

## SPECIAL ISSUE-LETTER

**Dynamic processing of DOM: Insight from exometabolomics, fluorescence spectroscopy, and mass spectrometry**H. J. Smith,<sup>1</sup> M. Tigges,<sup>2,a</sup> J. D'Andrilli,<sup>3</sup> A. Parker,<sup>1,4</sup> B. Bothner,<sup>2</sup> C. M. Foreman <sup>1,5\*</sup>

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**Scientific Significance Statement**

Glaciers are a significant reservoir of dissolved organic matter (DOM), with the potential to impact global carbon cycling. Current understanding of biological DOM processing is dominated by oceanographic studies, with a significant knowledge gap regarding how individual organisms interact with complex DOM from freshwater sources. We show that although DOM from glaciers is chemically labile, it does not support sustained respiration, thus lability alone is not sufficient to predict downstream effects on carbon cycling.

**Abstract**

Dissolved organic matter (DOM) in freshwater environments is an important source of organic carbon, supporting bacterial respiration. Frozen environments cover vast expanses of our planet, with glaciers and ice-sheets storing upwards of 6 petagrams of organic carbon. It is generally believed that DOM liberated from ice stimulates downstream environments. If true, glacial DOM is an important component of global carbon cycling. However, coupling the release of DOM to microbial activity is challenging due to the molecular complexity of DOM and the metabolic connectivity within microbial communities. Using a single environmentally relevant organism, we demonstrate that processing of compositionally diverse DOM occurs, but, even though glacially derived DOM is chemically labile, it is unable to support sustained respiration. In view of projected changes in glacier DOM export, these findings imply that biogeochemical impacts on downstream environments will depend on the reactivity and heterogeneity of liberated DOM, as well as the timescale.

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**Author Contribution Statement:** HJS, MT, BB, and CMF designed the research. HJS, MT, and JD performed the research. HJS, MT, JD, AP, BB, and CMF analyzed the data. HJS, MT, JD, AP, BB, and CMF wrote the paper.

**Data Availability Statement:** Data is available in the Antarctic Master Data repository at <https://gcmd.gsfc.nasa.gov/search/Metadata.do?entry-USAP-1141978>.

Additional Supporting Information may be found in the online version of this article.

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**This article is part of the Special Issue: Carbon cycling in inland waters**

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The transport of organic carbon (OC) through freshwater ecosystems is central to the global carbon cycle. Rivers and estuaries play a fundamental role in the transport of carbon as they link freshwater and marine ecosystems, transporting 0.9 petagrams of OC per year to the oceans (Cole et al. 2007). Within freshwater habitats, dissolved organic matter (DOM) is an important source of OC supporting microbial respiration. DOM, a heterogeneous mixture of labile and recalcitrant compounds (Mopper et al. 2007; Vahatalo et al. 2010), is present along a reactivity spectrum in all freshwaters. DOM reactivity is determined by both the molecular composition of DOM constituents and the metabolic capacity of the ecosystem, ultimately affecting trophic interactions and influencing aquatic food web dynamics.

DOM reactivity is not only a consequence of its composition and the in situ microbial communities, but also different physical and biological processes at play across aquatic environments. Laboratory experiments examining the utilization of DOM generally use glucose or combinations of amino acids (Nelson and Carlson 2012; Nikrad et al. 2012; Jørgensen et al. 2014; Lechtenfeld et al. 2015). Unfortunately, these commercially available substrates lack environmental relevance and the chemical complexity of naturally occurring DOM. To address the linkage between the decomposition and chemical reactivity of freshwater DOM, this study used different sources of environmental end-member DOM, including: microbially derived DOM from the Cotton Glacier stream, Antarctica (CG); microbially derived DOM from the eutrophic Pony Lake, Antarctica (PL); and as a counterpoint terrestrially derived DOM from the Suwannee River, U.S.A. (SR). The two Antarctic carbon sources were selected because the lack of higher order plants and simplified foodwebs makes Antarctica an optimal environment to study the processing of microbially derived freshwater DOM. Further, investigations from diverse environments show that glacially derived DOM can be highly bioavailable to microorganisms (Hood et al. 2009; Bhatia et al. 2013; Lawson et al. 2014; Smith et al. 2017), suggesting that glaciers are a reservoir of chemically reactive DOM.

While it is increasingly recognized that the intrinsic properties of DOM dictate the extent of microbial processing (Guillemette and del Giorgio 2011), it remains poorly resolved which fractions of DOM are degraded and how shifts in composition translate into rates of processing. Coupling changes in DOM composition to in situ microbial community processing is difficult due to the molecular complexity of DOM and the phylogenetic diversity of natural microbial assemblages. Single organism studies provide a way to resolve the contributions of individual microorganisms to bulk processing. Recent evidence indicates that individual species of marine organisms can affect ecosystem-wide processes, and may be responsible for significant DOM fluxes and nutrient mineralization (Pedler et al. 2014). Currently, our understanding of biological DOM processing is

dominated by oceanographic studies (Mou et al. 2008; Jiao et al. 2010; Kujawinski 2011; Nelson and Carlson 2012; Jiao et al. 2013; Hansell and Carlson 2014), with far less known in freshwater environments. Thus, there is a significant gap in knowledge regarding how individual organisms interact with complex DOM from freshwater sources.

The aim of this study was to determine the relationship between the intrinsic reactivity of environmentally isolated sources of freshwater DOM and microbial decomposition over time. To characterize these complex interactions, a combination of exometabolomic, microbiological, and biogeochemical techniques were employed.

## Materials and methods

### Experimental organism

*Janthinobacteria* are gram negative, motile, aerobic, rod-shaped microorganisms found in soil and aquatic environments globally. They are members of the Proteobacteria phylum and class Betaproteobacteria. *Janthinobacterium* sp. strain CG3 (CG3) was isolated from a supraglacial stream on the Cotton Glacier, Antarctica. The CG3 genome is 6.12 Mbp (Smith et al. 2013) and specifically chosen because it possesses genetic evidence for several central carbon metabolisms, both aerobic and fermentative (see Supporting Information for more details).

### Experimental setup

To investigate the biological transformation of freshwater DOM of varying reactivity, we conducted extended incubations under environmentally relevant conditions. Incubations remained axenic for the duration of the experiment (see Supporting Information). CG3 cells were inoculated (final concentration  $10^5$  cells/mL) into a carbon free minimal M9 media (Difco). The carbon source amendments used included: microbially derived DOM from the oligotrophic supraglacial Cotton Glacier stream, Antarctica (CG), microbially derived DOM from the eutrophic coastal pond, Pony Lake, Antarctica (IHSS Pony Lake Fulvic Acid; PL), and terrestrially derived DOM from the Suwannee River, U.S.A. (IHSS Natural Organic Matter; SR). The three amendments (CG, PL, and SR) were added to combusted amber bottles, to a mass-balanced final concentration of 5 mg/L C. All samples were incubated for 98 d at 15°C, in the dark, and shaken at 30 rpm. Samples were monitored for bacterial contamination and cell abundances four times over the course of the experiment (see Supporting Information Table S2). A destructive sampling design was used for extracellular emission matrices (EEMs) and exometabolite analysis with five replicates and corresponding abiotic controls.

### Cellular respiration

A Micro-Oxymax closed-circuit respirometer (Columbus Instruments) was used to continuously measure O<sub>2</sub> concentrations during the extended incubations. Each carbon source plus bacterial cells were run in triplicate and included

a blank without cells. Blank values were subtracted at each timepoint. Details of the statistical analyses of the respirometry data are provided in the Supporting Information.

### Fluorescence spectroscopy

DOM fluorescence signatures were analyzed by EEMs according to (D'Andrilli et al. 2013) on a Fluoromax-4 spectrofluorometer (HORIBA Jobin-Yvon). Samples were analyzed for UV-Vis absorbance (190–1100 nm) with a ThermoScientific Genesys10 scanning spectrophotometer on optically dilute samples (absorbance < 0.3 at 254 nm). EEMs data were post-processed to correct for instrument-specific bias using manufacturer-generated correction files for excitation, emission, and media blank subtraction (carbon free M9 minimal media). For temporal samples, EEMs were normalized according to the fluorescence intensity normalization calculation in the DOMFluor Toolbox (Stedmon and Bro 2008). Specific regions of fluorescence were defined for each carbon source corresponding to previously identified natural OM fluorophores (Coble 1996). For statistical analysis of OM fluorescence, EEMs were decomposed into individual fluorescing components using parallel factor (PARAFAC) (analysis with decomposition routines for excitation emission matrices; drEEM, v. 0.3.0) and the N-way scripts in MATLAB R2016b (Stedmon and Bro 2008; Murphy et al. 2013; see Supporting Information).

### External metabolite extraction

CG3 cells were removed from the microbiological media with 0.2  $\mu\text{m}$ , low-carbon binding filters. The filtered external metabolites were passed through Solid Phase Extraction PPL cartridges (Agilent Bond Elut) to concentrate DOM components and exometabolites and remove inorganic salts (Dittmar et al. 2008). Retained exometabolites were eluted into combusted glass vials with HPLC grade methanol, dried under nitrogen, and stored dark at  $-20^\circ\text{C}$  prior to analysis.

### UPLC-Q-TOF MS based exometabolite molecular constituent analysis

Mass spectra of external metabolites were obtained on a 1290 Ultra Performance Liquid Chromatography system coupled to a 6538 Ultra High Definition Accurate-Mass Quadrupole-Time of Flight mass spectrometer (UPLC-Q-TOF MS) operated in positive mode with an electrospray ionization (ESI) source (Agilent Technologies, see Supporting Information for details).

### FT-ICR MS

CG, PL, and SR DOM lyophilized powders were reconstituted in HPLC grade methanol prior to Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Mass spectra were obtained on a 9.4 Tesla FT-ICR mass spectrometer at the National High Magnetic Field Laboratory. Negative mode ESI and FT-ICR MS instrumental parameters are described in D'Andrilli et al. (2013). Prior to analysis, negatively charged gaseous ions were produced with a custom-built ESI source (Emmett et al. 1998) and transferred into the mass

spectrometer. Experimental parameters, mass spectral calibration, and peak list generation were optimized based on previous natural DOM analysis (D'Andrilli et al. 2013, 2015).

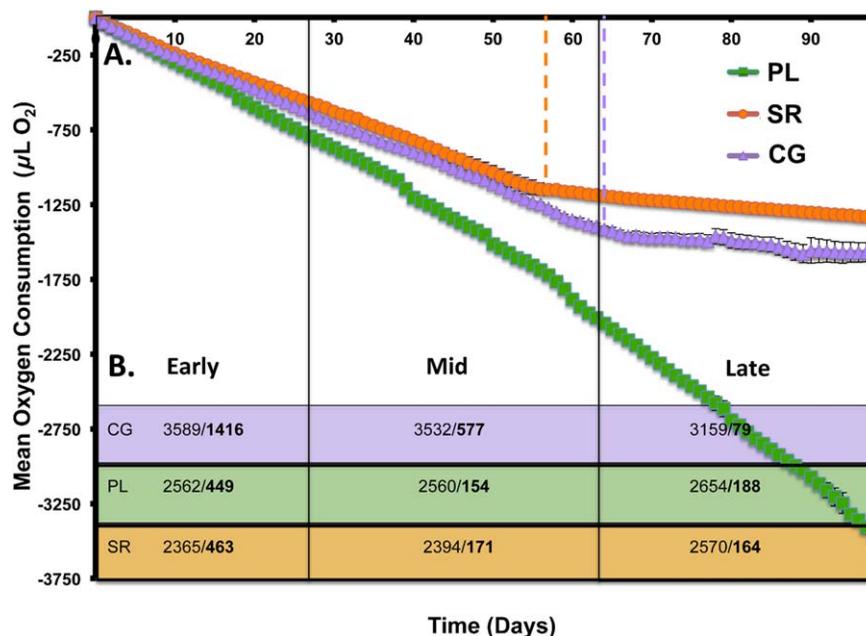
## Results

### Cellular respiration

Cellular respiration is a direct measure of metabolism, linking bacterial growth (see Supporting Information) to carbon utilization. To investigate specific metabolic rates, CG3 was grown with each of the carbon sources (CG, PL, and SR) with respiration measured every 24 h for 98 d (Fig. 1A). All treatments supported microbial respiration. No significant difference in mean  $\text{O}_2$  consumption among treatments was observed during the first 17 d of the experiment ( $p > 0.156$ ). After 17 d, conversion rates of the carbon sources to  $\text{CO}_2$  differed between PL and both CG and SR ( $p < 0.001$ ). The first statistically significant difference in  $\text{O}_2$  consumption between CG and SR occurred at day 59 ( $p = 0.0352$ ), with  $\text{O}_2$  consumption remaining significantly different ( $p < 0.001$ ) for the duration of the experiment. Broken line regression analysis of all carbon sources identified significant break-points, indicating a difference in the rate of  $\text{O}_2$  consumption by CG3. Break-points were identified for CG at day 64 ( $p < 0.001$ ) and for SR at day 56 ( $p < 0.001$ ). No significant break-point was identified for PL ( $p > 0.076$ ). Overall, PL DOM supported the greatest rate of respiration for CG3 at 32.76  $\mu\text{L O}_2$  consumed/day, followed by CG at 21.78 and SR at 20.17. After each break-point, CG3 respired the CG and SR DOM at reduced rates (18.82 and 16.02  $\mu\text{L O}_2$  consumed/day). These break-points in linearity were used to inform sampling timepoints for UPLC-Q-TOF MS molecular constituent characterization.

### Fluorescent characterization of DOM transformations

CG amended samples showed regions of maximum fluorescence (Excitation [Ex]: 240–280 nm, Emission [Em]: 300–350 nm; Supporting Information Fig. S1) corresponding to the proteinaceous fluorophores B and T (Coble 1996; Stedmon et al. 2003). DOM fluorescence was found at longer emission wavelengths (Ex: 240–280 nm, Em: 400–450 nm), representing humic-like fluorophores A and C in both PL and SR DOM. Fluorescence intensity values in the proteinaceous regions (B and T fluorophores) were summed and classified as more reactive, while humic-like fluorescence regions (A and C fluorophores) were combined and classified as less-reactive and more recalcitrant. The fluorescence in both regions was summed and converted to the percentage change relative to the previous sampling point (Fig. 2). Throughout the 98-d incubations, dynamic fluctuations were detected by EEMs showing the generation or loss of specific fluorophores. These results were further confirmed (Supporting Information Fig. S4) using PARAFAC analysis, a statistical approach that considers individual fluorescing OM regions.



**Fig. 1.** Measures of biological transformation of DOM over time. **(A)** Mean cumulative oxygen consumption ( $\mu\text{L O}_2$ ) by CG3 for Cotton Glacier (CG), Pony Lake (PL), and Suwannee River (SR) under constant incubation conditions, error bars  $\pm 1$  SD.  $\text{O}_2$  measurements were taken every 24 h for 98 d ( $N = 3$ ). Dashed lines indicate a significant break-point in linearity for CG (day 64) and SR (day 56). **(B)** Number of molecular constituents measured/constituents with significantly changed abundance in bold (adjusted  $p < 0.05$ ) between timepoints (Early = days 0–27, Mid = d27–62, and Late = d62–98) for CG3 incubations with CG, PL, and SR DOM sources.

### Analysis of DOM molecular constituent transformations

Liquid chromatography coupled mass spectrometry (UPLC-Q-TOF MS) is a powerful platform for the untargeted relative quantitation of small molecules over time (Want et al. 2005; Smith et al. 2006). While EEMs analysis provided bulk level characterization, UPLC-Q-TOF MS was used to track changes in abundance of individual molecular constituents at four timepoints: days 0, 27, 63, and 98. 5040 distinct molecular constituents were detected across all carbon sources and timepoints. Pairwise comparisons between timepoints (time ranges: Early = days 0–27; Mid = days 27–63, Late = days 63–98) were used to calculate variances in abundances of constituents over time. While significant (adjusted  $p < 0.05$ ) changes in abundance occurred throughout the incubations, the number of transformed constituents was highest in the early time range (Fig. 1B). The late time range contained the least transformed constituents for CG and SR, coinciding with decreased respiration rates.

To highlight biologically relevant molecular constituents, a one-way ANOVA was carried out for each carbon source. Overall, 3057 molecular constituents showing a significant (adjusted  $p < 0.01$ ) abundance change over time were retained. The unique mass/retention time of each constituent was used to determine similarities between carbon sources (Supporting Information Fig. S2A). Over the course of the experiment, 38% of significantly changed constituents were found in all three

carbon sources, while 26% were unique to CG. PL (8%) and SR (5%) had fewer uniquely transformed molecular constituents.

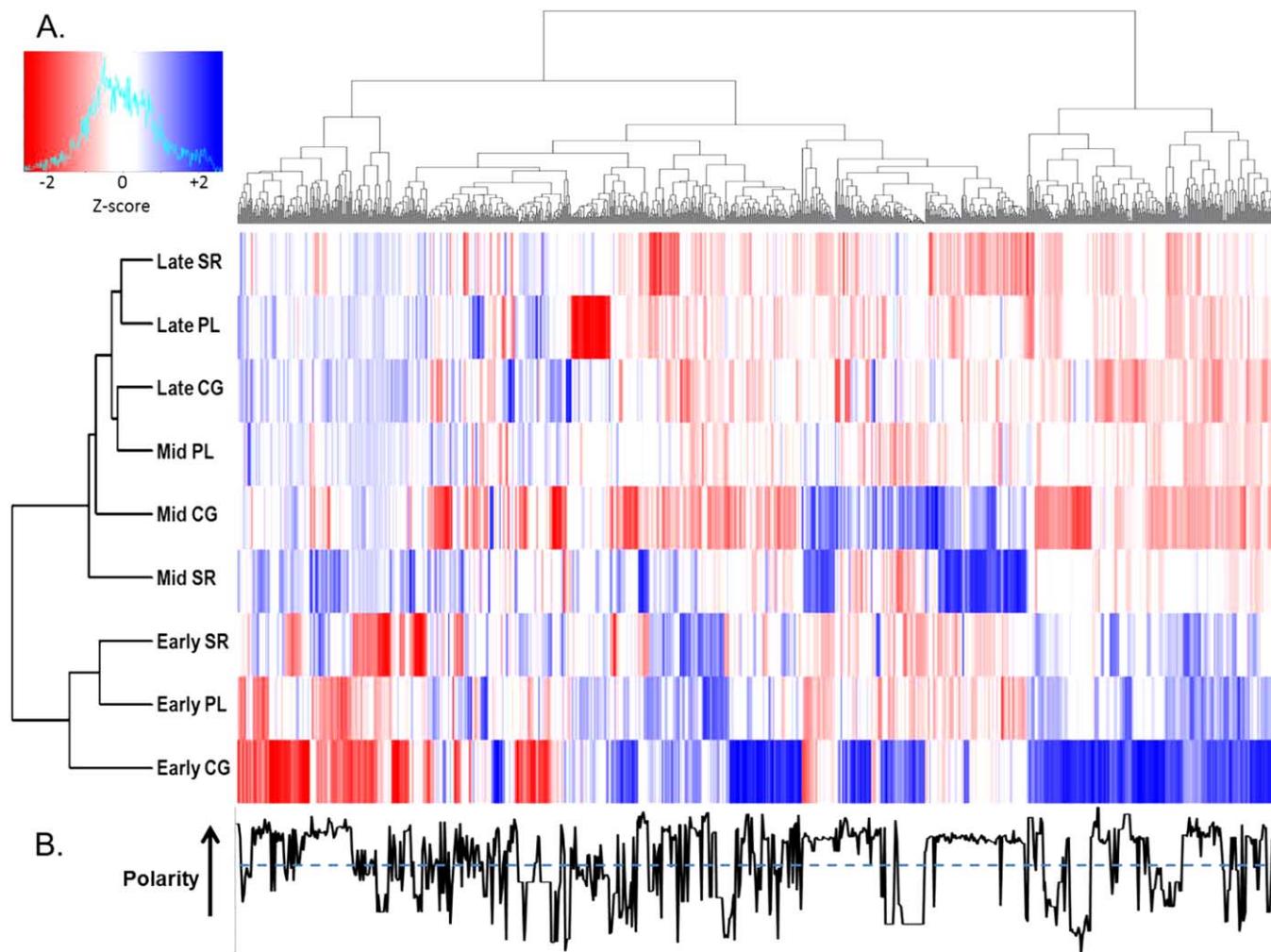
Two-way ANOVAs (timepoint, carbon source) were used to assess how trends in constituent abundance over time compared between carbon sources. Of these, 749 demonstrated a significant change by carbon source (adjusted  $p < 0.01$ ). Cluster analysis demonstrated both source and time dependence of DOM transformation by CG3 (Fig. 3). Only the early time range clustered exclusively by time, indicating that these transformations were more similar across all carbon sources.

Retention time and  $m/z$  values were used to identify exo-metabolite molecular constituents. Of 150 commonly identified metabolites, 26 were confirmed across all carbon sources and timepoints. Of those identified, 31% were amino acids, with the remaining being metabolic degradation products.

### Characterization of DOM source material intrinsic properties

Intrinsic chemical properties of the DOM source materials were determined to provide comparisons between the DOM sources used to predict processing rates. Datasets of EEMs, FT-ICR MS, and UPLC-Q-TOF MS data were divided into chemical classes consisting of relatively more- or less reactive molecular components. Each of these methods captures a different portion of the DOM pool, providing a more comprehensive analysis. Collectively, the analyses generated the





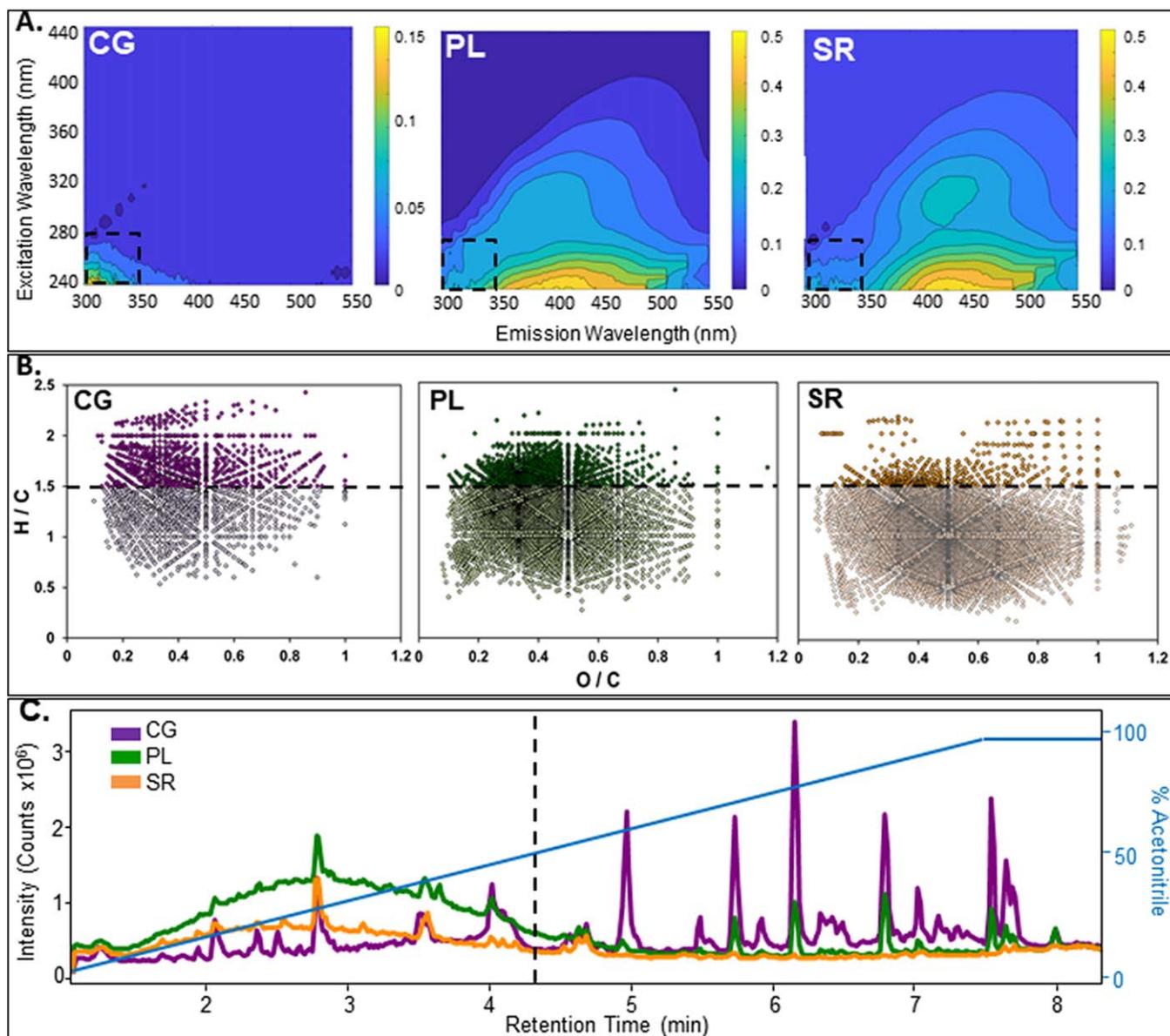
**Fig. 3.** Profile of significant (adjusted  $p < 0.01$ ) molecular constituent abundance changes in CG3-DOM incubations detected by UPLC-Q-TOF MS analysis. **(A)** Heatmap of CG3 molecular constituent abundance fold changes across time range (Early: d0–27, Mid: d28–63, Late: d64–98) and carbon source (Cotton Glacier: CG, Pony Lake: PL, Suwannee River: SR). Abundance increases and decreases are shown in blue and red, respectively. Constituents (columns) that cluster together have similar abundance patterns over time with respect to each carbon source. **(B)** Profile of the UPLC retention time based relative polarity of constituents. The dashed line represents the cutoff used to classify more- (below) and less- (above) reactive constituents.

same DOM chemical reactivity continuum with  $CG > PL > SR$  (Figs. 4, 5; Supporting Information Table S3). The chemical classifications of CG and SR DOM based on H/C and O/C ratios are provided on van Krevelen diagrams (Kim et al. 2003) in Fig. 5A,B. PL data are shown in D’Andrilli et al. (2013). Molecular formulae common to all carbon sources are present within the lipid-, protein-, carbohydrate-like, and highly unsaturated chemical species including both: phenolic-, and polyphenolic-like regions of the van Krevelen diagram (Fig. 5C). Further information on molecular components of the DOM source materials is presented in the Supporting Information and can be found in the Antarctic Master Data repository at <https://gcmd.gsfc.nasa.gov/search/Metadata.do?entry=USAP-1141978>

## Discussion

Glaciers, positioned at the top of the cryosphere hydrological cycle, are reservoirs of atmospherically deposited and biologically produced, chemically labile DOM (Stubbins et al. 2012; Smith et al. 2017). Studies linking microbial metabolism to both the ancient (Hood et al. 2009) and newly synthesized (Smith et al. 2017) fractions of DOM exist; however, the molecular mechanisms responsible for the rapid assimilation and transformation of glacial DOM are largely unresolved.

Across natural environments, DOM heterogeneity promotes ecosystem stability by providing diverse compounds as energy sources to heterotrophic bacteria (Wetzel 2003). Ecologically, it remains to be determined which community

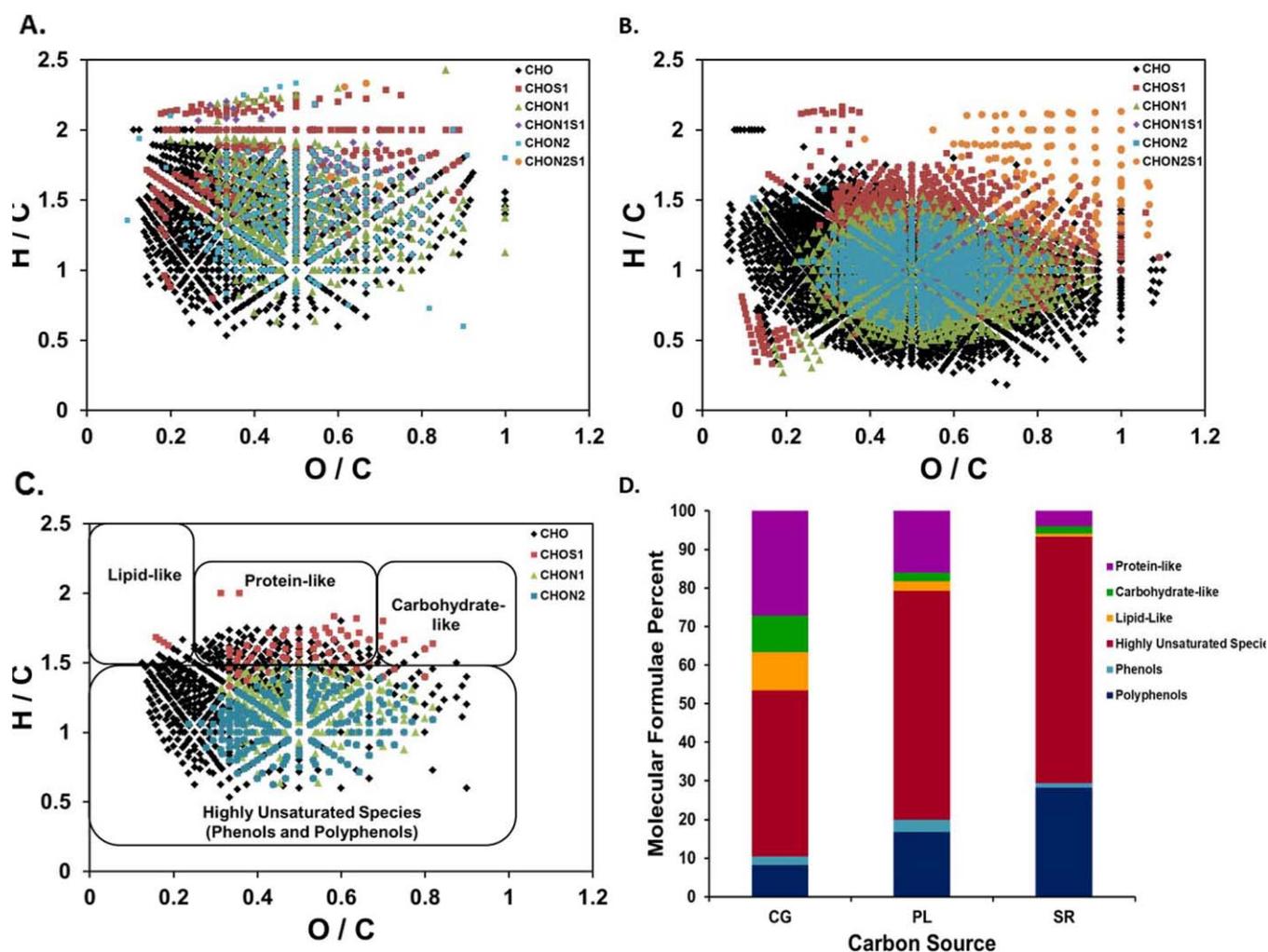


**Fig. 4.** Cotton Glacier (CG), Pony Lake (PL), and Suwannee River (SR) DOM source material characterization detected by (A) EEMs, where dashed lines represent the boundary of the more-reactive fluorescence region, fluorescence intensity in Raman Units (R.U.). (B) FT-ICR MS where the molecular lability boundary is highlighted as a dashed line, with chemically labile constituents having  $H/C \geq 1.5$ , and (C) reverse-phase UPLC-Q-TOF MS total ion chromatograms overlaid with the solvent elution profile where the dashed line represents the cutoff utilized for more-(right) and less-(left) reactive classification.

members, either rare or abundant, have an influential role in DOM processing. It is not known if a single organism is capable of processing compositionally diverse DOM, or if transformations rely on a cascade of events between community members. To understand this complicated network, single organism studies in combination with environmentally isolated DOM sources can help elucidate our understanding of the dynamic nature of DOM processing. *Janthinobacterium* sp. strain CG3 was selected based on its global ubiquity and

genome-enabled predicted metabolic flexibility (see Supporting Information). All three DOM sources in the present study supported respiration by CG3, despite their different starting points along the chemical reactivity continuum.

Microbially mediated DOM transformations and loss ultimately dictate the reactivity of DOM across aquatic ecosystems. The spectrum of environmental DOM lability (labile, semi-labile, recalcitrant) has typically been characterized by the extent of microbial interaction over time (Jiao et al.



**Fig. 5.** DOM molecular composition on van Krevelen diagrams for organic matter isolated from (A) Cotton Glacier DOM and (B) IHSS Suwannee River natural organic matter. Molecular formulae detected in all carbon sources (CG, PL, and SR) are shown in (C) with labeled chemical class regions (modified from Hodgkins et al. 2016), corresponding to the boundary conditions presented in (D), which shows the chemical class percentages contributing to each carbon source calculated from the molecular formulae.

2010), however, based on the techniques used, incubation times, and analytical limitations it is likely that these classifications are not applicable to all ecosystems and experimental questions. It is therefore more realistic to consider OM reactivity along a continuous spectrum (e.g., Koehler et al. 2012; Mostovaya et al. 2016). For this study, we characterized the chemical character of each carbon source to form hypotheses about processing and then analyzed the data based on a reactivity continuum. We defined the carbon components having the highest degree of change as the most reactive. Due to the highly dynamic fluctuations in amino acid-like fluorescence over time, these constituents were the most reactive across all source materials and time (Fig. 2).

For all carbon sources, exometabolite changes were greatest during the early time range, indicative of the greatest degree of DOM transformation. While the late time range contained the

least transformed constituents for CG and SR, coinciding with decreased respiration rates. Results from EEMs/PARAFAC and exometabolite analyses confirmed the importance of amino acids as a dominant fraction of the reactive DOM pool, consistent with the findings of (Cory and Kaplan 2012), while also showing that they experienced the most dynamic range of changes in our system. Interpreting the differences in respiration and exometabolites among carbon sources suggests that reactivity is contextual, being linked to both the microbial response to their energy source and the chemical composition of the DOM itself.

While chemical analyses (i.e., EEMs, FT-ICR MS, and UPLC-Q-TOF MS) identified CG DOM as the most chemically reactive carbon source, CG DOM did not support an initial spike in cellular respiration, rather, the first significant change in the rate of cellular respiration was identified for CG DOM at day

64 ( $p < 0.001$ ). These results are not indicative of a highly reactive source of DOM as suggested by simple sugar studies (Gruber et al. 2006; Hartley et al. 2010; Rousk et al. 2014). However, there were measurable changes in net fluorescence intensity and abundance of exometabolites, representing bacterial interactions with each carbon source. Over time, when combined with extended studies of biological processing, CG DOM did not support greater rates of cellular respiration or carbon turnover compared to other representative end-member more chemically heterogeneous DOM sources (PL and SR). The carbon source with the greatest extent of chemical heterogeneity was PL, which was steadily processed over the course of the experiment (no significant break-point could be identified  $p > 0.076$ ), and also supported the greatest rate of respiration ( $32.76 \mu\text{L O}_2$  consumed/day). Thus, our findings are consistent with the idea that while glacial DOM is more compositionally labile than the other two sources, the low degree of chemical heterogeneity may not necessarily support long-term metabolism. Therefore, our data indicate that caution is warranted when extrapolating from short-term studies.

In marine environments the accumulation of recalcitrant carbon has been linked to microorganisms (Ogawa et al. 2001; Jiao et al. 2010), and recent findings suggest that bacteria are responsible for the formation of structurally complex and persistent molecules (Lechtenfeld et al. 2015). Similar trends have not been established for freshwater habitats. Both EEMs and UPLC-Q-TOF MS measurements over 98 d provide evidence for dynamic cycling of DOM. The largest fluorescent changes over time were associated with the proteinaceous region, demonstrating the preferential usage of more reactive compounds. In contrast to previously reported data for marine systems, no overall increase in less-labile fluorescent material was observed over time. Fewer fluctuations in fluorescent DOM were observed for more humic-like compounds over time, indicating that this fraction is still biologically altered, but to a lesser extent.

Microbes have been shown to interact with DOM in a variety of ways, ranging from ignoring, consuming, producing, and fragmenting via extracellular enzymes (Kujawinski 2011). These interactions present a range of potential processing pathways an organism may employ, providing an explanation for the diversity of molecular and fluorescent DOM changes seen throughout this study. DOM transformations can occur very rapidly at the community level, masking carbon cycling intermediates and products generated by organisms. As the importance of metabolic intermediates and the effect on overall DOM processing is poorly understood, it is valuable to study individual organisms in controlled experiments to reduce the effect of confounding ecosystem variables. Studies of this type ultimately lead to greater insight of the contribution of an individual organism's metabolism to community level DOM processing. The metabolic flexibility of CG3 is key to its ability to survive on a range of carbon substrates. Perhaps organisms in cold temperature environments require extensive flexibility based on

the intermittent nature of available energy sources in these extreme environments.

It has been proposed that as glacial carbon enters aquatic ecosystems, microbial metabolism will be stimulated (Hood et al. 2009; Singer et al. 2012). The Cotton Glacier stream is an expansive hydrologic feature (Foreman et al. 2013), where meltwater transported across the glacial surface eventually flushes into the Ross Sea. Discharge of glacial DOM and its biological implications will have heightened ecological relevance as we face a warming climate with increasing glacial loss. This study is the first to propose that although glacially derived DOM is compositionally labile in nature, lability alone may not be the best predictor of downstream ecosystem effects. Rather, lability and compositional heterogeneity combined may more accurately predict the response of microorganisms to the released material and its long-term impacts on downstream ecosystems. These findings argue for a re-evaluation of the biological implications of glacially released carbon on nearby ecosystems. To date, the majority of the glacial OC research conducted has been in Arctic regions, while these areas are undergoing rapid warming leading to increased carbon loss, it is important to recognize that the Antarctic Ice Sheet contains  $\sim 93\%$  of glacially stored OC (Hood et al. 2015). Based on the sheer amount of carbon in Antarctic ecosystems more information is needed about the chemical composition and biological response to these sources of glacial OC. This information will enable more accurate predictions about the role of glacial DOM in stimulating microbial metabolism of aquatic environments.

Our ability to capture the dynamic nature of DOM processing was made possible by a combination of methods. In particular, UPLC-Q-TOF MS is well suited for high throughput studies of DOM processing, and can also be used to measure the internal metabolic response of organisms. Internal metabolite response will allow for the reconstruction of the specific pathways involved in the processing of complex and environmentally relevant DOM sources. When evaluating the impact of carbon release from glaciers, it is important to survey not only the quantity, but also the shifts in composition and chemical heterogeneity over time to allow for more accurate predictions about the role of DOM sources in the microbial loop, DOM transport, and ultimately  $\text{CO}_2$  release to the atmosphere.

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