Ancient Low-Molecular Weight Organic Acids in Permafrost Fuel Carbon Dioxide Upon Thaw

Travis William Drake
University of Colorado Boulder, travis.drake@colorado.edu

Follow this and additional works at: https://scholar.colorado.edu/envs_gradetds

Part of the Environmental Sciences Commons

Recommended Citation
https://scholar.colorado.edu/envs_gradetds/1

This Thesis is brought to you for free and open access by Environmental Studies at CU Scholar. It has been accepted for inclusion in Environmental Studies Graduate Theses & Dissertations by an authorized administrator of CU Scholar. For more information, please contact cuscholaradmin@colorado.edu.
ANCIENT LOW-MOLECULAR WEIGHT ORGANIC ACIDS
IN PERMAFROST FUEL CARBON DIOXIDE
PRODUCTION UPON THAW

by

TRAVIS WILLIAM DRAKE

B.A. Carleton College, 2010

A thesis submitted to the
Faculty of the Graduate School of the
University of Colorado in partial fulfillment
of the requirement for the degree of
Master of Science

Environmental Studies Program

2014
This thesis entitled:
Ancient Low-Molecular Weight Organic Acids in Permafrost Fuel Carbon Dioxide Upon Thaw
written by Travis William Drake
has been approved for the Program of Environmental Studies

________________________________________
Diane M. McKnight

________________________________________
Kimberly P. Wickland

Date ________________

The final copy of this thesis has been examined by the signatories, and we
find that both the content and the form meet acceptable presentation standards
of scholarly work in the above mentioned discipline.
Drake, Travis William (M.A., Environmental Studies)

Ancient Low-Molecular Weight Organic Acids in Permafrost Fuel Carbon Dioxide Upon Thaw
Thesis directed by Professor Diane M. McKnight

Northern permafrost soils contain an estimated 1,672 Pg of carbon (C), nearly twice that of the present atmosphere. Current and projected amplification of polar warming threatens to destabilize and thaw these frozen, organic carbon (OC) rich soils. Upon thaw, mobilized permafrost OC can enter streams and rivers, which are now recognized as important processors of terrestrial organic matter and conduits for carbon dioxide (CO₂) to the atmosphere. In this study, a series of biodegradation experiments sampled at high temporal resolution were used to assess the quality and mineralization of permafrost C in a simulated aquatic setting. Over a 200 hour incubation period, dissolved organic carbon (DOC) concentrations decreased by an average of 53%, whereas aqueous dissolved inorganic carbon (DIC) concentrations increased by an average of 660%. Ion Chromatography revealed that 87% of the DOC lost were low molecular weight organic acids, acetate and butyrate. Specific UV Absorbance at 254 nm increased over the incubation period, corresponding to the loss of these low-molecular weight organic molecules. This finding is corroborated by the simultaneous loss of fluorescence signatures attributed to low-molecular weight phenols. Collectively, these results are among the highest biolability values reported for permafrost C and are the first to directly link CO₂ production with low-molecular weight DOC loss. The high biolability and rapid respiration documented in these experiments suggests that permafrost C is quickly metabolized upon thaw and outgassed to the atmosphere as CO₂ in upstream ecosystems, either during transit through soils or within headwater streams. This finding may help to account for both the elevated levels of CO₂ in Arctic headwater streams and the lack of an aged isotopic signal indicative of the mobilization of permafrost to the mouths of higher order Arctic rivers.
CONTENTS

SECTION

I. INTRODUCTION ................................................................................................................. 1

II. METHODS .......................................................................................................................... 5
   1 Sampling Location ............................................................................................................ 5
   2 Leachate Preparation ....................................................................................................... 5
   3 Experimental Setup ......................................................................................................... 6
   4 Analyses .......................................................................................................................... 8
      2.4.1 UV-Visible Absorption .......................................................................................... 8
      2.4.2 DOC Analyses ........................................................................................................ 9
      2.4.3 DOC Fluorescence ............................................................................................... 9
      2.4.4 CO₂ Production .................................................................................................... 10
      2.4.5 pH, Temperature, and Dissolved Oxygen ............................................................. 10
      2.4.6 Ion Chromatography and Nitrogen....................................................................... 10
      2.4.7 Carbon Balance .................................................................................................... 11
      2.4.8 Visual MintEQ pH Analysis .................................................................................. 12

III. RESULTS ......................................................................................................................... 13
   1 DOC Age and Yield ......................................................................................................... 13
   2 pH, Dissolved Oxygen, and Temperature ..................................................................... 13
   3 DOC Loss Rates and Biolability ................................................................................... 16
   4 DIC Production ............................................................................................................... 18
   5 Absorbance and EEMs .................................................................................................. 18
   6 Acetate, Butyrate, and Nitrogen ................................................................................... 20
   7 Carbon Balance ............................................................................................................. 20

IV. DISCUSSION ...................................................................................................................... 22
   1 DOC Biolability ............................................................................................................. 22
2 Permafrost Microbes..................................................................................................................23
3 CO₂ Production and Outgassing......................................................................................................24
4 Secondary Processing of Permafrost OC..........................................................................................25
5 Upstream Processing and Conceptual Model .....................................................................................25
6 Acetate, Butyrate, and DOC Loss Rates..........................................................................................27
7 Optical Measurements and Remaining Labile DOC Pool.................................................................28

V. CONCLUSION ................................................................................................................................29

VI. REFERENCES ................................................................................................................................30
TABLES

Table

1  Dissolved Organic Carbon, Acetate, Butyrate, Nitrogen, and pH .......................... 14
2  Dissolved Organic Carbon Biolability over 204 hour incubation.......................... 17

FIGURES

Figure

1  Experimental setup with stainless steel chamber and flow-through system ............... 7
2  pH, Dissolved Organic Carbon, Dissolved Inorganic Carbon, and Specific UV Absorbance at 254nm over 204 hour incubation .............................................. 15
3  Excitation-Emission Matrix plots at 0, 70, and 204 hours.................................... 19
4  Conceptual diagram for the mobilization and mineralization of permafrost-derived DOC in upland ecosystems and headwater streams .................................................... 26
I. INTRODUCTION

Ongoing amplification of polar warming threatens to destabilize and thaw northern permafrost soils, which are estimated to contain 1,672 Pg of carbon (C) (Tarnocai et al. 2009, Schuur et al 2008, Grosse et al. 2011). Recent simulations estimate that by 2100, active layer thaw will release between 68 and 508 Pg of organic carbon (OC) (MacDougall et al. 2012). These estimates may be revised upward with the inclusion of additional mechanisms for OC release such as fire degradation, erosion, talik formation, and thermokarst processes (Vonk and Gustafsson 2013). Areas of discontinuous permafrost, including large swaths of interior Alaska, may be particularly vulnerable to climate-induced destabilization as a result of higher thermal insulation from a deep winter snowpack (Smith and Riseborough 1983), more ice-free days, and warmer winters (Hinzman et al. 2005).

The recent Intergovernmental Panel on Climate Change (IPCC) report states that it is virtually certain that the permafrost will experience thawing over the coming centuries but that there is low confidence as to the magnitude of frozen carbon losses (IPCC: AR5 2013). Many key processes relevant to the decomposition of terrestrial permafrost carbon are missing in the most recent global climate models, despite the large amount of C stored in these ecosystems and their vulnerability to rising temperatures (IPCC: AR5 2013). It is therefore a crucial task for the scientific community to constrain and develop an estimate of C emissions resulting from permafrost thaw and decomposition that can be incorporated into the latest climate models.

Generally, it is assumed that the majority of thawed OC from the permafrost will be microbially decomposed, converted to carbon dioxide (CO\textsubscript{2}) or methane (CH\textsubscript{4}), and contribute to further
greenhouse warming in what is known as the Permafrost-Climate Feedback (PCF). The exact mechanisms and locations of this conversion, however, remain elusive. The decomposition rate of permafrost OC depends on the quality of the thawed organic matter and the environmental conditions into which it is released (Schuur et al. 2008). Although recent studies have demonstrated high biodegradability of permafrost OC upon thaw (Dutta et al. 2006, Vonk et al. 2013), they have not characterized the biolabile fraction of permafrost OC nor provided insight as to where this decomposition might occur on the landscape. As a result, it remains unclear how the presumed gaseous by-products of decomposition (CO₂ and CH₄) might ultimately end up in the atmosphere.

Recently, a number of studies have illuminated the role of water-soluble organic carbon in respiration from arctic soils (Roehm et al. 2009, Michaelson et al. 1996, Dia et al. 2000, Michaelson and Ping 2003). These findings suggest that permafrost thaw releases DOC (Dissolved Organic Carbon), along with either free or OC-bound inorganic nutrients, into recipient aquatic ecosystems where they can stimulate or fuel microbial metabolism (Roehm et al. 2009).

Dissolved organic matter derived from terrestrial soils generally constitutes a diverse mixture of compounds comprised largely of humic substances, but also amino acids, carboxylic acids, and simple carbohydrates (Roehm et al. 2009, Fellman et al. 2008). Historically, this terrestrially exported carbon was considered to be largely recalcitrant (Geller 1986) but recent studies have shown that it can be readily bioavailable (Volk et al. 1997, Fellman et al. 2008). To complicate matters, the labile fraction of exported DOC often exhibits a fast rate of turnover (Søndergaard et al 2000) and is therefore missed by bioavailability measurements made on the remaining bulk
fraction (Roehm et al. 2009). This rapidly metabolized labile fraction is likely to be comprised of low-molecular weight (LMW) organic acids (Berggren et al. 2010) which can be readily assimilated by bacteria (Tranvik and Jorgensen 1995, Bertilsson and Tranvik 1998, Rosenstock and Simon 2001) and are associated with high a bacterial growth efficiency (BGE).

On the aquatic side, inland waters (rivers, streams, lakes) have gained attention as important processors of terrestrial organic matter and significant sources of CO$_2$ to the atmosphere (Aufdenkampe et al. 2011, Battin et al. 2008, Raymond et al. 2013). Although a few studies have demonstrated the mobilization of thawed permafrost C into lakes and wetlands (Berggren et al 2010), a definitive permafrost signal (DOC depleted in 14-C) has not been found at the mouths of large Arctic rivers (Raymond et al. 2007, Neff et al. 2006). A likely explanation for this disparity is upstream processing and evasion of CO$_2$ from the soil matrix, porewaters, and headwaters, which are generally supersaturated with CO$_2$ and considered important yet overlooked sites for the respiration of organic matter (Benstead et al. 2012). If highly biodegradable LMW C is leached from thawed permafrost soils, then it may be mineralized in upland ecosystems and small streams long before reaching larger rivers. Indeed, the rate of export of OC from the permafrost along with the degree of biodegradation that occurs within the soil matrix or porewaters prior to export will directly influence the quality of OC received by aquatic ecosystems (Roehm et al 2009).

Through the use of a simulated aquatic setting, this study aims to assess the biodegradability of permafrost derived DOC and characterize the biolabile fraction of DOC in order to gauge the response of permafrost soil organic carbon (SOC) to warming. A more detailed picture of the fate of permafrost derived DOC as well as the overall rate of DOC loss will inform future studies
as to the probable location on the landscape where permafrost C is lost to the atmosphere and thereby contributing to the Permafrost-Climate Feedback (PCF).
II. METHODS

2.1. Sampling Location

Frozen permafrost soils were collected on July 6th, 2013 from The Permafrost Tunnel in Fox, Alaska, which is operated by the U.S. Army Engineer Research and Development Center’s Cold Regions Research and Engineering Laboratory (CRREL). The samples were collected from soil that was set aside and covered after recent work on a new wing of the tunnel. These homogenized soils represent a range of depths from 5 to 15 meters. Across this range of depths, permafrost in Fox, AK is mostly uniform in composition and isolated from surface thaw conditions.

Permafrost at the Fox tunnel site is comprised of fossiliferous silt and alluvium deposited by eolian processes in the Pleistocene (Hamilton 1988). These loess sediments are characterized by high organic carbon (OC) content and a variety of ice formations. Samples for this study were collected from relatively ice-free soils. All soil samples were packaged into airtight zip-lock bags, shipped frozen to Boulder, CO, and stored in a freezer prior to experimentation.

2.2. Leachate Preparation

For each experiment, 600 g of frozen permafrost soil were leached into 2.8 L of deionized water for 24 hours at 2°C. Over the 24 hours, the slurry was agitated regularly to ensure full dissolution of the frozen soil. After 24 hours, a 40 mL sample of raw water was collected and filtered with a 1.6 μm pre-combusted glass fiber filter for use as the inoculant. The remainder of the leachate was decanted through a sieve and centrifuged at 8000 RPMs for 25 minutes to remove fine particulates and suspended clays. 2 L of the centrifuged leachate was then filtered through a 0.45 μm capsule filter and returned to the refrigerator until the beginning of the
2.3. Experimental Setup

The experimental setup utilized a gas-tight, 12 L, stainless steel chamber with inlet and outlet ports (Figure 1). A masterflex peristaltic pump drew water from the chamber and pumped it through a gas-tight exterior flow-through system at a rate of 1.1 L min$^{-1}$ to ensure continuous internal mixing (turnover time = 10 mins). The flow-through system was equipped with a YSI Professional Plus Multimeter, a WET Labs WETStar FDOM probe, a Liqui-Cel MiniModule membrane gas equilibrator connected to an PP Systems EGM-4 CO$_2$ Analyzer, and a three-way valve for taking inline discrete water samples (Figure 1).
Figure 1. Experimental setup with stainless steel chamber and flow-through system.
To initiate an experiment, the 2 L of leachate was added to the stainless steel chamber, diluted to 11 L with DI water, inoculated with the 40 mL 1.6 μm filtered raw sample, and mixed vigorously for 1 minute. Initial water samples (t0) were then taken before sealing the chamber and connecting the flow-through system. 50 mL water samples were taken at 1, 3, 6, and 12 hours followed by every 12 hours for a total of 204 hours. For each water sample taken from the chamber, 50 mL of CO₂ free air was returned to maintain constant pressure. Data from the CO₂ Analyzer, YSI, and the FDOM probe were collected continuously for the duration of the experiment.

2.4. Analyses

All analyses were conducted at the U.S.G.S. National Research Project laboratories located in Boulder, Colorado.

2.4.1. UV-Visible Absorption Analyses

For each timepoint, a 25 ml water sample was collected via the discrete sampling port and analyzed immediately using a Hewlett-Packard Model 8453 photo-diode array spectrophotometer (wavelength = 200-800 nm) and a 1-cm path-length quartz cell. The instrument was blanked using DI water prior to analyses. Results are reported for absorption at wavelength 254 nm, which is the wavelength associated with aromatic compounds (Chin et al. 1994). Specific UV absorbance (SUVA) of DOC, a measure of DOC aromaticity, was calculated by dividing UV-Visible absorbance at wavelength 254 nm by DOC concentration (Chin et al. 1994, Weishaar et al. 2003). SUVA is reported in L mg C⁻¹ m⁻¹.
2.4.2. **DOC Analyses**

For each timepoint, an additional 25 ml sample was collected via the discrete sampling port, acidified with 30 uL of HPO₄⁻, and stored in a refrigerator to await analysis. At the conclusion of each experiment, all DOC samples were analyzed on a O.I. 700 TOC Analyzer via the platinum catalyzed persulfate wet oxidation method (Aiken et al. 1992). Each run was calibrated using 5 standards that spanned the range of sample concentrations (0 - 30 mg C L⁻¹).

2.4.3. **DOC Fluorescence**

Three-dimensional fluorescence Excitation-Emission Matrices (EEMs) were generated for t0, t70, and t200 timepoints using unacidified water extracted via the discrete sampling port and run on a Jobin-Yvon Horiba Fluoromax 3 fluorometer. EEMs were collected over an excitation range of 240-450 nm every 5 nm, and an emission range of 300-600 nm every 2 nm. DI blank EEMs were subtracted from sample EEMs, instrument-specific excitation and emissions corrections were applied, and then sample EEMs were normalized using the area under the Raman scatter peak (350 nm excitation wavelength) from the DI blank. Sample EEMs were then corrected for inner filter effects using the UV-Visible absorbance spectra. Resulting corrected EEMs were plotted using Matlab with 30 contour lines. EEM subtractions (t0 minus t70 and t0 minus t200) were performed in Matlab to isolate the areas that changed in the t70 and t200 time points relative to the initial t0 EEM.

The fluorescence index (FI), a ratio of the intensities at excitation 370/emission 470 and excitation 370/emission 520, was determined from each EEM. FI can help determine whether the fluorescing aquatic fulvic acids are microbially-derived (FI = 1.7-2.0) or terrestrially-derived (FI < 1.4) (McKnight et al. 2001, Cory and McKnight 2005).
2.4.4.  CO₂ Production

Continuous in-situ dissolved CO₂ concentrations were measured using a Liqui-Cel MiniModule Membrane gas Equilibrator connected to a PP Systems EGM-4 Infrared CO₂ Analyzer (Figure 1). The EGM-4 analyzer was calibrated using a single-point 1969 ppm standard prior to the experiment. Color-indicating Silica Gel was used as a desiccant inline between the gas equilibrator and the EGM-4 to reduce the amount of moisture transported into the instrument. Dissolved Inorganic Carbon (DIC) concentrations were calculated using temperature-corrected carbon equilibrium constants and in-situ pH measurements from the YSI multimeter.

2.4.5.  pH, Temperature, and Dissolved Oxygen

In-situ pH, temperature, and dissolved oxygen (DO) were measured using a YSI Professional Plus multimeter connected inline via a YSI flow-through unit (Figure 1). Prior to each experiment, the YSI multimeter was calibrated for pH using a standard 3 point calibration and DO using 100% saturation values for the elevation of Boulder, Colorado. DO values are presented in mg O L⁻¹.

2.4.6.  Ion Chromatography and Nitrogen

At the start and end of each experiment, a 15 mL sample was collected and frozen. After all experiments had been run, all 10 frozen samples were allowed to thaw at room temperature and were immediately analyzed for acetate and butyrate concentrations on a Dionex DX-600 Ion Chromatograph. The instrument was calibrated using five standards for both acetate and butyrate that spanned the range of sample concentrations (0 - 20 mg L⁻¹). All acetate and butyrate values were carbon-normalized and presented as μmol C L⁻¹.
Additionally, 20 mL and 40 mL initial and final samples were collected, acidified with H3PO4 and H2SO4-, and stored in a refrigerator to await analysis. Upon completion of all five experiments, these samples were analyzed for Total Dissolved Nitrogen (TDN) and Ammonium (NH4+). TDN concentrations were measured on a Skalar Model FormacsTN Total Nitrogen Analyzer equipped with a ND25 Total Nitrogen detector using chemiluminescence detection. Samples were combusted at 900°C in a reactor tube containing cobalt/chromium and cerium oxide catalysts. NH4+ concentration was measured on a Thermo Scientific Dionex Model ICS-5000 ion chromatograph equipped with conductivity detection, CS12A and CG12A columns, and methanesulfonic acid (MSA) mobile phase

2.4.7. **Carbon Balance**

Carbon balance calculations were undertaken in order to track and account for the amount of C lost as DOC. In the closed incubation system, C can exist in 2 main reservoirs: dissolved in the water and as a gas in the headspace. In the aqueous phase, DOC is the only source of OC. Initial and final DOC concentrations and the volume of water provide the total amount of mineralized OC. As dissolved inorganic C (DIC) is produced via mineralization of DOC, it can exist as aqueous CO2, deprotonate to bicarbonate (HCO3-), and escape to the headspace as gaseous CO2. All of these species and concentrations can be derived from the CO2 concentration measured in the aqueous phase by the EGM-4, the pH, and the volumes of both the water and the headspace. In addition to DIC, particulate organic carbon (POC) is another byproduct of DOC mineralization and bacterial assimilation. POC concentrations were assumed to be zero at the beginning of each experiment. At the end of the experiments, final POC concentrations were ascertained by scraping the sides of the chamber to resuspend microbial biomass, vigorously shaking the
chamber, filtering the water through pre-weighed 0.45 μm pre-combusted glass fiber filters, and re-weighing the filters after they had been allowed to dry. The change in filter weight was attributed to the increase in POC captured by the filter assuming that roughly 50% of the bacterial biomass was C (Bratback and Dundas 1984). Given that it was not feasible to resuspend the microbial biomass that accumulated along the flow-through tubing and instrumentation, these POC methods likely yielded conservative POC concentrations.

2.4.8. Visual MintEQ pH Analysis

Visual MintEQ chemical equilibration software was used to verify and account for the increase in pH trends observed in the incubations. All measured inorganic and organic constituents were entered with their initial concentrations (T0). Initial SUVA values were used to bind the concentrations of humic DOC. Chloride concentrations were adjusted proportionate to acetate and butyrate concentrations to balance the charge between anions and cations. Initial runs were made with a fixed initial pH (measured) and iteratively adjusted for chloride until charges were balanced. After a series of iterations, initial runs were rerun with a calculated pH that resulted in the same pH as was measured at T0. Next, the concentrations of acetate and butyrate were reduced to zero (T200), DIC concentrations were adjusted to their final concentrations (T200), and any other measured cation or anion concentration changes measured at T200 were made. Running Visual MintEQ under these new conditions and allowing it to calculate pH resulted in a very similar pH as was measured at T200. This exercise demonstrated that the increasing pH trends observed in this study were a result of the loss of organic acids and subsequent production of DIC.
III. RESULTS

3.1. DOC Age and Yield

The average age of the carbon from the permafrost soil was 35,800 y.b.p. (Rob Spencer, PC). The permafrost leachates yielded an average of $38.40 \pm 3.69$ μmol C as DOC per gram soil. The average C content of the permafrost soil was 1.36% by weight (Rob Spencer, personal communication) and thus the average DOC yield per g C was 3.39%. Final incubated leachate after dilution had an average DOC concentration of $18.24 \pm 2.17$ mg L$^{-1}$.

3.2. pH, Dissolved Oxygen, and Temperature

The pH increased by an average of 0.49 pH units over the course of the 200 hour incubation (Table 1, Figure 2a). This range, from 6.45 to 6.94, spans the equilibrium pKa for carbonic acid (CO$_2$) and bicarbonate (HCO$_3^-$) for the laboratory temperature of 21º C and atmospheric pressure in Boulder, CO (pKa = 6.37; Stumm and Morgan 1996). This means that below a pH of 6.37, carbonic acid (CO$_2$) will be the dominant carbonate species whereas above 6.37, bicarbonate (HCO$_3^-$) will dominate. In general, dissolved oxygen levels decreased slightly over the course of the experiments but the closed incubation systems remained oxic for the entire 200 hours (DO > 4 mg/L). The temperature of each incubation started slightly below laboratory temperature (18º C) owing to the 2 L of refrigerated leachate used to make the final incubated volume. After 3 hours, the incubations equilibrated to the laboratory temperature (21º C) and did not fluctuate more than half a degree for the remainder of the incubation period.
<table>
<thead>
<tr>
<th>Experiment</th>
<th>DOC</th>
<th>Acetate</th>
<th>Butyrate</th>
<th>Ammonium</th>
<th>TDN</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>200</td>
<td>0</td>
<td>200</td>
<td>0</td>
<td>200</td>
</tr>
<tr>
<td>PD1</td>
<td>1629.17</td>
<td>753.33</td>
<td>389.69</td>
<td>56.6</td>
<td>366</td>
<td>65.7</td>
</tr>
<tr>
<td>PD2</td>
<td>1500.00</td>
<td>626.67</td>
<td>542.98</td>
<td>140.5</td>
<td>135</td>
<td>118</td>
</tr>
<tr>
<td>PD3</td>
<td>1306.67</td>
<td>300.00</td>
<td>435.95</td>
<td>131.1</td>
<td>146</td>
<td>112</td>
</tr>
<tr>
<td>PD4</td>
<td>1768.33</td>
<td>812.50</td>
<td>432.14</td>
<td>119.4</td>
<td>156</td>
<td>120</td>
</tr>
<tr>
<td>PD5</td>
<td>1379.17</td>
<td>732.5</td>
<td>381.51</td>
<td>114.5</td>
<td>151</td>
<td>114</td>
</tr>
<tr>
<td>Mean</td>
<td>1520.67</td>
<td>705.00</td>
<td>412.85</td>
<td>127.5</td>
<td>369</td>
<td>31.4</td>
</tr>
</tbody>
</table>

Data other than pH are reported in umol L⁻¹ (% change). Acetate and Butyrate data are reported in umol-Cl⁻¹. Average values reported as mean ± s.d. (mean % change), in umol L⁻¹.

**Table 1.** Dissolved Organic Carbon, Acetate, Butyrate, Nitrogen, and pH
Figure 2. pH, Dissolved Organic Carbon, Dissolved Inorganic Carbon, and Specific UV Absorbance at 254 nm over 204 hour incubation
3.3. **DOC Loss Rates and Biolability**

Over the course of the 200 hour incubation period, DOC concentrations decreased by an average of 53.5 ± 4.1% (Table 1). Generally, DOC levels started to decrease after 24 hours and leveled off around 150 hours (Figure 2b). The average consumption rate among all 5 replications was 1.373 ± 0.15 mg C L$^{-1}$ d$^{-1}$ (Table 2). Consumption rates were generally low for the first 24 hours, and tended to stabilize between 24 and 96 hours (Table 2). PD1 and PD2 displayed a slightly faster loss and leveling out of DOC than PD3, PD4 and PD5 (Table 2).
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Time (h)</th>
<th>Consumption Rate (mg L⁻¹ d⁻¹)</th>
<th>Consumed per Time Step (%)</th>
<th>Consumed per hour (%)</th>
<th>Total Cumulative Consumed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD1</td>
<td>0-24</td>
<td>1.015</td>
<td>6.52</td>
<td>0.272</td>
<td>6.52</td>
</tr>
<tr>
<td></td>
<td>24-96</td>
<td>2.883</td>
<td>45.69</td>
<td>0.635</td>
<td>49.24</td>
</tr>
<tr>
<td></td>
<td>96-204</td>
<td>0.201</td>
<td>8.86</td>
<td>0.082</td>
<td>53.73</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td><strong>1.461</strong></td>
<td></td>
<td><strong>0.263</strong></td>
<td></td>
</tr>
<tr>
<td>PD2</td>
<td>0-24</td>
<td>0.894</td>
<td>3.3</td>
<td>0.138</td>
<td>3.30</td>
</tr>
<tr>
<td></td>
<td>24-96</td>
<td>2.778</td>
<td>47.13</td>
<td>0.655</td>
<td>48.87</td>
</tr>
<tr>
<td></td>
<td>96-204</td>
<td>0.283</td>
<td>18.31</td>
<td>0.170</td>
<td>58.24</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td><strong>1.491</strong></td>
<td></td>
<td><strong>0.285</strong></td>
<td></td>
</tr>
<tr>
<td>PD3</td>
<td>0-24</td>
<td>0.212</td>
<td>0.15</td>
<td>0.006</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>24-96</td>
<td>1.270</td>
<td>24.01</td>
<td>0.333</td>
<td>24.12</td>
</tr>
<tr>
<td></td>
<td>96-204</td>
<td>1.179</td>
<td>40.36</td>
<td>0.374</td>
<td>54.74</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td><strong>1.174</strong></td>
<td></td>
<td><strong>0.268</strong></td>
<td></td>
</tr>
<tr>
<td>PD4</td>
<td>0-24</td>
<td>0.968</td>
<td>3.41</td>
<td>0.142</td>
<td>3.41</td>
</tr>
<tr>
<td></td>
<td>24-96</td>
<td>1.815</td>
<td>25.18</td>
<td>0.350</td>
<td>27.73</td>
</tr>
<tr>
<td></td>
<td>96-204</td>
<td>1.177</td>
<td>36.42</td>
<td>0.337</td>
<td>54.06</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td><strong>1.496</strong></td>
<td></td>
<td><strong>0.265</strong></td>
<td></td>
</tr>
<tr>
<td>PD5</td>
<td>0-24</td>
<td>0.039</td>
<td>0.58</td>
<td>0.024</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>24-96</td>
<td>1.091</td>
<td>16.53</td>
<td>0.230</td>
<td>17.02</td>
</tr>
<tr>
<td></td>
<td>96-204</td>
<td>1.415</td>
<td>40.24</td>
<td>0.373</td>
<td>50.42</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td><strong>1.245</strong></td>
<td></td>
<td><strong>0.247</strong></td>
<td></td>
</tr>
</tbody>
</table>

*Table 2.* Dissolved Organic Carbon biolability over 204 hour incubation
3.4. DIC Production

Calculated DIC concentrations increased by an average of 660% and generally corresponded to the change in DOC concentrations (Figure 2c). While CO₂ consistently rose throughout the experiment, the majority was assumed to be deprotonated instantly and converted into bicarbonate (HCO₃⁻) given the pH after the start of the experiments. Carbon-normalized DIC production accounted for an average of 50% of the DOC lost. As with the DOC, DIC levels for PD1 and PD2 displayed a sharper increase relative to the other replicates.

3.5. Absorbance and EEMs

For all experiments, average UV absorbance at 254 nm did not change significantly over the 200 hour incubation period. Average specific UV absorbance at 254 nm (SUVA 254) increased from 0.58 to 1.29 over the 200 hours (Figure 2d). This increase in SUVA 254 is driven by the 53.5% loss in DOC and not a change in UV absorbance at 254. For each experiment, EEM plots and subtractions revealed a loss of a low-molecular weight peak at emission 310 / excitation 375 (Figure 3). FI values (average = 1.59) did not change significantly over the course of the incubations. In-situ measurements taken with the FDOM sensor at wavelength ex370/em460 did not change significantly over the course of the experiments.
Figure 3. Excitation-Emission Matrices (EEMs) at 0 (A), 70 (B), and 200 (C) hours. Panels (D) and (E) are subtraction EEMs 70-0 and 200-0 respectively. Note the loss of the peak at ex275/em310.
3.6. Acetate, Butyrate, and Nitrogen

Over the course of the 200 hour incubations, acetate and butyrate concentrations dropped to nearly zero (98.4 and 98.9% loss respectively, Table 1). Average initial acetate concentrations were 305 μmol L\(^{-1}\) and comprised 20% of initial DOC and 37% of the DOC lost over the 200 hour incubation period (Table 1). Average initial butyrate concentrations were 413 μmol L\(^{-1}\) and comprised 27% of initial DOC and 50% of the DOC lost over the 200 hour incubation period (Table 1). Collectively, consumption of acetate and butyrate accounted for the vast majority (87%) of the DOC lost.

Ammonium concentrations dropped by an average of 53.4% over 200 hours whereas TDN concentrations decreased by an average of 26.5% (Table 1).

3.7. Carbon Balance

Over 90% of the C lost as DOC was accounted for by DIC and POC production in the incubation chamber. Within the closed incubation system, DIC production resulted from bacterial respiration (BR) of biolabile DOC. On average, DIC production accounted for 50% of the DOC lost. The majority of the remaining DOC lost was accounted for by bacterial production (BP), i.e. the growth of bacterial biomass. On average, POC production accounted for 40% of the DOC lost. Assuming that all of the DOC lost was due to bacterial assimilation (A, BP + BR), the bacterial growth efficiencies (BGE, BP / A) for the incubations were estimated to be 30-40%, which correspond closely to values measured in freshwater ecosystems (Cole et al. 1988, Del Giorgio and Cole 1998). The remaining 10% of DOC unaccounted for by DIC or POC production is likely due to the conservative methods used to gauge POC concentrations (i.e. unmeasured bacterial biomass growth along the flow-through pathway and instrumentation) or
the combined error in the measurement of the various organic and inorganic C species. Nevertheless, the high recovery of lost DOC ensures that there were no large C reservoirs or consumption pathways that were unaccounted for in these experiments.
IV. DISCUSSION

4.1. DOC Biolability

DOC yields from permafrost soils from the Permafrost Tunnel in Fox, AK were consistent with other permafrost studies (Wickland, *personal communication*). The 35,800 y.b.p. average age is among the oldest measured for permafrost soils to be examined for DOC biolability and characterization (Vonk et al. 2013, Dutta et al. 2006). DOC loss over the incubation period was attributed entirely to biological uptake given the lack of alternative consumptive processes in the closed system. DOC biolability (50% loss) measured in these incubations is the highest recorded value for permafrost soils and suggests that older soils do not necessarily yield more recalcitrant material than younger soils (Schmidt et al. 2011, Marín-Spiotta et al. 2014). Furthermore, results from this study showed permafrost DOC is respired relatively rapidly (~100 hours), compared to most biolability studies (~30 days). It is inherently difficult to compare DOC decomposition rates across studies that employ different methods given the fact that different setups (bioreactor columns, bottle bioassays, or large chamber bioassays) generate different physio-chemical conditions. Nevertheless, the average DOC consumption rate (1.373 ± .15 mg C L\(^{-1}\) d\(^{-1}\)) observed in this study was both high relative to other studies (Roehm et al 2009, Kalbitz et al. 2003, Wickland et al. 2007) and relatively stable across experiments (stdev = .15 mg C L\(^{-1}\) d\(^{-1}\)). Consumption rates varied across time periods (0-24, 24-96, 96-204 hours) from .039 to 2.883 mg C L\(^{-1}\) d\(^{-1}\). The lowest rates were universally observed in the first 24 hours, which is unsurprising given the relatively small inoculation volumes used, growth and reproduction lag time of the bacteria, and the lower temperatures at the beginning of the experiments. Variability in consumption rates across the different time steps was attributed to different starting conditions in the bacterial community present in the inoculant, either in number or variety.
4.2. **Permafrost Microbes**

The inoculum for each incubation consisted of only microbes indigenous to the frozen soils themselves. The high DOC loss with the isolated inoculum indicates that dormant microbes exist within permafrost soils, are reactivated upon thaw, and are immediately able to decompose permafrost OC. There is not much known about the metabolic potential of these dormant microbes, and very few studies have explored the temperature-dependent activities of enzymes used to degrade permafrost C (Graham et al. 2012, Mann et al. 2013). RNA gene sequencing has shown an abundance of acetotrophic methanogen phylotypes (*Methanosarcina*) in Arctic soils that obtain energy by converting acetate to methane and carbon dioxide (Wagner and Liebner 2010). If the same genus of bacteria is responsible for the mineralization of low molecular-weight organic acids in these experiments, then permafrost thaw and OC mobilization under anoxic conditions would likely produce methane, a more potent greenhouse gas. Regardless, the results of this study suggest that upon thaw, permafrost C need not be transported into new ecosystems in order to undergo microbial decomposition. However, subsequent transport into new ecosystems (porewaters and headwaters) upon thaw may or may not lead to more rapid and complete biodegradation of permafrost C given the diversity and abundance of heterotrophic microbes found in such recipient ecosystems (Judd et al. 2006, Zak and Kling 2006). Mobilization pathways may in fact subject permafrost C to a cascading set of microbial communities and physico-chemical parameters (Marín-Spiotta et al. 2014, Schmidt et al. 2011), which may be specifically adapted to decompose the different remnant DOC pools (Zak and Kling 2006, Vannote et al. 1980). Overall, the effect of this decompositional cascade may be that more total DOC is respired than previously assumed. If such a process occurs in freshwater ecosystems, the biolability results presented here may in fact be conservative.
4.3. CO₂ Production and Outgassing

The majority of DOC lost over the course of the incubations was mineralized to CO₂ (50%) whereas the bulk of the remaining DOC was used for biomass growth by microbes (40%). The DOC converted to CO₂ represents a potential direct source of CO₂ to the atmosphere. It is important to note that this study is the first to directly link permafrost DOC lability to CO₂ production. Given the pH range in this study, most of the CO₂ produced via DOC respiration was hydrolyzed to carbonic acid, quickly deprotonated, and converted to HCO₃⁻. In the closed incubation system, pH depends on the production of carbonic acid and the loss of organic acids. Since organic acids are stronger acids than carbonic acid, the net effect of these two processes was an increase in pH in all experiments. This pH trend was verified using Visual MintEQ chemical equilibration software and the measured concentrations of organic and inorganic constituents from the incubations. In natural waters, the relative proportion of CO₂ to HCO₃⁻ will depend on the pH, although there will be a flux to the atmosphere whenever there is supersaturation relative to CO₂. This flux or outgassing of CO₂ from natural waters depends on the concentration gradient between the water and the atmosphere, the gas exchange coefficient (k), and the surface area of the water (Butman and Raymond 2011, Raymond et al. 2013). These variables change from ecosystem to ecosystem, but it has been widely observed that natural waters are supersaturated with CO₂ and thus net sources to the atmosphere (Raymond et al. 2013, Battin et al. 2008, Aufdenkampe et al. 2011). In particular, Arctic rivers and streams exhibit high CO₂ concentrations (Denfeld et al. 2013, Kling et al. 1992). Indeed, it is possible that these high concentrations observed in Arctic streams are sustained in part by the respiration of biolabile permafrost C released during late summer thaw of the deep active layer (Frey and Smith 2005, Hinzman et al. 2005).
4.4. Secondary Processing of Permafrost OC

While not explored in this study, bacterial production of OC via assimilation of permafrost C likely represents a secondary form of ancient biolabile C. Higher-order heterotrophic grazing by flagellates and microzooplankton of this secondary OC is therefore another potential source of ancient permafrost C to the atmosphere as CO₂, assuming that these higher-order organisms are respiring the bacterial OC (Oevelen et al. 2006). In short, once assimilated into the biosphere, permafrost C is transferred up through the aquatic food web – leaking CO₂ at each trophic level via heterotrophic respiration.

4.5. Upstream Processing and Conceptual Model

Collectively, the transitional microbial communities that receive DOC, the varying physico-chemical parameters (pH, redox, temperature, etc.) of natural waters, and the secondary respiration of bacterial biomass comprised of assimilated permafrost C all exemplify the conditional nature, translocation, and cascading pattern of both permafrost decomposition and permafrost-derived CO₂ release to the atmosphere (Vonk et al. 2013). After travelling through sequential downstream ecosystems, it is likely that the majority of permafrost C is mineralized to CO₂ and outgassed long before reaching the ocean or even larger rivers. A conceptual model for the process of DOC mobilization and subsequent translocated outgassing from soils, pore waters, and streams is depicted in Figure 4.
Figure 4. Conceptual diagram for the mobilization and mineralization of permafrost-derived DOC in upland ecosystems and headwater streams. Yellow circles represent organic carbon (OC) in solid and dissolved phases. Blue circles represent porewaters and percolating precipitation.
Previous studies have suggested that warming in the Arctic increases flow paths, residence times, and microbial mineralization of DOC in the soil active layer (Striegl et al. 2005, Koch et al. 2013). Building from these findings and incorporating data from this study, this new conceptual model highlights the overall effect of these climate-driven changes to hydrologic controls, which is that DOC is respired in soils and pore waters and DIC is outgassed from upstream ecosystems. This fact poses a challenge for researchers seeking to quantify permafrost C mobilization given the general inaccessibility, heterogeneity, and widespread nature of headwater ecosystems. The fact that the terrestrial and hydrologic carbon cycles exhibit high spatiotemporal variability and that this causes problems for researchers attempting to capture spatial hotspots and hot moments of C processing is not new (McClain et al. 2003, Andrews et al. 2011, Cole et al. 2007).

4.6. Acetate, Butyrate and DOC Loss Rates

Acetate and butyrate comprised the vast majority of DOC lost (87%) over the incubation period. These low molecular-weight (LMW) organic acids have been shown to be extremely biolabile (Berggren et al. 2010), and are likely the byproducts of fermentation during the burial and subsequent anoxia of permafrost sediments 30k years ago. Recent studies have shown that these LMW compounds can be easily assimilated by bacteria (Tranvik and Jorgensen 1995, Rosenstock and Simon 2001) and are typically metabolized with high bacterial growth efficiencies (BGE > 0.4; Berggren et al. 2010). These lability patterns of LMW compounds correspond well to both the high biolability of acetate and butyrate and the high BGE observed in this study. The high biolability of these molecules and their relative abundance in the face of indigenous microbes that preferentially degrade them suggests that there is a temperature threshold below which metabolic respiration is unfavorable. This finding is somewhat contrary to recent studies that have shown activity of permafrost microorganisms at extremely low
temperatures (Rivkina et al. 2000), although it is possible that such activity proceeds at extremely slow rates.

The decrease but not exhaustion of biolable nitrogen (N) over the course of the experiment suggests that nitrogen was not a limiting nutrient. The relatively high concentrations of biolable N in these soil leachates suggests there are enough inorganic nutrients to sustain the respiration of biolable OC. In short, it is likely that the 50% DOC loss threshold observed in this study is a result of the complete consumption of biolable LMW organic acids, and not the depletion or limitation of inorganic nutrients to sustain bacterial metabolism.

4.7. Optical Measurements and Remaining Labile DOC Pool

The increase in SUVA 254 values and the loss of low molecular weight protein-like fluorescence corresponded well with acetate and butyrate consumption as the dominant change in the DOC pool. The fact that in-situ FDOM measurements at ex370/em460 did not change over the course of the experiment suggests that in-situ probes set to measure high excitation/emission wavelengths will not detect the presence of labile permafrost-derived DOC, much less any changes to it.
V. CONCLUSION

The high biolability and rapid respiration of permafrost-derived DOC demonstrated in this study suggests that the production and outgassing of greenhouse gases follow soon after thaw. The terrestrial-aquatic interface – including porewaters, groundwater, and headwaters – is a likely hotspot for this processing. Future field studies should prioritize sampling in headwater ecosystems for $^{14}$C-depleted DOC and CO$_2$. Furthermore, future iterations of these incubations could include anoxic replications to assess the methanogenic pathway as well as identification of indigenous microbial assemblages to better understand how they will respond to thaw. Respiration, fermentation, methanogenesis, and CH$_4$ oxidation may all play important roles in the production of GHGs along the terrestrial-aquatic pathway (Schurr et al. 2008). Research into the source and conditions that produce and concentrate low-molecular weight acids such as acetate and butyrate, as well as the reason for their stability at low temperatures, would also provide crucial information for predicting the responses of different permafrost-dominated areas to warming.
VI. REFERENCES


