with ANOVA using the aov function in R. Mass to charge ratios (*m/z*) are the largest (pseudomolecular) ion (including TMS derivatization) observed for each compound. Fold change (FC) > 1.0, or positive values for Log2 FC indicate greater relative abundance in glaciers versus rock glaciers, and FC < 1.0 or negative Log2 FC values indicate greater relative abundance in rock glaciers versus glaciers.

Compound	**Pseudomolecular ion (*m/z*)**	**FC (glacier/rock glacier)**	**Log2 FC (glacier/rock glacier)**
C884	565.3	9.93	3.31
C996	294.2	8.07	3.01
C1140	569.8	5.09	2.35
C815	419.1	4.37	2.13
C1719	540.8	4.20	2.07
C1612	633.5	3.55	1.83
C1599	516.1	2.71	1.44
C367	562.3	2.46	1.30
C1890	613.1	2.33	1.22
C1660	573.3	2.27	1.18
Maltose	292.2	2.19	1.13
Glutamate	365.2	1.99	1.00
C1655	480.2	1.80	0.85
C1739	489.1	1.75	0.81
C1840	640.1	1.57	0.65
C1870	641.9	1.23	0.30
C1414	507.2	1.15	0.20
C589	649.5	0.86	-0.22
C1942	535.1	0.80	-0.33
C1376	440.2	0.73	-0.46
C1439	406.2	0.69	-0.53
C2146	303.2	0.69	-0.54

C35	348.2	0.65	-0.62
C1827	624.1	0.61	-0.72
C1125	468.1	0.47	-1.08
C2153	261.2	0.44	-1.18
C1226	634.1	0.44	-1.18
C531	343.2	0.33	-1.62
C1463	360.2	0.29	-1.81
Quinic Acid	409.2	0.27	-1.89
C2052	533.2	0.25	-1.97
Glycolic Acid	185.1	0.17	-2.52
Threonic Acid	410.7	0.04	-4.54

Table 3 Characteristics of DOM and post incubation DOM from glaciers (G) and rock glaciers (RG). 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}), 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 ($p<0.01$) using the Wilcoxon signed rank test for paired nonparametric samples. Standard deviations of mean values are listed in parentheses.

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

Table 4 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 BDOM 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 ($p<0.01$) using the Wilcoxon signed rank test for paired nonparametric samples. Standard deviations of mean values are listed in parentheses.

Site	*Type*	B_0	B_1	*k*	*% BDOM*	*% Recalcitrant*	*BGE*
Andrews	Glacier	1.6	2.8	0.001	63.64	36.36	0.37
Arapaho	Glacier	1.86	2.6	0.001	58.3	41.7	0.39
Isabelle	Glacier	0.67	3.51	0.001	83.97	16.03	0.39
Peck	Glacier	1.89	2.62	0.001	58.09	41.91	0.30
Arapaho	Rock Glacier	2.66	1.9	0.002	41.67	58.33	0.13
Navajo	Rock Glacier	2.82	1.71	0.001	37.75	62.25	0.06
Peck	Rock Glacier	1.66	2.62	0.001	61.21	38.79	0.13
Taylor	Rock Glacier	2.35	1.91	0.001	44.84	55.16	0.07
Mean G	Glacier	**1.51 (0.54)**	**2.88 (0.53)**	0.001 (0.00)	**66.00 (10.61)**	34.00 (7.03)	**0.263 (0.13)**
Mean RG	Rock Glacier	2.37 (0.63)	2.04 (0.56)	0.001 (0.00)	46.37 (8.93)	**53.63 (6.84)**	0.157 (0.16)

Table 5 The top 25 compounds present in pre- and post-incubation samples organized by fold change (FC). Glaciers and rock glaciers had the same top 25 compounds before and after incubation (as shown in SI5). Microbial metabolism altered compounds present, resulting in a different set of compounds post incubation that were similar in rank and composition to the experimental control. Molecular Rank (MR) as well as FC, and log2 FC are included in order to compare FC on the same scale both pre/post and post/pre.

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Compound Number	Annotation	Compound Superclass	Pre Incubation MR	Post Incubation MR	LV water MR	FC (pre/post)	log2 FC (pre/post)	FC (post/pre)	log2 FC (post/pre)
C0397	Succinic acid, 3-chlorophenyl 4-methoxybenzyl ester	Benzenoids	1	1549	1786	7.72E+03	12.9	1.30E-04	-12.9
C1991	2-Thiophenecarboxylic acid, phenyl ester	Benzenoids	16	1988	2065	1.43E+03	10.5	6.98E-04	-10.5
C0046	Pentadecan-1-ol	Lipids and lipid-like molecules	2	767	1344	9.25E+02	9.9	1.08E-03	-9.9
C0056	p-Cyanophenyl 4'-heptyl-4-biphenylcarboxylate	Phenylpropanoids and polyketides	5	1030	1818	8.70E+02	9.8	1.15E-03	-9.8
C0053	Homosalate	Benzenoids	4	688	751	4.44E+02	8.8	2.25E-03	-8.8
C0700	Diiodoacetylene	Organohalogen compounds	12	817	1033	3.57E+02	8.5	2.80E-03	-8.5
C1393	3-Methyl-3-(N-methyl-2-pyrrolyl)-1,2-diphenylcyclopropene	Phenylpropanoids and polyketides	11	794	800	3.52E+02	8.5	2.84E-03	-8.5
C0391	Succinic acid, 2-fluorophenyl 2,4-dimethylpent-3-yl ester	Benzenoids	21	1013	956	2.05E+02	7.7	4.89E-03	-7.7
C0007	Benzenamine, 4,4',4''-methylidynetris[N,N-diethyl-3-methyl-	Benzenoids	25	940	859	1.69E+02	7.4	5.90E-03	-7.4
C0713	1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purine-8-carbaldehyde-oxime	Organoheterocyclic compounds	24	745	773	1.19E+02	6.9	8.37E-03	-6.9
C0018	Dibenz[d,f]cycloheptane, 5-amino-1,2,3,8-tetramethoxy-	Benzenoids	18	622	774	9.76E+01	6.6	1.02E-02	-6.6
C0027	Unknown	Unknown	8	278	379	8.36E+01	6.4	1.20E-02	-6.4
C1422	p-Cyanophenyl p-(2-butoxyethoxy)benzoate	Phenylpropanoids and polyketides	19	394	175	4.60E+01	5.5	2.17E-02	-5.5
C0013	Hydroxylamine	Homogeneous non-metal compounds	3	95	158	3.69E+01	5.2	2.71E-02	-5.2

C0350	Methanone, (2-amino-5-chlorophenyl)(2-fluorophenyl)-	Benzenoids	10	152	115	2.78E+01	4.8	3.60E-02	-4.8
C2134	Furan-2-carboxylic acid [2-(2,2,6,6-tetramethyl-piperidin-4-yl)-ethyl]-amide	Organic acids and derivatives	22	282	300	2.43E+01	4.6	4.11E-02	-4.6
C0479	Unknown	Unknown	14	110	2187	1.50E+01	3.9	6.67E-02	-3.9
C1035	Unknown	Unknown	23	175	182	1.27E+01	3.7	7.89E-02	-3.7
C0179	Scopoletin	Phenylpropanoids and polyketides	6	60	58	1.11E+01	3.5	9.04E-02	-3.5
C0135	Phosphoric acid	Homogeneous non-metal compounds	9	47	82	5.73E+00	2.5	1.74E-01	-2.5
C0040	Unknown	Unknown	15	61	108	5.56E+00	2.5	1.80E-01	-2.5
C2165	Unknown	Unknown	20	82	57	4.19E+00	2.1	2.38E-01	-2.1
C0071	Phenylethylmalonamide	Benzenoids	17	42	28	3.32E+00	1.7	3.01E-01	-1.7
C0037	Stearic acid	Lipids and lipid-like molecules	13	22	61	1.74E+00	0.8	5.73E-01	-0.8
C0029	Palmitic acid	Lipids and lipid-like molecules	7	13	31	1.40E+00	0.5	7.12E-01	-0.5
C0125	Xylopyranoside, methyl 4-azido-4-deoxy-3-O-methyl-, .alpha.-D-	Organic oxygen compounds	200	25	26	1.25E-01	-3.0	7.97E+00	3.0
C0285	Phenol, 2,4,6-tri-tert-butyl-	Benzenoids	309	17	153	4.34E-02	-4.5	2.30E+01	4.5
C0015	1,2-Ethenediol	Organic oxygen compounds	347	18	16	3.70E-02	-4.8	2.70E+01	4.8
C0001	Sucrose	Organic oxygen compounds	65	5	5	2.88E-02	-5.1	3.47E+01	5.1
C0016	2-Amino-2-methyl-1,3-propanediol	Organic nitrogen compounds	628	24	24	1.82E-02	-5.8	5.50E+01	5.8
C0010	Threonine	Organic acids and derivatives	584	21	22	1.50E-02	-6.1	6.67E+01	6.1
C0011	Glycerol	Organic oxygen compounds	87	4	4	9.84E-03	-6.7	1.02E+02	6.7
C0060	1-Ethoxy-2-propanol	Organic oxygen compounds	210	7	7	8.93E-03	-6.8	1.12E+02	6.8

C0073	Beta-alanine	Organic acids and derivatives	281	8	8	8.00E-03	-7.0	1.25E+02	7.0
C0019	Glycine	Organic acids and derivatives	426	11	9	7.68E-03	-7.0	1.30E+02	7.0
C0009	Pyroglutamate	Organic acids and derivatives	549	9	11	7.20E-03	-7.1	1.39E+02	7.1
C0092	Unknown	Unknown	1207	20	27	4.40E-03	-7.8	2.27E+02	7.8
C0067	Norleucine	Organic acids and derivatives	1399	23	39	4.04E-03	-8.0	2.47E+02	8.0
C0425	Unknown	Unknown	1364	15	13	2.79E-03	-8.5	3.58E+02	8.5
C0169	2-Methyl-6-nitro-4-quinolinol	Organoheterocyclic compounds	1640	19	21	2.33E-03	-8.7	4.28E+02	8.7
C0017	3,5-Di-tert-butyl-4-hydroxybenzoic acid	Benzenoids	1540	14	12	1.63E-03	-9.3	6.14E+02	9.3
C0070	1,2,4-Triazole, 3-mercapto-4-phenyl-5-methyl-	Organoheterocyclic compounds	250	2	2	1.28E-03	-9.6	7.80E+02	9.6
C0047	Aspartate	Organic acids and derivatives	1049	10	10	1.24E-03	-9.7	8.05E+02	9.7
C0126	l-Alanine, N-(2-methoxyethoxycarbonyl)-, octyl ester	Organic acids and derivatives	1929	16	14	1.10E-03	-9.8	9.13E+02	9.8
C0024	Lactate	Organic acids and derivatives	115	1	1	1.05E-03	-9.9	9.49E+02	9.9
C0281	2-Phenoxathiinamine-10,10-dioxide	Organoheterocyclic compounds	1567	12	134	1.05E-03	-9.9	9.54E+02	9.9
C0779	Benzenecarbothioic acid, 2,4,6-triethyl-, S-(2-phenylethyl) ester	Benzenoids	480	3	3	7.46E-04	-10.4	1.34E+03	10.4
C0014	Serine	Organic acids and derivatives	1099	6	6	5.52E-04	-10.8	1.81E+03	10.8