Impacts of wildfire on dissolved organic matter in boreal headwater streams

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Abstract

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Erin Matula

Increasing wildfire frequency, driven by climate change, can change the concentration and composition of dissolved organic matter (DOM) exported from land into receiving waters by removing terrestrial vegetation, changing soil hydrology, and interrupting microbial degradation. In this thesis, I tested how wildfire impacts the molecular composition and reactivity of DOM. I compared DOM from boreal headwater streams in northwestern Ontario, Canada between 10 catchments recently affected and 10 comparable catchments that were undisturbed for at least 20 years. Using optical spectroscopy, ultra-high-resolution mass spectrometry, and incubation experiments, I found that burned streams had 29% higher average DOM concentrations and contained less bioavailable DOM, which resulted in microbes respiring more CO_2 in burned streams rather than using carbon to build biomass. These results indicate that the impacts of wildfire on carbon sequestration have been underestimated and highlight the need to consider wildfire in forest carbon budgets.

Keywords: Wildfire, dissolved organic matter, boreal forest, headwater streams, Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS), carbon flux

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List of abbreviations:

BC: Black Carbon-like molecules

C: Carbon

CO_2: Carbon dioxide

DOC: Dissolved organic carbon

DOM: Dissolved organic matter

fDOM: Fluorescent Dissolved organic matter

FT-ICR MS: Fourier-transform ion cyclotron resonance mass spectrometry

KNO_3: Potassium nitrate

N: Nitrogen

Na_3PO_4: Trisodium phosphate

OM: Organic matter

P: Phosphorus

pyDOM: Pyrogenic dissolved organic matter

Chapter 1

1. General introduction

Boreal ecosystems are prominent in Canada, and boreal forests make up about one-third of global forest carbon sinks (Tagesson et al., 2020). Globally, between 30% and 70% of organic carbon that moves into inland waters is from terrestrial sources (Jansson et al., 2007), totaling 5.1 Pg of carbon per year and increasing at an average rate of 0.3 Pg per year (Drake et al., 2017). While carbon movement, or flux, is an important piece of the carbon cycle, it is unclear how it will be affected by the compounding impacts of climate change. Climate change directly through increasing temperatures and indirectly, through more frequent disturbances, like wildfires, increases variability in carbon movement (De Groot et al., 2019; Stocks et al., 1998). Microbes, in particular, play a key role in carbon movement from soil and inland waters into greenhouse gases (D'Andrilli et al., 2019; Hu et al., 2022). Additionally, greenhouse gases function as positive feedback into climate change. Understanding how carbon in inland waters varies spatially and temporally is important to consider when monitoring the effects of climate change on boreal ecosystems. Increasing climate change fueled disturbances, like wildfires, threaten the ability of boreal ecosystems to perform as a carbon sink, emphasizing the importance of considering wildfires for accurate carbon accounting.

1.1 The Movement of Carbon from Land into Water

Boreal terrestrial–headwater stream interactions are mediated through energy and matter fluxes, with contributions from dissolved organic matter (DOM) that move through leaching and overland flow. This is only one piece of the carbon cycle, which is the movement of carbon from

sources, emitters, to sinks or reserves of carbon. Carbon in the terrestrial sphere can dissolve in water, which is part of a broader pool of dissolved organic matter (DOM). Then, water above ground flows overland or leaches through the soil to inland waterbodies. In the past 150 years, organic carbon leaching has increased 28% in models of boreal regions due to the changing climate (Nakhavali et al., 2020). Observed DOC changes after disturbance are variable in inland waterbodies; for instance, there was a 27% decrease in lakes after the forest defoliator outbreak (Woodman et al., 2021) and a 40% increase in mountain streams after bark beetle outbreaks (Mikkelson et al., 2013). Once carbon enters inland waters, it can be consumed by microbes, transported downstream, or incorporated into sediment or higher trophic levels (Vachon et al., 2020). When the two major pathways—overland flow and leaching— encounter disturbance, forests can become less efficient as carbon sinks.

Allochthonous and autochthonous are the two categories of carbon origins that constitute DOM in inland waters. Allochthonous inputs like leachate, runoff, and forest litter originate outside the waterbodies. Autochthonous inputs originate in waterbodies, including primary production by macrophytes, algae, and phytoplankton. There is spatial variation in allochthonous (synonymous to terrestrial) and autochthonous DOM from littoral to limnetic zones in waterbodies (Murphy et al., 2008). Terrestrial DOM is more prominent in nearshore areas. Autochthonous DOM is present in nearshore in addition to offshore areas but is usually near sources of primary production (Murphy et al., 2008). Since carbon compounds can be autochthonous or allochthonous in origin, they can be grouped and identified using fluorescence spectroscopy or mass spectrometry to explain DOM composition in stream water better.

DOM, comprised of many different carbon compounds, is consumed by microbes and respired as carbon dioxide (CO_2) at variable rates depending on its composition. One way to

measure DOM composition is based on its optical properties. Fluorescence spectroscopy utilizes the property of DOM that it fluoresces when exposed to ultraviolet light, resulting in fluorescent DOM (fDOM) measurement. The composition highlighted in fDOM can be broken down into humic substances, like lignin and tannin from terrestrial sources, and protein-like substances. Fluorescence spectroscopy provides broader groupings, while mass spectrometry can specify the biochemical structure at a finer scale (Fellman et al., 2010). The composition of DOM, whether there is proportionally more recalcitrant versus bioavailable or easily broken-down compounds, affects its ability to be utilized efficiently by microbes. More specialized microbes are necessary to utilize recalcitrant DOM (Hu et al., 2022). Temperature, in addition to DOM composition, affects microbial respiration rate. For microbes, higher temperatures require less activation energy to use or degrade DOM (Hu et al., 2022). With higher temperatures, there is more respiration of CO_2 and CH_4 (Scholz et al., 2021). Other factors that affect CO_2 emissions from waterbodies include wildfire, precipitation, forestry management activities, wetland presence in catchments, and flooding (Chi et al., 2020; Scholz et al., 2021).

1.2 Effects of climate change on composition, transport, and fate of carbon

Climate change impacts microbial respiration through environmental drivers that control the respiration rate. Environmental drivers include temperature, precipitation, and the timing of phenological events. Higher temperatures increase microbial activity (Guggenberger & Zech, 1993). As temperatures and respiration rates increase, there is more CO_2 as a byproduct. Precipitation increases the inputs of DOM into waterbodies through runoff and leaching (Gielen et al., 2011; Kalinin et al., 2017; Sharma et al., 2023). Because there is more DOM for microbes to consume, there is more respiration. Changing phenology can include earlier spring thaw,

shorter winters, and longer drought seasons. With shorter winters, periods of slowed or dormant microbial respiration are reduced, and microbes have more time to respire during warmer months. Spring thaw is a period of increased DOM flux, particularly in permafrost regions, where it mobilizes ancient DOM previously stored in deep soil layers (Mann et al., 2015; Spencer et al., 2015; Textor et al., 2019). Direct impacts of climate change are not the sole drivers of respiration.

Climate change indirectly affects C flux through disturbances, including wildfires, which change the composition of carbon sources. It is important to study C flux changes due to the increased frequency and severity of wildfires in boreal regions (De Groot et al., 2019; Stocks et al., 1998). In Canada, 2023 was a record year for wildfires, with 15 million hectares burned (Jain et al., 2024). Continued warming trends and droughts will turn boreal ecosystems from sinks to sources of C, primarily through the burning of soil legacy C (Walker et al., 2019). After the initial loss of soil C, there is continued loss to inland waterbodies through overland flow and leaching groundwater.

After a fire, runoff increases because there is less vegetation to inhibit overland flow during precipitation events, and deeper flow paths leach through the soil. Additionally, decreased evapotranspiration in fire affected areas can further promote hydrological connectivity (Spence et al., 2020). From runoff, DOC can increase immediately, which takes two or more years. There are overall observed increases of up to 36% of dissolved organic carbon (DOC) in inland waters in the Northern Hemisphere, particularly boreal regions (Burd et al., 2018; Loiselle et al., 2020; Minshall et al., 2001; Monteith et al., 2007; Richardson et al., 2024). Other studies find no change or a decrease in DOC often attributed to the loss of organic matter due to fire offsetting inputs (Granath et al., 2021; Olefelt et al., 2013). While there is more carbon in waterbodies

postfire, it consists of charred or black carbon (BC) that is recalcitrant and not easily used by microbes (Dittmar et al., 2012). Because of the decreased bioavailability after a fire and the increased energy it takes to degrade, respiration of CO_2 has been found to increase in fire affected soil, but there is a knowledge gap in waterbodies affected by wildfire.

1.3 Analyzing Carbon and its Flux through Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS)

DOM consists of a complex mix of chemically distinct compounds with unique properties that influence their fate within an ecosystem and can be shaped by fire through bioavailability and thermodynamic favorability (Tanentzap & Fonvielle, 2024). FT-ICR MS provides an opportunity to characterize how the molecular composition of DOM changes after a wildfire and its fate. Literature on how recalcitrant DOM is after a fire seems variable, but findings can be narrowed down into decreases in bioavailability and increases in thermodynamic favorability. DOM experiences molecular alterations, becoming more aromatic and oxidized, as noted by a lower ratio of hydrogen to carbon (H:C) atoms, a higher ratio of oxygen to carbon (O:C), and a higher aromaticity index (AI_{mod}) (Nelson et al., 2022). H:C, O:C, and AI_{mod} have been found to decrease bioavailability in streams and soils after a fire (Cao et al., 2024; Xu et al., 2024; Zhang et al., 2024). Though seeming contrary, DOM in semiarid watersheds have been found to contain simpler molecules that take less energy to break down (Crandall et al., 2021). Thermodynamic favorability is explained through metrics including the nominal oxidation state of carbon (NOSC), Gibbs free energy (GFE), which indicates the potential energy of a molecule, and putative biotransformations, which indicate potential transformations it can go through (LaRowe & Van Cappellen, 2011; Danczak et al., 2020). While DOM may be difficult to access

or less bioavailable after a fire, molecules can still provide more energy or may be more thermodynamically favorable for microbes than in unburnt areas. These are all direct measures of change in DOM composition that can only be accomplished through FT-ICR MS, a novel approach that avoids the approximation of chemical attributes. FT-ICR MS is a valuable tool in fire research that will continue to provide new insights into how DOM degrades after disturbances.

2. Thesis aims

This thesis aims to address how wildfire changes DOM concentration, composition, and fate. Applying a novel approach with FT-ICR MS to remote field stream sampling from observational and experimental studies, I address four main questions that map onto two chapters:

2.1. How does wildfire change DOM concentration (Chapter 2)?

2.2. How does wildfire change DOM composition (Chapter 2)?

2.3. Do microbes degrade DOM from streams draining burned catchments differently than unburned catchments (Chapter 3)?

2.4. Do microbes respire more CO_2 from streams of burned catchments than unburned catchments (Chapter 3)?

More specifically, in Chapter 2, I investigate the effects of wildfire on DOM concentration and composition in boreal headwater streams throughout the summer. Using a paired natural experiment with ten burned and ten unburned streams, I measured water chemistry

monthly over the summer of 2023, including via FT-ICR MS and optical spectroscopy, to determine the impacts of wildfire on DOM composition. This chapter provides a foundation for understanding wildfire's impact on headwater streams since they more closely reflect the characteristics of the watersheds from which they originate and emphasizes the need to incorporate these effects into forest carbon budgets. In Chapter 3, I explore the degradation of DOM in fire-impacted headwater streams. I measured CO_2 flux and changes in DOM concentration and composition using FT-ICR MS over 28 days through incubation experiments with water paired from five burned and five unburned streams. This chapter underscores that DOM composition influences microbial respiration and carbon cycling in fire-impacted streams. In Chapter 4, I summarize the key findings of this thesis and conclude with suggestions for future research directions.

Chapter 2

Wildfires change dissolved organic matter in boreal headwater streams

Abstract

Boreal forests export large amounts of terrestrial carbon annually into downstream waters as dissolved organic material (DOM), but how this flux is affected by increasing wildfire frequency remains unknown. If this DOM is more readily consumed and respired by microbes, its export into water can offset carbon sequestration on land. Here we investigated how wildfire changes the concentration and composition of DOM exported from boreal forests into headwater streams in northwestern Ontario, Canada over a summer growing season. We compared the composition of DOM between 10 catchments that were burned in the last four years with 10 catchments that been undisturbed for at least 20 years using optical spectroscopy and ultra-high-resolution mass spectrometry. We found a 29% increase, on average, in dissolved organic carbon concentrations in burned streams by August. Burned streams had lower H:C and higher modified aromaticity index, indicating less bioavailable DOM, likely because of the 55% more black carbon-like molecules in the burned catchments entering the streams. However, we found that these more complex compounds could release more energy for microbial degradation if they became accessible, and they were as likely to be biochemically transformed as unburned DOM during July and August. Our results show that wildfire can change both concentration and composition of DOM exported from land into receiving waters. Overall, our results suggest that wildfires may lead to greater terrestrial carbon losses than previously estimated, highlighting the need to include the impacts of wildfire on receiving waters in carbon accounting.

Keywords: Wildfire, Dissolved organic matter, boreal forest, headwater streams, Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS), carbon flux

Introduction

The movement of dissolved organic matter (DOM) from land into freshwaters is a relatively large flux in the global carbon cycle, transporting between 2.4 to 5.1 Pg C per year (Drake et al., 2017; IPCC, 2021), but there is limited understanding of how this flux is impacted by natural disturbances in surrounding catchments. This knowledge gap is especially important to address because natural disturbances like wildfires are growing in frequency and intensity (De Groot et al. 2019; Stocks et al. 1998). In the USA, wildfires have impacted between 3- to 6-times more catchments in the past 30 years (McCullough et al. 2019). In Canada, the area burned by wildfire has increased by between 75 to 120% since the 2000s and is predicted continue rising through the end of the century (Flannigan et al. 2005; Hanes et al., 2019; Parisien et al., 2023; Cunningham et al., 2024). The year 2023, in particular, was a record-breaking year in Canada for burned area, fire season length, and temperature (Jain et al., 2024). Thus, understanding how natural disturbances impact DOM export from land into water is crucial, especially as events like insect outbreaks and drought can alter DOM concentrations by many times more than background inter-annual variability (Woodman et al., 2021; Tiwari et al., 2022). Natural disturbances may also alter the composition of DOM in ways that impact its microbial and physicochemical reactivity (Hu et al., 2022b; Hu et al., 2024b), and thus its eventual fate (D'Andrilli et al., 2019). Predicting how natural disturbances impact the quantity and composition of DOM exported from land into freshwaters is therefore essential to constrain carbon budgets and wider biogeochemical cycles.

Wildfires have been found to increase DOM concentrations in streams, primarily through the accumulation of organic material from overland flow and soil leaching (Carignan et al., 2000). Studies on the effects on stream DOM concentration vary, with some estimating increases

by as much as 67% (Minshall et al., 2001; Burd et al., 2018; Loiselle et al., 2020; Richardson et al., 2024, Uzun et al., 2020) and others finding no change (Granath et al., 2021; Wampler et al., 2024). The mechanisms of these differences are not fully understood but may relate to differences in the proportion and size of the catchment affected and stream order (Stanley et al., 2016; Maavara et al., 2021; Wampler et al., 2024). Fire intensity also likely plays a role, and deposition of ash immediately around a wildfire can further increase DOM concentrations, with the magnitude of increases dependent on fire temperature that influences the oxidation and pyrolysis of organic matter (Revchuk & Suffet, 2014; Cao et al., 2023; Farruggia et al. 2024). When increases in concentration are observed, it can take at least 2 and 4 years to return to pre-fire conditions (Allen et al., 2003; Carignan et al., 2000; McCullough et al., 2019; McEachern et al., 2000; Scrimgeour et al., 2001). These potential long-term increases in DOM export suggest wildfires have effects that are large enough to impact catchment -scale carbon balances, yet little is known about the fate of this DOM.

Once dissolved in water, the fate of DOM depends partly on its molecular composition (Kothawala et al., 2021). DOM is a complex mixture of thousands of individual compounds, each with their own unique properties that influences how they interact with physicochemical and biological processes (Tanentzap & Fonvielle, 2024). After wildfire, the DOM exported from burned vegetation and soils has a higher proportion of charred pyrogenic or black carbon (BC), which is less bioavailable for microbial degradation because of its more complex structure and greater hydrophobicity (Dittmar et al. 2012). Changes to biogeochemical processing and hydrology, as well as the absence of vegetation caused by wildfire, can further shift the composition of exported soil DOM towards compounds with lower bioavailability, such as condensed aromatics and tannins (Cao et al., 2024). While these compounds may be less

reactive, they can contain more energy for microbes to metabolize. Specifically, DOM affected by wildfire often experiences molecular alterations that reflect those seen in upland soils, resulting in more aromatic and oxidized compounds, such as characterized by a lower ratio of hydrogen to carbon (H:C) atoms, higher ratio of oxygen to carbon (O:C), higher aromaticity index (AI_{mod}), and higher nominal oxidation state of carbon (NOSC) (Nelson et al., 2022). Together, these changes in both the molecular composition and properties of individual compounds after wildfire can make DOM more thermodynamically favorable for microbial transformation, such as measured by a lower Gibbs free energy (GFE) (Cao et al., 2024). However, to our knowledge, no study has characterized the potential biochemical transformations of DOM found in receiving waters impacted by wildfire. Advances in computational analyses of ultra-high-resolution mass spectrometry data now provide an opportunity to characterize how the molecular properties of DOM change with wildfire, including the number of putative biochemical transformations, offering new insights into the fate of terrestrial carbon and its potential to be respired to the atmosphere.

In addition to DOM quantity and composition, environmental conditions impacted by wildfire, such as temperature and oxygen concentrations, influence how individual compounds interact with microbes and thus the fate of DOM (Dittmar et al., 2021). For example, increasing water temperatures after wildfire because reduced canopy cover can accelerate microbial metabolism and thus the amount of DOM degradation (Hu et al., 2024; Rhoades et al., 2011; Scholz et al., 2021). Both pH and conductivity have also been found to increase in stream water after wildfire, such as from leaching of alkaline ash (Earl & Blinn, 2003; Oliver et al., 2011). In nutrient-poor or oligotrophic boreal regions where wildfires overlap with hydrologically connected systems, the degradation of DOM is often limited by nitrogen (N) availability

(Findlay, 2010; Gulis et al., 2004). Even with increased or more bioavailable DOM, microbial activity can still be constrained by N (Findlay, 2010; Gomez et al., 1999; Vitousek & Howarth, 1991). In contrast, in nutrient-rich systems (e.g. with readily available N), elevated DOM inputs may be efficiently processed by microbes, resulting in lower respiration rates but higher biomass production (Findlay, 2010). These changes can shift the functional composition of microbial communities to degrade preferentially different organic matter sources (Fitch et al., 2018; Winder et al., 2023). Finally, wildfire, by definition, oxidizes organic matter, which can lead to more oxidized compounds being exported downstream. Any subsequent increase in oxidation-reduction potential (ORP) could constrain microbial degradation of DOM (Miller et al., 2009; Williams et al., 2010), but, to our knowledge, no studies have compared ORP between streams draining land affected versus unaffected by wildfire.

Here we tested how wildfire changes DOM concentration and composition over the summer season in boreal headwater streams. Headwaters comprise most stream length on Earth (Downing et al., 2012) and are widespread across boreal forests that sequester one-third of global forest carbon (Tagesson et al., 2020), so play a key role in the wider carbon cycle due to their extensive length and unique position at the land-water interface. However, despite their importance, headwaters remain largely overlooked in terrestrial carbon accounting (Smyth et al., 2023). To determine how wildfire impacts DOM and the fate of carbon, we used a “natural” experiment to pair 10 streams from recently burned catchments with 10 streams from environmentally similar but unburned catchments. We measured water chemistry monthly from June through August, including DOM concentration and composition with Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS) and fluorescence and absorbance spectroscopy. We predicted that wildfire would generate BC and other structurally complex

molecules that persist longer in downstream waters because they are less transformed by microbes, thereby causing DOM to accumulate and concentrations to increase. Our approach can ultimately improve estimates of how forest carbon budgets are impacted by wildfire and highlights the importance of monitoring small headwater streams to account fully for the fate of forest carbon.

Methods

Study sites

We sampled 20 headwater streams around Red Lake, Ontario, Canada (51.0° N, 93.8° W; Fig. A1). Wildfire is frequent in this area with about 1.5 fires per Mha per year (Erni et al., 2019), so even sites that have not been burned recently are likely to be recovering, to some extent, from wildfire. We selected the streams by first clipping annual maps of fires >40 ha collected by the Ontario Ministry of Natural Resources and Forestry (2023) to catchments of all headwater streams in Ontario delineated by the HydroSHEDS dataset at a 15-arc-second resolution (Lehner & Grill, 2013). We identified fires that had occurred in the four years before sampling (2019 to 2023) so that the landscape and downstream export of carbon would still be impacted along (Carignan et al., 2000; McCullough et al., 2019). We excluded areas where fire had occurred more than once in the preceding 20 years (2003 to 2023). Each retained fire area also had to encompass at least 10 headwater catchments that were within 200 m of a road to ensure accessibility. After applying these criteria, only a single fire area around Red Lake that occurred in 2019 was retained. The area contained the 10 focal catchments, none of which had a prior record of fire (fires were only mapped if >200 ha from 1960 to 1998; and >40 ha

thereafter). To identify the most environmentally similar but unburned control catchments, we focused on 42,829 headwater catchments in Ontario that had not experienced fire in the previous 20 years. We calculated the Gower's distance between each potential control and each burned site for latitude and longitude as proxies of climate and management history, catchment size (Lehner & Grill, 2013), percent wetland (Ontario MNRF, n.d.) and forest cover in the catchment (*Forest Resources Inventory*, 2023), and mean slope of the catchment estimated from a 30 m digital resolution model (Ontario MNRF, 2021). We then selected the unburned catchment with the smallest distance from each burned site that was within 200 m from a road. A principal coordinate analysis with two axes estimated using R v.4.2.2 (R Core Team, 2022) estimated that the distances between paired burned and unburned catchments was generally smaller than the distances between sites in the same treatment (Fig. A2).

To select sampling locations, we identified pour points (i.e. the drainage point of each catchment) by re-delineating catchments with a high-resolution (30 m) digital resolution model (DEM) developed for hydrological applications (Ontario MNRF, 2021). Flow accumulation and flow direction were generated using the D8 algorithm, where flow entering each 30 m pixel in the DEM was only routed to a single downstream neighboring pixel, using the whitebox v.2.4 package for R (Lindsay, 2016; Wu & Brown, 2022). The final sampling locations were located as close as possible to the highest flow accumulation value in each catchment, that is, pour point. At these locations, the streams were 0.2 to 1.5 m wide and 5 to 40 cm deep with rocky and silty bottoms. Riparian areas were generally surrounded by speckled alder (*Alnus incana*) and occasionally an open grassy delta. Upland catchments contained black spruce (*Picea mariana*), jack pine (*Pinus banksiana*), balsam fir (*Abies balsamea*), quaking aspen (*Populus tremuloides*), and paper birch (*Betula papyrifera*).

Water sampling

Streams were sampled in June, July, and August 2023. We sampled in the middle of the channelized flow from the stream banks not to disturb any sediment. We first measured pH, dissolved oxygen, oxidation reduction potential (ORP), conductivity, and temperature in situ using a pre-calibrated YSI Professional Plus (YSI Inc., Ohio, U.S.A.) handheld meter fitted with YSI Pro Series 1001 pH Sensor, 1002 ORP Sensor, 2003 Polarographic Dissolved Oxygen Sensor, and 5560 Temperature/Conductivity sensor. We then immediately passed water from the central flow of each stream through 0.45µm glass fiber filters (EZFlow HP Syringe Filters, Foxx Life Sciences, New Hampshire, U.S.A.) into three separate pre-combusted (450 °C for 4 hrs) 40 mL amber glass vials with no headspace. Filters were pre-combusted and pre-rinsed with 40 mL of stream water prior to sample collection. Two of the vials were prefilled with 50 µL of 30% H_2SO_4 to acidify the water samples to pH 2 for characterization of DOM composition. The third unacidified 40 mL vial was used to measure concentrations of dissolved organic carbon (DOC) and dissolved nitrogen (DN) using a Shimadzu TOC-L with TNM-L analyzer (Shimadzu Corp, Japan) within 14 days of sampling.

DOM composition

We used fluorescence and absorbance spectroscopy to characterize the samples in terms of general origin, extent of humification and aromaticity, and molecular weight. Samples were analyzed for fluorescence on a Cary Eclipse (Agilent Technologies, California, U.S.A.) with three-dimensional scans at 5 nm excitation steps from 250 to 450 nm, reading emissions at 2 nm

steps from 300 to 600 nm (with a 5 nm slit width). Absorbance was measured from 220 to 600 nm on a Varian Cary 60 UV–vis spectrophotometer (Agilent Technologies, California, U.S.A.). We then calculated the biological index (BIX), fluorescence index (FI), the humification index (HIX), specific UV absorbance (SUVA), E2:E3, and the spectral slope ratio (S_R). High BIX and FI are indices of autochthonous biological activity (Huguet et al. 2009; Cory et al. 2010), while high HIX indicates organic matter is more processed by microbes (Ohno, 2002). Larger SUVA values indicate greater humification and aromaticity (Weishaar et al. 2003), and increased E2:E3 and decreased S_R indicate high molecular weight (Helms et al., 2008; Peuravuori & Pihlaja 1997). All calculations were performed using the stardom v.1.1.28 package in R (Pucher et al., 2019).

We characterized the molecular composition of DOM using FT-ICR MS. First, DOM was extracted from each sample using cartridges filled with a styrene-divinylbenzene copolymer sorbent (1 g Bond Elut PPL cartridges, Agilent Technologies, U.S.A.) following an established protocol (Dittmar et al, 2008). The cartridges were filled with HPLC-grade methanol the night before extraction to soak and sequentially rinsed with one volume of ultrapure water, one volume of methanol, and one volume of ultrapure water acidified with HCl to pH 2. Then, we loaded 0.5 mg of carbon onto each cartridge and rinsed them with one volume of acidified ultrapure water to remove salts. We removed the excess water by drying cartridges under a constant flow of nitrogen gas for ~30 minutes. Finally, we passed 4 mL of methanol through each cartridge to elute the DOM into pre-combusted amber vials. We calculated the concentration in the extract by measuring diluted samples on the Shimadzu TOC-L analyzer and found we recovered a mean ± standard error of 34.0% ± 11.5% of the DOM we loaded onto the cartridge in 6 representative samples. We diluted each extract to a final concentration of 5 mg C L^{-1} in 1:1 methanol:water

before injecting them in a solariX 7 Tesla FT-ICR MS (Bruker Daltonics, Germany). The system was equipped with an electrospray ionization source (ESI, Bruker Apollo II) and set to negative mode. Each sample was injected at a rate of 120 uL h^{-1} and processed twice. For each measurement, we collected 250 transients, and we let ions accumulate for 0.25 s. We calibrated the instrument using a mixture of 0.1 mg mL^{-1} NaTFA in methanol. We processed acidified ultrapure water stored in bottles as samples as a control and a Suwanee River natural organic matter standard (International Humic Substances Society, batch number 2R101N) alongside all extractions.

Masses ranging from 100 to 1000 m/z were exported from the Bruker Data Analysis software, and we assigned molecular formulae using the online formula assignment and analysis tool ICBM-OCEAN (freely available at https://rhea.icbm.uni-oldenburg.de/geomol/; Merder et al., 2020). Briefly, we applied a method detection limit of 2, combined peaks within 0.5 ppm, recalibrated spectra using general additive models, and limited formula attributions to $C_{0–100}$, $H_{2–200}$, O_{0-70}, $N_{0–4}$, $S_{0–1}$, $P_{0–1}$ with a tolerance of 1 ppm (Merder et al., 2020). Only peaks detected in both duplicate measurements were retained. We normalized intensities of the peaks with an assigned molecular formula to the sum of all peak intensities in a sample. We further classified formulae into tentative compound classes after Kim et al. (2003) as lipid-like (O:C = 0–0.3, H:C = 1.5–2.0), amino sugar-like (O:C = 0.3–0.67, H:C = 1.5–2.2), carbohydrate-like (O:C = 0.67–1.2, H:C = 1.5–2), unsaturated hydrocarbon-like (O:C = 0–0.1, H:C = 0.7–1.5), lignin-like (O:C = 0.1–0.67, H:C = 0.7–1.5), tannin-like (O:C = 0.67–1.2, H:C = 0.5–1.5), and condensed aromatic-like (O:C = 0–0.67, H:C = 0.2–0.7).

We calculated 8 metrics from the assigned molecular formulae to compare the molecular weight, bioavailability, and thermodynamic favorability of DOM between treatments. First, we

calculated the molecular weight of each sample from the intensity-weighted mass to charge ratio (*m*/*z*). The intensity-weighting was calculated as the sum of the product of each formula's *m*/*z* and its normalized intensity, thereby accounting for differences in evenness in the prevalence of molecular formulae. Additionally, we calculated the intensity-weighted H:C and O:C ratios and AI_{mod} as measures of bioavailability, and GFE and NOSC as complementary measures of the thermodynamic favorability of DOM. Lower H:C, higher O:C, and higher AI_{mod} tend to indicate more aromatic, and highly oxidized molecules that are less bioavailable (D'Andrilli et al., 2023). Lower NOSC indicates molecules that release less potential energy during oxidation, while higher GFE suggests molecules are less thermodynamically favorable for microbial degradation (LaRowe & Van Cappellen 2011). All metrics were calculated using the function "compound_calcs" in the ftmsRanalysis v.1.0 package after Bramer et al. (2020) and LaRowe & Van Cappellen (2011). We also summed the relative intensity of all black carbon-like molecules (BC) per sample, defined as those with $AI_{mod} > 0.66$ (Bao et al., 2023; Koch and Dittmar 2006; Merder et al. 2020; Seidel et al. 2015). Finally, we estimated the number of putative biochemical transformations in each sample using the approach described by Danczak et al. (2020). A list of 1255 pairs of molecular formulae associated with commonly observed biotic and abiotic transformations, such as enzymatic reactions that add or subtract CH_2 groups and physical processes like condensation or dehydration (Breitling et al. 2006), was compared with our samples. The total number of pairs from this list detected in our sample was used to estimate the number of transformations per sample. As the number of transformations can increase simply because more formulae are present, we normalized this value by the number of molecular formulae in each sample.

Statistical analyses

We tested the effect of wildfire on stream biogeochemistry using mixed effects models. For each response variable, including water chemistry and DOM metrics, we estimated the effects of wildfire (burned vs unburned) and sampling month as fixed effects, and allowed for a statistical interaction between these, such as if differences between burned and unburned catchments only arose at certain times of year. We accounted for repeated measurements of the same stream by including catchment identity as a random effect. The models were fitted using restricted maximum-likelihood with the lmer function from the lme4 package v.1.1-35.5 in R (Bates et al., 2015). Only conductivity was not normally distributed and needed to be log transformed before analysis. We also refitted all models including wetland area, testing for improved model fit with Akaike information criterion (AIC) values (Table A2). The wetland area was calculated by intersecting the Ontario Ministry of Natural Resources and Forestry Wetlands dataset (2024) at a spatial resolution of 30 m with each delineated catchment. We also compared model fit (with AIC) where we replaced the binary burned/unburned predictor with a continuous measure of wildfire severity. Burn severity was derived using Landsat 5, 7, 8, and 9 imageries from Collection 2 Level-2 Surface Reflectance Tier 1 (EROS, 2020). Reflectance was averaged across all Landsat pixels in a catchment across a composite of all valid growing season imagery pre-fire in 2018 and immediately post-fire in 2019 using the script provided by Parks et al. (2018). Differences in reflectance between these years were used to calculate the relativized burn ratio based on the composite hybrid method described by Holsinger et al. (2021).

We also compared the molecular properties of formulae that were uniquely detected in the burned versus unburned catchments. We assumed that compounds unique to burned sites were direct outcomes of wildfire whereas those unique to unburned sites were no longer

produced after wildfire. For each month, we used modified $\chi 2$ tests of independence called G-tests to identify molecular formulae unique to burned and unburned sites. G-tests are more suited than $\chi 2$ tests for datasets with a small number of samples, such as ours (Bramer & White 2023). The analysis was implemented with the function "uniqueness_gtest" in the ftmsRanalysis R package. We then compared whether the mean H:C and O:C ratios of molecular formulae unique to burned sites differed from those in unburned sites in each month using t-tests.

Results

Wildfire increases headwater DOC concentrations

We found that burned streams had higher DOC concentrations during August, concurrent with more acidic and reduced conditions that suggests greater runoff through soil layers. The estimated mean ± standard error for DOC concentrations in burned streams of 45.1 ± 3.63 mg L^{-1} was 29% higher than 32.2 ± 3.63 mg L^{-1} in unburned streams by the end of the summer ($t = 4.70$, $df = 36.0$, $p < 0.001$; Fig. 1a). Both pH and conductivity were also an estimated 0.79 ± 0.34 units ($t = 2.35$, $df = 21.0$, $p = 0.029$) and 0.68 ± 0.28 µs cm^{-1} ($t = 2.40$, $df = 18.6$, $p = 0.027$) lower in burned than unburned streams in August, respectively (Fig. 1b, c), with a resulting decrease in ORP by 99.8 ± 28.76 mV ($t = 3.47$, $df = 53.5$, $p = 0.001$; Fig. 1d, e). Throughout the summer, burned streams were an estimated 3.53 ± 1.11 °C warmer ($t = 3.19$, $df = 34.9$, $p = 0.003$), likely from the loss of riparian shading, but there was no difference in dissolved nitrogen concentration between treatments ($t = 0.461$, $df = 21.7$, $p = 0.650$; Fig. 1f). Models that replaced the binary categorization of burned and unburned catchments with a continuous remotely sensed measure of

wildfire severity and models with the binary burn categories that considered continuous variation in surrounding wetland area were not better supported (Table A2).

Burned streams had unique and more persistent molecular formulae

Burned and unburned streams were dominated by plant-derived organic matter. In total, we assigned 11,285 unique molecular formulae across samples, with a mean ± standard error (SE) of 5194 ± 1647 per sample in burned and 4997 ± 1525 in unburned sites (t = -0.479, df = 58.0, p-value = 0.634). Across all sites and months, lignin-like compounds predominated, accounting for 55.0% and 56.3% of all formulae within burned and unburned sites, respectively (Fig. A3). Tannin-like (representing phenol derivatives) and condensed hydrocarbon-like compounds constituted the subsequent most abundant classes, accounting for 15.6% and 13.8% of all formulae in burned sites, respectively, and 15.4%, and 12.2% of all formulae in unburned sites, respectively (Fig. A3).

Despite similar bulk composition between treatments, DOM in burned streams was generally less bioavailable and more thermodynamically favorable for microbial degradation. The intensity-weighted H:C ratio was a mean of 2.2% lower in burned than unburned sites across all months with a mean ± SE of 1.05 ± 0.01 versus 1.07 ± 0.01, respectively (t = 2.07, df = 54.0, p = 0.043; Fig. 2a). As the intensity-weighted O:C ratio did not differ between treatments (t = -1.33, df = 54.0, p = 0. 191; Fig. A4), the difference in H:C ratios could have arisen from the higher proportion of aromatic unsaturated compounds in burned sites (Fig. A3). In support of this interpretation, DOM in burned sites had higher values of the modified aromaticity index across all months by a mean of 5.7% (t = 3.31, df = 54.0, p = 0.002) and contained 55.3% (t = 2.70, df =

53.5, p = 0.009) more formulae that could be classed as BC (Fig. 2b, c). Consequently, we estimated that there were 8% fewer, on average, biochemical transformations for each molecular formula in the burned as compared with unburned sites (t = -2.40, df = 54.0, p = 0.012; Fig. 2d), but this difference disappeared by July and August potentially as DOM was transformed in unburned sites (t = -1.80, df = 54.0, p = 0.078 and t = 1.62, df = 54.0, p = 0.111, respectively). As expected given the shift towards aromatic and highly oxidized compounds, DOM was also more thermodynamically favorable for microbial degradation in burned streams in July as indicated by a greater NOSC and lower GFE (t = -2.30, df = 54.0, p = 0.025 and t = 2.30, df = 54.0, p = 0.025, respectively). NOSC was 60.4% higher and GFE was 2.4% lower, on average, in burned versus unburned streams (Fig. 2e, f). Together, these results indicate that DOM in burned catchments may remain reactive, especially if microbes can access and degrade more complex but energetically favorable compounds (Fig. 2).

The optical data generally supported the differences in molecular composition identified using FT-ICR MS. BIX and FI were, on average, 10.1% (t = 2.35, df = 35.5, p = 0.024) and 5.8% (t= 2.20, df = 42.7, p = 0.033) lower in burned streams in July, respectively, indicating less autochthonous DOM production that is typically more bioavailable (Fig. 3a, b). Across all months, the E2:E3 ratio was also 6.5% lower, on average, in burned streams (t = -2.38, df = 22.2, p = 0.026), as expected if molecules were larger and more aromatic when DOM was highly processed (Fig. 3c). Other optical properties did not differ between sites (Fig. 3d, e, f).

Individual compounds unique to burned DOM appeared less bioavailable than those in unburned sites and reflected the accumulation of aromatic unsaturated compounds. We discovered that 435 molecular formulae across all months were exclusive to burned streams, that is, absent from unburned streams, and 209 were exclusive to unburned streams. These unique

molecular formulae varied in number and composition by month. In June, molecular formulae unique to burned streams had a lower mean H:C ratio by a mean (95% confidence interval) of 0.16 (0.07 to 0.25) and lower O:C ratio by 0.06 (0.01 to 0.12) compared to formulae unique to unburned streams, indicative of their reduced bioavailability ($t = 3.43$, $df = 1166$, $p < 0.001$ and $t = 2.18$, $df = 1166$, $p = 0.029$, respectively; Fig. 4a). In July, molecular formulae unique to burned streams still had a lower H:C ratio by 0.32 (0.25 to 0.38) but higher O:C ratio by 0.12 (0.16 to 0.07), suggesting accumulation of BC ($t = 9.31$, $df = 1026$, $p < 0.001$ and $t = -5.12$, $df = 1026$, $p < 0.001$, respectively; Fig. 4b). Similarly, in August, molecular formulae unique to burned streams had a lower H:C ratio by 0.25 (0.19 to 0.31) and higher O:C ratio by 0.10 (0.14 to 0.06) ($t = 8.09$, $df = 477$, $p < 0.001$ and $t = -4.62$, $df = 477$, $p < 0.001$, respectively; Fig. 4c).

Discussion

Here, we found that streams draining recently burned catchments had greater concentrations and complexity of DOM, consistent with our predictions of physicochemical changes to landscapes after wildfire. These changes resulted in DOM that was more energetically rewarding for microbes but more difficult for them to access, thereby challenging the widely accepted paradigm that BC will persist as a long-term sink of atmospheric CO_2 because it is intrinsically recalcitrant (Bowring et al., 2022; Coppola et al., 2018). Instead, our study offers a new and nuanced understanding of the potential persistence of wildfire-derived DOM by suggesting that it depends on environmental conditions that influence microbial activity (Dittmar et al., 2021). Specifically, our results indicate that the release of energetic constraints, such as through increasing concentrations of oxidizing agents and limiting nutrients, may enable microbes to access otherwise complex and refractory compounds, ultimately increasing the

carbon emissions associated with wildfire. These findings provide a mechanism to explain recent observations from isotopic data that BC in DOM is actually continuously degraded and respired in freshwaters (Qi et al., 2020) and add to evidence of its photochemical lability (Ward et al., 2018). Given the ca. 18 Tg C exported annually from land due to biomass charring (Jones et al., 2020), models of its fate must consider the environmental conditions into which it is released.

Increased DOM concentrations and other physicochemical changes after wildfire (e.g. reduced conductivity, lower pH, warmer temperatures) can be explained by burning of surrounding vegetation. Loss of vegetation from fire can increase overland flow and result in higher DOM concentrations in streams (Freeman et al., 2023; Cao et al., 2024). Likewise, fire deepens groundwater flow paths by removing canopy vegetation and roots and creating channels in soils that collectively promote deeper water flow (Cao et al., 2024). Water passing through more soil is consequently able to accumulate more DOM before entering streams (Sebestyen et al., 2008; Seibert et al., 2009; Burd et al., 2018; Gommet et al., 2022). Decreased conductivity, which can reflect greater resistance to flow, further supports a deepening of flow paths through more compact and smaller pore sized soils that can also elevate DOM concentration (Burd et al., 2018, Cao et al., 2024). The additional inputs of organic acids and anions from overland flow and leaching associated with the higher DOM concentrations could also explain why we observed lower pH in burned catchments (McEachern et al. 2000). Finally, loss of riparian shading can explain the warmer temperatures that we observed (Moore et al., 2005; Dunham et al., 2007; Rhoades et al., 2011). While some previous studies have found no effect of wildfire on downstream DOM concentrations (Rodríguez-Cardona et al., 2020; Granath et al., 2021; Wampler et al., 2024), we could have detected an effect because we sampled headwater streams. In headwater catchments, a greater proportion of the draining land area is impacted by fire than

higher order streams where a smaller proportion of the land is likely to be burned (Wampler et al., 2024). Given the large number of headwater streams that are expected to occur in boreal forests with frequent wildfires, large changes to their physicochemical conditions can have widespread impacts on stream ecology and water supply at regional scales (Williams et al., 2022).

The molecular composition of DOM in headwaters draining recently burned catchments was consistent with the effects of pyrolysis and altered flow paths. As expected in the burned streams, we found more BC and higher AI_{mod}, both of which can arise from the formation of lignin-derived aromatic structures (Dittmar et al., 2012; Jones et al., 2020; Zhang et al., 2024). DOM from burned streams can also appear highly aromatic and heat-altered because vegetation and soil microbiome loss results in fewer opportunities for degradation of these molecules (Cao et al., 2024; Xu et al., 2024). By contrast, we suspect O:C ratios did not differ between treatments because the burn severity was too low for pyrolysis to consume many oxygen-containing functional groups despite being impactful enough to change DOM concentration and other metrics (Sun et al., 2007; Flanagan et al., 2020; Zhang et al., 2024; Hu et al., 2024a). In all sites, our estimates of burn severity could be categorized as medium impact for Canadian boreal forests (Holsinger et al. 2021). Partial combustion and oxidation can also transform organic matter into more oxygenated compounds that are more energetically favorable for microbial degradation, such as with higher NOSC and GFE, without changing O:C ratios (Lin et al., 2020; Xu et al., 2024). For example, the addition of peroxides or carbonyls during partial oxidation, or the contributions of heteroatoms, such as involving nitrogen from plant biomass, could potentially increase NOSC without changing O:C. However, more energetic molecules can remain inaccessible, such as because of more reduced conditions that limit microbial activity,

consistent with the lower ORP that we observed. Consequently, we found small differences in the likelihood that DOM was transformed between burned and unburned sites, despite a clear signature of wildfire on the molecular composition of DOM.

Although wetland area—standardized by catchment area—was included in the linear models, the AIC showed that its inclusion was not supported (Table A1). This result suggests that fire impacts occur regardless of the wetland area in our sites. Wetland cover varied from 14.0-57.7% in our study catchments, but where wetlands are more abundant, they may mitigate fire impacts by trapping sediment and regulating excess overland flow (Richardson, 2011). Furthermore, estimating the effects of cryptic wetlands, measured through digital elevation and flow models, could strengthen our conclusions.

Our results suggest that the impacts of wildfire on forest carbon stocks are likely underestimated because wildfire promotes the fluvial export of forest carbon for at least four years, and this carbon can remain reactive. To assess the magnitude of these effects, we multiplied the reported increase in DOM concentration by estimated stream flow. Stream flow was estimated using runoff ratios (range: 0.00 to 0.41) measured in 16 nearby headwater catchments between 2019 to 2021 (Harrow-Lyle et al., 2023). The catchments had not been burned in at least 20 years, so these ratios are highly conservative given that wildfire is known to increase surface runoff (Harrow-Lyle et al., 2023; Noske et al., 2016; Spence et al., 2020). To derive the volume of water exported per unit area, the runoff ratios were multiplied by total precipitation recorded at a regional meteorological station during August 2023 (Dryden, Ontario: 49.83° N, 92.74° W). We then multiplied the volumes by the increase in DOM concentration observed in burned catchments. These calculations estimated that a mean of 0.07 g C m^{-2} more carbon was exported from burned catchments in August, with a standard error of 0.08 g C m^{-2}

from propagating uncertainty in mean runoff ratios and the mean increase in DOM concentrations. Although this value is small compared to the annual net primary productivity 60 g C m^{-2} for typical boreal forests (Gower et al., 1997), wildfire impacted 15 Mha of Canada in 2023 (Jain et al., 2024). Thus, our estimates can accumulate at a national scale with implications for carbon reporting. Our estimates are also four-years post-wildfire, so are expected to be larger immediately after fire. Overall, our comparison highlights the need to incorporate carbon losses from other forest types into carbon budgets and quantify the likelihood of this carbon being returned to the atmosphere.

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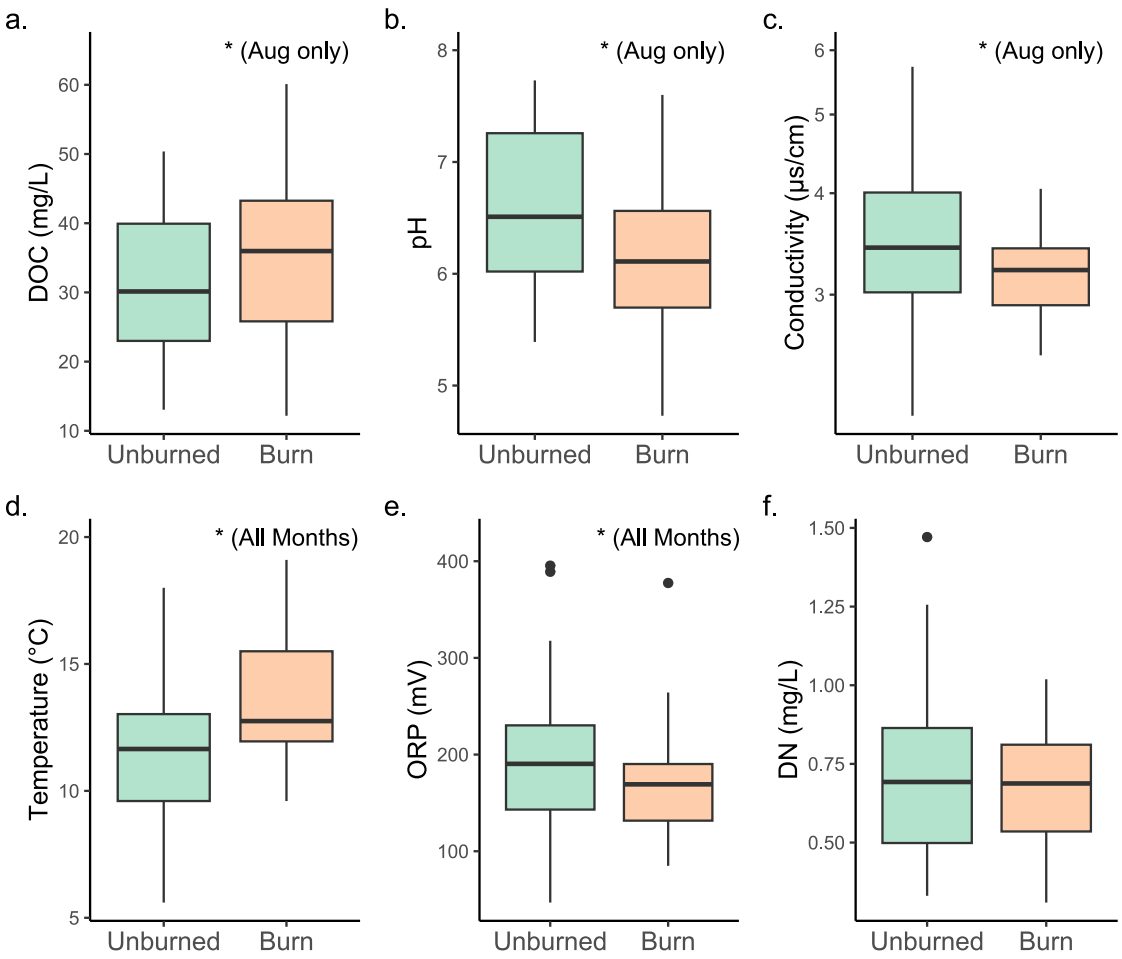

Fig. 1. Burned streams had higher DOC concentrations and distinct water chemistry. We compared: a. dissolved organic carbon (DOC) concentration, b. pH, c. conductivity, d. water temperature, e. oxidation-reduction potential (ORP), and f. dissolved nitrogen concentrations between streams draining unburned control catchments (n=10, green) and burned catchments (n=10, orange) in June, July, and August 2023. Boxes display interquartile range with whiskers extending to minimum and maximum values and outliers denoted by black circles. Medians are denoted by horizontal lines. Asterisks indicate statistically significant differences between treatments ($p < 0.05$) according to linear mixed effects models either across all months or August (Aug) only.

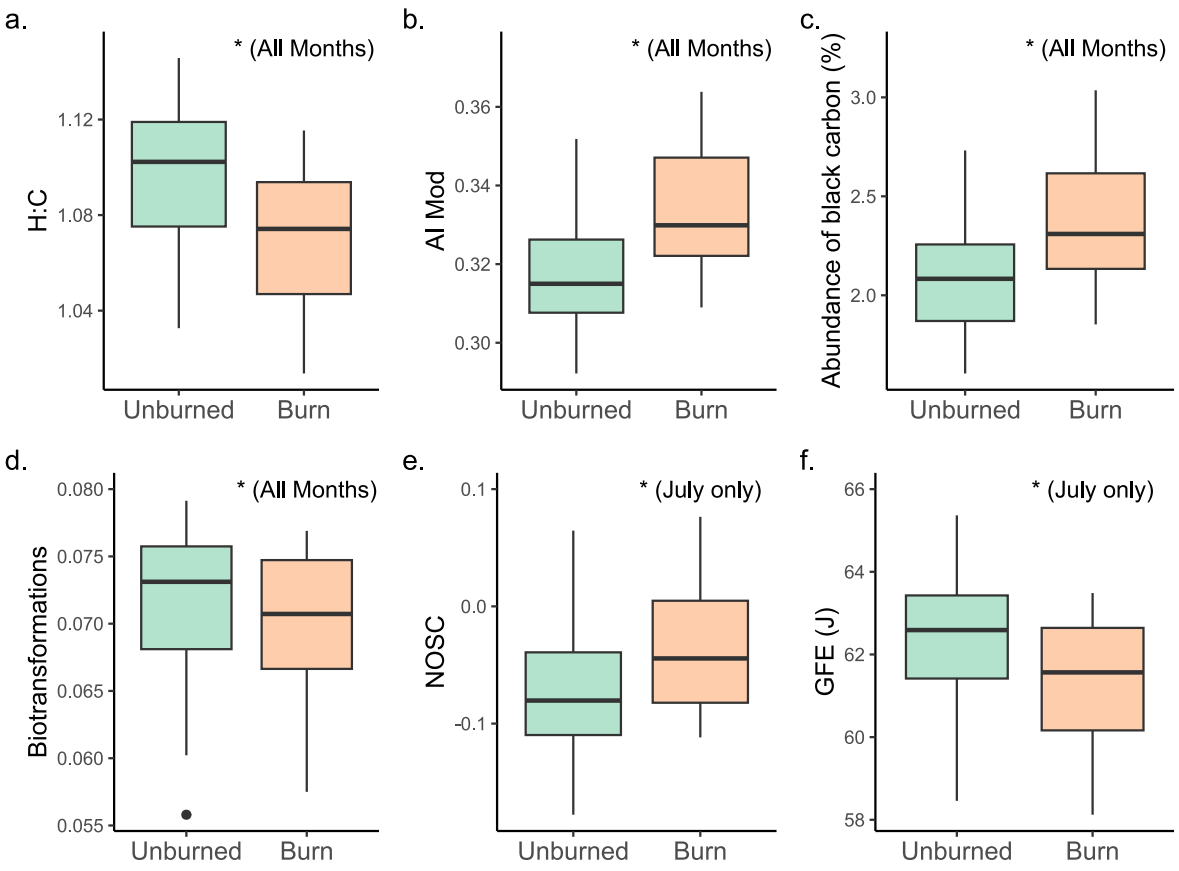

Fig. 2. Burned streams had less bioavailable but more thermodynamically favorable DOM. For each sample, we calculated the: a. intensity-weighted H:C ratio, b. intensity-weighted modified aromaticity index (AI Mod), c. summed relative intensity of black carbon-like molecules, d. number of putative transformations per formula, e. intensity-weighted nominal oxidation state of carbon (NOSC), and f. intensity-weighted Gibbs free energy (GFE, J). We compared values between streams draining unburned control catchments (n=10, green) and burned catchments (n=10, orange) in June, July, and August 2023. Boxes display interquartile range with whiskers extending to minimum and maximum values. Medians are denoted by horizontal lines. Asterisks indicate statistically significant differences ($p < 0.05$) between treatments according to linear mixed effects models either across all months, July only, or only June and July.

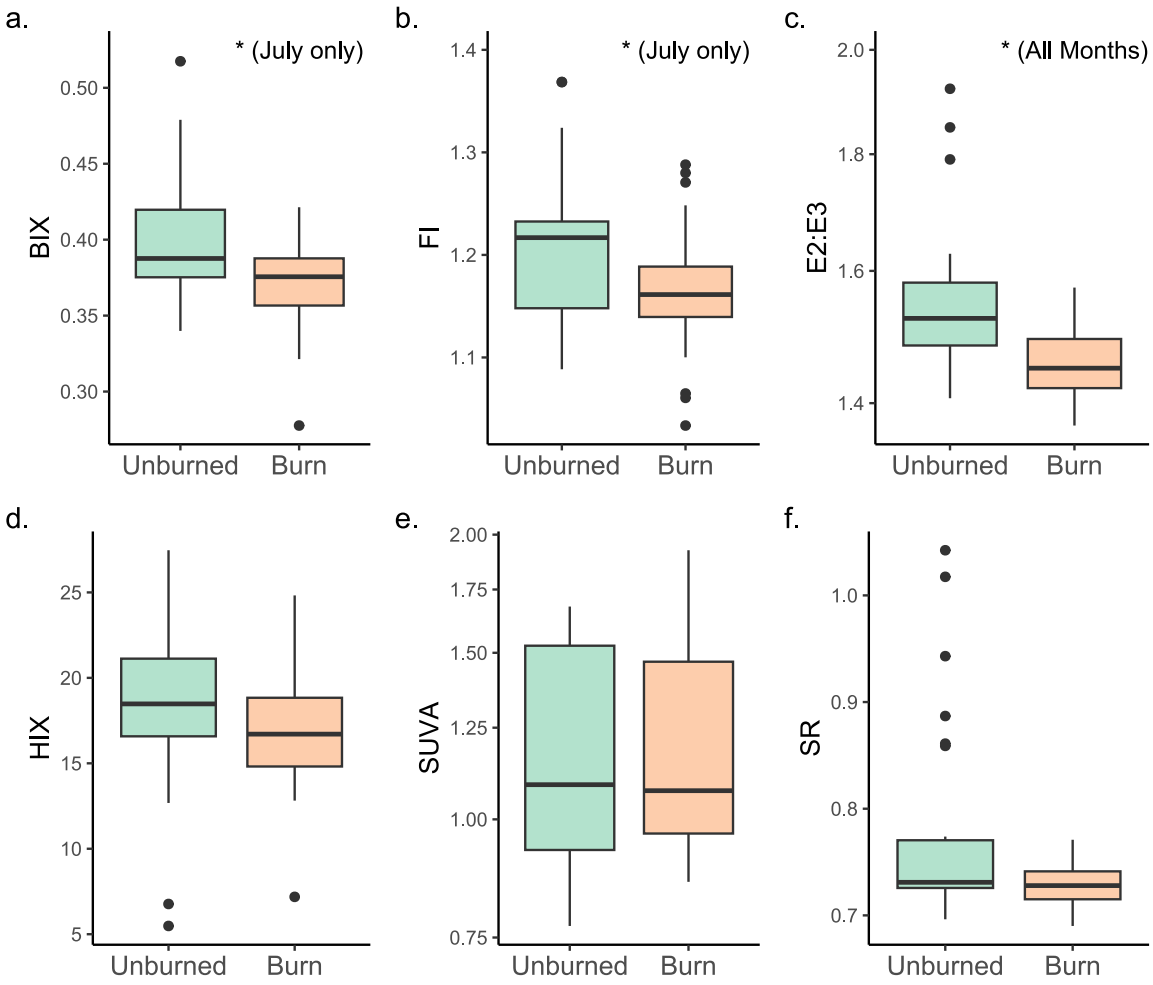

Fig. 3. Burned streams had larger and more aromatic DOM with less autochthonous production. We compared the: a. biological fluorescence index (BIX), b. fluorescence index (FI), c. E2:E3 ratio, d. humification index (HIX), e. specific ultraviolet absorbance (SUVA), f. and spectral slope ratio (SR) between streams draining unburned control catchments (n=10, green) and burned catchments (n=10, orange) in June, July, and August 2023. Boxes display interquartile range with whiskers extending to minimum and maximum values and outliers denoted by black circles. Medians are denoted by horizontal lines. Asterisks indicate statistically significant differences ($p < 0.05$) between treatments according to linear mixed effects models either across all months or July only.

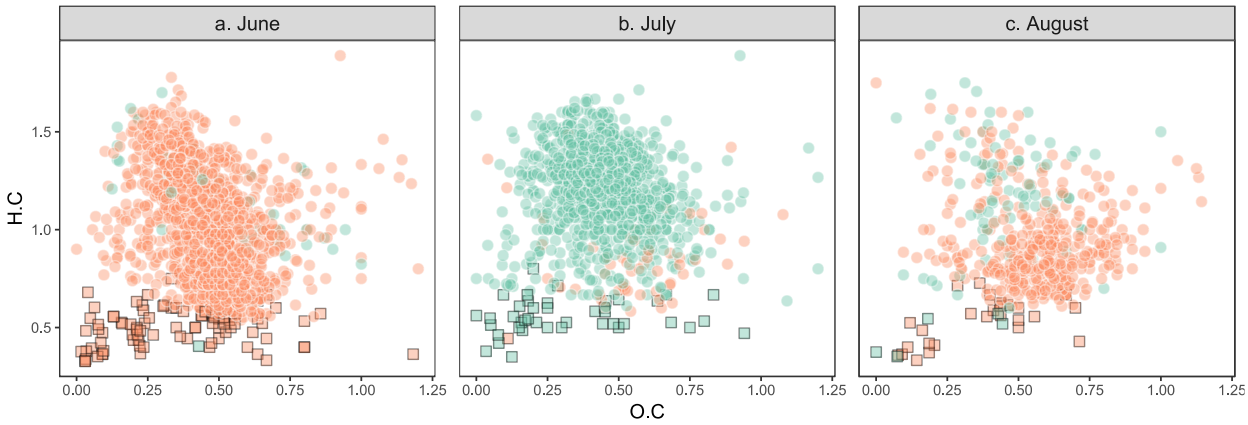

Fig. 4. Molecular formulae unique to burned streams were less bioavailable, i.e., more unsaturated and oxidized. Points are elemental ratios of molecular formulae uniquely detected in a. June, b. July, and c. August in burned (orange, n = 57 to 1,127 per month) and unburned (green, n = 41 to 971 per month) sites. Squares are black carbon-like formulae based on AI_{mod}.

Chapter 3

From burn to bioincubation: Boreal wildfire changes how headwater stream DOM degrades

Abstract

Wildfires are increasingly frequent disturbances that alter the carbon cycle by changing the composition and bioavailability of dissolved organic matter (DOM). Boreal headwater streams transport terrestrial carbon to aquatic ecosystems and are particularly sensitive to these changes. We investigated how wildfire affects DOM degradation in 10 recently burned and 10 unburned catchments near Red Lake, Ontario, Canada. Using a 28-day bioincubation experiment with nutrient addition treatments, we monitored DOM concentration and composition changes using Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Results showed that DOM concentrations decreased more rapidly in burned streams than in unburned, accompanied by elevated CO_2 production, suggesting enhanced microbial respiration. Nutrient addition did not increase CO_2 production, indicating microbes in burned streams respired recalcitrant DOM due to the extra energy it takes to degrade. Changes in DOM composition, including lower H:C and higher aromaticity, underscore a shift towards less bioavailable carbon after wildfire but still mostly able to be degraded by microbes. These findings highlight the importance of accounting for long-term wildfire-induced DOM dynamics in carbon flux models and provide insights into how climate-driven increases in fire frequency amplify carbon cycling feedback.

Keywords: Wildfire, Dissolved organic matter, boreal forest, headwater streams, Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS), carbon flux, nutrients

Introduction

Terrestrial disturbances, such as wildfires, alter the quantity and composition of dissolved organic matter (DOM) that moves from land into aquatic systems, with large implications for the global climate. Between 2.4 to 5.1 Pg C flows from land into freshwaters each year (Drake et al., 2017; IPCC, 2021; Regnier et al., 2013). Disturbances, such as insect defoliation outbreaks and drought, can cause DOM concentrations to increase due to enhanced leaching from soil vegetation structural damage (Chapter 2; Freeman et al., 2023; Tiwari et al., 2022; Woodman et al., 2021). These changes can also shift the chemical composition of DOM towards more aromatic and refractory compounds that resist degradation because they are less preferentially consumed by microbes (Burd et al., 2018; D'Andrilli et al., 2019; Loiselle et al., 2020). Less bioavailable DOM persists in the environment or is respired as CO_2 into the atmosphere instead of used for microbial growth, offsetting carbon (C) sequestration on land and acting as positive feedback to climate change (Guillemette et al., 2015). Because natural disturbances, such as wildfires, are increasing in frequency and intensity (Seidl et al., 2017), understanding their effects on land-water C cycling is increasingly important. For example, wildfires are projected to occur 2- to 11-times more often by the end of the century in Canada (Flannigan et al., 2005; Jones et al., 2024), which is home to boreal forests, one of the largest C sinks globally (Cunningham et al., 2024; Tagesson et al., 2020).

Wildfires can change the molecular composition of DOM with consequences for its bioavailability. Pyrolysis increases the presence of aromatic structures and produces dissolved black carbon (DBC), both of which can reduce the bioavailability of DOM (Dittmar et al., 2012; Forbes et al., 2006; Koch & Dittmar, 2006). Fire intensity also plays a role in how molecules change. Higher severity fires have been found to produce less bioavailable compounds in soils

(Knicker et al., 2012; Pellegrini & Jackson, 2020). Wildfire-induced changes to DOM bioavailability have complex implications for carbon cycling. While less bioavailable DOM can contribute to long-term carbon storage by resisting microbial degradation, an increase in bioavailable DOM downstream could amplify wildfire's effects on carbon cycling by accelerating CO_2 release via microbial respiration. This shift could transform otherwise stable carbon pools into more immediate sources of greenhouse gas emissions, potentially intensifying the role of wildfires as a climate feedback mechanism (Cory et al., 2010; Knicker et al., 2012; Mann et al., 2013; Pellegrini & Jackson, 2020). Since microbes are the link between DOM and atmospheric CO_2, we need to track how microbes use DOM over time to understand the consequences of wildfire-induced changes in molecular composition.

The chemical composition of receiving waters, particularly nutrient availability, is crucial in determining the fate of wildfire-derived DOM and its potential impact on microbial processing. Even when DOM concentrations increase or become more bioavailable, microbes can still be nitrogen (N) or phosphorus (P) limited because these nutrients are essential for their growth and metabolism (Findlay, 2010; Gomez et al., 1999; Vitousek & Howarth, 1991). In nutrient-rich systems, increased DOM inputs may be processed by microbes with low respiration and high biomass production when N and P are also abundant. However, in nutrient-poor or oligotrophic systems, such as boreal regions where wildfire intersects with large areas of freshwater, the breakdown of DOM is constrained by N and P limitation (Findlay, 2010; Gulis et al., 2004). If microbial degradation is limited, DBC and other less bioavailable DOM could persist longer in the ecosystem, disrupting carbon cycling and nutrient availability (Crandall et al., 2021). Global changes, such as cultural eutrophication and atmospheric N deposition, are

also likely to interact with future increases in wildfire frequency, but the combined effects of these processes on freshwater DOM remain uncertain (Heindel et al., 2022).

The primary objective of this study was to test how wildfire impacts the degradation of DOM composition in headwater streams. We incubated water from five streams draining recently burned catchments alongside water from five streams draining environmentally similar but unburned catchments. We measured changes in DOM concentration and composition with Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS) and CO_2 concentrations over the 28-day incubation period. We expected DOM concentrations to decrease while CO_2 concentrations would increase drastically in burned streams over the incubation period as microbes consumed and respired C. To test the effects of nutrient limitation, we replicated our experiment but added KNO_3 and Na_3PO_4 to supplement the oligotrophic systems. We expected that nutrient addition would increase microbial activity, as noted by more pronounced decreases in DOM concentration and increases in CO_2 concentration capacity. In both experiments, we expected that structurally complex and less bioavailable DOM would persist due to its lower thermodynamic favorability for microbial degradation. Our experiment highlights the importance of the molecular composition of DOM for determining the fate of carbon in burned catchments.

Methods

Field sites

We sampled 10 headwater streams around Red Lake, Ontario, Canada (51.0146° N, 93.8289° W; Fig. B1). These were 10 out of the 20 sites from Chapter 2. Refer to Chapter 2 for

initial site selection. To avoid samples degrading over the time it takes to sample sites and return to the lab, we selected these 10 because they were the most accessible sites based on proximity to a road and terrain to save time. At these locations, the streams were 0.2 to 1.5 m wide and 5 to 40 cm deep with rocky and silty streambeds. Riparian areas were generally surrounded by speckled alder (*Alnus incana*) and occasionally an open grassy delta. Upland catchments contained black spruce (*Picea mariana*), jack pine (*Pinus banksiana*), balsam fir (*Abies balsamea*), quaking aspen (*Populus tremuloides*), and paper birch (*Betula papyrifera*).

Water sampling

Streams were sampled in July 2024. We collected 500 mL of water from the central flow of each stream into duplicate high-density polyethylene bottles. We then immediately passed water from the central flow of each stream through 0.45µm glass fiber filters (EZFlow HP Syringe Filters, Foxx Life Sciences, New Hampshire, U.S.A.) into a pre-combusted (450 °C for 4 hrs) 40 mL amber glass vial with no headspace using a 60 mL syringe rinsed with stream water. Filters were pre-rinsed with 40 mL of stream water before sample collection. One vial for each site was prefilled with 50 µL of 30% H_2SO_4 to acidify the water samples to pH 2 and stored with ice packs until refrigerated. The acidified vial was used to measure concentrations of dissolved organic carbon (DOC), and dissolved nitrogen (DN) using a Shimadzu TOC-L with TNM-L analyzer (Shimadzu Corp, Japan). In the lab, the 500 mL bottles were poured through a 10 µm filter (Alpha Cotton Cellulous, Fisherbrand Q8) for the bioincubation experiment. 10 mL of the sample was put into a clean conical tube for phosphorus analysis (method 10210 with the Ultra Low Range Modification) on a Hach DR 3900 analyzer (Great Lakes Forestry Centre).

Bioincubations

To measure changes in gas flux and DOM composition, we incubated water for 28 days, in the dark, at 20°C (Fellman et al., 2010; Spencer et al., 2015; Vonk et al., 2013). Three replicate bottles per site were pre-combusted (450 °C for 4 hrs) 100 mL glass vials, filled with 70 mL of stream water that was filtered through a 10 µm cotton cellulose filter (Alpha Cotton Cellulous, Fisherbrand Q8) from the bulk 500 mL collection bottle. To test the effects of nutrient limitation, we spiked three additional replicates per stream from the same initial collection bulk 500 mL bottles with KNO_3 and Na_3PO_4 at twice the Redfield ratio in the corresponding stream based on measured DOC concentrations, as performed in bioincubation studies to mitigate nutrient limitations (Mann et al., 2015; Textor et al., 2019; Table B1). We used sample blanks to check for contamination. For blanks, we processed acidified ultrapure water that was stored in the same type of bottles as all samples and incubated three replicates with the nutrient addition and three replicates without. After filling each bottle with sample, we sealed each gastight with Teflon-lined stoppers and crimp seals.

We sampled the bottles four times throughout the incubation. On day 0 of the experiment, we transferred 40 mL of water filtered with 0.45µm glass fiber filters from each 500 mL bottle with the original field sample into 40 mL vials for FT-ICR-MS and DOC concentrations. Each vial was prefilled with 50 µL of 30% H_2SO_4 to acidify the samples to pH 2. After 7 and 14 days, we collected 5 mL for DOC concentrations. To collect the water, we pre-cleaned 10 mL syringes and sterile needles by rinsing them three times with MilliQ water adjusted to pH 2. After, we simultaneously extracted 7.5 mL of water for sampling with the syringe and injected 7.5 mL of nitrogen gas to balance the volume. We then filtered the water through a pre-combusted 0.45 µm

glass fiber filter into pre-combusted (450 °C for 4 hours) 40 mL amber glass vials for DOC concentrations. For DOC samples, 25 µL of 30% H2SO4 was added to acidify the water to pH 2, and the samples were diluted with 15 mL of Milli-Q water to have enough volume for analysis. On day 28, in addition to the 2.5 mL optical DOM and 5 mL DOC samples, we collected a 40 mL acidified sample in pre-combusted (450 °C for 4 hours) glass bottles for FT-ICR MS analysis. All FT-ICR MS samples were filtered using a 0.45 µm glass fiber filter.

Carbon fluxes

We monitored CO_2 fluxes from the bottles by measuring headspace air. To account for changes in CO_2 due to respiration in the workspace, before sealing each bottle gastight, we took a 1 mL sample of ambient room air, which we assumed represented the initial value for each bottle. On days 7, 14, and 28, we measured CO_2 emissions by injecting 1 mL of headspace air into a QUBIT Q-S151 CO_2 monitor (Qubit systems, Kingston, Ontario) calibrated to detect gases in a 0-22000 ppm range at a 50 mL/min flow rate with nitrogen carrier gas. After each measurement, we injected 1 mL of nitrogen gas into the sealed jar to maintain atmospheric pressure. We did not account for a 1 mL N_2 dilution at the beginning of each incubation period, since it was a small amount of gas, and would have required pressure measurements that we could not measure. To accurately assess CO_2 concentrations over the incubation period and account for changes in headspace volume, we corrected CO_2 concentrations at each time point based on DOC consumption and CO_2 production. First, we calculated the DOC reduction between time points to estimate the amount of CO_2 generated during each interval. Then, we determined the total CO_2 in the headspace at the start and end of each incubation interval by multiplying the CO_2 concentration (ppm) by the headspace volume for that time point. The CO_2

produced at each interval was then calculated by taking the difference between the total CO_2 values before and after each incubation period and dividing by the current headspace volume to yield the CO_2 produced in ppm. Finally, we added this volume-corrected CO_2 production to the CO_2 concentration from the previous time point to calculate a corrected CO_2 concentration, which accounted for cumulative CO_2 development over time. Filtered and acidified samples were analyzed for DOC concentrations as described above. Biodegradable DOC (BDOC) loss was calculated as the percent difference in DOC concentration between day 0 and day 28 for each bottle.

DOM composition

We analyzed the molecular composition of DOM using FT-ICR MS. First, DOM was extracted from each sample using cartridges filled with a styrene-divinylbenzene copolymer sorbent (1 g Bond Elut PPL cartridges, Agilent Technologies, U.S.A.) following an established protocol (Dittmar et al, 2008). The cartridges were filled with HPLC-grade methanol the night before extraction to soak and sequentially rinsed with one volume of ultrapure water, one volume of methanol, and one volume of ultrapure water acidified with HCl to pH 2. Then, we loaded 0.5 mg of carbon onto each cartridge and rinsed them with one volume of acidified ultrapure water to remove salts. We removed excess water by drying cartridges under a constant flow of nitrogen gas for ~30 minutes. Finally, we passed 4 mL of methanol through the cartridge to elute the DOM into pre-combusted amber vials (450 °C for 4 hrs). We calculated the DOC in the extract by measuring diluted samples on the Shimadzu TOC-L analyzer and found we recovered a mean ± standard deviation of 34% ± 11.5 of the DOM we loaded onto the cartridge measured in 6 representative samples. We then diluted each extract to a final concentration of 5 mg C L^{-1} in 1:1

methanol: water before injecting them in a SolariX 7 Tesla FT-ICR MS (Bruker Daltonics, Germany). The system was equipped with an electrospray ionization source (ESI, Bruker Apollo II) and set to negative mode. Each sample was injected at a rate of 120 uL h^{-1} and processed twice. For each measurement, we collected 250 transients, and we let ions accumulate for 0.25 s. We calibrated the instrument using a mixture of 0.1 mg mL^{-1} NaTFA in methanol. We processed ultrapure water as a control and a Suwanee River natural organic matter standard (International Humic Substances Society, batch number 2R101N) alongside all extractions. We used sample blanks that went through the same bioincubation experiment to check for contamination and found none.

Masses ranging from 100 to 1000 m/z were exported from the Bruker Data Analysis software, and we assigned molecular formulae using the online formula assignment and analysis tool ICBM-OCEAN (freely available at https://rhea.icbm.uni-oldenburg.de/geomol/; Merder et al., 2020). Briefly, we applied a method detection limit of 2, combined peaks within 0.5 ppm, recalibrated spectra using general additive models, and limited formula attributions to $C_{0–100}$, $H_{2–200}$, O_{0-70}, $N_{0–4}$, $S_{0–1}$, $P_{0–1}$ with a tolerance of 1 ppm (Merder et al., 2020). Only peaks detected in both duplicate measurements were retained. We normalized the peak intensities of the peaks with an assigned molecular formula to the sum of peak intensities. We further classified formulae into tentative compound classes after Kim et al. (2003) as lipid-like (O:C = 0–0.3, H:C = 1.5–2.0), amino sugar-like (O:C = 0.3–0.67, H:C = 1.5–2.2), carbohydrate-like (O:C = 0.67–1.2, H:C = 1.5–2), unsaturated hydrocarbon-like (O:C = 0–0.1, H:C = 0.7–1.5), lignin-like (O:C = 0.1–0.67, H:C = 0.7–1.5), tannin-like (O:C = 0.67–1.2, H:C = 0.5–1.5), and condensed aromatic-like (O:C = 0–0.67, H:C = 0.2–0.7).

We calculated 8 metrics from the assigned molecular formulae to summarize the molecular weight, stoichiometry, and bioavailability of each DOM sample. First, we calculated the intensity-weighted mass-to-charge ratio (*m*/*z*) to test whether burned streams contained compounds with higher molecular weight. The intensity-weighting was calculated as the sum of the product of the *m*/*z* for each formula and its normalized intensity and was used to account for differences in the prevalence of molecular formulae. Further, we calculated the intensity-weighted H:C ratio, O:C ratio, Gibbs free energy (GFE), the nominal oxidation state of carbon (NOSC), and the modified aromaticity index (AI_{mod}). A low H:C ratio is associated with resistance to microbial degradation, which with GFE when elevated, indicates molecules have more energy for microbes when they are consumed. High O:C ratios indicate more oxygenated molecules similar to high NOSC, which indicates formulae that are more degraded (Nelson et al., 2022). High AI_{mod} is associated with aromatic structures within molecules that make DOM resistant to microbial degradation (Cao et al., 2024; Xu et al., 2024). Double-bond equivalent (DBE) describes the degree of unsaturation in organic molecules and is another measure of the structural complexity and aromaticity of DOM (Xu et al., 2024). We calculated each metric using the function "compound_calcs" in the ftmsRanalysis package after Koch & Dittmar (2006) and LaRowe & Van Cappellen (2011). We also estimated the summed relative intensity of all black carbon-like molecules (BC) per sample, defined as formulae with $AI_{mod} > 0.66$ (Bao et al., 2023; Koch & Dittmar 2006; Merder et al., 2020; Seidel et al., 2015).

Statistical analyses

We tested the effect of wildfire on stream biogeochemistry using mixed effects models. For BDOC, DOM, and CO_2 concentrations and each DOM composition response variable, we

estimated the effects of wildfire (burned vs unburned), day of incubation, and nutrient addition as fixed effects, and allowed for statistical interactions. BDOC and CO_2 models allowed for a three-way interaction between burned vs unburned treatments, time, and nutrient additions. DOM composition models included 2 two-way interactions between time and burn treatment and burn treatment and nutrient addition because initial DOM samples were collected before the nutrient addition. A quadratic model was applied to the CO_2 data to account for non-linear trends. We accounted for replicates of the same stream and repeated measurements of the same incubation bottle by including catchment and bottle identity as random effects. The models were fitted using restricted maximum likelihood with the lmer function from the lme4 package in R version (Bates et al., 2015). All response variables were normally distributed and there was no need to transform them. We used permutational analysis of variance (PERMANOVA) with the *adonis2* function in the R package *vegan* to compare DOM compound class composition between treatments and experiment time points.

Results

DOM concentration decreased over time and more strongly with water from burned catchments

We found that DOM concentrations decreased over the 28-day incubation, while CO_2 concentrations inversely increased, suggesting that microbial degradation drove carbon cycling in our experiment. DOM concentration declined throughout the incubation regardless of other main effects of burn and nutrient additions (time main effect: $t = -2.00$, $df = 166.61$, $p = 0.047$; Fig. 1). Neither the main effect of fire nor nutrient addition differed from unburned and ambient samples (Table B2). In burned catchments, DOM concentrations decreased marginally faster

over time (0.006 mg/L per day) compared to unburned catchments (burned × time: $t = -1.95$, $df = 166.55$, $p = 0.052$; Fig. 1). The estimated mean ± standard error (SE) for DOM concentrations in burned streams were 2.41 ± 1.19 mg/L lower across the entire sampling period where nutrients were added versus not added (burned × nutrients: $t = -2.02$, $df = 221.99$, $p = 0.044$; Fig. 1), potentially from sites being oligotrophic and needing nutrient addition to thrive because decomposition was limited by N and P (Fig. 1). Of the other interaction effects, including the interaction between time and nutrient addition and the three-way interaction between time, nutrient addition, and burn treatment, none were significantly different (Table B2).

Consistent with the declines in DOM, CO_2 production increased over the 28-day incubation ($t = 10.51$, $df = 189.00$, $p < 0.0001$), with quadratic effects indicating a slowing rate of increase at later time points ($t = -8.75$, $df = 189.00$, $p < 0.0001$; Fig. 2). Burned catchments had higher overall CO_2 concentrations in headspace than unburned sites by a mean ± SE of 1,236.0 ± 463.0 ppm after 28 days (burned main effect: $t = -3.01$, $df = 22.29$, $p = 0.007$; Fig. 2). This result could be explained by the observation that CO_2 concentrations increased at a 1.6 times faster rate in burned sites (burned × time: $t = 4.93$, $df = 189.00$, $p < 0.001$; burned × time^2: $t = -3.43$, $df = 189.00$, $p < 0.001$; Fig. 2). Nutrient addition did not increase CO_2 production and was 17% slower over time compared to ambient samples (nutrients × time: $t = -2.64$, $df = 189.02$, $p = 0.009$; nutrients × time^2: $t = 2.66$, $df = 189.02$, $p = 0.008$; Fig. 2). No other effects were statistically significant (Table B2).

Temporal changes in DOM composition reflect burn history and nutrient addition

Burned and unburned streams were dominated by plant-derived OM. In total, we assigned 6,830 molecular formulae across samples, with a mean ± SE of 3338 ± 370 per sample from burned and 3323 ± 354 from unburned sites (t = 0.028, df = 28.0, p-value = 0.978). Across all sites and incubation days, lignin-like formulae predominated, accounting for 45.5% and 45.4% of all formulae within burned and unburned sites, respectively (Fig. B2). Tannin-like (representing phenol derivatives) and condensed hydrocarbon-like formulae constituted the next most abundant classes, accounting for 25.2% and 13.8% of all formulae in burned sites, respectively, and 26.4%, and 13.3% of all formulae in unburned sites, respectively (Fig. B2). The distribution of compound classes changed over time across all treatments, as noted with increases in the prevalence of lignins and tannins and decreases in condensed hydrocarbons (F = 8.47, df = 1, p = 0.002; Fig. B2). However, there was no main effect of burn treatment or nutrient addition on compound class distribution (F = 0.23, df = 1, p = 0.837; F = 2.05, df = 1, p = 0.065) and the interactions between time × fire and nutrient addition × fire were not significant (F = 1.05, df = 1, p = 0.349; F = 1.09, df = 1, p = 0.353; Fig. B2).

Although DOM in burned streams remained relatively recalcitrant to biodegradation over the incubation, it shifted towards reflecting autochthonous DOM production from microbial processes. Both the intensity-weighted H:C ratio (t = 2.98, df = 9.19, p = 0.015) and GFE (t = 2.31, df = 8.15, p = 0.049) increased by the end of the incubation period in both bottles from burned and unburned streams by a mean ± SE of 0.001 ± 0.0004 units and 0.06 ± 0.02 J respectively (Fig. 3a, b). We found decreases across all samples in AI_{mod} by 0.001 ± 0.0002 units (t = -3.04, df = 16.01, p = 0.008), BC by 0.04 ± 0.01 % (t = -3.30, df = 16.03, p = 0.005), DBE by 0.02 ± 0.008 units (t = -2.99, df = 16.01, p = 0.009), and the NOSC by 0.002 ± 0.0009 units (t = -2.31, df = 8.15, p = 0.049), suggesting DOM was being diluted by the production of more

bioavailable compounds (Fig. 3c, d, e, f). O:C across all sites, did not change over time (Fig. 3g; Table B2). DBE was an estimated 0.83 ± 0.36 units lower overall in burned streams (t = -2.30, df = 20.37, p = 0.032; Fig. 3e). There was no difference in all other metrics with the effect of fire (Table B2).

Interactions in the models showed differences in the direction of change based on the burn treatment. In burned catchments, the effects of time on the H:C ratio were larger by a mean ± SE of 0.20 % ± 0.001 units (burned × time: t = -2.95, df = 9.18, p = 0.016; Fig. 3a) however, the change in H:C ratio in burn over time did not differ from 0 (t = -1.19, df = 8.23, p = 0.268). Similarly, compared to unburned samples, burned AI_{mod} was marginally elevated by 0.22 % ± 0.001 units (burned × time: t = 2.08, df = 16.00, p = 0.054), DBE elevated by 0.33 % ± 0.03 units (burned × time: t = 3.19, df = 16.00, p = 0.006), and the NOSC elevated by 2.22 % ± 0.004 units (burned × time: t = 3.07, df = 8.14, p = 0.015), but their slopes over the incubation period in burned samples did not differ from 0 (t = -0.09, df = 8.49, p = 0.931; t = 1.52, df = 8.42, p = 0.166; t = 2.03, df = 8.09, p = 0.077 respectively; Fig. 3c, e, f). O:C increased in burned sites over the incubation period by 0.15 % ± 0.001 units (burned × time: t = 2.81, df = 7.64, p = 0.024; Fig. 3g). Additionally, GFE increased 0.2 % ± 0.04 J slower by the end of the incubation in burned catchments compared to unburned sites (burned × time: t = -3.07, df = 8.14, p = 0.015; Fig. 3b) but the change in GFE in burn samples over time does not differ from 0 (t = -2.03, df = 8.09, p = 0.077). However, BC did not decrease any more in burned sites than in unburned sites over the incubation period (burned × time: t = 0.66, df = 16.01, p = 0.518; Fig. 3d).

Across fire history and time, nutrient addition decreased H:C by 0.05 ± 0.01 units (t = -3.16, df = 12.55, p = 0.008) and increased all of AI_{mod} by 0.03 ± 0.01 units (t = 3.67, df = 16.00, p = 0.002), DBE by 0.60 ± 0.22 units (t = 2.71, df = 16.00, p = 0.015), NOSC by 0.07 ± 0.03

units (t = 2.23, df = 11.51, p = 0.047), and GFE by 1.99 ± 0.89 J (t = -2.23, df = 11.51, p = 0.047) (Fig. 3a, b, c, e, f). There were no increases in O:C ratios or BC with nutrient additions (burned × nutrients: Table B2; Fig. 3d, g). Also, there were no significant interactions between burn and nutrient addition (Table B2).

Discussion

Contrary to expectations, we found that stream water draining recently burned catchments was still bioavailable, but aligning with what we expected, we found elevated CO_2 production compared to unburned streams (Cao et al., 2024; Graham et al., 2023). Furthermore, we found that microbial communities in these headwater streams were not limited by N and P even though sites are oligotrophic systems which may be linked to DOM bioavailability (Bechtold et al., 2012; Crandall et al., 2021; Peters et al., 1987). We see recalcitrance trends in burned DOM through increases in AI_{mod}, DBE, and the NOSC likely because microbes use recalcitrant carbon less efficiently than more bioavailable carbon and that recalcitrant carbon produces more respiration before biomass (Fitch et al., 2018; Guillemette et al., 2015). Conversely, soil CO_2 respiration has been found to decrease even three years after a fire (Michelsen et al., 2004). We could have found different results because our unburned sites have not seen wildfire in at least 20 years while the low-fire sites have an indeterminate time since the last fire in Michelsen et al. (2004). Our in-depth FT-ICR-MS approach to DOM composition reveals that C released from soils into streams in the years after a wildfire is underestimated, as it is rapidly respired into the atmosphere, bypassing long-term storage.

DOM in burned sites appears more bioavailable after an incubation period because microbial communities either change BC and recalcitrant DOM into something new or they produce other bioavailable molecules, diluting the effects of fire. We cannot determine the difference without exploring microbial communities and their growth. A similar incubation experiment with lab-made BC in soil showed a resistance to degradation by microbes (Bruun et al., 2008). Bruun et al. (2008) also found that higher pyrolysis temperatures lead to higher resistance of BC. Bioavailability increasing with increases in H:C and GFE and decreases in $AI_{mod,}$ BC, DBE, and the NOSC over the incubation period in burned sites suggest that microbes are still using it even though it is recalcitrant (Fitch et al., 2018; Graham et al., 2023). With nutrient addition associated with lower bioavailability through H:C, AI_{mod}, BC, DBE, and the NOSC changes, microbes have been primed with nutrients to degrade bioavailable DOM first, leaving recalcitrant molecules (Bruun et al., 2008; Crandall et al., 2021; Fitch et al., 2018). Microbes in burned streams continue to use less bioavailable DOM, with the key difference from unburned streams being a higher rate of CO_2 respiration. Because the molecules are more complex, it takes more energy to degrade DOM in burned streams meaning increased respiration rather than using the C for biomass efficiently (Fitch et al., 2018; Guillemette et al., 2016; Graham et al., 2023). However, nutrients did not increase CO_2 production because microbes may have more energy to use C efficiently instead of respiring it. These findings underscore the role of microbes in fire-impacted streams: while they still consume recalcitrant DOM, they may do so with altered efficiency, leading to heightened CO_2 flux, affecting how long after wildfire catchments are a source of C acting as positive feedback into climate change.

Our findings suggest that DOM from fire-affected streams is being utilized less efficiently by microbes leading to increased respiration of CO_2 even if DOM is less bioavailable

compared to its counterpart in unburned streams. If more CO_2 is emitted from streams after a fire, less C is stored in stocks on the boreal landscape, acting as positive feedback into climate change (Battin et al., 2009; Graham et al., 2023). Because C released from soil by fire into streams is more quickly respired into the atmosphere even four years later, current C accounting models need to be updated to include this prolonged effect. A faster turnover time of C into the atmosphere is more pronounced in inland waters compared to terrestrial sources and in warmer compared to colder climates (Catalán et al., 2016). This novel take on fire disturbance affecting C fluxes directly addresses the need for more field studies outlined in the 2023 Canadian Blueprint for Forest Carbon Science (Smyth et al., 2023). As wildfires intensify with climate change, their role in shifting DOM composition, particularly by decreased molecule bioavailability that microbes still use but respire more, suggests a critical need to adjust forest carbon frameworks to appropriately accommodate the influence of fire (Graham et al., 2023; Smyth et al., 2023). To gain a deeper understanding of how microbial communities interact with pyrogenic DOM over time and how it transforms, we must focus on temporal studies and incubation experiments. Microbial growth, C use efficiency, and assays are the next steps in strengthening our understanding of how wildfire changes how C moves through ecosystems.

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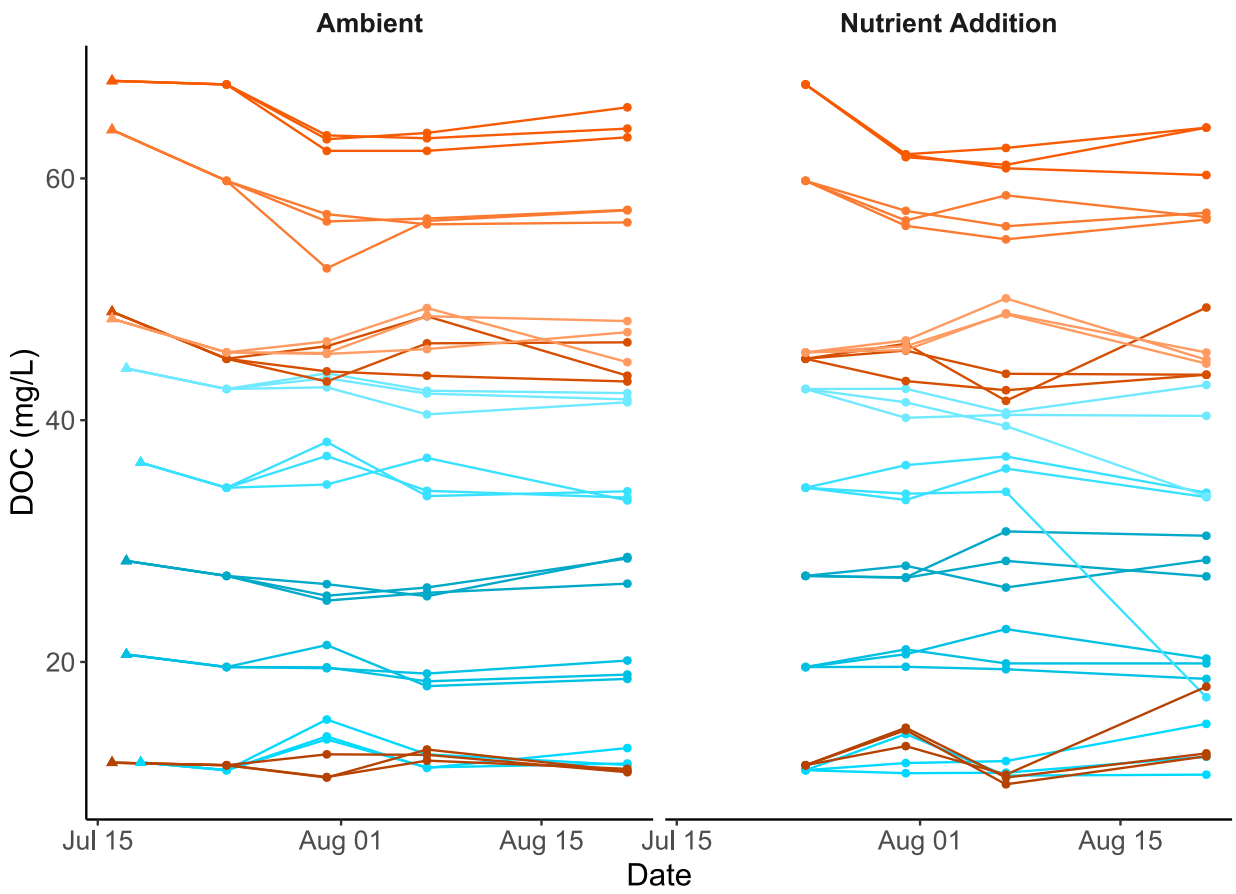

Fig. 1. DOM concentrations decreased more in bottles from burned streams. We compared dissolved organic carbon (DOC) concentrations in water from streams draining unburned (n=5, blue) and burned catchments (n=5, orange). Water was incubated for 28-days in three replicates bottles per stream times as shown by lines of the same color. Triangle points indicate in-stream measurements, and all circles were from bottles in the incubation experiment. We replicated this experiment adding nutrients, KNO_3 and Na_3PO_4, to test the effect of nutrient limitation and found the DOC concentrations were an estimated mean ± standard error (SE) 2.41 ± 1.19 mg/L lower across the entire sampling period where nutrients were added in burned streams (burned × nutrients: t = -2.02, df = 221.99, p = 0.044).

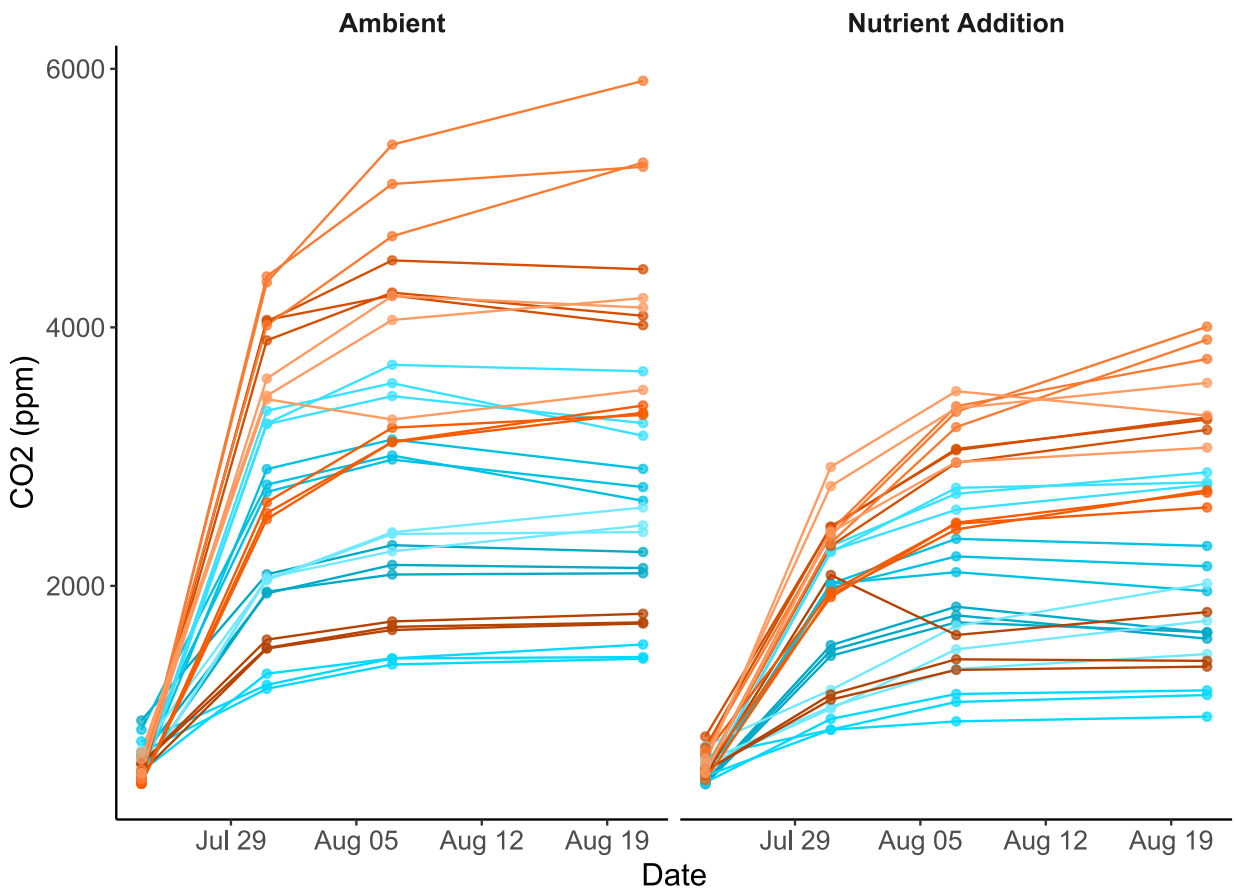

Fig. 2. CO_2 concentrations accumulated more quickly in bottles from burned streams faster. We compared CO_2 concentration in the headspace of bottles with water from streams draining unburned catchments (n=5, blue) and burned catchments (n=5, orange). Water was incubated for 28-days in three replicates bottles per stream times as shown by lines of the same color. We replicated this experiment by adding nutrients, KNO_3 and Na_3PO_4, to test the effect of nutrient limitation and found nutrient addition decreased CO_2 production 17% by the end of the incubation period (nutrients × time: t = -2.64, df = 189.02, p = 0.009).

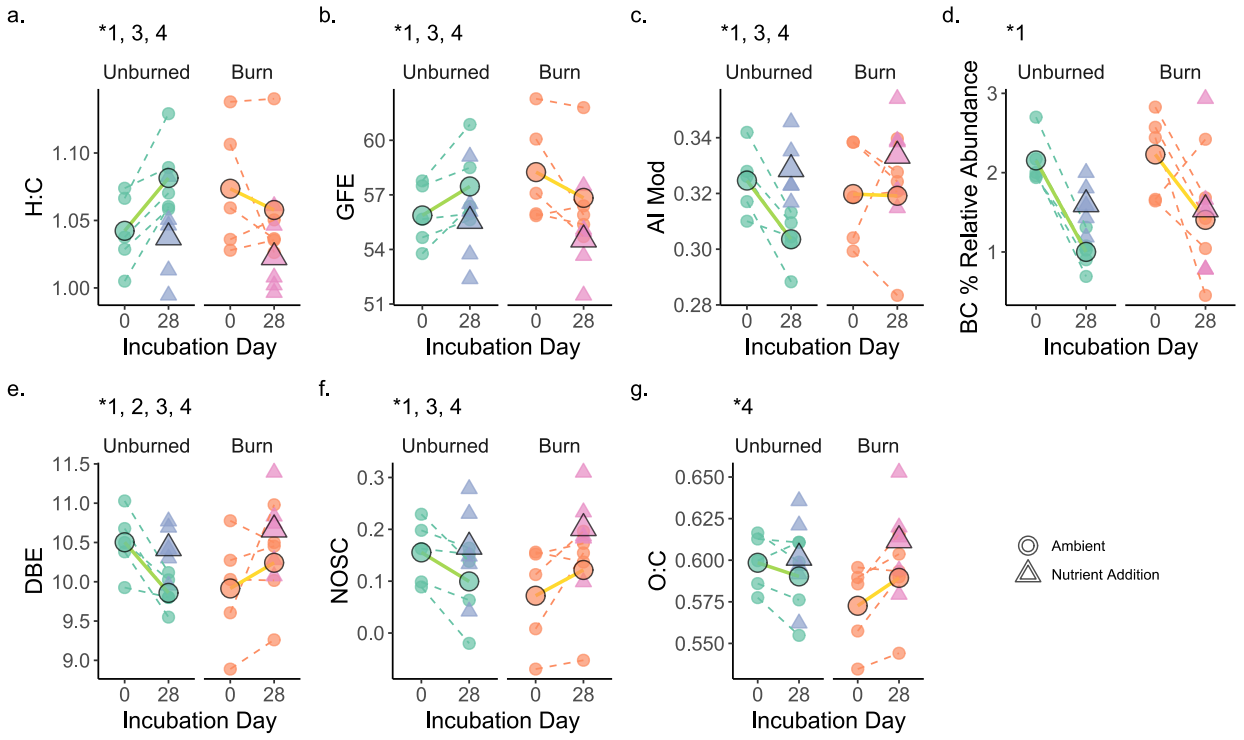

Fig. 3. Burned streams had more aromatic and less bioavailable DOM after 28-days of incubation. For one replicate per stream, we calculated the: a. intensity-weighted H:C ratio, b. intensity-weighted Gibbs free energy (GFE, J), c. intensity-weighted modified aromaticity index (AI Mod), d. percent relative intensity of black carbon-like (BC) formulae, e. intensity-weighted double-bond equivalent (DBE), f. intensity-weighted nominal oxidation state of carbon (NOSC), and g. intensity-weighted H:C ratio. We compared streams draining unburned catchments (n=5, green = ambient, and purple = nutrient addition) and burned catchments (n=5, orange = ambient, and pink = nutrient addition). Larger symbols outlined in black are treatment means. Model significance is denoted with an asterix and 1. time, 2. fire, 3. nutrient addition, 4. time × fire, and no metric was statistically different with the interaction between fire and nutrient addition.

General Discussion

In Chapter 1, I laid out four questions that this thesis aimed to answer:

1. How does wildfire change DOM concentration (Chapter 2)?

2. How does wildfire change DOM composition (Chapter 2)?

3. Do microbes degrade DOM from streams draining burned catchments differently than unburned catchments (Chapter 3)?

4. Do microbes respire more CO_2 from streams of burned catchments than unburned catchments (Chapter 3)?

In this final chapter, I reflect on how the analyses conducted throughout this thesis have addressed the key research questions. I also provide a critical assessment of limitations and suggest potential directions for future research based on the findings.

I have shown that wildfires increased DOM concentrations in streams, and DOM is less bioavailable but more thermodynamically favorable for microbial degradation (Chapter 2). The increase in DOM concentrations and associated physicochemical changes in streams are largely attributed to the loss of surrounding vegetation, which mobilizes organic matter from the soil into streams (Cao et al., 2024; Freeman et al., 2023). Wildfire also deepens groundwater flow paths by altering soil structure from decimated vegetation, allowing water to pass through more soil and accumulate higher DOM concentrations before reaching streams (Burd et al., 2018; Cao et al., 2024; Gommet et al., 2022; Sebestyen et al., 2008; Seibert et al., 2009). Altered flow paths decreased residence time in the terrestrial sphere, limiting opportunistic degradation of pyrogenic DOM (pyDOM) left after a fire leading to the accumulation of highly aromatic and heat-altered

molecules in streams (Cao et al., 2024; Dittmar et al., 2012; Jones et al., 2020; Xu et al., 2024; Zhang et al., 2024). These findings demonstrate that wildfire shapes terrestrial-aquatic C fluxes and its effects can be found at a molecular level.

I have also demonstrated that wildfire changes the fate of DOM as it is still used by microbes in burned streams at the cost of enhanced CO_2 respiration (Chapter 3). Burned DOM shows trends of lower bioavailability throughout the incubation experiment because microbes use pyDOM less efficiently than other molecules. Microbes respire more because of the difficulty of accessing complex molecules with double bonds and aromatic rings (Fitch et al., 2018; Guillemette et al., 2015). Increased CO_2 flux from streams is an unexpected long-term impact of wildfire that will make it difficult for boreal ecosystems to transition back into a C sink with increasing fire frequency and severity (Battin et al., 2009; Graham et al., 2023). Perhaps even more bamboozling is how nutrient addition could affect C flux because it primes microbes to degrade bioavailable DOM first, leaving pyDOM till the end of the incubation (Bruun et al., 2008; Crandall et al., 2021; Fitch et al., 2018). This makes it seem like nutrient addition, eutrophication, and N deposition would be loosely linked with decreased CO_2 respiration in oligotrophic systems. Still, I suspect more complex dynamics are at play that can only be examined through the lens of microbiology.

This thesis will inform forest management policy and improve C accounting in Canada to meet international obligations by providing novel results on carbon movement from terrestrial to aquatic systems in the context of wildfires (Smyth et al., 2023). The result that wildfire-affected DOM is difficult to use and less bioavailable yet still provides microbes energy (thermodynamically favorable) informs us that products of wildfire are not inherently detrimental to ecosystems, but the increasing frequency and severity of wildfires are (Chapter 2).

The result that wildfire accelerates carbon losses from boreal forests in headwater streams reinforces that headwater streams should be included in C budgets (Chapter 3).

While this thesis explored direct measures of DOM with microbial context, I did not directly measure microbial C use efficiency or growth. Microbes and DOM are two faces of the same coin that both need to be explored to understand wildfire effects in inland waterbodies fully. Chapter 3 was limited by gas sampling equipment as I originally intended to investigate CH_4, but the gas analyzer only provided reliable data on CO_2. Monitoring interactions between CH_4 and wildfire is an important future direction in incubation experiments. A notable strength of this thesis was the focus on headwater streams as a greater proportion of the catchment is impacted by fire than higher-order streams (Wampler et al., 2024). Future research on wildfire impacts on streams should prioritize studying headwater streams for clearer results.

In a broader lens, in wildfire management, there must be initiatives on both federal and provincial scales to move watershed sensitivity into the list of priorities. Fire management with a long-term scope for protecting life and ecosystems incorporates carbon sequestration, bioenergetics, and raw water quality. Alberta ranks soils and watersheds third and other natural resources fourth in their fire response priorities even though it is much less hydrologically connected than Boreal Shield provinces (Tymstra et al., 2020). While the Canadian Boreal Shield is important globally for C flux in the context of watershed connectivity, it is also important to explore DOM composition changes across other biomes affected by wildfires.

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Appendix A

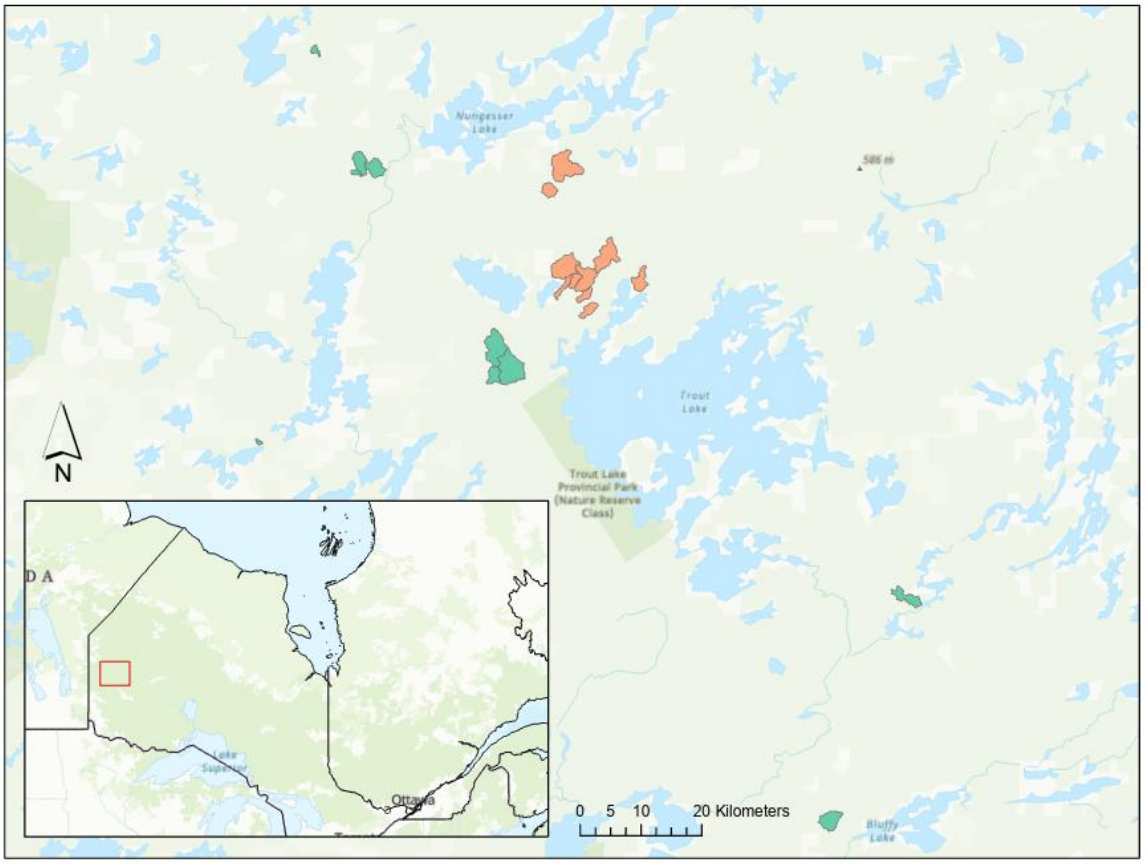

Fig. A1. Map of study sites in northwestern Ontario, Canada. The 10 burned catchments are represented in orange and the 10 unburned catchments are in teal.

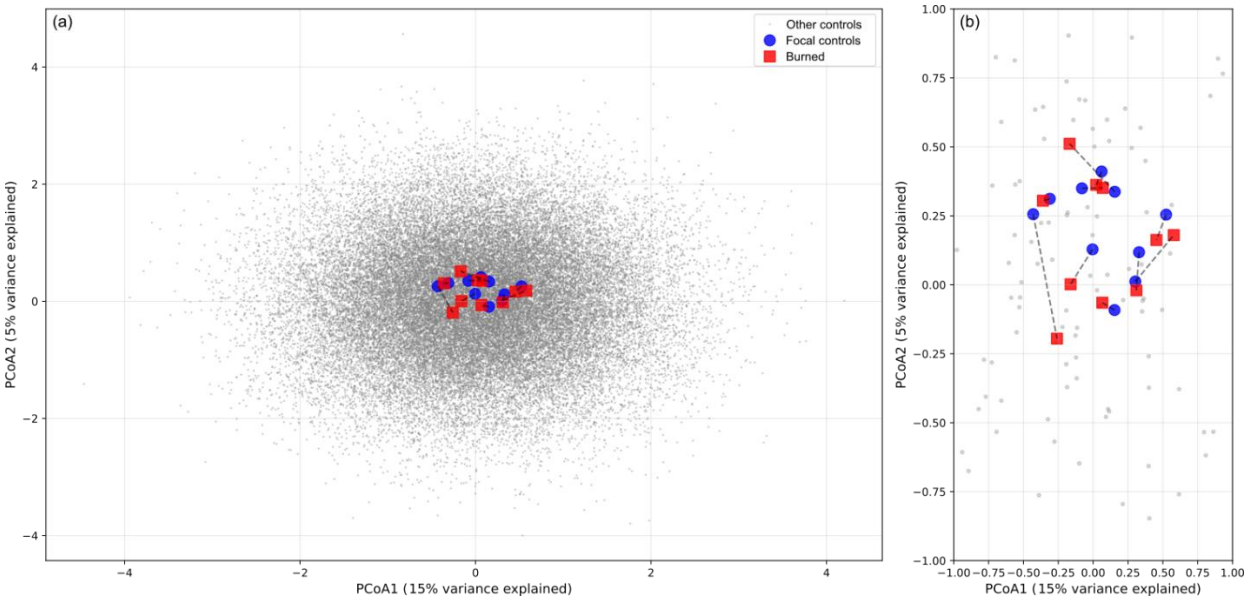

Fig. A2. Burned catchments are paired with unburned catchments that are more environmentally similar than adjacent burned catchments. Principal coordinates analysis on Gower's distance for latitude, longitude, catchment size, percent catchment wetland and forest cover, and mean slope in 42,829 unburned catchments and 10 burned catchments displaying (a) all sites and (b) enlarging only sampling sites. Lines connected pair unburned and burned catchments sampled in this study.

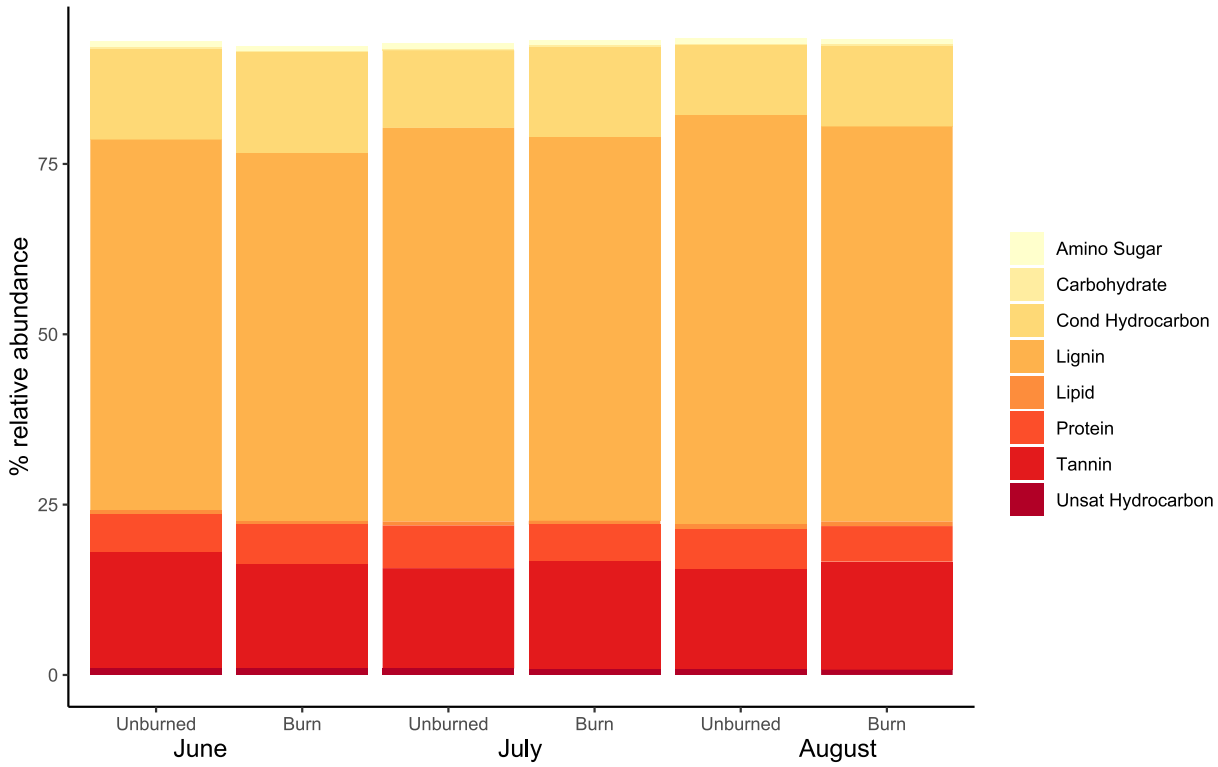

Fig. A3. All sites were dominated by lignin-like molecules. Bar plots are the relative abundances of putative compound classes across all burned and unburned sites by month.

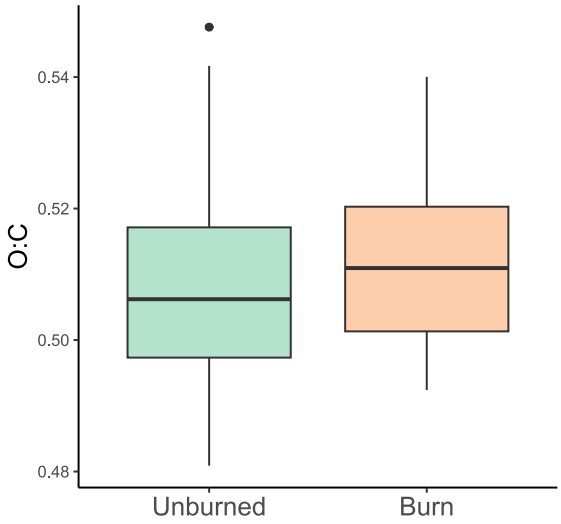

Fig. A4. Burned and unburned streams have similar O:C ratio. We compared the intensity-weighted O:C ratio of stream water between streams draining unburned control catchments (n=10, green) and burned catchments (n=10, orange) in June, July, and August 2023. Boxes display interquartile range with whiskers extending to minimum and maximum values and a single outlier is denoted by a black circle. Medians are denoted by horizontal lines.

Table A1. Linear models predicting DOM concentration and predictive metrics with the presence of wildfire. Bolded values are statistically significant. Values in cells are mean estimated effects ± standard error relative to the intercept, with the intercept expressed relative to zero. Df = 52 for all response variables. R^2_M represents marginal and R^2_C represents conditional R^2 values.

	Fixed Effects							**Random Effects**		
	Predictors	Intercept	Treatment: Burn	Trip: July	Trip: August	Burn × July	Burn × August	Random	Catchment	R^2_M / R^2_C
DOC	Mean Effect	7.89	0.02	1.21	1.87	0.26	4.7	18.63	112.82	0.198 / 0.886
	p	**<0.001**	0.987	0.233	0.068	0.794	**<0.001**			
DN	Mean Effect	8.67	-0.46	1.8	3.66	-0.17	0.96	0.01	0.05	0.089 / 0.875
	p	**<0.001**	0.647	0.078	**0.001**	0.863	0.341			
pH	Mean Effect	27.21	-0.73	0.99	2.61	0.19	-3.38	0.06	0.50	0.103 / 0.897
	p	**<0.001**	0.469	0.328	**0.012**	0.848	**0.001**			
ORP	Mean Effect	12.23	-3.47	-1.66	-4.07	2.45	3.29	3865.88	269.59	0.263 / 0.311
	p	**<0.001**	**0.001**	0.102	**<0.001**	**0.018**	**0.002**			
Conductivity	Mean Effect	15.48	-0.87	17.07	20.85	-2.66	-7.13	0.01	0.39	0.284 / 0.983
	p	**<0.001**	0.39	**<0.001**	**<0.001**	**0.01**	**<0.001**			
Temperature	Mean Effect	12.65	3.19	2.62	3.38	-0.72	-3.39	2.92	3.20	0.259 / 0.646
	p	**<0.001**	**0.002**	**0.012**	**0.001**	0.474	**0.001**			
Dissolved O_2	Mean Effect	9.54	-0.95	-2.6	-3.76	1.76	1.35	2.29	6.84	0.077 / 0.769
	p	**<0.001**	0.346	**0.012**	**<0.001**	0.085	0.184			

H:C	Mean Effect	136.22	-2.07	2.74	3.75	-0.69	-0.4	0	<0.01	0.434 / NA
	p	**<0.001**	**0.044**	**0.008**	**<0.001**	0.492	0.688			
O:C	Mean Effect	126.7	-1.32	-3.03	-3.79	2.05	1.87	0	<0.01	0.238 / NA
	p	**<0.001**	0.191	**0.004**	**<0.001**	**0.046**	0.067			
GFE	Mean Effect	133.83	-0.32	2.92	3.82	-1.4	-1.15	2.07	<0.01	0.320 / NA
	p	**<0.001**	0.747	**0.005**	**<0.001**	0.168	0.254			
AI_{mod}	Mean Effect	81.94	3.31	-2.41	-3.47	0.05	-0.35	0	<0.01	0.498 / NA
	p	**<0.001**	**0.002**	**0.019**	**0.001**	0.96	0.73			
BC	Mean Effect	25.42	2.7	-2.47	-2.55	0.02	-0.66	0	<0.01	0.386 / 0.430
	p	**<0.001**	**0.009**	**0.017**	**0.014**	0.984	0.512			
NOSC	Mean Effect	-1.25	0.32	-2.92	-3.82	1.4	1.15	0	<0.01	0.320 / NA
	p	0.217	0.747	**0.005**	**<0.001**	0.168	0.254			
Putative transformations	Mean Effect	43.65	-2.40	1.08	3.60	2.97	0.55	0	0.01	0.705 / 0.909
	p	**<0.001**	**0.020**	0.286	**0.001**	**0.005**	0.583			
BIX	Mean Effect	33.07	-1.4	1.87	2.52	-0.97	-0.16	0	<0.01	0.209 / 0.613
	p	**<0.001**	0.169	0.066	**0.015**	0.338	0.87			
HIX	Mean Effect	13.28	-1.02	0.43	3.19	0.62	-0.22	12.18	3.56	0.233 / 0.406
	p	**<0.001**	0.312	0.669	**0.002**	0.536	0.825			
SUVA	Mean Effect	12.93	1.28	-0.02	-1.41	-1.8	-1.83	0.05	0.04	0.148 / 0.542
	p	**<0.001**	0.205	0.985	0.164	0.077	0.073			

SR	Mean Effect	36.92	-1.43	-1.99	-0.24	0.71	-1.18	0	<0.01	0.114 / 0.911
	p	**<0.001**	0.159	0.052	0.812	0.478	0.243			
FI	Mean Effect	53.4	-1.1	1.94	1.28	-0.98	0.48	0	<0.01	0.146 / 0.456
	p	**<0.001**	0.277	0.057	0.206	0.332	0.633			
E2:E3	Mean Effect	52.02	-2.38	0.77	-0.62	-0.15	0.97	0	0.01	0.206 / 0.878
	p	**<0.001**	**0.021**	0.442	0.536	0.882	0.335			

Table A2. Binary fire categories were the best supported linear models based on AIC. The binary fire model used burned and unburned categories while continuous fire was burn severity data from LANDSAT imagery. We also calculated burn severity of the catchment by weighting individual pixels in a catchment by their distance to the focal streams using the R package hydroweight (https://github.com/GLFC-WET/hydroweight). We undertook these calculations by weighing pixels by flow length across the land to the stream (iFLS) and by considering the flow accumulation of each pixel (HAiFLS). The + Wetlands model added a wetland effect to the binary fire model. Bolded values were the best supported model, that is, with the lowest AIC. If AIC values were within 2 units, we picked the simplest model.

Parameter	Binary Fire Categories	Continuous Fire	iFLS	HAiFLS	+ Wetlands
DOC	**414.51**	413.71	414.32	415.12	416.50
DN	**-53.65**	-53.89	-54.00	-53.80	-51.71
pH	**78.96**	80.04	81.29	82.03	80.63
ORP	**679.35**	679.47	679.96	680.15	679.43
Conductivity	**-3.98**	-4.01	-1.10	0.16	-1.99
Temp	**273.32**	276.59	275.53	276.14	275.32
DO2	**275.59**	275.90	275.66	275.87	277.59
H:C	**-263.13**	-262.95	-262.77	-262.46	-261.49
O:C	**-340.93**	-342.23	-342.49	-342.32	-339.22
GFE	**223.56**	223.04	222.94	223.17	225.21
AI Mod	**-344.19**	-343.37	-342.61	-342.29	-342.60
BC	**-523.44**	-523.30	-522.48	-522.23	-521.82
NOSC	**-178.43**	-178.95	-179.05	-178.82	-176.78
Putative transformations	**-458.22**	-455.70	-453.47	-453.41	-456.64
BIX	**-231.18**	-231.09	-230.06	-230.15	-229.21
HIX	**342.54**	343.01	342.78	343.07	343.86
SUVA	**25.04**	26.18	25.94	26.58	27.01
SR	**-215.99**	-215.76	-215.98	-215.91	-214.09
FI	**-146.48**	-146.02	-145.80	-145.84	-144.81
E2:E3	**-158.32**	-157.76	-157.04	-156.97	-156.39

Appendix B

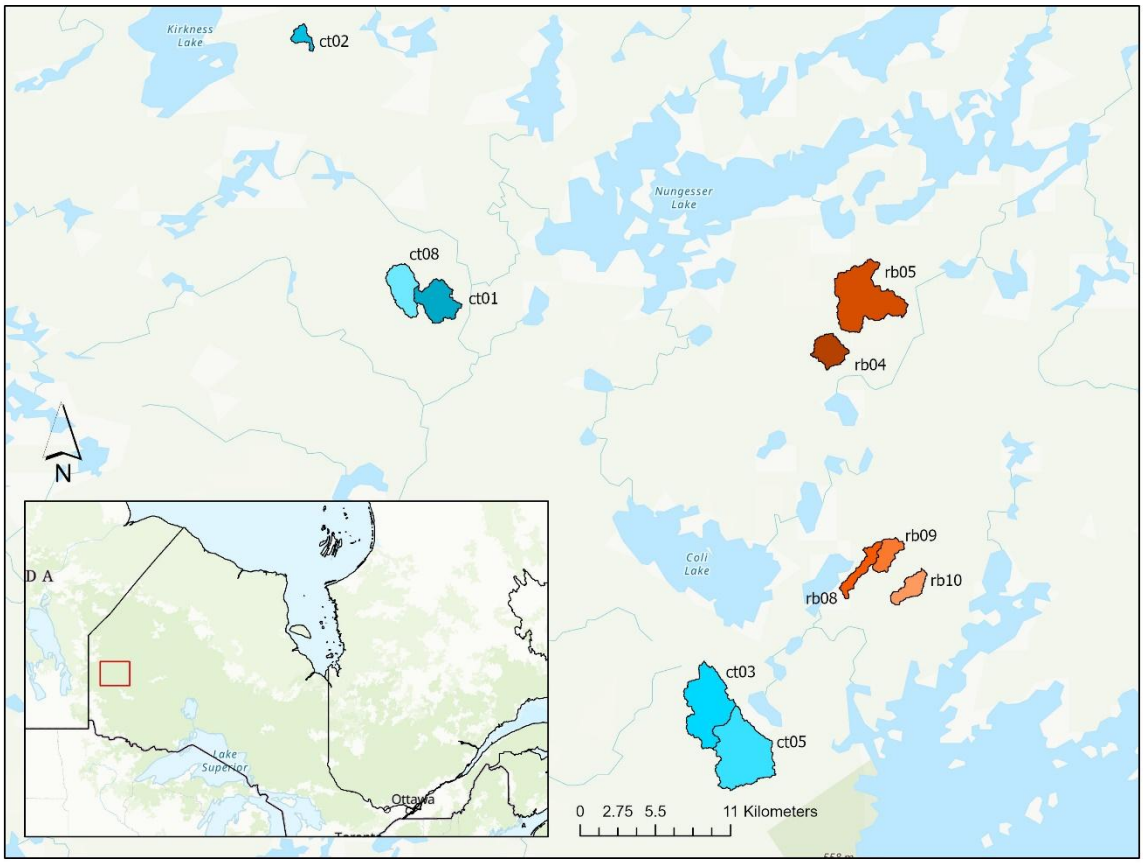

Fig. B1. Map of Northwestern Ontario with catchments sampled near Red Lake, ON. The 5 burned catchments are represented in orange and the 5 unburned catchments are in blue. Gradations of colors correspond to line colors in figures 1 and 2.

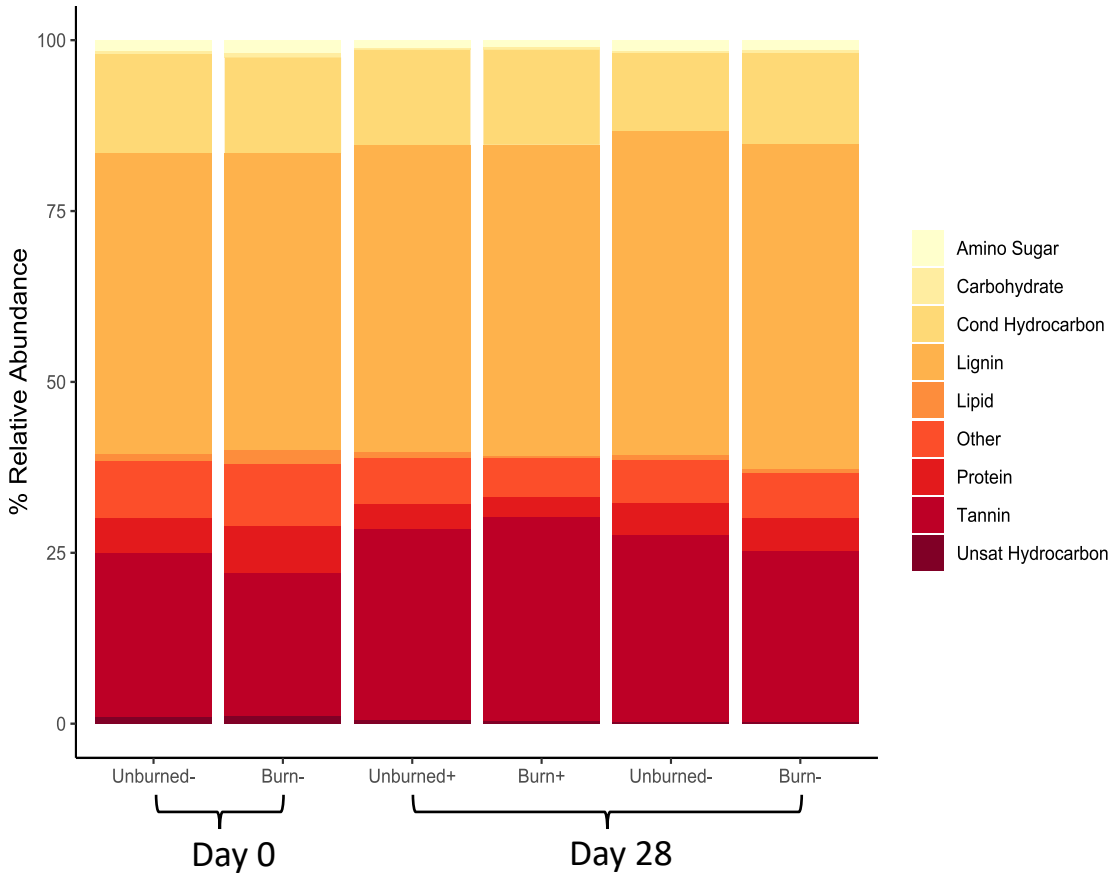

Fig. B2. All sites were dominated by lignin-like molecules over an incubation period. Bar plots are the relative abundances of putative compound classes across all burned and unburned sites by incubation day. Nutrient treatments are denoted by + for nutrient addition and – for ambient.

Table B1. KNO_3 and Na_3PO_4 used during the bioincubation experiment. Quantities reflect the amount added to each replicate sample.

Stream	KNO_3 (g)	Na_3PO_4 (g)
rb04	0.0067	0.0006
rb05	0.0645	0.0065
rb08	0.0551	0.0056
rb09	0.0407	0.0041
rb10	0.0256	0.0026
ct01	0.0571	0.0058
ct02	0.0742	0.0075
ct03	0.0592	0.0060
ct05	0.0728	0.0074
ct08	0.0428	0.0043

Table B2. Linear models predicting DOM and CO_2 concentration and DOM composition with the presence of wildfire. Bolded values are statistically significant. A quadratic model was applied to the CO_2 data to account for non-linear trends. Values in cells are mean estimated effects ± standard error relative to the intercept, with the intercept expressed relative to zero. R^2_M represents marginal and R^2_C represents conditional R^2 values. Blank cells are interactions that were not included in each model.

	Fixed Effects													**Random Effects**			
	Predictors	Intercept	Time	Treatment: Burn	Nutrient Addition	Time × Burn	Time × Nutrient Addition	Burn × Nutrient Addition	Time × Burn × Nutrient Addition	$Time^2$	Burn × $Time^2$	Nutrient Addition × $Time^2$	Burn × Nutrient Addition × $Time^2$	Random	Stream	Catchment	R^2_M / R^2_C
DOC	Statistic	3.78	-2	1.83	-0.45	-1.95	0.47	-2.02	1.71					3.16	0.19	271.37	0.229 / 0.991
	p	**<0.001**	**0.047**	0.068	0.65	0.052	0.639	**0.044**	0.09								
	df	228	228	228	228	228	228	228	228								
CO_2	Statistic	-2.34	10.51	-3.01	1.83	4.93	-2.64	1.1	-1.12	-8.75	-3.43	2.66	0.97				
	p	**0.02**	**<0.001**	**0.003**	0.069	**<0.001**	**0.009**	0.271	0.263	**<0.001**	**0.001**	**0.008**	0.335	194751.8	<0.01	499640.8	0.810 / NA
	df	194	194	194	194	194	194	194	194	194	194	194	194				
H:C	Statistic	57.85	2.98	1.77	-3.16	-2.94		0.54						0	<0.01	<0.01	0.247 / 0.752
	p	**<0.001**	**0.007**	0.091	**0.005**	**0.008**		0.591									
	df	21	21	21	21	21		21									
O:C	Statistic	49.92	-1.32	-1.91	1.21	2.81		0.81						0	<0.01	<0.01	0.187 / 0.878
	p	**<0.001**	0.201	0.07	0.24	**0.011**		0.429									
	df	21	21	21	21	21		21									
AI_{mod}	Statistic	42.23	-3.04	-0.86	3.67	2.08		-1.19						0	<0.01	<0.01	0.433 / NA
	p	**<0.001**	**0.006**	0.397	**0.001**	**0.05**		0.247									

	df	21	21	21	21	21		21									
Black Carbon-like	Statistic	7.76	-3.3	0.09	1.83	0.66		-1.05						0	<0.01	<0.01	0.381 / NA
	p	**<0.001**	**0.003**	0.928	0.081	0.516		0.306									
	df	21	21	21	21	21		21									
GFE	Statistic	48.16	2.31	1.94	-2.23	-3.07		-0.24						1.22	0.71	4	0.211 / 0.838
	p	**<0.001**	**0.031**	0.067	**0.037**	**0.006**		0.812									
	df	21	21	21	21	21		21									
NOSC	Statistic	4.18	-2.31	-1.94	2.23	3.07		0.24						0	<0.01	<0.01	0.211 / 0.838
	p	**<0.001**	**0.031**	0.067	**0.037**	**0.006**		0.812									
	df	21	21	21	21	21		21									
DBE	Statistic	42.09	-2.99	-2.3	2.71	3.19		-0.58						0.12	<0.01	0.14	0.440 / NA
	p	**<0.001**	**0.007**	**0.032**	**0.013**	**0.004**		0.568									
	df	21	21	21	21	21		21									