EVALUATING THE LEGACY OF SEVERE WILDFIRE ON AQUATIC CARBON DYNAMICS AND MICROBIOMES IN MONTANE WATERSHEDS

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ABSTRACT

As the climate continues to warm, wildfires are expected to increase in frequency and severity, particularly across the western United States, likely impacting the global carbon (C) balance. Fire influences C stocks via shifts in vegetation, hydrologic flow paths, and microbial communities. These disturbances alter C partitioning across landscapes, connectivity between hillslopes and streams, and microbial function. However, potential linkages between disturbance, C cycling, and terrestrial and aquatic microbiomes require further investigation. This study explored the long-term legacy of wildfire on watershed C dynamics and aquatic microbial communities, while evaluating how changes to the terrestrial landscape are reflected in stream systems. During the summer of 2019, dissolved inorganic and organic carbon pools were measured along with estimates of stream metabolism and characterization of aquatic microbiomes for three streams draining catchments that burned during the 2002 Colorado wildfire season and two streams unaffected by fire. Compared to unburned watersheds, aquatic C exports and microbiomes were characteristically different in burned watersheds; specifically, C stocks were less aromatic and less bioavailable, which could exert controls on microbial communities. Although stable isotope analyses revealed that all streams were dominated by allochthonous sources, trends in dissolved C pool composition in streams draining unburned landscapes suggest shifting flow paths and/or differences in in-situ sources and transformation between watersheds. Overall, wildfire appears to create a feedback loop between C quality, processing, and microbiomes due to changes in vegetation and hydrology in the terrestrial environment as well as differences in microbiomes across the landscape. These alterations could affect recovery dynamics in burn scars and imply that wildfires in warmer, drier climates could result in steady state shifts.

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INTRODUCTION

Effects of Wildfire on Aquatic Systems

Wildfire represents a dominant disturbance that influences landscapes on a global scale (Bowman *et al*., 2009), and, as the climate continues to warm, fires are expected to increase in both frequency and severity (IPCC, 2018). The negative impacts of increasing wildfire are numerous and extend to many groups of people and ecosystem services. Air pollution from smoke (Reid *et al*., 2016), habitat loss (Morton *et al*., 2003), changes to water quality (Chow *et al*., 2019), and increased hazard for subsequent natural disasters (Moody & Martin, 2001) during and after fire pose great risks for many populations in the face of climate change (IPCC, 2018). Such disturbances can also reduce biodiversity (Abella & Fornwalt, 2015) and alter long-term nutrient storage (McLauchlan *et al*., 2014), endangering the longevity and health of ecosystems that offer needed services (IPCC, 2018). Additionally, fire destroys forests that naturally sequester significant amounts of carbon (C) from the atmosphere, which threatens the global C balance and augments the greenhouse gas effect (IPCC, 2018).

As drier and warmer conditions persist in the western United States (U.S), fire return intervals will likely shorten, yielding little recovery time for these forested environments (Enright *et al*., 2015). Since the 1980s, fire season in this region has become longer, and fires have increased in numbers and duration (Westerling *et al*., 2006; Abatzogloua & Williams, 2016). Montane forests in the northern Rocky Mountains are at particular risk for fire as spring and summer temperatures rise, spring snowmelt occurs earlier, and drought-related impacts increase (Westerling *et al*., 2006; Enright *et al*., 2015; Abatzogloua & Williams, 2016; Westerling *et al*., 2016). For example, the Hayman Fire of 2002, which prior to 2020 was the largest wildfire in recorded Colorado history, burned 560 km^2 of land southwest of Denver, CO.

However, in the summer and fall of 2020, the Cameron Peak Fire, East Troublesome Fire, and Pine Gulch Fire all grew much larger than the Hayman Fire, burning over 2,100 km^2 in northern and western parts of the state (Johnson, 2020; Kirk, 2020; USFS, 2020). These fires directly harmed local Colorado populations, causing over $195 million in damage, unsafe air quality levels, and the relocation of many communities (Miller, 2020); still, the lasting socio-economic costs of the 2020 fire season will likely be much more. The short- and long-term effects, some of which are enumerated here, only emphasize the imminent dangers posed by increasing wildfires in the west.

Understanding the consequences of changing fire regimes in the western U.S. on C partitioning is particularly important as current research suggests that forests in this region constitute 20 to 40% of all carbon sequestration in the country (Pacala *et al*., 2001; Huber *et al*., 2006; Westerling *et al*., 2006). However, persistent changes to vegetation and soil organic matter (SOM) following fire limit the ability of these systems to effectively sequester and store C (Buma *et al*., 2014; Fornwalt *et al*., 2016). In essence, Ponderosa pine (*Pinus ponderosa*) forests, like those studied here, historically function as carbon sinks, but new fire activity and slow rates of recovery may shift these systems toward serving as carbon sources. Losses of these C sinks will only exacerbate the negative repercussions of climate change. These landscapes will not be able to efficiently remove C emitted by anthropogenic sources (e.g., burning fossil fuels) from the atmosphere, resulting in significant management implications: if wildfire disturbs a majority of global forests so they no longer effectively store C, the global C balance may be altered for centuries.

By modifying terrestrial landscapes, fire also changes nutrient export (Knicker, 2007). Fire yields greater runoff and higher stream discharges in response to changes in vegetation

(Buma *et al*., 2013; Ebel & Moody, 2013) and/or soil structure (González-Pérez *et al*., 2004; Rhoades *et al*., 2011), which can greatly alter hydrology by changing flow paths and decreasing rates of evapotranspiration (Hallema *et al*., 2017). Such disturbances allow for the formation of preferential flow paths and overall decreased soil water holding capacity (Gilbertson 2018; Barnes & Gilbertson, 2018, unpublished data). Lack of vegetation and thus belowground biomass also reduce demand for nutrients within the watershed, increasing transport to stream systems (Certini *et al*., 2005). Given that nutrient and C mobilization is strongly linked to hydrologic transport through the landscape (Xu & Saiers, 2010) and the magnitude of hydrological responses to fire (Moody & Martin, 2001), these differences likely control C export—and storage—as well.

Allochthonous C sources (e.g., C inputs from the watershed) dominate small headwater streams (Vannote *et al*., 1980; Palmer *et al*., 2001; Hotchkiss *et al*., 2018), like those studied here. In many systems, concentration-discharge relationships suggest a transport limitation or a chemostatic response of dissolved organic carbon (DOC) to hydrologic fluctuations (i.e., mobilization is proportional to changes in watershed discharge) (Godsey *et al*., 2009; Godsey *et al*., 2019). Given that DOC concentrations often respond positively to flow events (Striegl *et al*., 2005; Raymond & Saiers, 2010; Dawson *et al*., 2008), hydrology serves as a significant driver for headwater stream DOC fluctuations (Guarch-Ribot *et al*., 2016).

The hydrologic connectivity of hillslopes and streams links terrestrial and aquatic environments, and the amount of C held within vegetation and soils (i.e., terrestrial C sequestration) influences lateral C flux to streams. Prior to a decade ago, the transport of C to aquatic environments was often disregarded in net ecosystem C balance calculations (e.g., Foley *et al*., 1996; Cramer *et al*., 2001; Prentice *et al*., 2001). Rivers were thought of as “pipes” to transport C (Cole *et al*., 2007; Hotchkiss *et al*., 2015), as DOC accounts for most of the total annual export from watersheds (Mei *et al*., 2012) and the majority fate of C in lentic systems is export (Hotchkiss *et al*., 2018). Yet, lateral flux to inland waters affects C storage, processing, and transport (Cole *et al*., 2007; Butman *et al*., 2016). For example, freshwater C fluxes—C exported downstream or emitted to the atmosphere—provide 106 Tg C per year in the contiguous U.S. (Butman *et al*., 2016), which represents a much larger contribution than previously considered in C budgets. When leaching and export of C is ignored in the equation, terrestrial net ecosystem production (NEP) and CO_2 uptake and storage by the landscape can be overestimated (Hotchkiss *et al*., 2015); export could represent up to 27% of NEP estimations (Butman *et al*., 2016). Given how fire changes terrestrial C stocks and the importance of inland waters to global C cycling, it is necessary to understand how fire affects lateral C export to streams draining burned watersheds and the subsequent fate of this C.

Although terrestrially derived sources dominate small streams, in-stream processes, such as microbial respiration, also influence the C pool (Hotchkiss *et al*., 2015), and fire may shift autochthonous processing of C by altering in-situ conditions (Rhodes *et al*., 2011; Murphey *et al*., 2018). Estimates of stream metabolism—ecosystem respiration (ER), gross primary production (GPP), and net ecosystem production (NEP = GPP - ER)—are effective proxies for measuring stream productivity and processes (Betts & Jones, 2009; Hotchkiss & Hall, 2014). For example, ecosystem respiration has been shown to be highest in streams draining burned watersheds compared to those draining unburned watersheds (Betts & Jones, 2009). Moreover, differences in photosynthetically active radiation (PAR), primary productivity (Mulholland *et al*., 2001), and discharge (Finlay, 2003), all of which may be impacted by severe fire, can change ecosystem respiration rates and other in-stream processes.

Post-fire Shifts in Microbial Community Composition

Fire transforms the size, activity, and composition of microbial biomass in the terrestrial environment (Hart *et al*., 2005) and both directly and indirectly affects the stream microbiome. Shifts in microbial community composition immediately after fire are often due to heat-induced mortality during heat transfer that occurs with the combustion of organic matter (OM) (Hart *et al*., 2005; Pressler *et al*., 2019). Altered environmental conditions (e.g., SOM character, etc.) following fire may also lead to differences in communities (Knicker, 2007). Given that the composition of stream microbiomes may be influenced by transport of terrestrial microorganisms from soil and upslope communities into fluvial systems (Crump *et al*., 2003; Crump *et al*., 2012), documented shifts in the terrestrial environment may be reflected in the aquatic microbiome (Leibold *et al*., 2004; Vellend, 2010).

Still, little is known regarding the influence of wildfire on both terrestrial and aquatic microbial communities (Pressler *et al*., 2019). Most bacterial phyla are not immediately resilient to disturbance regardless of taxonomic complexity or severity of disturbance events (Allison & Martiny, 2008). Thus, the environmental conditions that persist following fire (e.g., elevated pH, altered vegetation, changes in nutrient availability, etc.) likely influence microbial community composition (Vellend, 2010). In general, fire is expected to reduce microbial biomass, abundance, and diversity (Pressler *et al*., 2019), but much of this research rests on the assumption that stream and soil communities respond similarly to fire. Thus, the response of terrestrial and aquatic microbiomes to disturbance needs to be further studied (Langenheder & Lindström, 2019).

Changes in microbiome composition and activity due to disturbance may directly affect ecosystem processes, such as respiration, given the reliance of heterotrophic microbial community members on DOC (Kaplan *et al*., 2008). As such, surveys of bacterial diversity in freshwater have identified many populations that are involved in the degradation of OM and other processes related to C cycling, including members of the Bacteroidetes, Cyanobacteria, Patescibacteria, Actinobacteria, and Verrucomicrobia phyla (Glöckner *et al*., 2000; Zwart *et al*., 2002; Eiler *et al*., 2002). Overall, it is well understood that biogeochemical (e.g., DOM supply, Judd *et al*., 2006) and environmental factors (e.g., pH, Fierer *et al*., 2007) influence these aquatic microbial communities, and differences in community attributes (e.g., diversity) can govern ecosystem processes (Langenheder *et al*., 2010; Frossard *et al*., 2012).

The composition and activity of microbial assemblages is often linked to C quality and/or quantity (Bernhardt & Likens, 2002; Crump *et al*., 2003; Eiler *et al*., 2003; Judd *et al*., 2006; Strickland *et al*., 2009; Zeglin, 2015). Different physical and nutritional habitats in the water column may select for different microbial assemblages (Zeglin, 2015; Stelzer *et al*., 2003) and microbial functions (Bernhardt & Likens, 2002). Organic matter lability and quality can also regulate communities, with the addition of labile C stimulating bacterial production and community shifts (Crump *et al*., 2003; Judd *et al*., 2006; Crump *et al*., 2012). Since disturbances transform C transported to streams, fire likely impacts stream microbial assemblages indirectly.

Research Questions

While many studies address how wildfire alters different watershed characteristics, such as flow paths (Moody & Martin, 2001; Ebel & Moody, 2013; Hallema *et al*., 2017), nutrient cycling (Smith *et al*., 2011; Santos *et al*., 2016; Rhoades *et al*., 2018), and microbial community composition (Hart *et al*., 2005; Knicker, 2007; Pressler *et al*., 2019), further investigation is still required to synthesize the positive feedback loops between disturbance, C cycling, and microbiomes. Even research that considers how wildfire influences C sequestration and partitioning across the landscape (Buma *et al*., 2014) often overlooks linkages between hillslopes and streams. And, although some studies address differences in the terrestrial microbiome, few studies have been conducted surrounding aquatic microbial community structure and function following fire. Therefore, additional work needs to be done to understand the long-term legacy of fire on overall ecosystem function.

The goal of this study was to understand how C cycling and microbial community structure differ post-fire and to begin a discussion on the ways fire collectively alters terrestrial and aquatic C stocks and microbial communities. Building off the work of Jones (2020) and Bonwell (2020), who looked at the legacy of fire on carbon dynamics and export across terrestrial and aquatic environments respectively, I investigated how shifts in C cycling regulate microbial assemblages in three streams draining landscapes burned during the Hayman Fire, CO as compared to two streams draining unburned landscapes. Research questions included: (1) How does fire change C quality and export in aquatic systems? (2) Are C sources different between burned and unburned watersheds? (3) In what manner is the fate of C in these landscapes determined by fire? (4) Does fire shift the composition of stream microbiomes? (5) How do changes in biogeochemical factors influence stream microbial assemblages? (6) Do shifts in microbial community structure predict shifts in ecosystem functioning (e.g., ecosystem respiration)?

METHODS

Site Description

The sites for this study are in and around Deckers, CO, USA in the Colorado Front Range (Table 1; Fig. 1). This region receives an annual average of 60 cm of precipitation and has an average temperature of 9.4°C (Stevens, 2013). Pikes Peak batholith underlies the majority of this area (Bryant *et al*., 1981), and soils are weakly developed, excessively drained, coarse sandy loams (Stevens, 2013). Forests are in the lower montane zone (1980–2750 m elevation) and are dominated by Ponderosa pine (*Pinus ponderosa*) and Douglas fir (*Psuedotsuga menziesii*) with understory plants including forbs, shrubs, and graminoids (Fornwalt *et al*., 2016).

In May 2002, the Schoonover Fire burned 16 km^2, and in June 2002, the Hayman Fire burned over 560 km^2 of the South Platte River drainage; both fires were stand-replacing. Seventeen years post-fire, watersheds in the burn scars experienced little recovery due to slow rates of tree colonization and forest regrowth and were characterized by grasses and forbs (Rhoades *et al*., 2011; Fornwalt & Kaufmann, 2014). Unburned watersheds generally exhibited greater tree cover (Jones, 2020) and resembled the pre-fire conditions of burned counterparts, containing more live trees, woody plants, conifers, and litter cover (Fornwalt & Kaufmann, 2014). However, vegetation differed noticeably between the two unburned watersheds, Sugar and Painted Rocks—see Jones (2020) for vegetation characterization. Specifically, Painted Rocks (12.65 ± 0.03%) had significantly more grass cover than Sugar (4.2 ± 0.02%) ($p = 0.019$). Therefore, watershed land cover (i.e., vegetation) and site location possibly affected results in manners not quantified in the study.

Five first- or second-order streams were studied, each draining an independent catchment (Fig. 1). Three streams—Cabin, Pine, and Fourmile—drain catchments that burned in the 2002 fires, and watershed sizes span from 11.1 to 24.9 km^2, with the total area burned varying across the three catchments. Overall burn extent ranges from 28% in Cabin, 54% in Pine, and 82% in Fourmile (Table 1). Two streams—Sugar and Painted Rocks—drain landscapes that have not burned in over 100 years but are geographically close to the burned sites to serve as reference systems. Unburned watershed areas encompass that of the burned watersheds, ranging from 4.5 km^2 in Painted Rocks to 36.2 km^2 in Sugar.

Sampling and Measurements

Stream Water Collection

During the summer of 2019 (May 31-July 30), the five streams were sampled. Stream chemistry as well as velocity, depth, and width were measured weekly. Velocity was determined via one of two methods: (1) using a Swoffer Model 3000 Current Velocity and Stream Discharge Indicator in streams greater than 3 in deep or (2) estimating flow with a ping pong ball (distance traveled / time = v). Instantaneous stream discharge (Q) was then calculated using corresponding stream channel measurements (i.e., depth and width). Stream temperature, dissolved oxygen (DO), pressure, pH, specific conductivity, and salinity were measured with a YSI Professional Plus Multiparameter Instrument. Standard pH solutions were used to calibrate YSI instruments prior to use. PME miniDO$_2$T Loggers II were placed in all streams excluding Painted Rocks (due to flow constraints) to continuously measure DO and temperature in 10-minute intervals for the entire study period.

Each week, water samples were collected for the analysis of the following stream constituents: total dissolved nitrogen (TDN), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), specific UV absorbance at 254 nm (SUVA$_{254}$), dissolved CO_2, $\delta^{13}C$-DOC, $\delta^{13}C$-DIC, $\delta^{13}C$-CO_2, and $\delta^{18}O$-H_2O. At the time of collection, all samples were filtered through 0.45 µm syringe filters in the field. DIC samples were taken by injecting 1 mL of filtered stream water into prepared evacuated exetainers with 0.2 mL H_3PO_4. To measure stream CO_2 concentrations, 30 mL of stream water was equilibrated with 30 mL of ambient air while submerged in the stream, and 15 mL of headspace from the syringe was added to a 12 mL exetainer. Field duplicates of all gas (including ambient air) and DIC samples were obtained. To analyze aquatic microbial communities, stream water was filtered until water could no longer exit through 0.22-µm Sterivex filters (MA, USA), biweekly—totaling five samples per site. Microbial sampling coincided with the biweekly field incubations described below.

All samples were stored on ice for transport back to Colorado College (Colorado Springs, CO). CO_2, DIC, and DOC samples—which were processed within two weeks of collection—were promptly stored at 5°C, while isotope samples were frozen until analyses could be completed. Filters for DNA extraction and 16S ribosomal RNA (rRNA) gene sequencing were stored at -80°C.

Solute and DOC Analysis

The above solute samples and DOC quality analyses were completed during the summer of 2019 at Colorado College. TDN and DOC samples were analyzed on a Shimadzu TOC-L/TNM-L with standards using KHP and KNO_3 (0.1 to 10 mg L^{-1} C and N). Each sample's specific UV absorbance at 254 nm was measured using an Agilent UV-Vis Spectrometer. These values—when normalized to DOC concentrations (absorbance at 254 nm/[DOC]*100, in L mg C^{-1} m^{-1})—function as a proxy for DOC aromaticity (Weishaar *et al.*, 2003). Gas samples (ambient air and stream CO_2) were measured on an SRI-8610C gas chromatograph. Standard gases of 100 ppm, 1000 ppm, 10000 ppm CO_2, and ambient lab air were run alongside samples for instrument calibration and equilibrium calculations.

Results from the above analyses were used to complete calculations for further study. Given the TDN and DIN (which consists of nitrite, nitrate, and ammonium) concentrations, dissolved organic nitrogen (DON) concentrations were determined (DON = TDN - DIN). The C:N of DOM was calculated using DOC and DON molar concentrations. Solute yields were calculated by dividing solute fluxes (flux = [solute]*Q) by respective watershed areas (yield = g m^{-2} $year^{-1}$). DOC yields were also converted to grams of C $year^{-1}$ and compared to estimates of grams of C exported from the soil using data from Jones (2020) to calculate DOC:soil C $year^{-1}$ ratios.

Isotopic Analysis

Samples were sent to the Yale Analytical and Stable Isotope Center lab (Yale University, New Haven, CT, USA) for isotopic characterization. Analyses for $\delta^{13}C$-CO_2, $\delta^{13}C$-DOC, $\delta^{13}C$-DIC, and $\delta^{18}O$-H_2O were performed on a Thermo DeltaPlus XP isotope ratio mass spectrometer. Carbon isotopic signatures indicated C sources to and within streams for CO_2, DOC, and DIC. Additionally, $\delta^{18}O$-H_2O measurements helped characterize differences in source waters and terrestrial processing prior to stream export.

Metabolism Measurements

In-situ three-hour dark incubations were completed according to Bogard *et al*. (2019) to measure planktonic respiration and indirectly assess DOC quality. These incubations were repeated five times throughout the summer at Sugar, Cabin, Pine, and Fourmile. Due to extremely low flows, incubations could not be conducted at Painted Rocks. At each site, stream water was added to a plastic five-gallon bucket until completely full to limit gas exchange with ambient air. Stream water in the bucket was clear of obvious aquatic plants, debris, litter, and sediment. A PME miniDO_2T Logger II was suspended in the bucket to record temperature and

DO concentrations every minute for 2.5-3 hours. While stream water incubated, the bucket remained submerged in flowing water as much as possible and covered with white plastic to ensure minimal temperature change from stream conditions.

The stream incubation mesocosm experiment measured DO uptake, the rate of which is representative of rapid mineralization of labile C in the water column (Bogard *et al.*, 2019). The difference in oxygen concentration over time (mg O_2 L^{-1} day^{-1}) in the bucket was calculated relative to DO saturation to account for changes in water temperature over the course of the incubation. However, since the bucket was not characteristic of stream conditions (i.e., it was often deeper and had no sediment), this rate demonstrates the relative bioavailability of C in the water column but not in-situ heterotrophic respiration rates (Bogard *et al.*, 2019). Molar ratios of O_2:DOC day^{-1} were then quantified for each stream incubation by comparing the incubation respiration rate and respective stream water DOC concentrations given the volume of the bucket (18.93 L). This ratio serves as a proxy for stream DOC biodegradability.

Using the continuous 10-minute interval DO concentrations and temperature measurements from the miniDO$_2$T loggers, weekly whole stream metabolism metrics—net ecosystem production (NEP = GPP - ER), ecosystem respiration (ER), and gross primary productivity (GPP)—were calculated for Sugar, Cabin, Pine, and Fourmile following the inverse modeling method described by Hotchkiss & Hall (2014). Estimations of reaeration coefficients (k_{600}) were calculated based on stream temperature, the scaling equation, and the Schmidt number conversions for O_2 (Wanninkhof, 1992; Jähne *et al.*, 1987). Photosynthetically active solar radiation and barometric pressure characteristics used in this method were modeled based on latitude, longitude, and elevation data of the sites (Yard *et al.*, 1995; Colt, 2012). Metabolism metrics were estimated in R using the streamMetabolizer package (Appling *et al.*, 2018). In cases where modeled GPP outputs were negative, values were corrected to zero given that negative productivity cannot occur.

Microbial Community Characterization

DNA extractions from the Sterivex filters were performed using Quick-DNA Fecal/Soil Microbe Kits (Zymo Research, CA, USA) in the fall of 2019 at the Wilkins Lab at Colorado State University (Fort Collins, CO). 16S rRNA gene sequencing and amplification was completed at Argonne National Laboratory (Lakewood, CO) on an Illumina HiSeq2500 sequencing system using the bacterial/archaeal primer set 515F/806R. This primer targets the V4 region and ensures maximum coverage of bacteria and archaea species and produces polymerase chain reaction (PCR) products of adequate length. Subsequent reads were grouped into exact sequence variants (ESVs) at 100% similarities via the QIIME 2 pipeline (QIIME2-2018.11). Sequences were archived in the NCBI sequence read archive under BioProject ID PRJNA659031.

Data Analysis

Statistical analysis and data visualization were completed using R Version 3.6.1. Pearson correlations were calculated to determine direction and strength of relationships between water quality measurements (including those of solutes, DOM quality, metabolism, and microbial community diversity metrics) and percent watershed burned, as well as other relevant variables such as water yield. This metric was also quantified for in-stream isotopes and qualitative stream variables. Isotopic measurements were considered temporally and compared to known signatures to determine sources. Two-sample t-tests were used to assess differences between streams draining burned or unburned catchments, and relationships with p-values less than or equal to

0.05 were considered statistically significant. Linear regression with a 95% confidence interval was used to assess relationships already identified as significant.

Alpha diversity, evenness, and richness metrics—Shannon's Diversity Index (H), Simpson's Index (D), Pielou's Evenness (J), and species richness—were calculated for further analysis of microbial communities within samples. Shannon's Diversity Index combines both abundance and evenness measurements with 0 being the least diverse, as does Simpson's Index (values = 0-1). Pielou's evenness (values = 0-1) considers how close in numbers each species in the sample are with 1 being the most even. Species richness is based on how many different species are present in the sample.

The vegan (Oksanen *et al*., 2007) and phyloseq (McMurdie & Holmes, 2013) packages in R were used to analyze differences in 16S rRNA gene datasets between streams and sampling dates. To assess differences in community composition between samples, Bray-Curtis dissimilarities were calculated. Bray-Curtis dissimilarity quantifies how dissimilar the microbial structures are. These values were plotted on a non-metric multidimensional scaling (NMDS) ordination to visualize differences in beta diversity between samples. The envfit function in R identified environmental variables and stream characteristics associated with microbial community composition shifts. The proximity of points to a vector illustrates a correlation, while vector length represents the strength of this relationship. In addition, analyses of similarity (ANOSIM) were computed to evaluate community differences between sites, burned and unburned watersheds, and burn extent.

RESULTS

Watershed Characterization

Stream discharge (ft^3 sec^{-1}) and water yield (mm year^{-1}) variability are the result of flow paths and catchment water export, which can both be altered by fire (Hallema *et al.*, 2017). At all five sites, peak discharge occurred by mid-June and declined throughout the study period, as temperature increased. This trend is expected as discharge of these montane streams is dominated by spring snowmelt. Additionally, discharge and water yield varied significantly among sites throughout the summer (Table 2; Fig. 2). The average discharge in the most extensively burned watershed (Fourmile) was the highest of the streams studied (1.58 ± 1.42 ft^3 sec^{-1}), and average discharge was relatively lower in streams draining unburned catchments (Sugar: 0.70 ± 0.63 ft^3 sec^{-1}; Painted Rocks: 0.063 ± 0.079 ft^3 sec^{-1}). Average water yields were significantly higher in burned (41.99 ± 34.33 mm year^{-1}) than unburned watersheds (15.01 ± 15.50 mm year^{-1}); however, this relationship ($p < 0.001$; Fig. 2) was largely driven by Fourmile's high (and variable) water yields (56.56 ± 51.09 mm year^{-1}).

Other stream parameters measured in the field did not consistently vary between sites, with fire history, or temporally. pH remained circumneutral in all streams and ranged from 7.11 to 7.90. Synoptic measurements of DO were similar among four of the stream sites, ranging from 71.5% to 81.4%, while DO measurements at Painted Rocks were significantly lower ($p = 0.01$) and ranged from 36.0% to 76.9%.

Stream Solutes

Dissolved Organic Matter

DOC concentrations in streams are linked to allochthonous and autochthonous processes and illustrate net differences in C sources and processing between watersheds. Although average stream DOC concentrations did not vary with extent watershed burned, streams draining burned landscapes generally had lower DOC concentrations than those draining Ponderosa forest (Table 2). The average DOC concentration in Painted Rocks, an unburned watershed, was the highest among the sites studied (5.66 ± 0.89 mg C L^{-1}). The other unburned stream, Sugar, experienced the second lowest average DOC concentration (1.63 ± 0.38 mg C L^{-1}) among the five streams. Still, on average, DOC was lower at the three burned watersheds—Cabin, Pine, and Fourmile—than at Painted Rocks (3.45 ± 0.48 mg L^{-1}, 3.62 ± 1.10 mg L^{-1}, and 1.62 ± 0.43 mg L^{-1} respectively). Lastly, DOC yields, DOC exports normalized to respective watershed areas, increased with burn extent (percent watershed burned) ($r = 0.31$, $p = 0.026$), largely due to greater water yields ($r = 0.76$, $p < 0.001$) in burned catchments (average values shown in Fig. 2).

Overall, $SUVA_{254}$ values were significantly higher in unburned watersheds ($p < 0.001$) (Table 2) and decreased with percent burned ($r = -0.68$, $p < 0.001$; Fig. 3). $SUVA_{254}$ values were as low as 2.30 ± 0.38 L mg C^{-1} m^{-1} in the most extensively (82.36%) and severely burned (57.6%) site (Fourmile) and increased to 3.30 ± 0.53 L mg C^{-1} m^{-1} in the least extensively (28%) and severely burned (14.9%) site (Cabin). In streams draining unburned landscapes, $SUVA_{254}$ values over the summer were higher; Sugar averaged 3.37 ± 0.75 L mg C^{-1} m^{-1}, and Painted Rocks averaged 3.87 ± 0.44 L mg C^{-1} m^{-1}. Because $SUVA_{254}$ values are a proxy for DOC aromaticity (Weishaar *et al*., 2003), these results also imply that the C exported by burned watersheds was less aromatic (i.e., aromaticity and burn extent were negatively correlated). $SUVA_{254}$ values, and therefore aromaticity of C, in streams draining burned watersheds did not fluctuate as much over the study period as compared to the DOM in streams draining unburned catchments. Within unburned watersheds, $SUVA_{254}$ values increased over the course of the summer as discharge decreased ($r = -0.82$, $p < 0.001$) and experienced greater temporal variability throughout the summer (Sugar: range = 2.37 L mg C^{-1} m^{-1}; Painted Rocks: range = 1.49 L mg C^{-1} m^{-1}) (Fig. 3). The temporal differences in $SUVA_{254}$ between burned and unburned watersheds suggest that stream DOC may be from different sources and/or processed differently due to shifts in flow paths.

DOC to DON ratios within streams are important to consider as they can be indicative of hydrologic flow paths and are often determined from soil C:N ratios (Aitkenhead-Peterson *et al.*, 2005). The ratio of DOC:DON was greatest in the unburned watershed Sugar (76.91 ± 53.34). Although the three streams in burned watersheds all had lower DOC:DON ratios than Sugar, DOC:DON was not significantly different between streams based on fire history.

DOC to soil C ratios illustrate how export of C from the watershed differs and is dependent on %C soil and water yield. This ratio was on average higher in burned watersheds (0.00063 ± 0.00048 $year^{-1}$) than in unburned watersheds (0.00027 ± 0.00043 $year^{-1}$) and increased with extent watershed burned ($r = 0.75$, $p = 0.05$; average values shown in Fig. 4).

δ^{13}C-DOC

Conclusions can be made regarding C sources by comparing $\delta^{13}C$ signatures of DOC with known $\delta^{13}C$ values in the literature. The mean $\delta^{13}C$ in streams draining burned landscapes was -30.28 ± 0.95‰ versus -29.97 ± 1.80‰ in streams draining unburned landscapes (Table 3). Fourmile, an extensively burned watershed, experienced the most depleted $\delta^{13}C$-DOC signal on average (-30.93 ± 0.97‰), while Painted Rocks, an unburned watershed, experienced the most enriched $\delta^{13}C$-DOC signal (-29.06 ± 1.08‰). Stream $\delta^{13}C$-DOC did not vary significantly with burn extent or over the course of the summer. At all five streams, $\delta^{13}C$-DOC values remained steady throughout the study period (burned: CV = 0.03; unburned: CV = 0.06) and were not correlated with discharge. The stable isotope signatures indicate consistent sources of organic matter from May to August.

Inorganic Carbon

Small headwater streams are typically supersaturated in CO_2; therefore, CO_2 is a significant contribution to the aquatic DIC pool (Campeau *et al*., 2017; Finlay, 2003). CO_2 concentrations were significantly higher in streams draining unburned landscapes (1530.20 ± 819.72 ppm) than those draining burned landscapes (745.32 ± 236.28 ppm) ($p = 0.0004$). Average CO_2 concentrations ranged from 1945 ± 975 ppm in an unburned watershed (Painted Rocks) to 619 ± 87 ppm in the low extent burned watershed (Cabin) (Table 3). Moreover, CO_2 concentrations exhibited a negative relationship with discharge ($r = -0.37$, $p = 0.01$); stream concentrations increased over the course of the summer as water export decreased. CO_2 concentrations varied the most over the course of the summer in Painted Rocks, and unburned watersheds in general (Fig. 5). CO_2 concentrations were also positively correlated with stream DOC concentrations ($r = 0.36$, $p = 0.01$).

$\delta^{13}C$-DIC and $\delta^{13}C$-CO_2

The $\delta^{13}C$ values of DIC and CO_2 reveal input sources to/within streams and subsequent C processing. $\delta^{13}C$-DIC signatures in streams incorporate $\delta^{13}C$-CO_2 from biogenic inputs and atmospheric evasion, as well as geogenic and photooxidative sources of inorganic C (Campeau *et al*., 2017). Given that stream DIC and CO_2 are in equilibrium relative to pH and temperature, it was expected that $\delta^{13}C$-DIC and $\delta^{13}C$-CO_2 were significantly and positively correlated ($r = 0.90$, $p < 0.001$). On average, DIC and CO_2 were more enriched in ^{13}C in burned than unburned sites ($p < 0.001$; Fig. 5). $\delta^{13}C$-DIC in unburned watersheds had an average value of -10.48 ± 1.16‰ as compared to -12.39 ± 0.84‰ in burned watersheds (Table 3). DIC was most enriched in ^{13}C at Cabin, a moderately burned watershed, (-10.17 ± 0.62‰) and most depleted at Painted Rocks (-12.94 ± 0.80‰). $\delta^{13}C$-CO_2 values reached -16.61 ± 1.76‰ in burned watersheds, and like DIC, was most enriched in ^{13}C at Cabin (-15.88 ± 2.19‰; Table 3). In unburned sites, $\delta^{13}C$-CO_2 averaged -20.93 ± 2.43‰; Painted Rocks experienced the most depleted C signature as was the case with DIC (-22.19 ± 2.15‰). Average summertime $\delta^{13}C$-DIC and $\delta^{13}C$-CO_2 values additionally increased with percent burned ($r = 0.54$, $p < 0.001$; Fig. 5).

In burned and unburned streams, $\delta^{13}C$ signatures of DIC and CO_2 became more depleted throughout the summer, and $\delta^{13}C$-DIC was correlated with both discharge ($r = 0.56$, $p < 0.001$) and temperature ($r = -0.24$, $p = 0.10$). There was greater temporal variability in unburned watersheds (Fig. 5), and relationships with discharge were stronger in these streams as well (Sugar: $r = 0.87$, $p =0.001$; Painted Rocks: $r = 0.81$, $p = 0.005$). In all watersheds except for Cabin, $\delta^{13}C$-DIC increased with discharge, yet this relationship was only significant at Fourmile, a severely burned site ($r = 0.71$, $p = 0.02$). Similarly, $\delta^{13}C$-CO_2 was only significantly associated with discharge in unburned streams ($r = 0.74$, $p = 0.004$) and Fourmile ($r = 0.73$, $p = 0.03$).

Nitrogen

As observed in other studies (Rhoades *et al.*, 2011), burn extent and severity largely predicted variation in summer stream nitrogen (N) concentrations and the species of dissolved N present. Fourmile had the highest average TDN concentration (0.58 ± 0.36 mg N L^{-1}) over the summer, while Sugar had the lowest average (0.059 ± 0.064 mg N L^{-1}) (Table 2). However, TDN concentrations in Fourmile were greatly determined by DIN (average = 0.43 ± 0.19 mg L^{-1}), which was dominated by nitrate (average = 0.42 ± 0.19 mg N L^{-1}), while DON dominated the dissolved nitrogen pools in the other four streams (all DIN averages < 0.1 mg L^{-1}).

$\delta^{18}O$-H_2O

$\delta^{18}O$-H_2O measurements can be indicative of flow paths, as well as overall hydrology, and are dependent on temperature (Gat, 2010). On average, $\delta^{18}O$-H_2O was the most depleted in Sugar (-14.34 ± 0.23‰), an unburned site. H_2O was most enriched in ^{18}O in unburned site Painted Rocks (-12.52 ± 0.11‰) and the most severely burned watershed Fourmile (-13.22 ± 0.29‰) (Fig. 6). Excluding the smallest watershed, Painted Rocks, $\delta^{18}O$-H_2O values increased with extent watershed burned ($r = 0.58$, $p < 0.001$). Significant relationships were not found between $\delta^{18}O$-H_2O and temperature or discharge, which were also both strongly correlated with burn extent. However, $\delta^{18}O$-H_2O tended to increase through the study period from May to August ($r = 0.42$).

Stream Metabolism

In Situ Incubations

Incubation rates—which describe rates of labile C mineralization in streams—did not vary significantly between burned and unburned watersheds or between individual sites. Average rates also varied between streams. Both the maximum (5.66 mg O_2 L^{-1} day^{-1}) and minimum (0.61 mg O_2 L^{-1} day^{-1}) rates occurred at Cabin, indicating large temporal variability within a single site. Although similar amounts of variability occurred at the other four sites, incubation rates were not statistically correlated with temperature or discharge. Furthermore, incubation rates were not statistically correlated with any solute measured during the experiment (e.g., TDN, DOC, $SUVA_{254}$, $\delta^{13}C$-DIC).

Dissolved C Bioavailability

From bucket incubation oxygen consumption rates, molar ratios of O_2:DOC day^{-1} were calculated as a measure of stream DOC bioavailability. The ratio varied temporally at all sites.

Unlike incubation rates, which did not vary with disturbance history, O_2:DOC day^{-1} ratios were greater in unburned watersheds on average (0.65 ± 0.28) as compared to burned watersheds (0.45 ± 0.31) (Fig. 7). However, Fourmile, the most extensively burned watershed, had the highest C bioavailability on average among the five sites (0.77 ± 0.29), while Cabin, another burned watershed, experienced the lowest average ratio (0.29 ± 0.22). Among streams draining burned watersheds, O_2:DOC day^{-1} ratios significantly increased with extent watershed burned ($r = 0.66$, $p = 0.007$; Fig. 7). These ratios were negatively correlated with δ^{13}C-DOC ($r = -0.50$, $p = 0.03$; Fig. 8), and this relationship was most significant at Cabin ($r = 0.99$, $p = 0.0014$) and Sugar ($r = 0.90$, $p = 0.04$).

Modeled Daily Metabolism

Weekly whole stream metabolism estimations from in-situ O_2 sensor measurements illustrated that all five streams were dominated by ecosystem respiration (ER) and had low levels of gross primary productivity (GPP). Net ecosystem productivity was negative at all sites throughout the summer, as expected. ER was not significantly different between sites, with average ER estimates ranging from 1.49 ± 0.36 g O_2 m^{-2} day^{-1} to 1.89 ± 0.42 g O_2 m^{-2} day^{-1}. ER was positively correlated with water yield across all sites ($r = 0.50$, $p = 0.001$).

Rates of GPP were often higher in burned watersheds than unburned. However, significantly higher rates of GPP occurred at unburned site Sugar, which averaged 1.22 ± 0.40 g O_2 m^{-2} day^{-1}, while moderately burned site Cabin averaged 0.055 ± 0.17 g O_2 m^{-2} day^{-1}. Negative outputs occurred in 37% of the estimates, most often in Cabin and Pine, suggesting the model (Hotchkiss & Hall, 2014) did not capture all variability in the data. Mineralization of DOC rates calculated from the bucket incubations were not correlated with the modeled rates of whole stream respiration (ER).

Microbial Communities

Diversity Metrics

Community diversity in aquatic microbial communities may be influenced by factors such as OM pools and temperature (Zeglin, 2015). Accordingly, species richness, Shannon's Diversity Index, Simpson's Index, and Pielou's evenness varied according to burn severity, although not always significantly. Aquatic microbial communities from burned and unburned watersheds had similar Pielou's evenness. Still, burned watersheds experienced slightly higher Pielou's evenness on average (range = 0-1) (0.85 ± 0.087) than aquatic microbial communities from unburned watersheds (0.81 ± 0.078). Species richness was highest in Pine (2417 ± 432 ESVs), a moderately burned watershed, and lowest in Fourmile (1517 ± 493 ESVs), a severely burned watershed. Microbial species richness decreased with extent burned, but this was not a statistically significant relationship ($r = -0.20$, $p = 0.35$). No significant differences were found for Shannon's Diversity and Simpson's Index values between burned and unburned aquatic microbial communities ($p > 0.05$).

Both microbial community composition and diversity often show temporal heterogeneity as well as connections to ecosystem-scale responses (Zeglin, 2015). Exact sequence variants (ESVs) were most similar within sites (ANOSIM, Sugar vs. Painted Rocks vs. Cabin vs. Pine vs. Fourmile, 0.784, $p < 0.001$) and were also statistically similar between sites with similar fire history and burn extent (ANOSIM, burned vs. unburned watersheds, 0.558, $p < 0.001$; ANOSIM, % burned, 0.546, $p < 0.001$). When plotted on an NMDS ordination, Bray-Curtis dissimilarity values had a relatively low stress value (0.113), indicating a fair fit of the ordination (Fig. 9). Bray-Curtis dissimilarities illustrated significant differences between microbial assemblage composition based on site location and extent burned (Fig. 9). These results further reinforce relationships between site characteristics and fire history. Microbial communities differentiated based on $SUVA_{254}$ values and TDN concentrations across the burn gradient. Greater $SUVA_{254}$ were correlated with the microbial communities at unburned sites (Fig. 3), and greater TDN concentrations (Table 2) were correlated with the microbial communities at burned sites. Bray-Curtis dissimilarities confirmed differences between microbial communities as explained by temporal variables including hydrological characteristics (e.g., average velocity), inorganic C isotope signatures, and diversity metrics (e.g., species richness).

Differences in Relative Abundance

Differences in the relative abundances of microbial phyla between burned and unburned sites can demonstrate how the stream microbiome is altered by fire and fire severity. The six most abundant phyla in streams draining burned and unburned watersheds (in order of decreasing relative abundance in burned watersheds) included Proteobacteria, Bacteroidetes, Cyanobacteria, Verrucomicrobia, Patescibacteria, and Actinobacteria. On average, streams draining burned landscapes contained greater relative abundances of Proteobacteria, Bacteroidetes, Actinobacteria, and Verrucomicrobia than unburned streams, which contained greater relative abundances of Cyanobacteria and Patescibacteria (Table 4; Fig. 10a). Relative abundances of Bacteroidetes, Verrucomicrobia, and Actinobacteria were significantly different between burned and unburned sites ($p = 0.008, 0.001, 0.002$, respectively; Table 4). Also, certain families of Actinobacteria—Intrasporangiaceae, Nocardiaceae, and Mycobacteriaceae—were more abundant in streams from burned catchments (Fig. 10b).

DISCUSSION

Changes to C Export and Processing

Understanding linkages between terrestrial and aquatic environments following wildfire is important for assessing its legacy on carbon partitioning, and thus carbon sequestration, across the landscape. Seventeen years after the Hayman Fire, changes to the terrestrial environment persist. Vegetation significantly differed compared to unburned sites, resulting in shifting hydrology and a characteristically different soil and soil microbiome (Bonwell, 2020; Jones, 2020). Fire alteration of vegetation and soil properties plays a significant role in OM quality (González-Pérez *et al*., 2004; Knicker, 2007; Fornwalt & Kaufmann, 2014) and amount of C exported to streams (Moody & Martin, 2001; Rhoades *et al*., 2018). In burned watersheds, SOM appears to be more bioavailable (Jones, 2020) and was more likely to be laterally exported to streams than in unburned catchments (Bonwell, 2020); therefore, C likely has a shorter residence time in these terrestrial landscapes (Fig. 11).

Export of DOC is largely related to terrestrial C cycling and balance, and burned systems are no exception (Aitkenhead-Peterson *et al*., 2005). Amendments to the antecedent environmental conditions, which may be driven by fire, impact aquatic nutrient export in response to changes in temperature (Xu & Saiers, 2010), discharge, seasonality (Dawson *et al*., 2008), and overall DOM mobilization from the watershed (Raymond & Saiers, 2010). Wildfire often leads to elevated water export (Fig. 2) due to decreased vegetative cover and decreased evapotranspiration (Hallema *et al*., 2017), which occurred in the three burned watersheds here. Given that water yield was highest in burned catchments (Cabin, Pine, and Painted Rocks), DOC export was also greater in the moderately burned watersheds (Cabin and Pine) than the unburned watersheds (Sugar and Painted Rocks), consistent with previous studies (Rhoades *et al*., 2018).

Moreover, DOC export was greater per unit C in the watershed (Fig. 4), suggesting that a combination of factors, including increased water yield and reduced %C in burned soils (Jones, 2020), may lead to greater transport of C from terrestrial to aquatic environments.

Differences in the quality of C exported can also be understood in terms of riparian and upland vegetation differences between sites and the slow recovery of vegetation after fire (Rhoades *et al*., 2018). Lower $SUVA_{254}$ values indicate that DOM was less aromatic (Weishaar *et al*., 2003) in streams draining burned landscapes as compared to intact forests. In addition, pyrogenic carbon (highly aromatic C produced during fire) in soils did not vary statistically between burned and unburned watersheds 15 years following the Hayman Fire (Barnes & Gilbertson, 2018, unpublished data), potentially due to fire history and the residence time of black carbon. However, previous studies examining stream DOC exports, typically occurring within a decade following fire, report higher stream $SUVA_{254}$ values with the influx of pyogenic and other condensed aromatic C compounds (Santos *et al*., 2016; Olefeldt *et al*., 2013; Chow *et al*., 2019). Although $SUVA_{254}$ values remained elevated 14 years post-Hayman Fire in moderately burned watersheds (Chow *et al*., 2019), our results indicate that DOC export moved away from the aromatic C pools that typically dominate streams following fire (Fig. 3). Shifts in vegetation and thus C inputs (e.g., lignin, cellulose, etc.) after fire likely drive this pattern. Minimal forest recovery in 17 years led to the dominance of herbaceous cover (i.e., grasses and forbs, 19.0 ± 10.1% and 10.9 ± 5.39% cover respectively, Jones, 2020) in burned regions of the Hayman burn scar. In contrast, woody plants (i.e., Ponderosa pine and shrubs) dominate the unburned landscapes, contributing aromatic inputs of lignin, as compared to the less aromatic cellulose inputs within burned catchments.

The interaction between hydrology and vegetation also drives carbon dynamics in these watersheds and its lateral transport to streams. Isotopic analysis (Table 3) reveals that both inorganic and organic C pools in all streams were predominantly terrestrially derived, as expected for small headwater streams (Vannote *et al*., 1980; Palmer *et al*., 2001; Hotchkiss *et al*., 2018). For example, δ^{13}C-DIC signatures (-11.24 ± 1.35‰) generally fell between values for atmospheric CO_2 (~-8‰, Finlay & Kendall, 2007) and CO_2 from soil respiration (-14.11 ± 4.19‰, Jones, 2020). Integration of the atmospheric CO_2 signature occurred as small streams are typically supersaturated in CO_2, and this disequilibrium allows for the rapid evasion of CO_2 from stream surfaces to the atmosphere (Öquist *et al*., 2009). δ^{13}C-DIC values additionally reflect that stream inorganic C pools are influenced by terrestrial SOM decomposition (i.e., CO_2 from microbial respiration; Fig. 12), similar to other studies, which only emphasizes the importance of quantifying and qualifying DIC fluxes (Finlay, 2003; Campeau *et al*., 2017). Additionally, DIC and CO_2 were more enriched in ^{13}C in burned sites than in unburned sites, reflecting the more isotopically enriched, albeit smaller, soil CO_2 pools in burned watersheds (Jones, 2020). Lastly, average δ^{13}C-DOC values (-30.28 ± 0.95‰) in burned streams were more depleted than that of catchment SOM (~-24‰, Jones, 2020) (Fig. 5), implying preferential retention of ^{13}C following leaching (Hagedorn *et al*., 2004) or different sources of DOC in the aquatic systems, such as bacterial communities.

Fire history likely influences in-situ processes, which respond to fluctuations in C stocks (Langenheder *et al*., 2010) and changing abiotic conditions. At all sites, as dissolved CO_2 concentrations increased throughout the summer, δ^{13}C-CO_2 signatures became more depleted (Fig. 5), suggesting increased rates of respiration as temperatures rose (Aerts, 1997; Fierer *et al*., 2005). During the beginning of the study period in May and June, δ^{13}C-CO_2 closely reflected the

C signature of the CO_2 respired from soil at both burned and unburned catchments. However, the $\delta^{13}C-CO_2$ signature became noticeably more depleted over the course of the summer at unburned sites, trending toward the signatures of the watershed SOM and stream DOC (Fig. 5). This trajectory indicates a shift in flow path (or contributing source area) and/or differences in in-situ sources and processing between sites over time. For example, greater rates of evapotranspiration in forests could lead to greater temporal variability in flow paths, which was also evident in trends in $\delta^{18}O-H_2O$ (Fig. 6).

Oxidation of stream DOM may impact aquatic C stocks in streams draining unburned watersheds to a greater extent given that it was more aromatic and less processed than the DOM in streams draining burned landscapes (Fig. 3). According to measurements from in situ bucket incubations, DOM in burned streams was also less bioavailable (Fig. 7), and bacterial production may be reduced in these sites due to lower species richness in streams draining burned landscapes. These data may also explain the lack of temporal signals within burned streams as compared to unburned streams where DOM was more bioavailable. Furthermore, OM bioavailability and $\delta^{13}C$-DOC were negatively correlated (Fig. 8), illustrating a potential relationship between C availability to microbial communities and C character (Wiegner *et al.*, 2005; Kaplan *et al.*, 2008). Although SOM in burned soils was more bioavailable (Jones, 2020), C may be less bioavailable in burned streams as this C was processed more within the terrestrial landscape or because the SOM pool in burned soils was significantly smaller than that of unburned soils, and thus more likely to be processed before reaching the stream.

Shifts in the Stream Microbiome

Stream microbiomes were distinct from each other and were distinguishable by fire history. It is surprising that the aquatic microbiomes appear to be more distinct depending on site and burn extent as well as more responsive to environmental factors like C quality—as illustrated by how close samples cluster to each other and plotted vectors (Fig. 9)—than the soil microbiomes of these watersheds (Jones, 2020). Soil microbiomes did not distinguish between sites and only differentiated based on fire history (see Fig. 9 in Jones, 2020).

Many factors may explain these shifts in community structure, including biogeochemical (e.g., DOM supply, Judd *et al.*, 2006) or environmental characteristics (e.g., pH, Fierer *et al.*, 2007). However, the NMDS ordination used to quantify dissimilarities between microbial community samples illustrates that the microbial composition changed with shifts in hydrology (e.g., velocity), organic matter quality ($SUVA_{254}$), and inorganic carbon and nitrogen pools ($\delta^{13}C$-DIC, TDN) (Fig. 9). The fact that $SUVA_{254}$ values and $\delta^{13}C$-DIC were correlated with shifts in microbial assemblage implies that C supply exerts some control on the stream microbiome. Given the importance of DOC as the largest pool of organic matter in fluvial systems (Kaplan *et al.*, 2008) and as a source of C to stream microorganisms, fire indirectly drives differences in microbiome composition by altering DOC export and C quality.

The C pool and character select for different microbial taxa over a relatively short time period (Judd *et al.*, 2006). For example, microbiomes in streams draining unburned watersheds exhibited the most inter-site variability, which also experienced greater variability in water yield, DOC export, $SUVA_{254}$, and ^{13}C isotopic signatures than streams draining burned watersheds. Streams draining burned watersheds exported greater DOC concentrations per unit C in hillslope soils (Fig. 4), which could likewise yield differences in microbial communities (Eiler *et al.*, 2003). In essence, these shifts in DOM quality and export post-fire may elucidate the observed differences in stream microbiomes both in the short-term and long-term (Bernhardt & Likens, 2002; Crump *et al*., 2003; Eiler *et al*., 2003; Judd *et al*., 2006; Strickland *et al*., 2009; Zeglin, 2015).

Although aquatic C stocks may influence the microbiome, aquatic microbial communities and other in-stream processes also affect dissolved organic and inorganic C stocks. Different relative abundances of certain microbial phyla possibly inform the changes in C quality via increased microbial transformation of C, preferential degradation of less aromatic C compounds, or a greater effect of photodegradation on aromatic DOM within burned watersheds (Olefeldt *et al*., 2013; Hotchkiss *et al*., 2018). Heterotrophic taxa within the Bacteroidetes, Actinobacteria, and Verrucomicrobia phyla were statistically more abundant in burned watersheds (Table 4; Fig. 10) and may all play key roles in the degradation of organic matter (Thomas *et al*., 2011; Cardman *et al*., 2014; Chen *et al*., 2016). While it is challenging to predict functional properties of different taxa identified through marker gene analyses, it is likely that there are overlaps in the ecological functions of microorganisms within these phyla that result in minor differences in the aquatic C stock.

Actinobacteria—including three specific families (Fig. 10b)—that were more abundant in both burned soils (Jones, 2020) and streams draining those landscapes (Fig. 10) are associated with the degradation of aromatics, including pyrogenic carbon in burned watersheds (Khodadad *et al*., 2011; Chen *et al*., 2016). Soil pyrogenic C amendments have been shown to drive an increase in the relative abundance of bacteria in the phylum of Actinobacteria following fire (Khodadad *et al*., 2011). Given that greater amounts of pyrogenic C no longer remain in these watersheds this many years post-fire (Barnes & Gilbertson, 2018, unpublished data) but elevated Actinobacteria relative abundances persist (Fig. 9), the legacy of fire on burned landscapes appears to extend to the microbial communities beyond that of certain C modifications. Although the conditions which enriched this phylum have diminished, the persisting higher abundances of Actinobacteria in streams draining burned landscapes potentially illustrates the ability of wildfire to disturb ecosystems to a new steady state.

Even though microbial communities differed based on fire history, differences in in-stream processes (e.g., ER) did not reflect these community shifts. Given that ecosystem respiration did not vary significantly between streams, but the microbial community structure did, functional redundancy—the ecological phenomenon when different taxa assume the same functional niche—may exist in these systems. Therefore, fire seems to govern stream systems in ways that are either not detectable in measurements of ecological functioning, or that the communities function differently but still result in same process rates when considered at the community level (Allison & Martiny, 2008).

CONCLUSION

Overall, the legacy of wildfire on these landscapes extends to carbon sources, subsequent processing, and microbial community composition in both terrestrial and aquatic environments 17 years after the Hayman Fire (Fig. 11). C partitioning and residence times have been altered in the terrestrial environment due to differences in the dominant vegetation (Jones, 2020). Moreover, shifts in hydrology and the soil C stock post-fire led to increased lateral export of C to aquatic systems in burned landscapes. However, although allochthonous sources dominated these small headwater streams, C was less bioavailable and qualitatively different once transported. Compared to streams draining unburned landscapes, in-situ processing may have a smaller effect on burned systems, and microbial assemblages experienced significant shifts nearly two decades after the disturbance. Although differences in C character inevitably impact aquatic microbial composition, differences in stream microbiomes may alter aquatic C stocks and transformation as well. Thus, wildfire potentially forces a positive feedback loop between C export and aquatic microbiomes by furthering C alteration (Fig. 11). The altered recovery dynamics for both vegetation and microbiomes suggest that the Hayman Fire has shifted these systems to a new steady state in the face of severe disturbance (Stevens-Rumann *et al*., 2018).

Understanding how C is partitioned across watersheds like those studied here is pertinent when considering the global C balance in response to climate change. Expanding knowledge on the lateral export of C to inland waters is often disregarded in these assessments, and microbial mediation of C is typically ignored in small streams. As such, it is valuable to address these issues following disturbances like fire, which are expected to increase in both frequency and severity in the coming decades (Westerling *et al*., 2006; Abatzogloua & Williams, 2016).

TABLES AND FIGURES

	Sugar	**Painted Rocks**	**Cabin**	**Pine**	**Fourmile**
Latitude	39.30476	39.08249	39.23229	39.24051	39.24257
Longitude	-105.1818	-105.1039	-105.302	-105.2819	-105.2311
Area (km^2)	36.2	4.5	24.0	11.1	24.9
% Burned	0	0	28	53.7	82.3
% Severely Burned	0	0	14.9	34.1	57.6

Table 1. Burn site characteristics, including latitude and longitude data for site locations. The watershed area of each site is listed as well as the percent of the watershed that burned and was severely burned.

	Sugar	Painted Rocks	Cabin	Pine	Fourmile
% Burned	0	0	28	53.7	82.3
Water Yield (mm year^{-1})	17.35 ± 15.70	12.68 ± 15.77	24.19 ±14.68	45.21 ± 19.34	56.56 ± 51.09
DOC (mg C L^{-1})	1.63 ± 0.38	5.66 ± 0.89	3.448 ± 0.49	3.62 ± 1.10	1.62 ± 0.43
DOC Yield (g C m^{-2} year^{-1})	30.22 ± 31.54	74.36 ± 99.71	83.92 ± 51.93	173.46 ± 109.47	95.84 ± 89.60
SUVA$_{254}$ (mg C^{-1} m^{-1})	3.37 ± 0.75	3.87 ± 0.44	3.30 ± 0.53	2.97 ± 0.32	2.30 ± 0.38
TDN (mg N L^{-1})	0.06 ± 0.06	0.48 ± 0.15	0.29 ± 0.09	0.18 ± 0.09	0.69 ± 0.35
TDN Yield (g N m^{-2} year^{-1})	1.51 ± 3.22	4.89 ± 5.97	6.00 ± 3.99	8.05 ± 7.54	26.59 ± 15.14

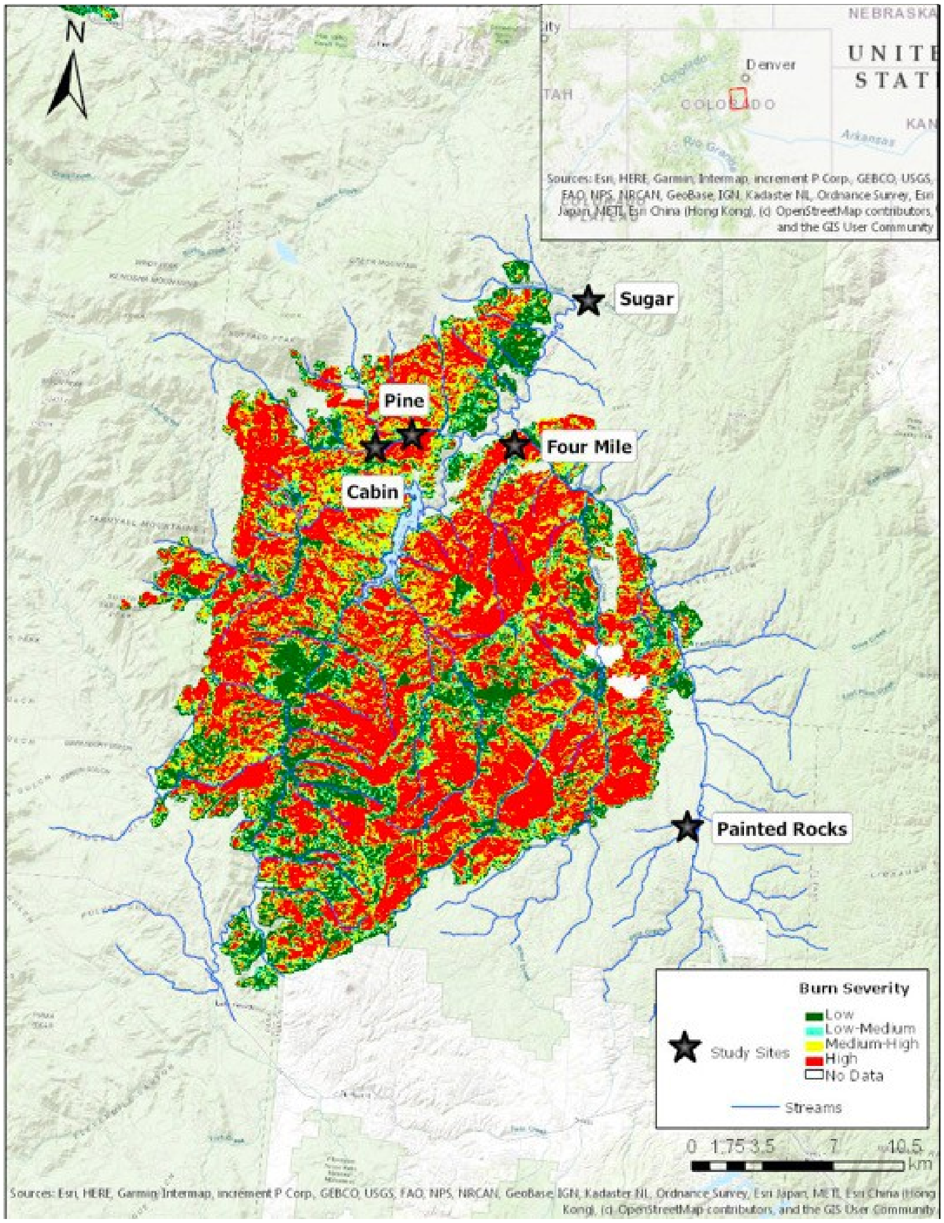
Table 2. Solute concentrations and quality measurements. Averages and standard deviations for water yields (mm year^{-1}), DOC and TDN concentrations (mg L^{-1}) and yields (g m^{-2} year^{-1}), and SUVA$_{254}$ values for each watershed are provided.

	Sugar	**Painted Rocks**	**Cabin**	**Pine**	**Fourmile**
% Burned	0	0	28	53.7	82.3
δ^{13}C-DOC (‰)	-30.88 ± 1.95	-29.06 ± 1.08	-30.11 ± 0.82	-29.80 ± 0.73	-30.93 ± 0.97
δ^{13}C-DIC (‰)	-11.83 ± 1.23	-12.94 ± 0.80	-10.17 ±0.62	-10.72 ± 0.79	-10.54 ± 1.05
δ^{13}C-CO_2 (‰)	-19.66 ± 2.08	-22.19 ± 2.15	-15.88 ± 2.19	-16.42 ± 1.32	-17.46 ± 1.52
CO_2 (ppm)	1116 ± 294	1945 ± 795	619 ± 87	719 ± 138	885 ± 331
δ^{13}C-SOM (‰)	-24.30 ± 0.95	-24.33 ± 0.42	-24.20 ± 0.36	-24.27 ± 1.01	-24.47 ± 0.49
δ^{13}C-CO_2, Soil Respiration (‰)	-14.85 ± 1.72	17.34 ± 2.01	-14.6 ± 0.95	-14.07 ± 0.89	-13.68 ± 0.67

Table 3. Isotopic analysis of aquatic and terrestrial C sources. Average ^{13}C signatures and standard deviations for DOC, DIC, and CO_2, compared to CO_2 concentrations (ppm) and ^{13}C signatures from the watershed (SOM and CO_2, Jones, 2020) for each catchment are provided.

Phylum	Average Relative Abundance (burned)	Average Relative Abundance (unburned)	p-value
Proteobacteria	0.394 ± 0.080	0.385 ± 0.098	0.806
Bacteroidetes	0.195 ± 0.061	0.129 ± 0.049	0.008*
Cyanobacteria	0.0841 ± 0.092	0.180 ± 0.147	0.104
Verrucomicrobia	0.0783 ± 0.027	0.0400 ± 0.012	0.0001*
Patescibacteria	0.0420 ± 0.029	0.0514 ± 0.036	0.5166
Actinobacteria	0.0517 ± 0.037	0.0148 ± 0.004	0.002*

Table 4. Average microbial relative abundances ± standard deviation for the six most abundant phyla found in all sites studied by burn history. Differences were tested with two-sample t-tests, and significance was defined as $p \leq 0.05$ (as indicated by *).



Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

Figure 1. Map of study site locations (indicated with black star and name label) and burn extent of 2002 Hayman and Schoonover fires. Based on the Monitoring Trends in Burn Severity (MTBS) scale, burn severity is shown low (green) to high (red). Map created in ArcGIS Pro. *Figure courtesy of Cheristy Jones.*

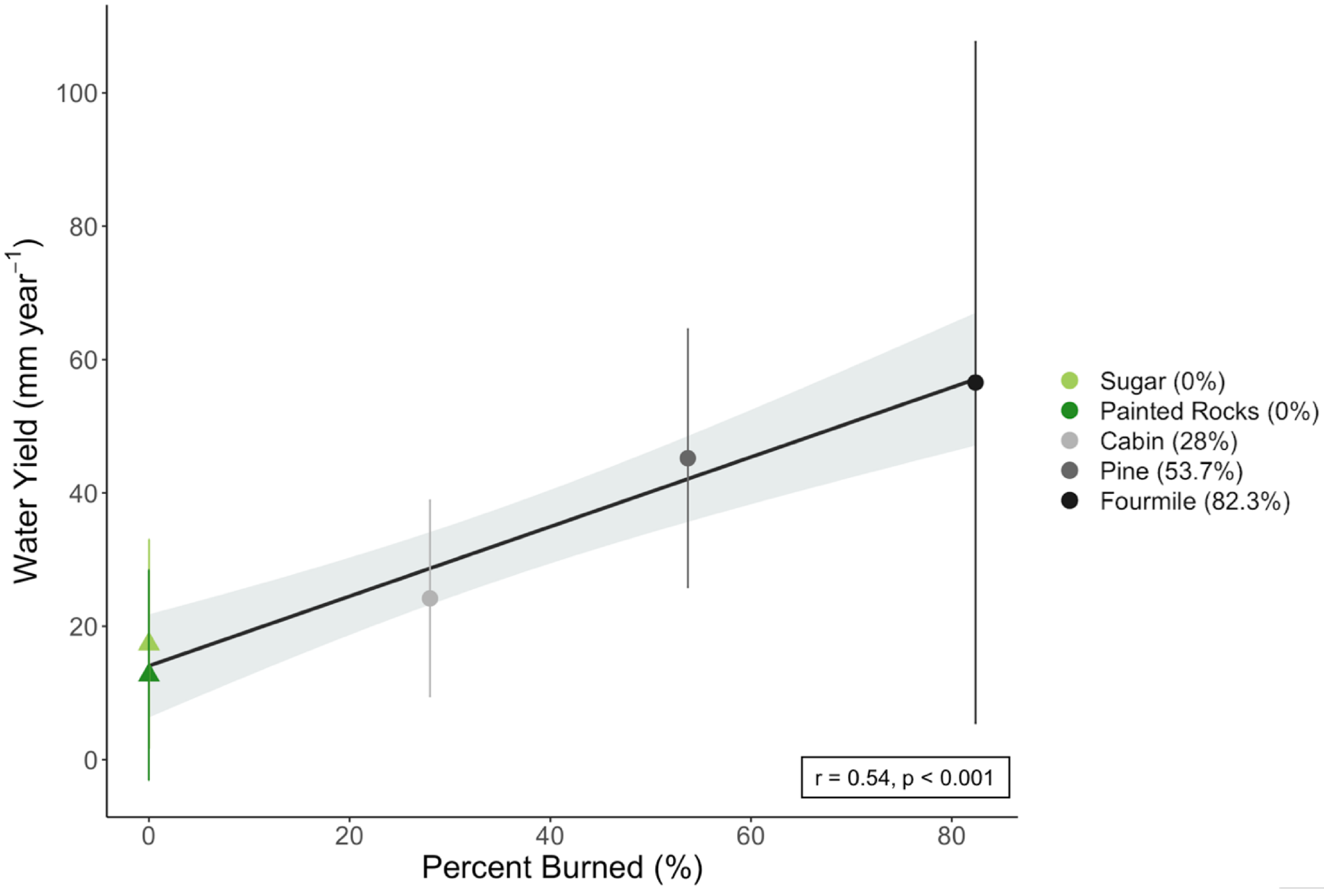


Figure 2. Average water yields (mm year^{-1}) by site based on percent watershed burned (%) with line of best fit. Gray coloration indicates the 95% confidence interval, and error bars are representative of ± standard deviation. Circle points correspond to burned watersheds, and triangle points correspond to unburned watersheds. Water yield increased significantly with percent burned.

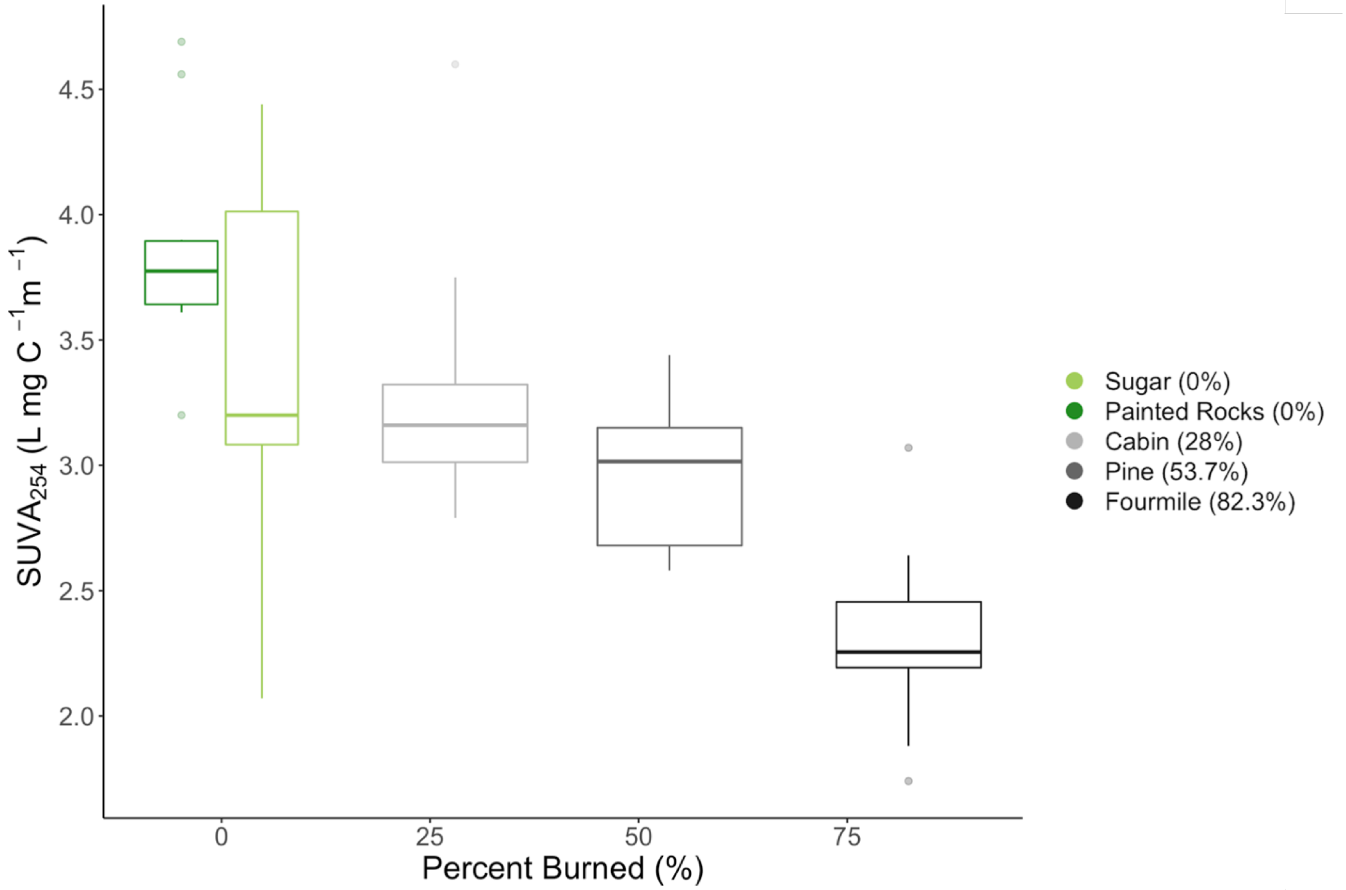


Figure 3. Box plot of $SUVA_{254}$ (L mg C^{-1} m^{-1}) values according to percent watershed burned (%). $SUVA_{254}$ significantly decreased with percent burned (r = -0.68, $p < 0.001$), indicating that aromaticity also was highest in unburned watersheds and lowest in severely burned watersheds (Weishaar *et al*., 2003).

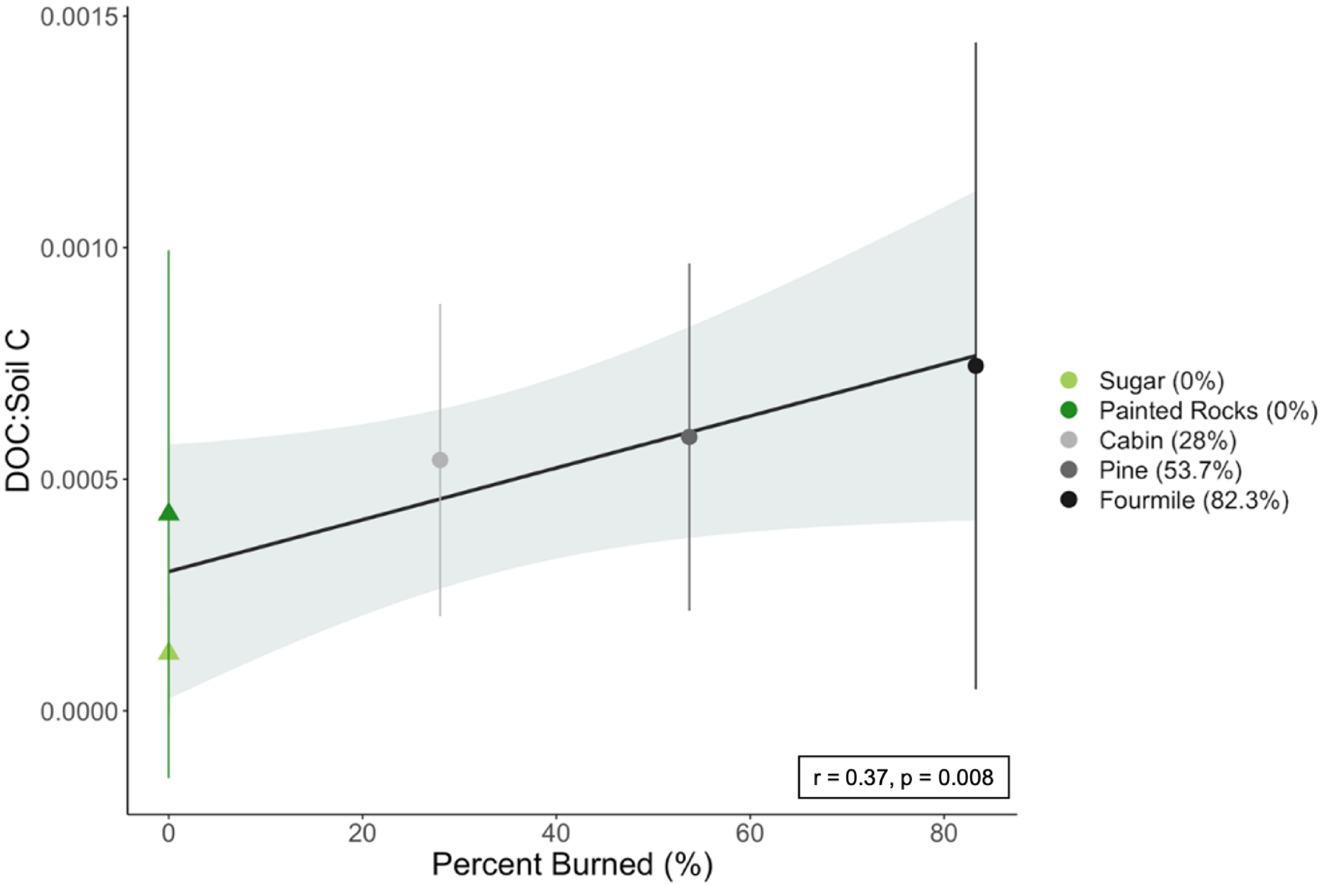


Figure 4. DOC:soil C (year^{-1}) ratios by percent watershed burned (%) with line of best fit. Gray coloration indicates the 95% confidence interval, and error bars are representative of ± standard deviation. Circle points correspond to burned watersheds, and triangle points correspond to unburned watersheds. DOC to soil C ratios shed light on how watershed C export differs and is dependent on %C soil and water yield. Average ratios increased with extent watershed burned (r = 0.37, p = 0.008).

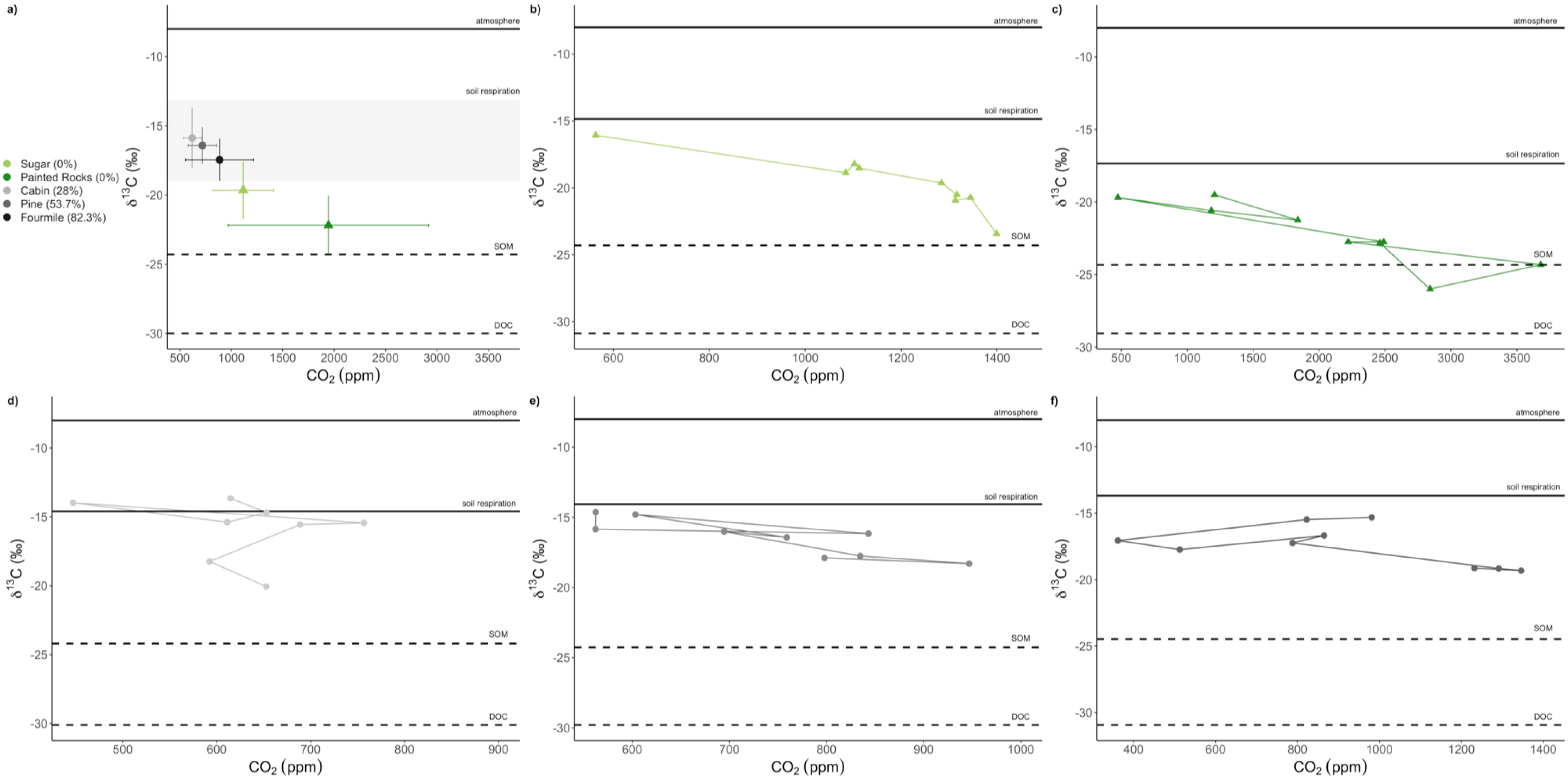

a)
atmosphere
soil respiration
SOM
DOC
δ13C (‰)
-10
-15
-20
-25
-30
500
1000
1500
2000
2500
3000
3500
CO2 (ppm)
Sugar (0%)
Painted Rocks (0%)
Cabin (28%)
Pine (53.7%)
Fourmile (82.3%)
b)
atmosphere
soil respiration
SOM
DOC
δ13C (‰)
600
800
1000
1200
1400
CO2 (ppm)
c)
atmosphere
soil respiration
SOM
DOC
δ13C (‰)
500
1000
1500
2000
2500
3000
3500
CO2 (ppm)
d)
atmosphere
soil respiration
SOM
DOC
δ13C (‰)
500
600
700
800
900
CO2 (ppm)
e)
atmosphere
soil respiration
SOM
DOC
δ13C (‰)
600
700
800
900
1000
CO2 (ppm)
f)
atmosphere
soil respiration
SOM
DOC
δ13C (‰)
400
600
800
1000
1200
1400
CO2 (ppm)

Figure 5. δ^{13}C-CO_2 (‰) values compared to CO_2 concentrations (ppm). Circle points correspond to burned watersheds, and triangle points correspond to unburned watersheds. The solid black line represents average δ^{13}C-CO_2 (atm) (~-8‰, Finlay & Kendall, 2007). Additionally, the two dashed lines represent average δ^{13}C-SOM and δ^{13}C-DOC values respectively, which may serve as sources to processes that produce CO_2. These lines or regions are indicated as these C sources may be potential terrestrial sources of CO_2. *(a)* The shaded grey region represents the range of values of δ^{13}C-CO_2 (soil respiration) in all five watersheds. Plotted points are indicative of an average δ^{13}C-CO_2 and average CO_2 concentrations in each site. Error bars are representative of ± standard deviation and illustrate the temporal variation in these variables, which was greatest in the unburned watersheds for CO_2 concentration and δ^{13}C-CO_2. *(b-f)* The solid black line at a more depleted C signature represents the average δ^{13}C-CO_2 (soil respiration) value in a single watershed. Plotted points are connected temporally to demonstrate how CO_2 concentrations generally increase as δ^{13}C-CO_2 becomes more depleted over the course of the summer. Unburned sites possibly reflect different in-situ sources or processing of C as the summer progresses.

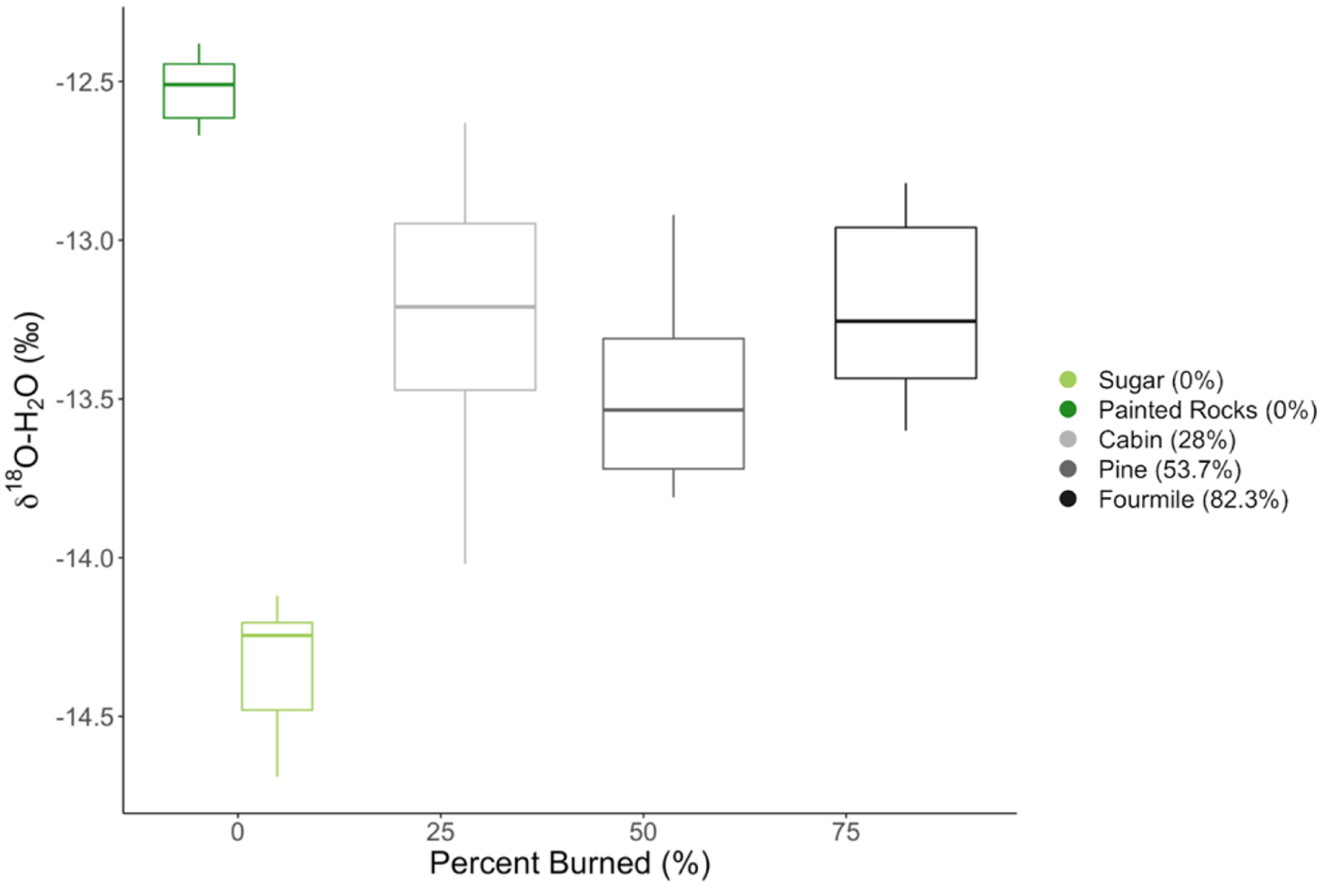


Figure 6. Box plot of $\delta^{18}O\text{-}H_2O$ (‰) according to percent watershed burned (%). $\delta^{18}O\text{-}H_2O$ values can be indicative of overall hydrology (Gat, 2010). When excluding the smallest watershed, Painted Rocks (an unburned site), $\delta^{18}O\text{-}H_2O$ values increased with extent watershed burned ($r = 0.58$, $p = 0.001$), thus becoming less depleted.

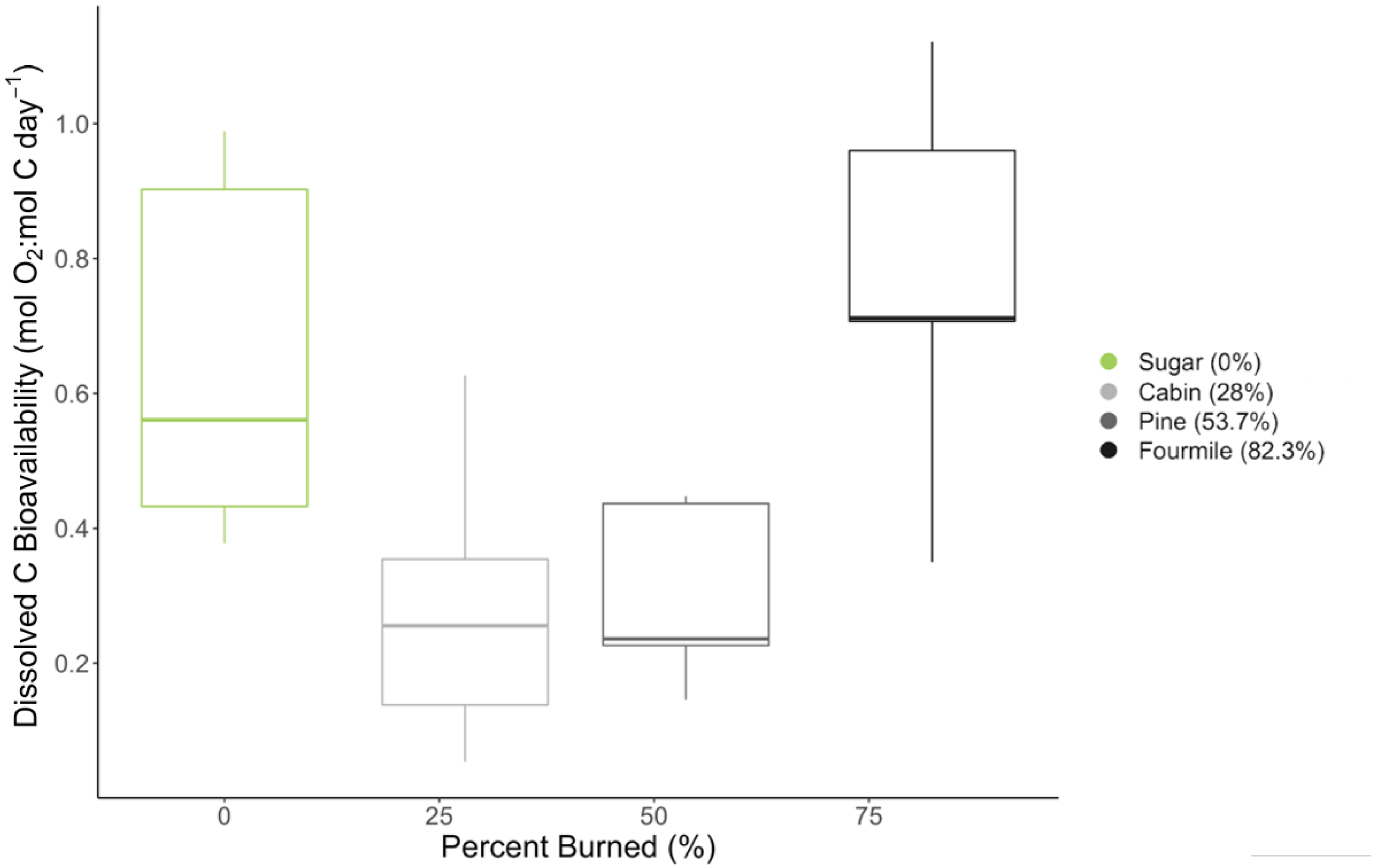


Figure 7. Box plot of dissolved C bioavailability (mol O_2:mol DOC day^{-1}) according to percent watershed burned (%). Measurements were not taken at the unburned site, Painted Rocks, due to extremely low flows. In general, O_2:DOC day^{-1} ratios were greater in unburned watersheds on average as compared to burned watersheds, but all four sites experienced significant temporal variation. However, this temporal variation was more prominent in the unburned stream, Sugar. Also, among the three streams draining burned landscapes, O_2:DOC day^{-1} ratios significantly increased with extent watershed burned ($r = 0.66$, $p = 0.007$).

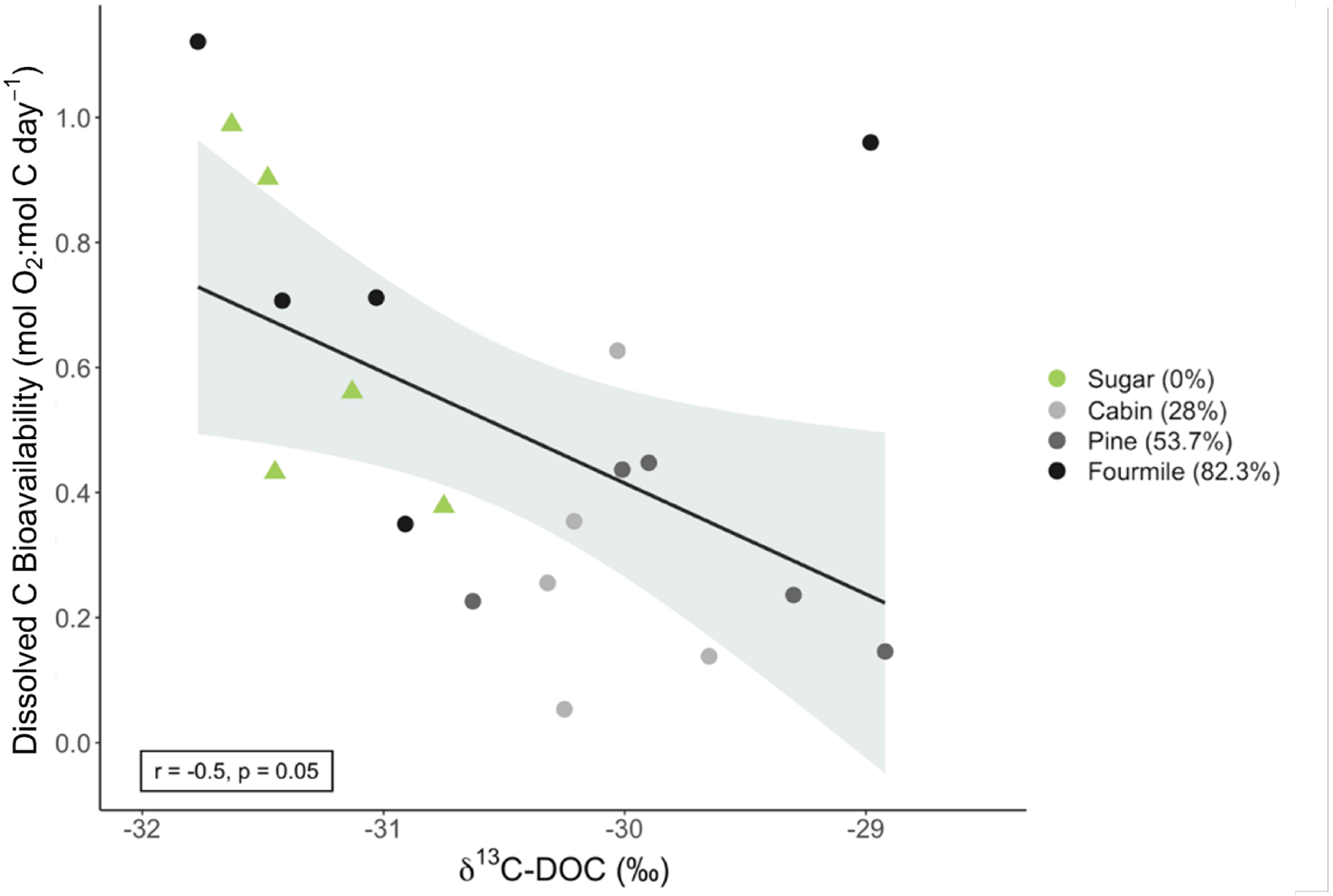


Figure 8. Dissolved C bioavailability (mol O_2:mol DOC day^{-1}) compared to δ^{13}C-DOC with line of best fit. Measurements were not taken at the unburned site, Painted Rocks, due to extremely low flows. Gray coloration indicates the 95% confidence interval, and error bars are representative of ± standard deviation. Circle points correspond to burned watersheds, and triangle points correspond to unburned watersheds. Dissolved C bioavailability was negatively correlated with δ^{13}C-DOC ($r = -0.50$, $p = 0.03$). This relationship was most significant at Cabin ($r = 0.99$, $p = 0.0014$) and Sugar ($r = 0.90$, $p = 0.040$).

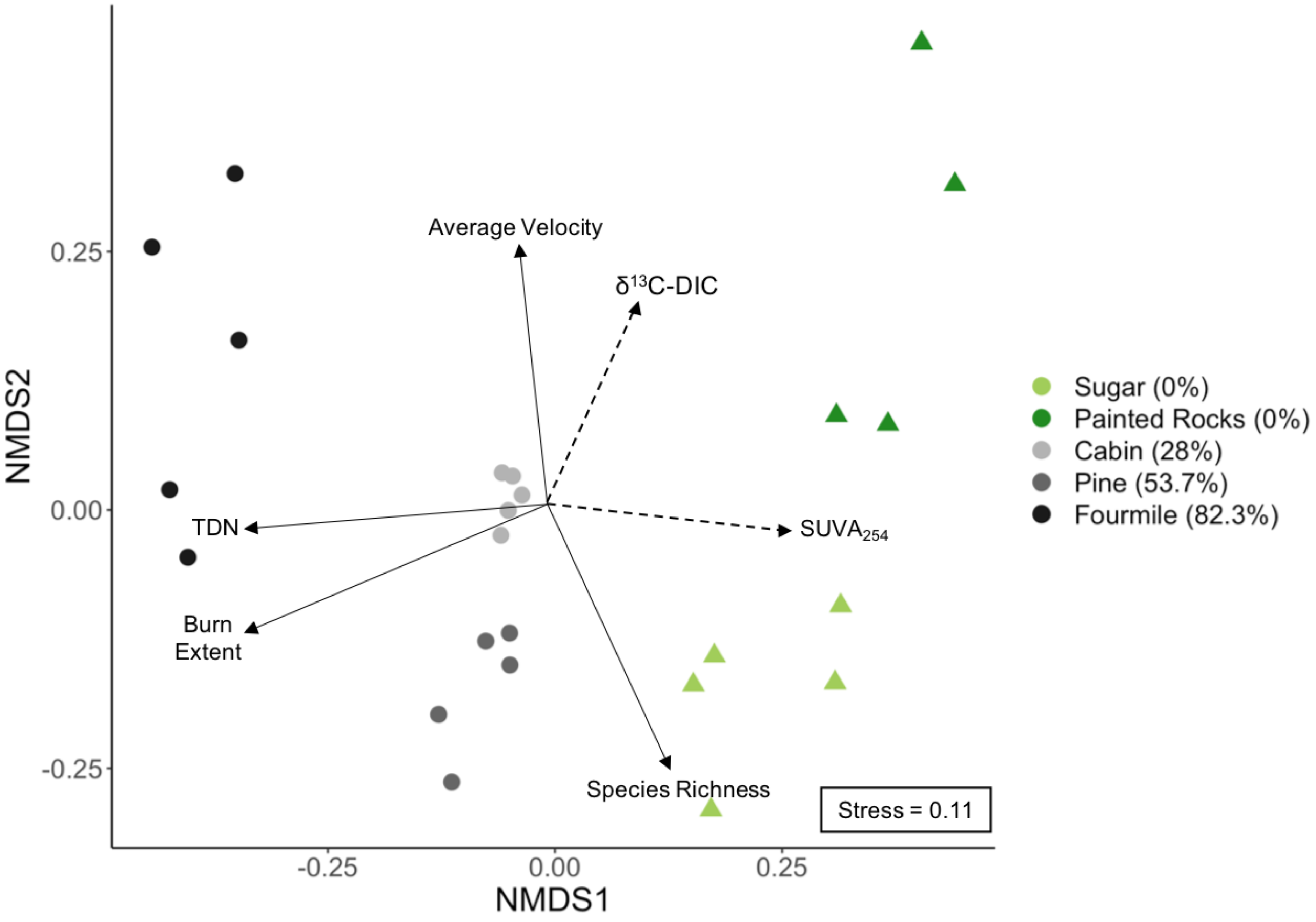


Figure 9. Non-metric multidimensional scaling (NMDS) ordination of exact sequence variants (ESVs) to assess beta diversity in aquatic microbial communities by watershed. Circle points correspond to burned watersheds, and triangle points correspond to unburned watersheds. The low stress value indicates a good fit of the ordination. Samples that are more similar (based on Bray-Curtis dissimilarities) are plotted closer together. Samples were most similar to those from the same stream (ANOSIM: 0.784, $p < 0.001$). Samples were also more similar to those with similar fire history or burn extent (ANOSIM, burned vs. unburned watersheds, 0.558, $p < 0.001$; ANOSIM, % burned, 0.546, $p < 0.001$). Vectors illustrate variables that were correlated with differences between samples. Solid vectors have a p-value < 0.05, whereas dashed vectors have a p-value > 0.05. The proximity of points to a vector illustrates a correlation, while vector length represents the strength of this relationship. As such, stream chemistry and burn extent appear to be associated with the microbial communities in the five streams studied here.

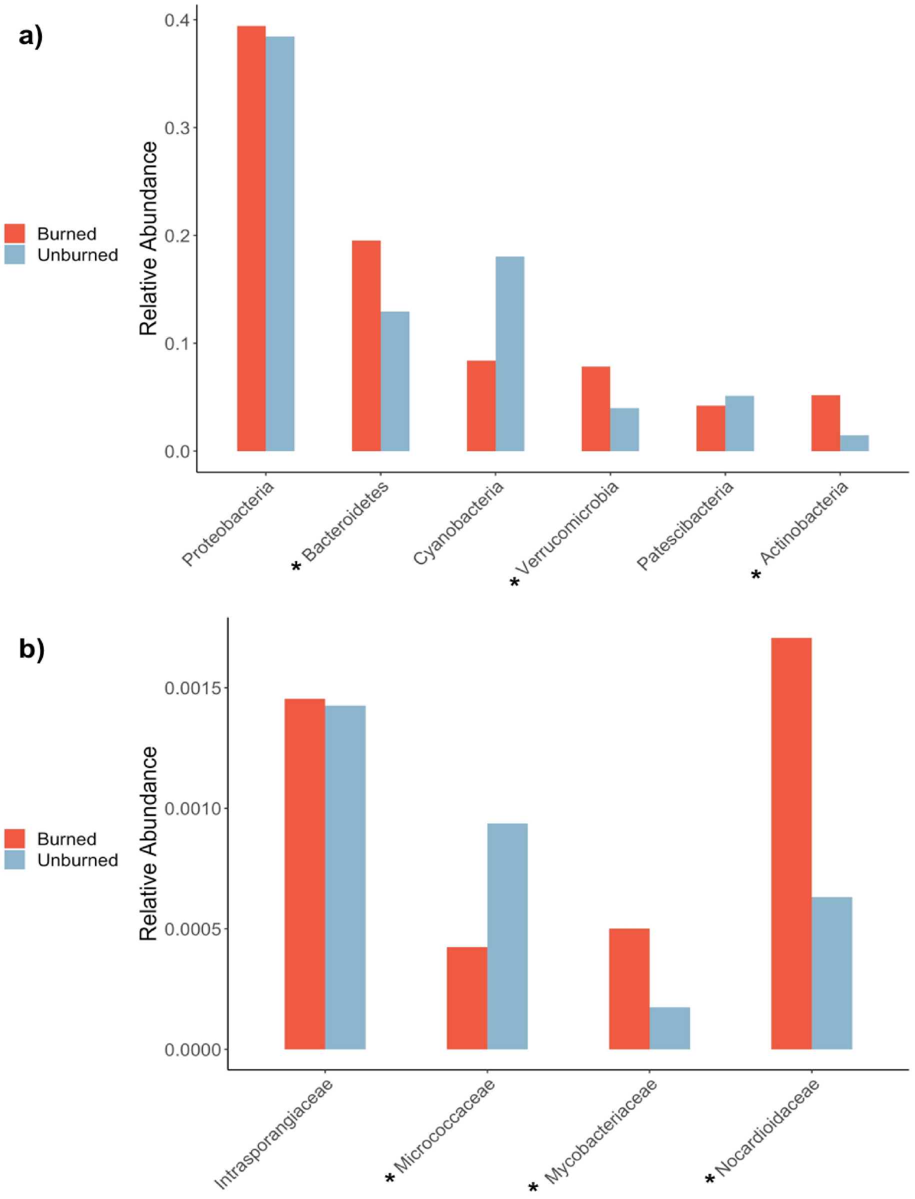


Figure 10. Relative abundances for ecologically relevant microbial taxa present in streams draining both burned and unburned landscapes. Stars next to phyla or family indicate that the difference in relative abundance between burned and unburned watersheds was statistically significant ($p \leq 0.05$). *(a)* Average relative abundances between burned and unburned watersheds for the six most abundant phyla across streams. Proteobacteria, Bacteroidetes, Verrucomicrobia, and Actinobacteria were more abundant in burned watersheds, whereas Cyanobacteria, and Patescibacteria were more abundant in unburned watersheds. *(b)* Average relative abundances between burned and unburned watersheds for four families of the Actinobacteria phyla that may be related to the degradation of aromatics. Three out of four of these families were more abundant in burned watersheds, excluding Micrococcaceae.

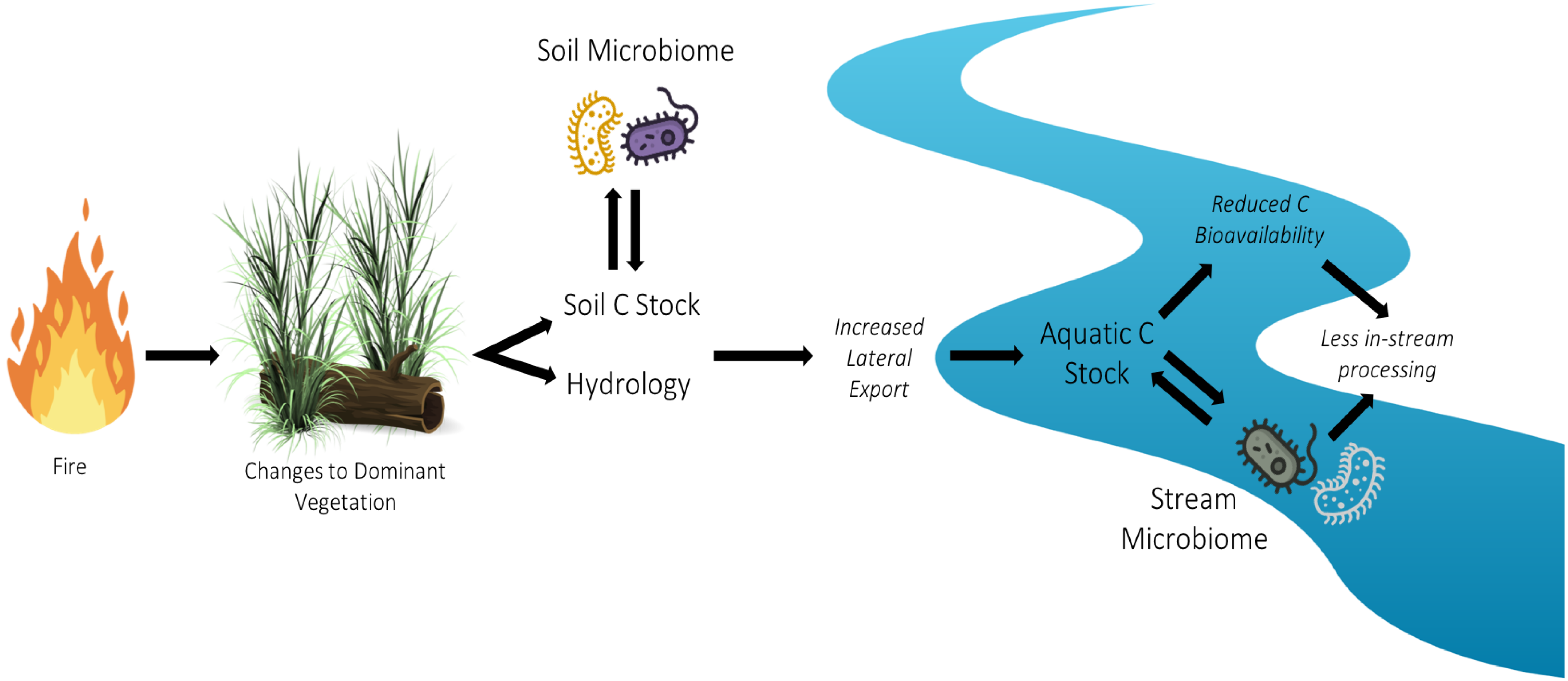


Figure 11. The effects of wildfire on C partitioning and processing across a montane landscape. Wildfire drastically affects the dominant vegetation present in a watershed, and the burned watersheds studied here were predominantly covered by grasses and forbs. Such changes to vegetation directly affect the soil and soil C stock; at these sites, soil C was more bioavailable (Jones, 2020), and C experienced shorter residence times (Bonwell, 2020). Soil microbial communities both influence this C pool and have also shifted in structure in response to SOM quality. Together, these impacts to the watershed led to increased lateral export of terrestrial C to aquatic systems. DOC export was elevated as a result and less bioavailable compared to that of streams draining unburned landscapes. C was qualitatively different in these watersheds (as evidenced in $SUVA_{254}$ and $\delta^{13}C$ values), potentially leading to diminished in-stream processing. Additionally, changes to the aquatic C stock may shift the stream microbial assemblage, and microbial processes can further determine DOC character.

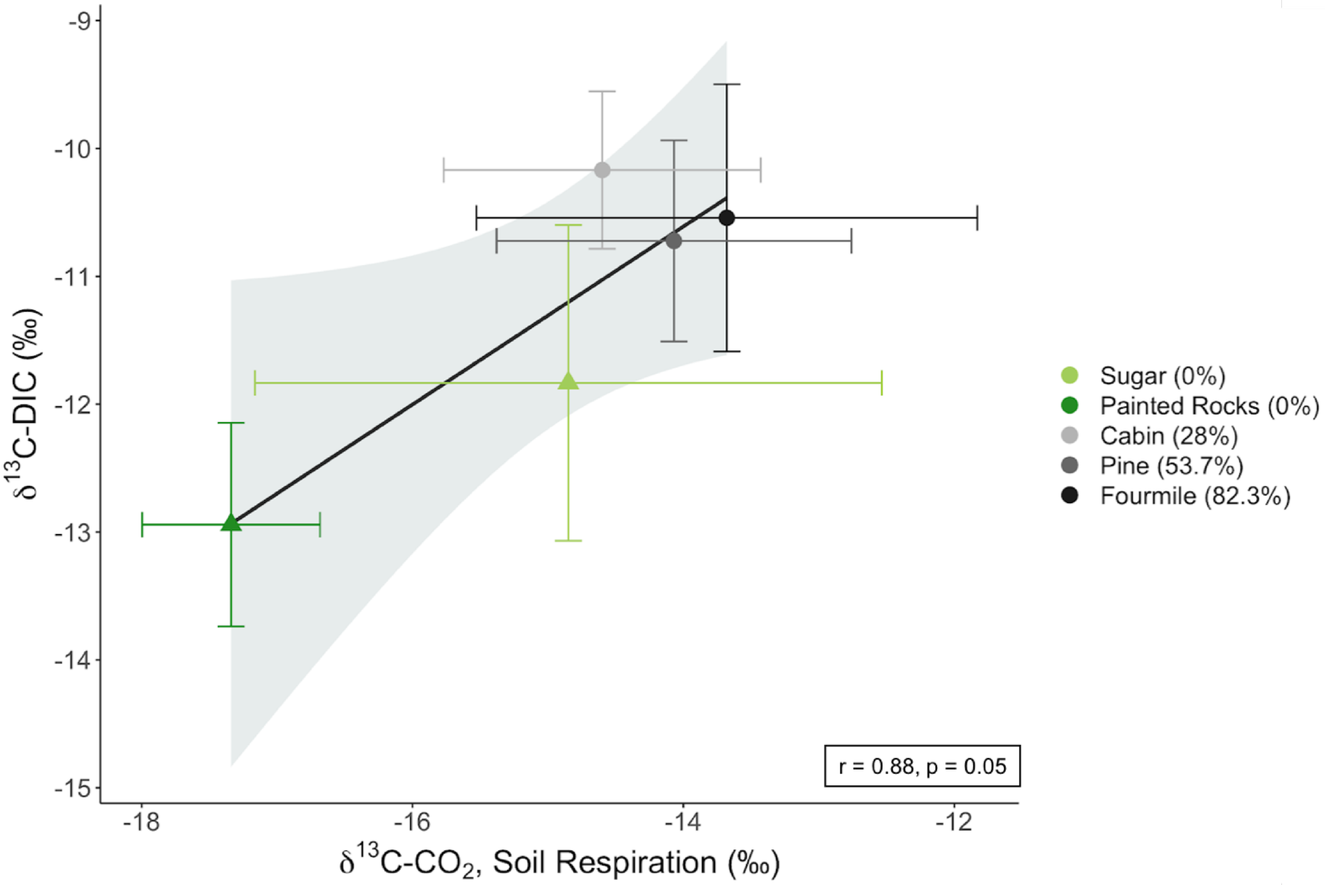


Figure 12. Average stream δ^{13}C-DIC (‰) values compared to average δ^{13}C-CO$_2$ (‰) respired through soil microbial respiration in each watershed with line of best fit. Gray coloration indicates the 95% confidence interval, and error bars are representative of ± standard deviation. Circle points correspond to burned watersheds, and triangle points correspond to unburned watersheds. Stream δ^{13}C-DIC values were correlated with δ^{13}C-CO$_2$ from soil respiration ($r = 0.88$, $p = 0.05$). This relationship illustrates the influence of terrestrial sources on the aquatic DIC pool.

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