1 **Chemical composition of dissolved organic matter draining**

2 **permafrost soils**

3 *Collin P. Ward^a and Rose M. Cory^{a*}*

4 ^aDepartment of Earth and Environmental Sciences, 2534 C.C. Little Building, 1100 North

5 University Avenue, University of Michigan, Ann Arbor, Michigan 48109, USA

6 * Corresponding author: phone: +1 734-615-3199; email: rmcory@umich.edu

Abstract

Northern circumpolar permafrost soils contain roughly twice the amount of carbon stored in the atmosphere today, but the majority of this soil organic carbon is perennially frozen. Climate warming in the Arctic is thawing permafrost soils and mobilizing previously frozen dissolved organic matter (DOM) from deeper soil layers to nearby surface waters. Previous studies have reported that ancient DOM draining deeper layers of permafrost soils was more susceptible to degradation by aquatic bacteria compared to modern DOM draining the shallow active layer of permafrost soils, and have suggested that DOM chemical composition may be an important control for the lability of DOM to bacterial degradation. However, the compositional features that distinguish DOM drained from different depths in permafrost soils are poorly characterized. Thus, the objective of this study was to characterize the chemical composition of DOM drained from different depths in permafrost soils, and relate these compositional differences to its susceptibility to biological degradation. DOM was leached from the shallow organic mat and the deeper permafrost layer of soils within the Imnavait Creek watershed on the North Slope of Alaska. DOM draining both soil layers was characterized in triplicate by coupling ultra-high resolution mass spectrometry, ^{13}C solid-state NMR, and optical spectroscopy methods with multi-variate statistical analyses. Reproducibility of replicate mass spectra was high, and compositional differences resulting from interfering species or isolation effects were significantly smaller than differences between DOM drained from each soil layer. All analyses indicated that DOM leached from the shallower organic mat contained higher molecular weight, more oxidized, and more unsaturated aromatic species compared to DOM leached from the deeper permafrost layer. Bacterial production rates and bacterial efficiencies were significantly higher for permafrost compared to organic mat DOM; however, respiration rates were similar

30 between DOM sources. Increased release of permafrost DOM from arctic soils to surface waters
31 will change the chemical composition of DOM and its lability to bacteria, but this study suggests
32 that these shifts in DOM composition and lability may not increase the carbon dioxide produced
33 by bacterial respiration of permafrost DOM exported to arctic surface waters compared to DOM
34 currently draining the shallow active layer.

1. INTRODUCTION

Northern circumpolar permafrost soils contain ~1700 Pg of organic carbon (OC; Ping et al., 2008; Tarnocai et al., 2009), roughly twice the 800 Pg-C stored in the atmosphere today. The majority of this soil OC (88% or 1466 Pg-C) is perennially frozen (Tarnocai et al., 2009). Because the climate is warming rapidly in the arctic, permafrost soils are thawing and hydrologic flow paths are deepening, leading to the mobilization of ancient dissolved organic matter (DOM) from deeper horizons to nearby surface waters. For instance, the amount of ancient OC drained from deeper permafrost layers of the Eurasian Arctic soils to surface waters has been estimated to have increased by 3 - 6% from 1985 - 2004 (Feng et al., 2013). Once flushed to surface waters, permafrost DOM can be mineralized by sunlight or bacteria and released back to the atmosphere as carbon dioxide (CO₂) where it can positively reinforce climate warming, or it can be partially degraded, buried in lake sediments, or exported to the ocean (Vonk and Gustafsson, 2013). The current estimate is that 25 - 40% of CO₂ released from arctic surface waters to the atmosphere is produced through the mineralization of DOM by sunlight in the water column (Cory et al., 2014). The remaining CO₂ released from surface waters is likely generated through bacterial respiration of DOM in the water column or in aquatic sediments, or is directly transferred from soil waters (Kling et al., 1991; Ramlal et al., 1994; Kling et al., 2014; Cory et al., 2014). Thus, understanding the controls on the lability of DOM to degradation to CO₂ by bacteria in arctic soil and surface waters is critical for understanding how much permafrost DOM will be respired to CO₂ versus exported through rivers to the ocean.

The fate of DOM in arctic surface waters may change if permafrost sources comprise a larger fraction of DOM draining arctic soils because permafrost DOM has been found to be more quickly converted to CO₂ by aquatic bacteria compared to DOM currently draining the active

layer (Mann et al., 2014; Abbott et al., 2014; Spencer et al., 2015). Given the importance of substrate composition as a control on bacterial respiration in arctic surface waters (Crump et al., 2003; Judd et al., 2006), permafrost DOM has been proposed to be more labile to aquatic bacteria due to differences in chemical composition compared with DOM draining the shallower active soil layer. For example, permafrost DOM has been consistently reported to contain less aromatic C compared with DOM draining the active layer of arctic soils (Mann et al., 2012; Cory et al., 2013; Mann et al., 2014; Abbott et al., 2014), and proxies for aromatic C content were found to be inversely correlated with the lability of DOM to bacterial respiration in receiving waters (Mann et al., 2014; Abbott et al., 2014). However, a previous study found no difference in bacterial respiration between permafrost and modern DOM when degraded by aquatic bacteria in the dark, despite large differences in aromatic C content (Cory et al., 2013). Thus, aromatic C may control bacterial respiration in some waters, but the composition of the aromatics present may be as important as the abundance of aromatic C (Sleighter et al., 2014). For example, Mann et al. (2014) proposed that increased phenolic aromatics within modern DOM inhibited aquatic bacterial respiration (Freeman et al., 2001), compared to ancient permafrost DOM containing less aromatic C. Alternatively, aromatic C content may not be a causal factor in bacterial respiration; that is, other facets of DOM composition may contribute to variability in bacterial activity, such as molecular weight or oxidation state (Meyer, 1994; Amon and Benner, 1996; Vallino et al., 1996; Sun et al., 1997). In one study of Amazon River DOM, the high molecular weight fraction was more labile to bacterial degradation than the low molecular weight fraction (Amon and Benner, 1996). In contrast, lower molecular weight species were more susceptible to biological degradation in a temperate stream than higher molecular weight species (Sleighter et al., 2014). Furthermore, multiple studies have suggested that bacterial growth rates and

efficiencies are inversely correlated to the average oxidation state of DOM (Meyer, 1994; Vallino et al., 1996; Sun et al., 1997). Given that aromatic C, molecular weight, and oxidation state of DOM have been reported to differ between permafrost and organic mat DOM in arctic soil waters (Hodgkins et al., 2014; Spencer et al., 2015; Dubinenkov et al., 2015), a more detailed view of DOM chemical composition is needed to identify the organic compounds that govern the susceptibility of DOM to aquatic bacterial degradation.

The most resolved analysis of DOM chemical composition can be achieved using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Ultra-high resolution mass spectrometry (i.e., FT-ICR MS) confirms that DOM is a heterogeneous mixture and provides the molecular composition of thousands of individual formulas that comprise the DOM pool (e.g., Stenson et al., 2003). While FT-ICR MS provides the composition (i.e., the amount of CHONSP), molecular weight, oxidation state, and compound class (e.g., aromatic vs. aliphatic) of each formula detected, it does not provide the structural arrangement of the atoms within each formula. Without structural information, it is not possible to identify the distribution of functional groups within each formula, for example, whether any oxygen atom in a formula is present as a phenol or a ketone (Hertkorn et al., 2008; Remucal et al., 2012). Thus, FT-ICR MS alone can't identify the phenolic subset of aromatic compounds hypothesized to inhibit bacterial activity in arctic surface waters (Mann et al. 2014). To address this limitation, FT-ICR MS analysis of DOM is increasingly coupled with a measure of structural or functional group distribution, such as solid-state ^{13}C nuclear magnetic resonance (^{13}C NMR; Hockaday et al., 2009; Hertkorn et al., 2013).

FT-ICR MS is also increasingly coupled with optical proxies for average DOM composition (Jaffé et al., 2012; Stubbins et al., 2014; Sleighter et al., 2014) because these optical

proxies correlate well with molecular properties of DOM and the lability of DOM to aquatic bacteria (Cory and McKnight, 2005; Spencer et al., 2008; Cory and Kaplan, 2012; Sleighter et al., 2014). For example, optical proxies based on the light-absorbing and emitting fractions of the DOM pool (CDOM and FDOM, respectively) have been correlated with aromatic C content and composition (McKnight et al., 2001; Weishaar et al., 2003; Cory and McKnight, 2005), and average molecular weight (Helms et al., 2008). In addition, recent work coupled FT-ICR MS with CDOM and FDOM analyses, finding that aromatic-rich riverine DOM supports bacterial respiration in a watershed where the dominant source of DOM is plant and soil organic matter (Sleighter et al., 2014). Another reason CDOM and FDOM are commonly used as a measure of DOM lability is because this analysis requires little sample manipulation, rendering this approach relatively free of bias introduced during pre-isolation, which is often required for DOM analysis by FT-ICR MS or ^{13}C NMR.

DOM is often isolated using solid phase extraction (SPE) prior to FT-ICR MS analysis to minimize the influence of interfering species that suppress the ionization of organic compounds (e.g., salts; King et al., 2000). Pre-isolation of DOM is required for ^{13}C NMR to remove interfering impurities (i.e., paramagnetics). Recoveries of DOM by SPE are < 100% and compounds lost during SPE extraction are likely low molecular weight, less aromatic compounds (Dittmar et al., 2008; Sleighter and Hatcher, 2008). Thus, SPE may preferentially exclude compounds important for aquatic bacterial degradation. Additionally, due to time, labor, and instrument costs associated with SPE isolation and FT-ICR MS analysis, replicate FT-ICR MS spectra have rarely been reported. Good agreement between FT-ICR MS analysis of filtered, whole water and SPE-isolated DOM have been reported for riverine DOM (e.g., Sleighter et al., 2010); however, these comparisons and analyses of reproducibility of FT-ICR MS spectra have

not been reported for DOM in soils or surface waters of the Arctic, which exhibit different ratios of DOM vs. interfering constituents compared to the riverine DOM previously studied.

The objective of this study was to resolve the compositional features that distinguish DOM draining different horizons of permafrost soils using complimentary analytical and statistical analyses. To achieve this objective we characterized the chemical composition of DOM draining the shallower organic mat and the deeper permafrost layer of Alaskan Arctic soils by coupling FT-ICR MS, ^{13}C NMR, and optical spectroscopy methods with multi-variate statistical analyses. Because we expected differences in DOM composition to influence bacterial degradation of DOM leached from the organic mat and the deeper permafrost layer, we measured bacterial production, respiration, and growth efficiency for DOM inoculated with native bacterial communities.

2. METHODS

2.1 Study site

Sample collection took place between June and July 2013 in the Imnavait Creek watershed on the North Slope of Alaska (68.62° N, 149.28° W; elevation ~ 900 m). Imnavait Creek is a 1st order tributary of the Kuparuk River, the 4th largest fluvial source of OC to the Arctic Ocean in the Alaskan Arctic (McGuire et al., 2009). The Imnavait Creek watershed was glaciated during the Pleistocene (610 - 132 k yr BP), however, paludification of these soils started approximately 11,500 ± 140 years ago (Eisner, 1991; Walker et al., 2014). Characteristics of the soil column have been previously described in detail (Hinzman et al., 1991). Briefly, the top 20 cm is comprised of live and dead organic matter mixed with glacial till. Beneath the organic horizon, the soil is primarily glacial outwash with occasional evidence of frost-churned

organic matter. The entire basin is underlain with permafrost, with average peak thaw depth, measured in August from 2003 to 2012, ranging from 38 to 52 cm (43 ± 11 cm, $N = 2890$; Kling et al., 2014). The average thaw depth at Imnavait Creek watershed during sampling in July 2013 was 19 ± 2 cm ($N = 9$).

2.2 Experimental Design

The seven-step experimental design to collect dissolved organic matter from the shallower organic mat and deeper permafrost layer of the soil is shown in Figure 1. Each of the seven steps was completed in triplicate for both layers of the soil. (1) Three adjacent 1.5 m x 1.5 m soil pits (20 m apart) were constructed ~ 100 m up the west facing hillslope of the Imnavait Creek drainage basin (Pit #1: 68.6139 N, 149.3145 W; Pit #2: 68.6138 N, 149.3145 W; Pit #3: 68.6136 N, 149.3144 W). Soil organic matter (SOM) was collected at two depths, the organic mat (5 - 15 cm) and the permafrost mineral layer (95 - 105 cm), corresponding to the annually thawed and permanently frozen soil layers, respectively. At each depth equal amounts of frozen SOM from each pit were placed into three ziplock bags, transferred to a cooler, and transported to freezers at Toolik Lake Field Research Station (68.63° N, 149.60° W). Soil from the organic mat was collected on 15-June-2013, while soil from the permafrost mineral layer was collected on 8-July-2013. (2) The frozen soils were thawed prior to leaching. 3600 g of soil from the organic mat layer or 750 g of permafrost soil was added to acid and de-ionized water (DI) rinsed five gallon HDPE buckets and leached overnight in 15 L of laboratory grade DI at room temperature. (3) The dissolved fraction of the leachate was isolated using a series of pre-cleaned nylon filter screens (50, 20, and 10 μ m; Cole-Parmer, Inc.) and pre-cleaned 5 and 0.45 μ m high-capacity cartridge filters (Geotech Environmental Equipment, Inc.). The dissolved fraction of the

soil leachates are from here on referred to as organic mat DOM and permafrost DOM. (4) Splits of DOM were taken for water chemistry (i.e., pH, conductivity), UV-visible and fluorescence spectroscopy, and high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). (5) Additional splits of DOM were concentrated and inorganic impurities were removed using solid phase extraction (SPE). (6) SPE eluate from each replicate leachate was reserved for spectroscopic and FT-ICR MS analyses. (7) Functional group distributions of pooled, freeze-dried SPE eluates were measured by solid-state ^{13}C NMR.

2.2 Whole water DOM vs. solid phase extraction DOM

In this study, whole water DOM refers to the bulk DOM in a filtered soil leachate, and solid phase extraction (SPE) DOM refers to DOM isolated using Bond Elut PPL cartridges (Agilent Technologies; Dittmar et al. 2008). Briefly, organic mat and permafrost DOM were acidified to pH 2 and loaded onto LC-MS grade methanol rinsed (MeOH; Fisher Scientific) 5-g PPL SPE cartridges. All DOC loadings were less than the 2 mmol-C L^{-1} recommended threshold to avoid breakthrough (Dittmar et al., 2008). The cartridges were flushed with 0.01 M trace metal grade HCl (Fisher Scientific), dried with ultra-high purity N_2 (Airgas), and then eluted with MeOH. Mean recovery of chromophoric DOM by the PPL solid phase, quantified as absorbance at 305 nm of the column effluent divided by the initial DOM absorbance at 305 nm, was $93 \pm 3\%$ and $88 \pm 8\%$ for organic mat and permafrost DOM, respectively. Carbon recovery by the PPL solid phase was similar between DOM sources (organic mat = $57 \pm 1\%$; permafrost = $67 \pm 7\%$; $p = 0.13$).

2.3 DOC concentration and optical characterization

Dissolved organic carbon concentration (DOC) was quantified as CO₂ after high-temperature catalytic combustion using potassium hydrogen phthalate as the calibration standard (Shimadzu Corporation; Kling et al., 2000). UV-visible absorption spectra of whole water DOM were collected using a 1-cm pathlength UV-visible spectrophotometer (Aqualog; Horiba Scientific). Absorption spectra of SPE isolated DOM were collected by spiking 400 µL of SPE eluate into 40 mL pre-ashed ampules, evaporating the MeOH with ultra-high purity N₂, reconstituting in 25 mL DI, and analyzing using a 5-cm pathlength UV-visible spectrophotometer (Cary 300; Agilent Technologies). Splits of SPE DOM reconstituted in DI were reserved for fluorescence spectroscopy and DOC analyses. Napierian absorption coefficients were calculated by multiplying absorbance (A) by 2.303 and dividing by the pathlength (m) of the quartz cuvette. Spectral slope ratio (S_R) was calculated from the absorption spectra as the ratio of the slope from 275 to 295 nm to the slope from 350 to 400 nm, following Helms et al. (2008). Specific UV-visible absorbance at 254 nm (SUVA₂₅₄; L mg-C⁻¹ m⁻¹) was calculated following Weishaar et al. (2003), where decadic absorbance at 254 nm was divided by the pathlength (m) and by the DOC concentration (mg-C L⁻¹).

Fluorescence excitation-emission matrices (EEMs) of whole water and SPE DOM were collected over excitation and emission ranges of 240 to 600 nm by excitation/emission increments of 5/1.64 nm/nm, respectively, using integration times ranging from 3 to 4 s (Aqualog; Horiba Scientific). When necessary, DOM was diluted to less than 0.6 absorbance units (A) at 254 nm prior to analysis (Miller et al., 2010). EEMs were corrected for inner-filter and instrument specific excitation and emission effects in Matlab (version 2013b). Blank EEMs were collected using fluorescence free, laboratory-grade DI water, and were subtracted from sample EEMs to minimize the influence of water Raman peaks. Intensities of corrected sample

EEMs were converted to Raman units. Dominant peaks in the corrected EEMs were identified following Coble (1996): Peak A ($\lambda_{\text{ex}} = 250$ nm; $\lambda_{\text{em}} = 380 - 460$ nm), Peak C ($\lambda_{\text{ex}} = 350$; $\lambda_{\text{em}} = 420 - 480$ nm), and Peak T ($\lambda_{\text{ex}} = 275$ nm; $\lambda_{\text{em}} = 340$ nm). The fluorescence index (FI; McKnight et al., 2001) was calculated as the ratio of emission intensity at 470 nm to emission intensity at 520 nm at an excitation wavelength of 370 nm.

2.4 Fourier transform ion cyclotron resonance mass spectrometry

Whole water and SPE DOM were diluted with LC-MS grade MeOH and water, respectively, to give a final sample composition of 50:50 (v/v) MeOH:H₂O. Additionally, SPE samples were diluted to less than 50 mg-C L⁻¹ to minimize charge competition during ionization. DOM was continuously injected into an Apollo II ESI ion source of a Bruker Daltonics 12 Tesla Apex Qe FT-ICR MS housed at the William R. Wiley Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory. Samples were injected at a rate of 120 $\mu\text{L h}^{-1}$ and were analyzed in negative ion mode. Accumulation of ions in the hexapole ranged from 0.4 - 3 s before being transferred to the ICR cell, where 300 scans, collected with a 4 MWord time domain, were co-added for each sample. The summed free induction decay signal was zero-filled once and Sine-Bell apodized prior to fast Fourier transformation and magnitude calculation using Bruker Daltonics Data Analysis software. Similarly, a 50:50 (v/v) MeOH:H₂O blank spectrum was collected to test for contamination.

Mass spectra were externally calibrated using a polyethylene glycol standard and internally calibrated using fatty acids and other CH₂ homologous series naturally present in the sample (Sleighter et al., 2008). Peaks from 250 to 1000 m/z with a signal to noise ratio ≥ 4 were added to mass lists, and those peaks detected in the blank spectrum were discarded prior to

formula assignments. The presence or absence of peaks between spectra was quantified, where common peaks were defined as m/z values that agree within 0.5 mDa of each other (Sleighter et al., 2012). A molecular formula calculator (Molecular Formula Calc v.1.0 ©NHMFL, 1998) generated formulas using carbon, hydrogen, oxygen, nitrogen, and sulfur. Only formulas that agreed within an error of $\leq \pm 1$ ppm to the calculated exact mass of the formula were considered, although the majority of formulas ($> 85\%$) had an error of $\leq \pm 0.5$ ppm. All formula assignments were screened to meet the criteria described by Stubbins et al. (2010). For whole water DOM replicates, 70 - 78% of all peaks were assigned unique formulas (excluding contributions from ^{13}C isotopes), accounting for 69 - 76% of the spectral intensity. For SPE DOM replicates, 70 - 78% of peaks were assigned formulas (excluding contributions from ^{13}C isotopes), accounting for 75 - 86% of the spectral intensity.

2.5 Principal component analysis of whole water and SPE DOM

Principal component analysis (PCA) was performed on the twelve DOM samples (including triplicate analyses) to relate characteristics of organic mat and permafrost DOM determined using optical spectroscopy and FT-ICR MS. PCA was carried out on two data matrices. The first data matrix included averaged bulk characteristics from optical spectroscopy (i.e., S_R , FI, Peak C λ_{em} , and Peak C/A) and from FT-ICR MS (MW, O/C, H/C, AI_{mod} , DBE, and DBE/O). The second data matrix included the relative magnitudes of all formulas in the set of twelve spectra (Sleighter et al., 2010; Sleighter et al., 2014). If a formula was not detected in a spectrum, the relative magnitude was set to zero. PCA was conducted using IBM SPSS Statistics V.22.

2.6 Solid-state ^{13}C NMR analysis of SPE DOM

The remaining SPE DOM replicate eluates from the two DOM sources were pooled prior to freeze drying to ensure enough powdered DOM for solid-state ^{13}C NMR analysis (Step 7; Fig. 1). To avoid thawing throughout freeze-drying, the pooled eluate was evaporated in a drying oven at 50 °C and then diluted with DI water to a final concentration of 10:90 (v/v) MeOH:H₂O. The diluted eluate was then transferred to DI-rinsed, pre-combusted flasks and frozen to -80 °C prior to loading on to a FreeZone 4.5 L freeze drier (LabConoco). Solid-state ^{13}C NMR spectra of the powdered SPE DOM were obtained using a Varian Infinity CMX 300 MHz spectrometer at the William R. Wiley Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory. Powdered SPE DOM was packed into 4 mm zirconia rotors fitted with Teflon spacers and caps. A ramped (^{13}C pulse) cross polarization magic angle spinning (CPMAS) pulse sequence was used. The contact time was 1 ms, the spinning rate was 14 kHz, and the decoupling field was 71 kHz. The ^{13}C chemical shifts were referenced to tetramethylsilane (0 ppm) using an external reference, hexamethylbenzene (methyl C; 17.36 ppm). Functional group distributions were quantified using Mnova NMR software (Metrelab Research) following Cory et al. (2007).

2.7 Bacterial respiration, production, and growth efficiency

Bacterial respiration and production rates were quantified following previously described protocols (Cory et al., 2013; Cory et al., 2014). GF/F filtrate of each replicate leachate was inoculated with its native bacterial community (equivalent to 20% of the filtrate volume), which was prepared by GF/C filtering each replicate leachate. Incubations were carried out for six to seven days in the dark at 6 - 7 °C. Respiration incubations were carried out in air-tight, pre-

combusted 12 mL exetainer vials (Labco, Inc), while production incubations were carried out in 10-mL plastic vials. Respiration was measured as O₂ consumption and CO₂ production relative to killed controls (1% HgCl₂). Membrane inlet mass spectrometry was used to measure bacterial O₂ consumption, while a DIC analyzer (AS-C3; Apollo SciTech, Inc.) was used to measure CO₂ production. There was not a shift from aerobic to anaerobic conditions during the short-term incubations. Final O₂ concentrations in the bacterial incubations were $81 \pm 21 \mu\text{M-O}_2$ and $337 \pm 2 \mu\text{M-O}_2$ for organic mat and permafrost DOM, respectively. Bacterial production was determined by measuring ¹⁴C-labeled L-leucine incorporation into the cold trichloroacetic acid (TCA)-insoluble fraction of macromolecules in two subsamples and one TCA-killed control incubated for 2 - 4 hours at 6 °C in the dark. Bacterial growth efficiency was calculated as bacterial production divided by the sum of bacterial production and respiration. Bacterial respiration and production rates were normalized to initial DOC and initial bacterial concentrations. Bacterial concentrations were quantified from gluteraldehyde-fixed samples following Crump et al. (1998). Mann-Whitney U-tests were used to determine statistical significance in bacterial respiration, production, and growth efficiency between organic mat and permafrost DOM ($\alpha = 0.05$; IBM SPSS Statistics V.22).

3. RESULTS

3.1 DOC concentration and water chemistry of soil leachates

DOC concentrations were significantly higher for leachates containing soil from the organic mat compared to soil from the permafrost layer (Table 1). However, when DOC concentrations were normalized to the amount of soil added to each leachate and to the duration of the leaching process, the rate of DOC leached from both soil layers was similar (organic mat:

12.7 $\pm$ 0.8 mmol-DOC g-soil⁻¹ day⁻¹; permafrost: 13.3 $\pm$ 0.4 mmol-DOC g-soil⁻¹ day⁻¹). These leaching rates were not normalized to their dry weight contents, which could have influenced the leaching rates. However, both soil types were saturated prior to leaching, which likely minimized the influence of soil moisture on the leaching rates. Leachates from both organic mat and permafrost layers were mildly acidic (organic mat: pH = 5.7 $\pm$ 0.1; permafrost: pH = 5.8 $\pm$ 0.1). Conductivity was low for both leachates but was 4-fold higher for whole water leachate of organic mat compared to permafrost soils (organic mat: 37 $\pm$ 3 μ S cm⁻¹; permafrost: 9 $\pm$ 1 μ S cm⁻¹). When normalized to the amount of soil added to each leachate and to the duration of the leaching process, conductivity was similar between the leachates (organic mat: 10 $\pm$ 1 nS cm⁻¹; permafrost: 8 $\pm$ 1 nS cm⁻¹).

3.2 Optical characterization of DOM in whole water leachates

There were significant differences in the characteristics of chromophoric (light-absorbing) and fluorescent (light-emitting) DOM in the whole water organic mat and permafrost leachates (Table 1). The mean slope ratio (S_R) of organic mat DOM (0.72 $\pm$ 0.03) was lower than the mean S_R of permafrost DOM (0.96 $\pm$ 0.06), suggesting that organic mat DOM exhibited a higher average molecular weight than permafrost DOM (Helms et al., 2008). DOC normalized specific UV absorbance at 254 nm (SUVA₂₅₄; L mg C⁻¹ m⁻¹) was higher for organic mat DOM (mean = 2.7 $\pm$ 0.1) compared to permafrost DOM (mean = 1.2 $\pm$ 0.2), indicating that organic mat DOM contained more aromatic C than permafrost DOM (Weishaar et al., 2003). EEMs of whole water organic mat and permafrost DOM exhibited similar characteristic humic peaks A and C and protein-like peak T (Coble, 1996). As expected for terrestrially-derived DOM (e.g., Cory et al., 2007), emission intensities were highest for the humic peaks, i.e., peaks A and C > peak T

(Table 1). There were no differences in the ratio of peak C to peak A between organic mat and permafrost whole water DOM (Table 1). The emission maximum (λ_{em}) of peak C for organic mat DOM was shifted to higher wavelengths by 4 ± 3 nm compared to permafrost DOM, suggesting that organic mat DOM was more condensed than permafrost DOM (Stedmon and Cory, 2014). Average FI was significantly lower for organic mat DOM (1.49 ± 0.01) compared to permafrost DOM (1.55 ± 0.02), indicating that organic mat DOM contained more aromatic C than permafrost DOM (Table 1; McKnight et al., 2001).

3.3 FT-ICR MS characterization of whole water DOM

High resolution mass spectrometry analysis of whole water organic mat and permafrost DOM showed that interfering species such as salts contributed significantly to the peak distribution and intensity. At a maximum, 625 salt ions were detected in organic mat DOM and 367 salt ions were detected in permafrost DOM, accounting for 20 and 18% of organic mat permafrost whole water DOM spectral magnitude, respectively (Fig. 2). Salt ions, shown as black peaks in Fig. 2, were detected across the mass spectrum from 250 - 785 Da, but the majority of salt ions were detected at $m/z < 500$ Da. On average, 2794 ± 55 and 2390 ± 190 non-salt ions were detected in organic mat and permafrost whole water DOM, respectively (excluding contributions from ^{13}C isotopes; Table 2).

The reproducibility between mass spectra of whole water DOM from triplicate experimental leachates was high for both DOM sources. For instance, 74% of all peaks identified in any replicate leachate of organic mat DOM were common to all three replicates, where common peaks were defined as m/z values that agreed within 0.5 mDa. A similar result was obtained for triplicate experimental leachates of permafrost DOM, that is, 76% of observed

peaks were common to each of the three experimental replicates. Of the subset of peaks common to all replicate spectra, there was a strong, positive correlation between peak intensities (Figs. 3 and S1). Thus, most of peaks identified in a sample were detected at similar intensities in each of the three replicates, demonstrating a strong similarity in the distribution of the peaks common in each set of replicates of either organic mat or permafrost DOM.

Four major differences were observed between whole water mass spectra of organic mat from permafrost DOM. Firstly, nearly half (49%) of the 2573 individual peaks detected were unique to either organic mat and permafrost DOM, with the remainder of peaks detected present in both organic mat and permafrost DOM. That is, 29% of the 2573 individual peaks detected were found only in organic mat DOM, and 20% were found only in permafrost DOM. Of the peaks common to organic mat and permafrost DOM (51% of all peaks), we expected significantly different distributions of these peaks between DOM sources. To test this hypothesis, we used we used Spearman's rank correlation to identify the degree to which peak intensities were ranked in the same order between organic mat and permafrost DOM. A direct comparison of absolute intensities between organic mat and permafrost DOM was not possible due to differences in DOM concentration and in the presence of salt ions. Spearman's rank correlation between the intensities of the peaks common to both sources of whole water DOM was weak, with coefficients ranging from 0.32 to 0.43 (Table S1). Thus, nearly one quarter to one third of all peaks identified were unique to either organic mat or permafrost DOM, and the distribution of the 51% of peaks common to both DOM sources differed significantly between DOM sources.

Secondly, peak distributions and intensities within nominal masses indicated that organic mat whole water DOM was more oxidized and less saturated compared to permafrost whole water DOM. For instance, comparing peaks detected at nominal m/z 503 between whole waters

of organic mat vs. permafrost DOM, the majority of peaks detected only in organic mat DOM exhibited more negative mass defects (< 0.1 m/z ; blue peaks in Fig. 4), while the majority of peaks detected only in permafrost DOM exhibited more positive mass defects (> 0.1 m/z ; red peaks in Fig.4). Because oxygen exhibits a negative mass defect (exact mass = 15.9949Da), and hydrogen exhibits a positive mass defect (exact mass = 1.0078 Da), peaks with lower mass defects in Fig. 4, such as those detected only in organic mat DOM, were more likely to contain oxygen and less likely to contain hydrogen. Thus, peaks unique to organic mat DOM were characterized by a lower mass defect and were thus oxidized and unsaturated compared to the higher mass defect peaks detected only in permafrost DOM. Additionally, of the peaks common to both DOM sources (i.e., black peaks in Figs. 4), those peaks with lower mass defects generally exhibited higher relative intensities in organic mat DOM spectra compared to permafrost DOM spectra. These patterns were evident across all odd nominal masses between 250 to 1000 m/z , demonstrating that the relative abundance of oxygen-rich and hydrogen-deficient peaks was higher in organic mat DOM compared to permafrost DOM.

Thirdly, formulas unique to organic mat DOM exhibited a higher average O/C ratio and lower average H/C ratio compared to permafrost DOM, demonstrating that the criteria used to convert peak masses to elemental formulas were consistent with the mass defect spacing patterns observed at nominal masses in each spectrum. Analysis in van Krevelen space revealed that formulas unique to organic mat whole water DOM were more oxidized (blue squares in Fig. 5a; mean O/C = 0.56) and less saturated (mean H/C = 0.87) compared to formulas unique to permafrost whole water DOM (red circles in Fig. 5a; mean O/C = 0.45, mean H/C = 1.39). Lastly, the average molecular weight of formulas in organic mat DOM was 23 Da higher compared to permafrost DOM (organic mat = 511 ± 7 Da; permafrost = 488 ± 3 Da; Table 2).

Comparing between the subset of formulas unique to each DOM source, the average molecular weight of formulas unique to organic mat DOM was 32 Da higher than the average molecular weight of formulas unique to permafrost DOM (organic mat = 509 Da, permafrost = 477 Da; Fig. 5a).

3.4 Optical characterization of SPE DOM

There were significant differences in the characteristics of chromophoric and fluorescent organic mat and permafrost SPE DOM (Table 1). Mean S_R of organic mat SPE DOM (0.64 ± 0.01) was lower than the mean S_R of permafrost SPE DOM (0.88 ± 0.10), suggesting that organic mat DOM exhibited a higher average molecular weight than permafrost DOM (Helms et al., 2008). Average $SUVA_{254}$ was higher for organic mat SPE DOM (3.3 ± 0.1) compared to permafrost SPE DOM (1.1 ± 0.2), indicating that organic mat DOM contained more aromatic C than permafrost DOM (Weishaar et al., 2003). EEM emission intensities were highest for the humic peaks C and A compared to protein-like peak T, and there were no differences in the ratio of peak C to peak A between organic mat and permafrost SPE DOM (Table 1). The emission maximum of peak C for organic mat SPE DOM was shifted to higher wavelengths by 11 ± 4 nm compared to permafrost SPE DOM, suggesting that organic mat DOM was more condensed than permafrost DOM (Stedmon and Cory, 2014). Average FI was significantly lower for organic mat SPE DOM (1.42 ± 0.01) compared to permafrost SPE DOM (1.58 ± 0.01), indicating that organic mat DOM contained more aromatic C than permafrost DOM (Table 1; McKnight et al., 2001).

3.5 FT-ICR MS Characterization of SPE DOM

In contrast to mass spectra of whole water DOM, high resolution mass spectrometry analysis of the SPE fractions of organic mat and permafrost DOM did not exhibit interference from salt ions, that is, interfering ions did not contribute significantly to the peak distribution and intensity. At a maximum, salt peaks, shown in black in Fig. 2, contributed 0.1 and 0.5% of total spectral magnitude in organic mat and permafrost SPE DOM, respectively. The two salt peaks in organic mat SPE DOM ranged in mass from 363 to 727 m/z , while the 36 salt peaks in permafrost SPE DOM ranged in mass from 295 to 727 m/z . On average, 4478 ± 153 and 3221 ± 22 non-salt ions were detected in organic mat and permafrost SPE DOM, respectively (excluding contributions from ^{13}C isotopes; Table 2).

Similar to the results from whole water DOM spectra, the reproducibility between mass spectra of SPE DOM from triplicate experimental leachates was high for both DOM sources. For instance, 71% of all peaks identified in any replicate leachate of organic mat SPE DOM were common to all replicates, while 73% of all peaks observed in permafrost SPE DOM were common to each of the three experimental replicates. As observed between replicate spectra of whole water DOM, peak intensities were strongly linearly correlated between replicates of SPE spectra for organic mat or permafrost DOM (Fig. S1).

Similar to whole water fractions of both DOM sources, four mass spectral features distinguished organic mat from permafrost SPE DOM. Firstly, 57% of the 3868 individual peaks detected were unique to either organic mat or permafrost SPE DOM; 39% were unique to organic mat SPE DOM, and 18% were unique to permafrost SPE DOM. Of the 43% of peaks common to both organic mat and permafrost SPE DOM, Spearman's rank correlation between their intensities was weak, with coefficients ranging from 0.26 to 0.39 (Table S1). Thus, while 43% of all peaks were common to both DOM sources, the intensities of these common peaks

differed significantly between DOM sources, indicating that organic mat SPE DOM had a different distribution of the common peaks compared to permafrost SPE DOM.

As observed for whole water DOM, peak distributions and intensities within nominal masses indicated that organic mat SPE DOM was more oxidized and less saturated compared to permafrost SPE DOM. Again comparing within nominal m/z 503, the majority of peaks detected only in organic mat SPE DOM exhibited defects < 0.1 (blue peaks in Fig. 4), while the majority of peaks detected only in permafrost SPE DOM exhibited defects > 0.1 (red peaks in Fig. 4). Additionally, of the peaks common to SPE fractions of both DOM sources (black peaks in Fig. 4), those peaks with lower mass defects generally exhibited higher relative intensity in organic mat DOM spectra compared to permafrost DOM spectra, suggesting that the relative abundance of oxygen-rich and hydrogen-deficient peaks was higher in organic mat SPE DOM compared to permafrost SPE DOM.

Consistent with peak distribution patterns within nominal masses, formulas unique to organic mat SPE DOM also revealed that organic mat DOM exhibited a higher average O/C ratio and lower average H/C ratio compared to permafrost SPE DOM. Analysis in van Krevelen space revealed that formulas unique to organic mat SPE DOM were more oxidized (blue squares in Fig. 5b; mean O/C = 0.61) and less saturated (mean H/C = 0.81) compared to formulas unique to permafrost SPE DOM (red circles in Fig. 5b; mean O/C = 0.29, mean H/C = 1.20). Lastly, the average molecular weight of formulas in organic mat SPE DOM was 71 Da higher compared to permafrost SPE DOM (organic mat = 585 ± 6 Da; permafrost = 514 ± 9 Da; Table 2). The average molecular weight of formulas unique to organic mat SPE DOM was 86 Da higher than the average molecular weight of formulas unique to permafrost SPE DOM (organic mat = 592 Da, permafrost = 506 Da; Fig. 5b).

3.6 The effects of salt ions and isolation on FT-ICR MS characterization of DOM

Differences between mass spectra of whole water and SPE fractions of each DOM source were likely due to the effects of interfering salt ions and the SPE isolation process. For instance, compared to SPE DOM, spectra of whole water DOM contained 15 and 20% more salt ions, and 60 and 35% fewer non-salt peaks for organic mat and permafrost DOM, respectively (Table 2). Because salt ions suppress the ionization of organic compounds (King et al., 2000), the increased presence of salt ions along with fewer organic peaks in whole water spectra (relative to SPE spectra) suggested that salt ions decreased the number of organic formulas detected in whole water DOM compared to SPE DOM. To determine the influence of salt ions on the distribution of peaks detected in the whole water DOM mass spectra, we used Spearman's rank correlation to test the similarities in peak intensities for the subset of peaks common to both whole water and SPE fractions of the same DOM source. This analysis showed that peak intensities were strongly correlated between whole water and SPE spectra for either organic mat or permafrost DOM, with coefficients ranging from 0.77 to 0.89 (Table S1). To determine if new compositional information was gained from the "additional" peaks detected in the SPE spectra, we compared formulas detected only in the whole water fraction to formulas detected only in the SPE fraction of each DOM source (Figs. 5c and d) with the assumption that "additional" formulas unique to SPE were those that were quenched by salts, and thus undetected in the whole water fraction. There was large overlap in van Krevelen space between formulas unique to the whole water fraction, formulas unique to the SPE fraction, and formulas common to both fractions of each DOM source (Figs. 5c and d). This result indicated that while fewer formulas were detected in the whole water spectra of each DOM source due to the effect of salts, the additional formulas

detected in the SPE fraction did not add new information on chemical composition because there was strong overlap in the chemical composition of formulas detected in whole water and SPE DOM, for both organic mat and permafrost DOM.

In addition to the influence of interfering salt ions, it was likely that isolation of DOM by SPE resulted in lower detection of highly oxidized formulas by mass spectrometry because these formulas have been shown to exhibit poor retention on SPE resins (Sleighter and Hatcher, 2008; Perminova et al., 2014). Consistently, the whole water fraction of both DOM sources contained a cluster of highly oxidized formulas ($O/C > 0.8$; Figs. 5c and d) that were absent in the SPE fraction, suggesting that these compounds exhibited poor sorption affinity to the PPL solid phase during extraction.

3.7 ^{13}C NMR Characterization of SPE DOM

Integration of resonance spectra of SPE DOM according to dominant functional or structural groups revealed compositional differences between organic mat and permafrost DOM (Figure 6). Organic mat SPE DOM was enriched in aromatic C compared to permafrost SPE DOM; the ratio of aromatic to aliphatic C (i.e., resonance from 110 - 160 ppm divided by resonance from 0 - 110 ppm) was 29% higher for organic mat compared to permafrost SPE DOM. Organic mat SPE DOM was enriched in oxygen-containing functional groups compared to permafrost SPE DOM; containing higher carboxyl C, and ketone and aldehyde C by 4% each. Organic mat SPE DOM was depleted in carbohydrate C (60 - 90 ppm) by 10% compared to permafrost SPE DOM.

3.8 Principal component analysis of whole water and SPE DOM

Principal component analysis of average optical and mass spectrometry chemical characteristics reduced the dataset into two components that distinguished organic mat from permafrost DOM and whole water from SPE DOM. Sample scores of experimental replicates were tightly grouped, and there was less sample score variability between replicates compared to the variability between DOM sources and fractions (Fig. 7). The first component (PC1) explained 71% of the sample variance, and was positively related to aromaticity. The second component (PC2) explained 21% of the sample variance, and was negatively related to oxygen content. Comparing organic mat to permafrost DOM, organic mat DOM was enriched in high molecular weight, low H/C, and high O/C formulas, as shown by positive PC1 scores and negative PC2 scores. Comparing whole water to SPE DOM, whole water DOM was enriched in low molecular weight, high H/C, and high O/C formulas, as shown by negative PC1 and PC2 scores relative to SPE DOM (Fig. 7).

Principal component analysis of the relative magnitude of all formulas detected in the twelve DOM spectra reduced the dataset into two components that distinguished formulas enriched in organic mat or permafrost DOM and formulas enriched in whole water or SPE DOM (Fig. S2a). Similar to the principal component analysis of average characteristics (Fig. 7), sample scores of experimental replicates were tightly grouped, and there was less variability between replicates compared to the variability between DOM sources and fractions. The first component (PC1) explained 31% of the sample variance and separated the DOM source (organic mat vs. permafrost; Fig. S2a). The second component explained 15% of the sample variance and separated whole water from SPE fractions of DOM (Fig. S2a).

Using the results from the principal component analysis, formulas that were relatively enriched in each DOM source were identified by comparing sample and formula scores (Figs.

S2a and b). In total, six-color coded regions were identified based upon the co-location of sample and formula scores, corresponding to formulas that are relatively enriched in those samples (Figs. S2a and b). For example, formulas in the light blue box in Fig. S2b had high PC1 scores (i.e., > 0.80), similar to organic mat SPE DOM replicates in Fig. S2a that also had high PC1 scores. Thus, formulas in the light blue box were relatively enriched in organic mat SPE DOM compared to all other DOM spectra. Accordingly, the summed relative magnitude of formulas in the light blue box was at a minimum 4-fold higher in the organic mat SPE DOM replicates compared to all other spectra (Table S2).

Plotting formulas enriched in each DOM source in van Krevelen space revealed the subset of all formulas that were most important in explaining differences between organic mat and permafrost DOM (i.e., blue squares vs. red circles in Fig. 8). For example, formulas of greater relative abundance in organic mat DOM were tightly clustered with low H/C that spanned O/C 0.45 - 0.8, while formulas of greater relative abundance (and thus more prominent) in permafrost DOM were clustered in the central region of the van Krevelen plot (Fig. 8). Additionally, the average molecular weight of formulas more prominent in organic mat DOM was 101 Da greater than the average molecular weight of formulas characteristic to permafrost DOM, further demonstrating the compositional differences between organic mat and permafrost DOM.

3.9 Dark bacterial respiration rates of organic mat and permafrost DOM

Volumetric rates of bacterial respiration, measured as O₂ consumption and CO₂ production per day, were higher for organic mat compared to permafrost DOM (organic mat DOM: O₂ consumption = 41.7 ± 4.9 $\mu\text{M-O}_2 \text{ d}^{-1}$, CO₂ production = 30.5 ± 8.0 $\mu\text{M-CO}_2 \text{ d}^{-1}$;

permafrost DOM: O_2 consumption = $1.9 \pm 0.3 \mu\text{M-O}_2 \text{ d}^{-1}$, CO_2 production = $1.6 \pm 0.7 \mu\text{M-CO}_2 \text{ d}^{-1}$). Average volumetric rates of bacterial production were similar between organic mat and permafrost DOM (organic mat DOM = $9.4 \pm 4.3 \mu\text{M-C d}^{-1}$; permafrost DOM = $6.2 \pm 0.5 \mu\text{M-C d}^{-1}$). When bacterial respiration and production rates were normalized to initial DOC concentration and cell abundance in organic mat and permafrost DOM (Table 1), respiration rates were similar between DOM sources, but production rates were significantly higher for permafrost compared to organic mat DOM (Fig. 9). Additionally, bacterial growth efficiency was significantly lower for organic mat DOM ($20 \pm 14\%$) compared to permafrost DOM ($80 \pm 7\%$; Fig. 9).

4. DISCUSSION

4.1 FT-ICR MS reproducibility and the effects of interfering species, isolation, and instrumentation on DOM chemical composition

Based on the strong overlap in peak mass and intensity between replicate mass spectra, DOM composition as analyzed by FT-ICR MS was highly reproducible (Tables 2 and S1, Figure 3). High reproducibility of DOM mass spectra has previously been reported (Sleighter et al., 2012), where 70% of peaks detected in mass spectra of riverine SPE DOM were common to experimental replicates, similar to the 71 - 76% overlap in peak detection reported here for replicates of organic mat and permafrost SPE DOM. The degree of reproducibility between replicate spectra served as a baseline for evaluating the much larger differences in DOM composition observed between DOM fractions (whole water vs. SPE) and sources (organic mat vs. permafrost). For example, there were fewer common peaks detected between different fractions of the same DOM source (43 - 45% for whole water vs. SPE), or between the different

DOM sources (43 - 51% for organic mat vs. permafrost), relative to the 71 - 76% detected among any set of replicate spectra. Furthermore, Spearman rank correlation of common peak intensities between experimental replicates was significantly stronger compared to correlation between DOM fractions and sources; correlation coefficients between replicates ranged from 0.95 - 1.00, correlation coefficients between fractions ranged from 0.77 - 0.89, and correlation coefficients between sources ranged from 0.13 - 0.43 (Table S1). These results demonstrated that the observed differences in chemical composition of DOM by fraction or by source were significant, consistent with the groupings in DOM composition by whole water vs. SPE or organic mat vs. permafrost DOM as separated by principal component analysis (Figs. 7 and S2a).

Smaller compositional differences between whole water and SPE fractions of DOM compared to differences between DOM sources is consistent with studies of freshwater DOM (Sleighter et al., 2010). Differences in DOM composition by FT-ICR MS between whole water and SPE were likely predominantly due to preferential loss of high O/C formulas during SPE isolation; by comparison, salts or other interfering species had minor influence on the mass spectra and thus DOM composition. Based on principal component analysis (Fig. 7), separation of DOM between whole water and SPE fractions occurred along the PC2 axis, which accounted for only 21% of the variability in DOM composition and suggested that the greater number of high O/C compounds in the whole water was the key distinction between whole water and SPE DOM. Fewer formulas with high O/C in the SPE fractions was likely due to preferential loss of highly oxidized formulas during SPE isolation, consistent with previous observations of incomplete recovery and fractionation of DOM during isolation (Dittmar et al., 2008; Sleighter and Hatcher, 2008).

While salts resulted in the detection of fewer DOM formulas in the whole water spectra compared to the SPE spectra, the “additional” formulas detected by SPE had similar chemical composition as formulas detected in the whole water, as evidenced by their overlap in van Krevelen space (Fig. 5). This result together with the loss of high O/C formulas during SPE isolation suggested that for DOM in soils similar to those studied here (i.e., low conductivity, relatively high DOM concentrations), SPE isolation may result in a stronger bias on DOM composition as analyzed by FT-ICR MS compared to the effects of salts when analyzing whole water DOM. However, independent of the fraction of DOM analyzed, FT-ICR MS analysis of DOM is “blind” to some compound classes, based on comparison to characterization by solid-state ^{13}C NMR.

Compared to compound class distributions measured by ^{13}C NMR, FT-ICR MS significantly overestimated aromatic C and underestimated carbohydrate C, which has previously been reported for freshwater and marine DOM (Hockaday et al., 2009; Jaffé et al., 2012; Hertkorn et al., 2013). For instance, compared to ^{13}C NMR, the fraction of aromatic C was 11 - 22% higher and the fraction of carbohydrate C (defined in Stubbins et al., 2010) was 22 - 33% lower when analyzed by FT-ICR MS (Figs. 5 and 6). Discrepancies in compound class distributions between ^{13}C NMR and FT-ICR MS have been attributed to greater ionization efficiency of aromatics compared to carbohydrates because the assumption is that ^{13}C NMR has less bias in compound class detection compared to FT-ICR MS (Hockaday et al., 2009; Hertkorn et al., 2013).

However, ^{13}C NMR is susceptible to biases that occur when DOM is isolated and concentrated from water prior to ^{13}C NMR analysis to remove interfering impurities (e.g., paramagnetics) present in water. For example, the presence of highly oxidized compounds in the

whole water mass spectra (i.e., those that were not retained by the solid phase resin during extractions), suggested that the abundances of oxygen-containing functional groups detected by ^{13}C NMR were likely a minimum (i.e., carboxyl, aldehyde, and ketone; Fig. 6). Despite biases in absolute amounts of compound classes or functional groups of DOM depending on the technique used, the relative differences in organic mat and permafrost DOM chemical composition were consistent across all methods and statistical analyses. The agreement in relative differences in DOM character across multiple characterization techniques has previously been reported for freshwater (e.g., Hockaday et al., 2009; Sleighter et al., 2014) and soil DOM (Tfaily et al., 2013).

4.2 Compositional differences between organic mat and permafrost DOM

Examining either optical proxies for DOM composition or direct measurements of DOM composition by FT-ICR MS or ^{13}C NMR, all analyses indicated that DOM leached from the shallower organic mat contained higher molecular weight, more oxidized, and more unsaturated, aromatic species compared to DOM leached from the deeper permafrost layer (Tables 1, 2, and S1 Figs. 4, 5, 6, and 7). These differences in DOM composition between organic mat and permafrost soil layers were consistent with previous observations in arctic (Mann et al., 2012; Cory et al., 2013; Hodgkins et al., 2014; Spencer et al., 2015; Dubinenkov et al., 2015) and sub-arctic soils (e.g., Kaiser et al., 1997; Cory et al., 2004; D'Andrilli et al., 2010; Tfaily et al., 2013). For instance, Cory et al. (2013) used optical proxies to show that organic mat DOM contained higher aromatic C and higher average molecular weight compared to permafrost DOM. That is, Alaskan Arctic surface waters that primarily drained the shallower organic mat of soils exhibited higher SUVA_{254} , lower slope ratio, and lower fluorescence index compared to surface waters impacted by thermokarsts or hillslope failures that primarily drained deeper

mineral-rich soils. In addition, DOM collected from a shallower active layer bog site in Swedish permafrost soils contained higher molecular weight, more oxidized, and more unsaturated species compared to DOM from a deeper active layer fen site (Hodgkins et al., 2014). Furthermore, the compounds distinguishing permafrost from organic mat DOM in the Siberian Arctic were unsaturated, aliphatics (Spencer et al., 2015; Dubinenkov et al., 2015).

These differences in soil DOM chemical composition between the shallower organic mat and deeper permafrost layer were consistent with a conceptual model proposed for DOM processing in soils. This “downward cycling” model for DOM predicts a shift in DOM composition between surface organic layers and deeper mineral layers due to the net effects of sorption to mineral phases, microbial degradation, and mobilization within the soil column (Kaiser and Kalbitz, 2012). Factors contributing to the downward cycling model include vegetation, soil acidity, and exchangeable iron and aluminum. Dominant vegetative species within the Imnavait Creek watershed include moss (*Sphagnum*) and tussock (*Eriophorum vaginatum*), both of which trap moisture and release organic acids, contributing to increased soil acidity (Walker et al., 2014). Consequently, lower soil pH significantly increases exchangeable iron and aluminum concentrations (Ping et al., 1998), which likely increases the relative importance of DOM sorption to mineral phases compared to other controls on DOM composition with depth. Compounds enriched in organic mat DOM in this study, i.e., higher molecular weight compounds, aromatics, or compounds rich in carboxyl functionalities, exhibit higher sorption capacities to iron and aluminum oxides and thus are typically immobilized, protected from microbial degradation, and enriched in shallow soils (Kaiser and Kalbitz, 2012). Compounds enriched in permafrost DOM, i.e., lower molecular weight compounds, aliphatics, or compounds poor in carboxyl functionalities, exhibit lower sorption capacities, and thus are not

protected from microbial degradation, leading to the accumulation of these compound classes and their degradation products in deeper soils (Kaiser and Kalbitz, 2012). Consistently, a study of DOM sorption processes in Siberian permafrost soils reported that hydrophobic, aromatic DOM was preferentially retained by shallower, acidic layers of the soil that were rich in iron oxides compared to deeper horizons (Kawahigashi et al., 2006).

4.3 Bacterial degradation of DOM draining arctic permafrost soils

Given the importance of substrate quality on bacterial degradation in arctic surface waters (Crump et al., 2003; Judd et al., 2006), we expected the substantial differences in chemical composition between organic mat and permafrost DOM to affect the bacterial degradation of DOM drained from these soil layers. However, there was no significant difference in rates of bacterial respiration (normalized to DOC and bacterial cell concentrations) between organic mat and permafrost DOM (Fig. 9), and both were within the range of previously reported rates for DOM draining pan-arctic soils (Cory et al., 2013; Mann et al., 2014; Cory et al., 2014; Abbott et al., 2014). In contrast, bacterial production rates and growth efficiencies were significantly higher for permafrost compared to organic mat DOM (Fig. 9). Bacterial growth rate and efficiency have been strongly, inversely correlated to the oxidation state of DOM (Meyer, 1994; Vallino et al., 1996; Sun et al., 1997), suggesting that compounds with a lower oxidation state may be used more efficiently by bacteria. Consistently, permafrost DOM had a lower average O/C and fewer compounds at the higher O/C range, and was more efficiently used by bacteria (i.e., less CO₂ respired per DOC consumed) compared to organic mat DOM (Fig. 9).

While not significant, higher respiration rates for permafrost DOM compared to organic mat DOM in this study were consistent with reported inverse correlations between proxies for aromatic C content and DOM lability in surface waters draining permafrost soils (Mann et al.,

2012; Abbott et al., 2014). Mann et al. (2014) suggested that phenolic compounds, i.e., aromatics substituted with hydroxyl functional groups, inhibited bacterial activity and thus slowed the respiration of DOM drained from the organic mat compared to the deeper permafrost layer. Consistently, organic mat DOM in this study contained more aromatics and more oxygen-containing functional groups than permafrost DOM (Figs. 6 and 8).

However, despite large differences in aromatic and phenolic content, no significant difference in respiration rates or relationship between aromatic C and respiration rates in some studies suggests that DOM degradation by aquatic bacteria is likely more complex than predicted based on aromatic or phenolic content alone (Cory et al., 2013 and this study). We offer two explanations for these contrasting relationships between aromatic C content and respiration rates. Firstly, aromatic compounds may concurrently inhibit and support bacterial respiration. An alternative to the phenolic inhibition hypothesis (Freeman et al., 2001; Mann et al., 2014) is that native bacterial communities degraded aromatics, including the phenolic fraction. Aromatic compounds are abundant in soil and surface waters in the Arctic (Cory et al., 2007; Waldrop et al., 2010; this study), the pathways used by microorganisms to metabolize aromatics (including phenolic compounds) are well characterized (Fuchs et al., 2011), and aromatics have been found to support bacterial respiration in other waters where the dominant source of DOM is plant and soil organic matter rich in aromatic C (Cory and Kaplan, 2012; Ward et al., 2013; Sleighter et al., 2014). Secondly, the content of aromatic C may not be a causal factor controlling rates of bacterial respiration. Other aspects of DOM composition that may co-vary with aromatic C may be important. For example, the distinguishing feature of permafrost DOM was low molecular weight, highly saturated aliphatic compounds depleted in oxygen (Fig. 8). Relative to aromatic compounds, these low molecular weight aliphatics are expected to be more labile to bacteria

(Meyer, 1994; Vallino et al., 1996; Sun et al., 1997; Sleighter et al., 2014). Consistently, oxygen-poor, aliphatic compounds comprising the DOM pool in Siberian Arctic permafrost melt streams exhibited higher lability to biological degradation compared to less saturated, aromatic compounds within the permafrost DOM pool (Spencer et al., 2015). Thus, the abundance of relatively more labile oxygen-poor, aliphatic compounds may account for the observed increased bacterial growth efficiency or respiration of permafrost DOM once thawed and exported to surface waters (Mann et al., 2014; Abbott et al., 2014; Spencer et al., 2015; Mann et al., 2015; this study).

4.4. Conclusions and implications

Several studies have demonstrated that warming surface temperatures in the Arctic is deepening hydrologic flow paths through soils and increasing the export of permafrost DOM to arctic surface waters (Keller et al., 2010; Feng et al., 2013; Kling et al., 2014). The results from this study demonstrated that an increase in the export of permafrost DOM to surface waters will result in DOM that has a lower SUVA₂₅₄ (absorbs less light per DOC), and is comprised of smaller, more saturated, and less oxidized species compared to DOM currently flushed to surface waters. Permafrost DOM is more labile to bacteria, as indicated by the significantly higher bacterial production rates and growth efficiencies compared to organic mat DOM. Thus a shift in DOM source is expected to change the rates and pathways of DOM degradation in surface waters compared to DOM currently draining the shallow active layer. However, conversion of permafrost DOM to CO₂ by bacterial respiration could be the same (this study) or up to 1.6 times greater (Mann et al., 2014) than respiration of DOM currently draining the organic mat of arctic soils. Taking the results from our study as a conservative estimate (i.e., no difference in rates of bacterial respiration between permafrost and organic mat DOM; Fig. 9), and assuming no

changes in the lateral transfer of DOM with permafrost thaw, a shift in the composition of DOM from organic mat to permafrost character may not change the amount of DOM that is respired by bacteria to CO₂ in soil waters, surface waters, and aquatic sediments, and subsequently released from arctic surface waters to the atmosphere (Kling et al., 1991; Ramlal et al., 1994; McGuire et al., 2009; Kling et al., 2014; Cory et al., 2014).

Thus, bacterial respiration of permafrost DOM will continue to contribute significantly to the CO₂ fluxes from freshwaters that currently account for up to 40% of the net land surface C exchange with the atmosphere (Kling et al., 1991; McGuire et al., 2009; Cory et al., 2014). Given that bacterial respiration may increase by a factor of 1.6 (Mann et al., 2014), and conversion of DOM to CO₂ by sunlight was most often three to 10-fold greater than rates of bacterial respiration for DOM in the water column of arctic lakes and streams (Cory et al., 2014), photo-mineralization of DOM may continue to dominate DOM degradation in the water column. However, “dark” bacterial respiration of DOM in soil waters and in aquatic sediments likely contributes more CO₂ released from surface waters to the atmosphere than photo-mineralization. Therefore, degradation of DOM by both bacteria and sunlight will continue to be important for DOM fate as more permafrost DOM is exported to surface waters (Cory et al., 2013; Cory et al., 2014), and understanding the controls on both bacterial and photochemical degradation of permafrost DOM is critical for predicting changes in the magnitude of CO₂ released to the atmosphere as permafrost soils continue to thaw.

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Tables:

Table 1. Summary of chemistry, optical spectroscopy, and bacterial cell count data for organic mat and permafrost whole water and solid phase extraction (SPE) DOM^a.

DOM Source	Organic Mat		Permafrost	
DOM Fraction	whole water	SPE	whole water	SPE
DOC (μM)	3054 ± 181	465 ± 29	998 ± 27	179 ± 13
A_{254} (m^{-1})	100 ± 9	19 ± 2	15 ± 3	2 ± 1
SUVA ₂₅₄ ($L\ mg^{-1}\ C^{-1}\ m^{-1}$)	2.7 ± 0.1	3.3 ± 0.1	1.2 ± 0.2	1.1 ± 0.2
Slope Ratio	0.72 ± 0.03	0.64 ± 0.01	0.96 ± 0.06	0.88 ± 0.10
Peak A (RU)	5.26 ± 0.36	1.75 ± 0.13	1.98 ± 0.51	0.49 ± 0.07
Peak C (RU)	2.32 ± 0.17	0.67 ± 0.06	0.83 ± 0.21	0.17 ± 0.01
Peak T (RU)	1.28 ± 0.05	0.25 ± 0.01	0.30 ± 0.08	0.08 ± 0.01
Peak C:A	0.44 ± 0.01	0.38 ± 0.01	0.42 ± 0.01	0.38 ± 0.01
Peak C λ_{ex} max (nm)	449 ± 2	456 ± 4	445 ± 1	445 ± 1
Fluorescence Index	1.49 ± 0.01	1.42 ± 0.01	1.55 ± 0.02	1.58 ± 0.01
^b Bacterial Cells ($cells\ mL^{-1}$)	$1.3 \pm 0.2 \times 10^7$	-	$1.4 \pm 0.5 \times 10^6$	-

^a A_{254} = decadic absorbance at 254 nm; SUVA₂₅₄ = specific UV absorbance at 254 nm; Values show $\pm$ standard deviation from the mean of experimental triplicates.

^bValues reflect abundances at the start of incubation with 20% GF/C filtered leachate.

782 **Table 2.** Summary of FT-ICR MS data for organic mat and permafrost whole water and solid
 783 phase extraction (SPE) DOM.^a

DOM Source	Organic Mat		Permafrost	
DOM Fraction	Whole Water	SPE	Whole Water	SPE
# of peaks	2794 ± 55	4478 ± 153	2390 ± 190	3221 ± 22
MW	511 ± 7	585 ± 6	488 ± 3	514 ± 9
O/C	0.53 ± 0.01	0.52 ± 0.01	0.49 ± 0.01	0.44 ± 0.01
H/C	1.01 ± 0.02	0.96 ± 0.01	1.16 ± 0.01	1.14 ± 0.01
DBE	13 ± 1	16 ± 1	11 ± 1	12 ± 1
AI _{MOD}	0.37 ± 0.01	0.40 ± 0.01	0.29 ± 0.01	0.32 ± 0.01
%Aliphatic	69 ± 1	65 ± 1	79 ± 2	77 ± 1
%Aromatic	31 ± 1	35 ± 1	21 ± 2	23 ± 1

^a # of peaks excludes contributions from ¹³C isotopes and salt ions; MW = molecular weight; DBE = double bond equivalents; AI_{MOD} = modified aromaticity index. Values show ± standard deviation from the number-averaged mean of experimental triplicates.

Figure descriptions:

Figure 1. Experimental design for leaching and isolating DOM from the shallower organic mat and deeper permafrost layer of arctic soils.

Figure 2. Mass spectra of whole water and solid phase extraction (SPE) organic mat and permafrost DOM. Black peaks are salt ions.

Figure 3. Linear correlations between FT-ICR MS peak intensities of experimental replicates of whole water organic mat DOM.

Figure 4. Nominal m/z 503, where blue peaks were detected only in organic mat DOM, red peaks were detected only in permafrost DOM, and black peaks were detected in organic mat and permafrost DOM.

Figure 5. van Krevelen diagrams demonstrating differences between **a)** whole water fractions of organic mat and permafrost DOM, **b)** SPE fractions of organic mat and permafrost DOM, **c)** whole water and SPE fractions of organic mat DOM, and **d)** whole water and SPE fractions of permafrost DOM.

Figure 6. Functional group distributions of organic mat SPE DOM (blue bars) and permafrost SPE DOM (red bars) determined using ^{13}C NMR. Integration regions are listed below each functional group following Cory et al. (2007).

Figure 7. Bi-plot from principal component analysis of number-averaged chemical characteristics determined using optical spectroscopy and FT-ICR MS. Black triangles are variable loading scores.

Figure 8. van Krevelen diagrams of formulas that were enriched in organic mat DOM (blue square on PCA loading plot; Fig. S2) and permafrost DOM (red square on PCA loading plot; Fig. S2). Summed spectral intensities of formulas are listed in Table S2.

807 **Figure 9.** DOC and cell concentration normalized bacterial respiration (O₂ consumption and
808 CO₂ production) and bacterial production rates. Error bars indicate $\pm$ standard deviation from the
809 mean of experimental triplicates. *designates statistical significance at $\alpha = 0.05$.

Figures:

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

Figure 8.

Figure 9.

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Appendix

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