

FROM TREE TO TAP: THE IMPACTS OF CLIMATE CHANGE ON BIOGEOCHEMICAL
PROCESSES DURING CONIFER NEEDLE DECOMPOSITION AND BROADER
IMPLICATIONS FOR WATER QUALITY IN COLORADO

by

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ABSTRACT

Recent climate change has contributed to large-scale tree mortality across forested regions in Colorado. As forest health declines, concern for associated terrestrial biogeochemical and hydrologic shifts is mounting. These shifts are related to reduced tree canopy cover, cessation of belowground rhizospheric processes, and increased organic inputs to the system. This can in turn affect terrestrial carbon and nitrogen cycling at the tree scale and downstream water quality. These processes within the forest are complex, however, and the observed changes in soil and stream chemistry can vary across watersheds depending on the land cover, local climate, and location. This dissertation explores biogeochemical processes associated with conifer litter decomposition, a source of terrestrial organic matter contributions, and more broadly the associations between forest health and water quality in Colorado. This work includes field-based experiments beginning at the tree-scale and concludes more broadly with watershed-scale evaluations of water quality. Results revealed the inherent chemistry associated with tree species has a significant influence on soil biogeochemistry during isolated needle decomposition. Further, biogeochemical shifts observed with bark beetle impact are likely driven by other changes (e.g., the cessation of rhizospheric processes and tree canopy loss). Comparisons of the roles of elevation, soil type, seasonal shifts in soil moisture, and snowmelt timing on litter decomposition processes revealed needle presence and seasonal variability of soil moisture are influential in soil carbon release and magnitude. In addition, soil carbon fluxes returned the greatest release associated with more bioavailable litter. Microbial community structure was a key variable that demonstrated sensitivity but also resilience to climate shifts. Extended work beyond the field using historical drinking water reports revealed increased organic disinfection byproducts (DBPs) across certain municipalities of Colorado. The trends correlated with climate variables of reduced total annual days of frost and ice conditions. Closer inspection of these trends in a town of interest revealed long-term decadal and seasonal trends of DBPs associated with spring snowmelt events. This work reveals that as terrestrial-sourced organic matter increases under a changing climate, drinking water facilities should monitor the watershed landscape and source water chemistry to proactively respond to heightened DBP production.

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To my grandparents:

Grandpa, for your stories of the days when you “drank straight from the river without a worry,”

Grandma, for always being my guardian angel.

The world has changed.

CHAPTER 1

INTRODUCTION

The Colorado River watershed provides municipal water to an estimated 40 million citizens in the western United States (USBR, 2012). With the fast growth of Colorado’s population expected to reach 7.7 million by 2050, increased demand compounded with climate change-induced drought, higher temperatures, and earlier spring snowmelt has strained our water resources (Rocky Mountain National Park, 2018). Stakeholders are focused on this major challenge of water scarcity in Colorado’s future; however, this is not our only challenge. Water quality is a key component of water resource management and growing research is revealing the sensitivity of water quality to climate change.

Colorado’s water supply is snowpack dependent on the Western slope. As a result, the quality of surface waters depends on forested ecosystems of the Rocky Mountains, which are changing with the climate. As of 2020 in Colorado forests, over 22% of standing trees are deadwood either killed by insects (65%), disease (23%), or fire (4%) (CSFS, 2021). These variables of tree death are driven by drought conditions, higher temperatures, and shifts in snowpack (EPA, 2016). Recently, Colorado was ranked as one of the five worst states in forest carbon emissions due to increased deadwood and wildfires releasing more carbon into the atmosphere than the forests can store (CSFS, 2021). This issue of increased carbon export indicates a broader issue of biogeochemical shifts with a changing climate linked to the forest, affecting not only the air but soil and water quality as well (Clow et al., 2011; Mikkelsen et al., 2013a; Rodríguez-Jeangros, Hering & McCray, 2018). Specifically, when drought and insect-induced stressors cause large-scale tree mortality, reductions in canopy cover, rhizospheric processes, and overall net productivity of the forest are observed (Schlesinger et al., 2016). These observations further shift tree carbon and nutrient uptake and storage (Schlesinger et al., 2016), soil respiration rates (Brouillard et al., 2017), soil nutrient levels (Clow et al., 2011; Cigan et al., 2015), and soil carbon concentration and character (Brouillard et al., 2017; Fraterrigo, Ream & Knoepp, 2018).

Interestingly, observations in biogeochemical cycling shifts have been variable across watersheds as a function of forest structure and tree mortality (Negrón and Cain, 2018; Fraterrigo, Ream, and Knoepp, 2018) as well as the heterogeneity of forest landscapes. Specifically, terrestrial responses to drought vary across slope and aspect as they relate to energy balances and soil moisture (Körner, 2007) while soil type impacts baseline geochemical signatures and seasonal soil moisture (Lawrence & Hornberger, 2007; Wilson et al., 2008). Additionally, snowpack dynamics that vary across elevations affect water availability and soil moisture (Tennant et al., 2017). A recent study aimed to broadly classify organic carbon release sensitivity

to climate change across biomes and identified highly sensitive biomes that encompass montane and subalpine ecoregions of Colorado (GeoNetwork, 2007; Kramer & Chadwick, 2018). These findings have highlighted a precedent to understand how specific terrestrial processes in sensitive forest ecosystems will continue to respond to climate change.

This dissertation is a culmination of a published paper and protocol, a paper accepted for publication, and one in preparation for submission. In addition, exploratory work is included that is not planned for publication. Together this work aims to further our understanding of climate change impacts on biogeochemical cycling during needle decomposition and more broadly associate climate variables with water quality. This begins in Chapter 2, a publication in *PeerJ*. The type of aboveground forest litter can impact biogeochemical processes, specifically as a function of litter quality. High quality litter is defined by high nitrogen composition, low C:N ratios, and low recalcitrance (Šantrůčková, Křišťůvková & Vaněk, 2006; Fraterrigo, Ream & Knoepp, 2018). Previous research has observed elevated terrestrial dissolved organic carbon (DOC), aromaticity, and ammonium associated with lodgepole tree mortality (Brouillard et al., 2017); however the role of needle litter chemistry in these observations was not clear. As a result, we explored these processes and isolated litter decomposition from the influence of the tree network to understand the discrete role of litter decomposition in organic carbon cycling. We hypothesized the most pronounced soil biogeochemical responses would be associated with beetle impacted litter in contrast to litter from healthy trees. We chose two major Colorado species of healthy conifer needles: lodgepole pine (*Pinus contorta*) and Engelmann spruce (*Picea engelmannii*) for comparison with bark beetle impacted spruce needles. The needles were collected from local Colorado forests and deployed on bare soil in an open meadow to measure soil biogeochemistry variables of gas flux, porewater chemistry, and microbial ecology over two years. Our findings revealed the inherent chemistry associated with tree species had a greater impact on soil biogeochemical cycling with increased CO₂ fluxes and soil DOC and organic nitrogen content under lodgepole in contrast to spruce. Interestingly, bark beetle-impacted needles had no discernable influence on the measured biogeochemical properties when compared to healthy spruce. As a result, the larger terrestrial shifts in carbon and nutrient cycling from beetle impacted forests are likely driven by the associated processes of tree death such as the cessation of rhizospheric and canopy processes.

We expanded our study of litter decomposition processes further in Chapter 3 which has been accepted for publication in *PeerJ*. We continued to monitor isolated conifer needle decomposition processes for lodgepole and spruce litter across climate variables of elevation, season, and early snowmelt. Soil characteristics can drive available carbon, nitrogen, microbial communities, and soil moisture (Delgado-Baquerizo et al., 2015); however trends across elevation can be inconsistent (Djukic et al., 2010). Further, snowmelt dynamics across elevation associated with freeze-thaw cycles can influence terrestrial

decomposition rates (Zhu, Yang & He, 2013) and increase carbon release (Arnold, Ghezzehei & Berhe, 2014). These variables along with responses to seasonal shifts in soil moisture can collectively impact biogeochemical cycling and observed soil, water, and air impacts associated with drought. As a result, Chapter 3 explores litter decomposition over three years from 2017-2019 and across three elevations ranging 2,800-3,500 meters. The goal of the study was to determine the role of soil type, elevation, and early snowmelt on biogeochemical processes during litter decomposition, as well as evaluate the resilience of soil microbial communities to low moisture conditions. Early snowmelt was induced by 2-3 weeks using a protocol created and published in *Bio-Protocol* (Appendix E). Further, large differences in precipitation across the study period occurred during a record warm summer in 2018, enriching the observed findings (CSFS, 2020). The results of the study indicated that elevation and soil type influenced baseline soil biogeochemistry; however, needle decomposition rates were consistent across the 700-meter elevation gradient. Interestingly, early snowmelt had little impact on the measured biogeochemical variables except for associated lodgepole porewater collections which suggested a potential increase of aqueous DOC export. During the pronounced period of lower moisture in 2018, the bacterial community shifted significantly from the structure observed the previous year but demonstrated ecological resilience with a return to pre-drought structure after snowmelt the following year. This study revealed the sensitivity but also the resilience of biogeochemical decomposition processes to seasonal shifts in climate and snowmelt.

We extended our work to the watershed-scale in Chapter 4 with exploratory work to determine if the projections of increased organic carbon release associated with tree mortality and climate change would be reflected in water quality reports. Organic carbon is a natural component of surface water but can impact water treatment processes. Specifically, disinfection byproducts (DBPs) are constituents of concern that form when aqueous organics react with chlorine during water treatment. As a result, past work has shown increased DBP presence in surface waters sourced from bark-beetle impacted watersheds (Mikkelsen et al., 2013b; Brouillard et al., 2017). This is a health concern with DBPs identified as potential carcinogens (Villanueva et al., 2015; Richardson & Plewa, 2020), and regulated by the Environmental Protection Agency (EPA). Thus, Chapter 4 explores climate trends to determine potential correlations of climate and DBP formation within municipal drinking water facilities treating surface water. Initial results revealed a subset of Colorado water treatment facilities harbored increased trends of two regulated groups of DBPs. Despite several significant climate shifts across regions of the state, no clear trends were observed across larger watershed delineations. This work revealed limitations in relating climate variables directly to DBP formation without including other important variables in the analysis which include the water treatment processes. Future work with these exploratory findings is encouraged with more time and resources spent on smaller watershed delineations and extensive variable characterization that includes the treatment

processes and local climate.

Chapter 5 is in preparation for submission to *Environmental Science & Technology Water* and addresses the limitations of Chapter 4. The goal of this work was to determine the spatial and temporal aspects of surface water organic carbon mobilization contributing to increased DBP formation during drinking water treatment. We hypothesized an export control point contributing to DBP formation downstream could be identified in the creek transect using organic carbon and aromaticity measurements. Further, lignin was expected to be the most reactive organic class during source water DBP formation potential tests. We used water quality reports from the treatment facility to understand the long-term DBP trends over the past 15 years in addition to a more detailed dataset from the past five years of regular dissolved organic carbon sampling and stream discharge. In addition, synoptic sampling was conducted to determine the source of reactive organic classes. The water quality reports revealed a long-term increase in the formation of two major classes of DBPs: haloacetic acids (HAA5) and trihalomethanes (TTHM) with seasonal trends consistently exceeding the maximum contaminant level guidelines during the spring snowmelt and early summer months. This revealed increased DOC release during peak discharge sourced from a confluence entering a wetland portion of the creek. Qualitative analyses of chlorine-reacted and unreacted samples indicated lignin, tannins, and condensed hydrocarbons to be the major reactive organic classes with chlorine oxidation. These results revealed a spatiotemporal hot moment that is contributing to increased DBP formation in the town of Crested Butte. These findings suggest a potential use of water quality archives on a broader scale as a forensic tool to discern ecosystem control points for organic export in analogous watersheds.

CHAPTER 2

A COMPARISON OF LODGEPOLE AND SPRUCE NEEDLE CHEMISTRY IMPACTS ON
TERRESTRIAL BIOGEOCHEMICAL PROCESSES DURING
ISOLATED DECOMPOSITION

A paper published in *PeerJ*¹

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2.1 Abstract

This study investigates the isolated decomposition of spruce and lodgepole conifer needles to enhance our understanding of how needle litter impacts near-surface terrestrial biogeochemical processes. Harvested needles were exported to a subalpine meadow to enable a discrete analysis of the decomposition processes over two years. Initial chemistry revealed the lodgepole needles to be less recalcitrant with a lower carbon to nitrogen (C:N) ratio. Total C and N fundamentally shifted within needle species over time with decreased C:N ratios for spruce and increased ratios for lodgepole. Differences in chemistry correlated with CO₂ production and soil microbial communities. The most pronounced trends were associated with lodgepole needles in comparison to the spruce and needle-free controls. Increased organic carbon and nitrogen concentrations associated with needle presence in soil extractions further corroborate the results with clear biogeochemical signatures in association with needle chemistry. Interestingly, no clear differentiation was observed as a function of bark beetle impacted spruce needles versus those derived from healthy spruce trees despite initial differences in needle chemistry. These results reveal that the inherent chemistry associated with tree species has a greater impact on soil biogeochemical signatures during isolated needle decomposition. By extension, biogeochemical shifts associated with spruce beetle infestation are likely driven more so by other changes such as the cessation of rhizospheric processes than by needle litter decomposition.

2.2 Introduction

Forest litter decomposition alters surrounding soil by influencing mechanisms that include terrestrial carbon (C) and nitrogen (N) cycling (Šantrůčková, Křišťůfková, and Vaněk, 2006; Hicks Pries et al., 2017). Several studies have looked further into these contributions to soil biogeochemistry; however, the observed impacts of decomposition on soil biogeochemistry differ between studies as a function of forest structure and tree mortality levels that fluctuate with time (Negrón and Cain, 2018; Fraterrigo, Ream, and Knoepp, 2018). Recent bark beetle outbreaks in association with climate change have resulted in increased tree mortality within forests and pulsed litterfall that collectively result in short and long-term impacts to the forest ecosystem (Edburg et al., 2012; Mikkelsen et al., 2013a). A discrete analysis of litter decomposition could help us better understand its relative contribution to broader processes such as why certain forests reveal different C and N signatures from others.

A unique aspect of bark beetle tree mortality is a pulsed release of conifer needles to the forest floor that adds additional organic inputs to the litter decomposition process (Edburg et al., 2012). In concert, reduced canopy cover causes shifts in tree hydrologic processes such as canopy interception and evapotranspiration (Mikkelsen et al., 2013a; Bearup et al., 2014) further altering the decomposition process with changes to soil temperature and moisture levels. This litter decomposition is coupled to the cessation of rhizospheric processes and collectively results in tree-scale biogeochemical shifts in net soil respiration (Brouillard et al., 2017), soil nutrient levels (Clow et al., 2011; Cigan et al., 2015), and soil carbon concentration and character (Brouillard et al., 2017; Fraterrigo, Ream & Knoepp, 2018). However, we do not fully understand how needle decomposition versus other variables such as cessation of rhizodeposits and reduced canopy alters the magnitude, character and timing of the biogeochemical response. Understanding the contributions of increased litter inputs to soil C and N cycling is important as endemic bark beetle species are prevailing at higher magnitudes globally. Increased outbreaks in conifer forests have been documented in Europe, Canada, and the United States causing concern for forest aesthetics and economics as well as altered ecosystem and watershed function (Natural Resources Canada, 2013; US Forest Service, 2019; Hlásny et al., 2019).

To this end, we designed a study to isolate spruce and lodgepole needle litter from the complexity of tree-scale processes. We hypothesized that the isolated decomposition of needles containing different organic carbon and nitrogen signatures would impart unique soil biogeochemical responses. Soil respiration, C, N, porewater composition, and microbial ecology were then compared as a function of tree species and bark beetle impact over two years of decomposition in a subalpine meadow environment (3,170 meters above sea level) in Crested Butte, Colorado. Recently, mountain pine beetle (*Dendroctonus*

ponderosae) outbreaks were cause for concern in Colorado with peak lodgepole pine mortality occurring in 2008 (Colorado State Forest Service, 2018). Spruce beetle (*Dendroctonus rufipennis*) infestation has intensified since 2011. This approach allowed us to decouple the effects of varying litter decomposition from the tree canopy and root network to better understand selective pressures exerted by needle decomposition on tree-scale biogeochemical processes and their relevance to ecosystem disruption.

2.3 Materials & Methods

2.3.1 Needle sample collection

Needle litter samples were collected in August 2016 from separate forests containing two tree species, lodgepole pine (*Pinus contorta*) and Engelmann spruce (*Picea engelmannii*). Throughout the paper these collected needles will be referred to as “harvested needles”. The lodgepole pine needles were collected in White River National Forest near Frisco, Colorado (39.54° N, 106.15° W, elevation 3,050 meters). The forest is mostly lodgepole pine and experienced mountain pine beetle infestation between 2007 and 2008 (Brouillard et al., 2017). The soil type of this location is a Leighcan family- Cryaquolls complex with metamorphic gneiss bedrock (United States Department of Agriculture, 2019; U.S. Geological Survey, 2005). This site was chosen for its large area of lodgepole pine trees that have not been affected by anthropogenic activities. Litter was collected under lodgepole trees that had naturally senesced. Throughout the paper, these needles will be referred to as “lodgepole.”

Spruce needles were collected from Monarch Pass (38.50 ° N, 106.33 ° W, elevation 3,440 meters) located in central Colorado between Gunnison and Chaffee county where the forest is predominantly Engelmann spruce. The bedrock of this location is granitic igneous intrusive (U.S. Geological Survey, 2005). The soil type of this location is not available. This site was chosen for its abundance of spruce trees with active spruce beetle infestation that intensified in the area during 2015 (Colorado State Forest Service, 2016). Visual observations included beetle boreholes, intensive sap release from pitch tubes along with live larvae and adult beetles underneath the outer bark layers. Within months of observing beetle infestation, the impacted spruce needles turned red, further confirming spruce beetle infestation. By August 2016 the majority of needles had fallen from the impacted trees and were then collected from both impacted and naturally senesced spruce trees from this forest. The needles collected under the impacted trees are referred to as “impacted” spruce needles throughout this paper and the needles collected under non-impacted naturally senesced spruce trees will be referred to as “healthy” spruce needles.

The harvested needles were stored in a dark and dry location at room temperature to air dry until deployment. The dry needles were sieved using a Tyler Equivalent 10 mesh, 1.7 mm to homogenize the collected litter from each respective sample type and remove rocks and dirt. The sieved needles were then

weighed into 250 gram subsets based on the three needle sample types and aliquoted for deployment at the study site described below. All litter composition results are presented based on air-dried weight.

2.3.2 Study site and needle deployment

The experimental plot used in this study is located in Crested Butte, Colorado. The plot is located within the subalpine region of the Washington Gulch drainage into the East River watershed located at 38.95 ° N, 107.03 ° W, with an elevation of 3,170 meters and a northeast aspect and average slope of 9-12 °. The plot is located in an open meadow to ensure isolation from surrounding tree canopies and roots. The subalpine meadow soil type is classified as a mixture of Tilton sandy loam and Cryaquolls and Histosols with sedimentary clastic Mancos shale bedrock (United States Department of Agriculture, 2019; U.S. Geological Survey, 2005). Conifer tree stands are within approximately 300 m of the experimental plot.

Climate data was collected from the nearest snow telemetry (SNOTEL) weather station on Mt. Crested Butte (38.89 ° N , 106.95 ° W) at 3,100 meters elevation (site number 380). Mean annual precipitation over the study years of 2016-2018 averaged 65 cm, most of which falls as snow starting in September/October and continues through May. The average daily temperatures recorded at the Butte SNOTEL location were separated into the snow-free months of May-September and snow-covered months of October-April. The temperatures averaged 11.6 °C and -1.1 °C, respectively. Hourly soil temperature was measured with unshielded microclimate sensors buried on-site at 12 cm soil depth. The mean soil temperatures recorded over 2016-2018 were 12.6 °C during the months of May-September, and 1.4 °C during the months of October-April.

Needles were deployed at the experimental plot in October 2016 in 25.4 cm diameter white polyvinyl chloride (PVC) collars before winter snowfall. The collars were 18 cm in height with a beveled edge at the end. Beveled end first, the collars were driven into the ground to leave approximately 10 cm of the height above ground level. Foliage was raked and pulled out of the ground to remove all native plants and roots. A total of 16 PVC rings were inserted into the ground to hold four sample types in quadruplicate. The plot matrix was established with quadruplicate samples as follows: 4x250 grams of healthy spruce, 4x250 grams impacted spruce, 4x250 grams lodgepole and 4x needle-free controls (bare soil). Randomization of harvested needle samples was achieved using R Studio to produce a 4x4 matrix with sample assignments. A schematic of this field deployment grid with the randomized samples can be found in Figure A.1.

Soil Moisture's 1905L06 15 cm lysimeters were installed in the middle of each ring with a bentonite seal over the surface. The needles were placed on top of the soil surface and surrounding the lysimeter. Each collar top was covered with deer netting and secured to minimize interference from native animals. Onset HOBO Data Loggers soil moisture (S-SMD-M005) and temperature (S-TMB-M006) probes were installed

per manufacturer instructions at 12 cm depth within the sample grid to monitor soil moisture and temperature hourly.

While the needle deployment limited emergent plant growth, any new growth was manually pulled out of the soil with as little disturbance as possible. Frequent monitoring of the plots similarly ensured that bare soil control rings were free of emerging plant growth. In October 2017, one quadruplicate ring from each of the four sample types was sacrificed for soil extractions. This reduced the number of sample replicates to three for subsequent samplings. In addition, shaded controls were added in May of 2018 prior to any 2018 measurements with randomized placement of three PVC rings on bare soil with two layers of permeable 50% black mesh shade fabric secured over the rings to understand the impact of solar radiation (Figure A.1). Biogeochemical attributes of needle decomposition were then measured with soil respiration, porewater composition, and microbial analysis as a function of tree species and bark beetle impact over two years of decomposition.

2.3.3 Needle composition: Total carbon, nitrogen and FTIR analyses

An initial subset of each harvested needle type (healthy spruce, impacted spruce, and lodgepole) was used for compositional analysis at the start of the experiment. This same compositional analysis was conducted on a subset of needles collected from each sample collar two years later (October of 2018). Needles were set in a dark and dry location at room temperature to air dry. Once air-dried, the needles were ground to a fine powder and submitted in triplicate for total carbon and nitrogen analysis at Colorado State University’s Ecocore facility with the LECO TruSpec CN analyzer (LECO Corporation) and Fourier Transform Infrared (FTIR) analysis at Lawrence Berkeley National Laboratory. The FTIR spectrometer used was equipped with an attenuated-total-reflection accessory (Nicolet IS50, Thermo Fisher Scientific Inc). The sample was pressed down uniformly on the top surface of the crystal and the reflected infrared signal from the sample was collected to a deuterated-triglycine sulfate detector. All collected absorption spectra were further preprocessed for baseline correction and peak-by-peak analysis was applied to quantify the concentration of each functional group which was linearly correlated to the absorbance following Lambert-Beer’s Law.

In addition to compositional analysis, water extraction tests were conducted with a subset of the harvested needles to determine the initial extractable carbon and nitrogen concentrations. Measurements of extractable dissolved organic carbon (DOC), UV_{254} , and total nitrogen (TN) were conducted after equilibrating in deionized water following one hour of shaking in 1:8 solid mass: liquid volume ratio. The extractions were filtered through 0.45 μm filters. Filtrate DOC and TN were tested using a Shimadzu TOC-550A Total Organic Carbon Analyzer after acidifying with hydrochloric acid. UV_{254} was measured

using a DU 800 spectrophotometer. Specific UV absorbance (SUVA) was calculated by normalizing the UV_{254} values with the respective DOC concentrations in mg/L for porewater samples and mg/g for the soil and litter extractions following Environmental Protection Agency Method 415.3 and (Eq. 4.1) (Potter & Wimsatt, 2009):

$$SUVA(L/mg - m) = UVA(cm^{-1})/DOC(mg/L) * 100cm/m \quad (2.1)$$

2.3.4 Gas flux analysis

Gas flux measurements were conducted above each soil collar during the snow-free months for a total of eleven sampling events over two years. Data was collected over an approximate two-hour window that fell midday between 10AM and 3PM. The collar sampling sequence was randomized each time to account for temporal fluctuations in variables such as temperature and moisture. Gas flux measurements were conducted using a Picarro G2508 cavity ring-down spectroscopy analyzer capable of analyzing CO_2 , CH_4 , N_2O , NH_3 and H_2O . Guaranteed spec ranges of the G2508 are 0.3-200 ppm N_2O , 1.5-12 ppm CH_4 , 380-5000 ppm CO_2 , 0-300 ppb NH_3 , and 0-3% H_2O . A closed system was implemented with two lengths of norprene tubing and an airtight PVC chamber to circulate the headspace for the duration of the sample period. One length of the tubing was attached to the Picarro gas inlet and the other was attached to the outlet. Each end of the tubing was then connected to an inlet and outlet valve in the PVC chamber that was placed over the permanently deployed PVC collars. After steady state was established by linear trends of production or removal, data collection was initiated for two minutes. The Hutchinson & Mosier method provided with the Picarro computer software was used to compute a best fit for each gas trend to calculate the fluxes.

2.3.5 Porewater and soil extraction analysis

Porewater was collected from within each PVC collar after snowmelt in the spring and high moisture events during early fall for a total of four collections over two years. Porewater was collected from the lysimeters using a Luer-lok 50mL vacuum syringe. The samples were filtered to 0.45 μ m and frozen the same day of collection for storage at -20°C until analysis. The samples were subsequently thawed for TN, DOC, and SUVA analyses as specified in Section 2.3.

Extractions were conducted with soil underlying the needles from single quadruplicate collars sacrificed for each sample type in the Fall of 2017. The extractions were conducted according to the methods outlined in Section 2.3 except with a 2:9 solid mass: liquid weight ratio. Soil samples were collected from the upper surface horizon in contact with decomposed matter (2-3 cm) and mixed for a homogenized sample from which five subsamples were collected. Geochemical extractions were conducted with DI water

following the methods of Brouillard et al., 2017. Resulting filtrate at 0.45 μm was analyzed for TN, DOC, and SUVA as described in Section 2.3. Ammonium concentrations were measured using the sodium salicylate method and absorbance at 650 nm with a DR 3900 Hach Spectrometer. Nitrite and nitrate were measured using an ICS-5000 ion chromatography analysis consisting of an ICS-5000 DC Conductivity Detector, an ICS-5000 DP Isocratic Pump, and an AS-DV Autosampler. All soil extraction results were normalized by air-dried weights.

2.3.6 DNA extraction and preparation

Soil samples for DNA extractions were collected within each PVC collar for microbial analysis at five time points over two years: August 2017, October 2017, May 2018, July 2018, and October 2018 to align with the early, mid, and late-season snow-free months. Samples were collected in the upper 1-2 cm soil layer under each representative needle type with sterilized scoopulas. Homogenization was achieved by collecting a minimum of ten subsamples of soil from the first 2 cm of the upper soil surface to fill a 2 mL test tube. This process was conducted throughout the full collar surface area to completely represent the sample. Care was taken to ensure samples were randomly collected across the soil surface and not biased towards the inside or outside edges of the collar. The soil samples were then frozen for storage at -20°C until analysis. DNA was extracted from the samples using the ZymoBIOMICS DNA Miniprep kit according to the manufacturer instructions using 0.25 g weighed out from each 2 mL sample tube.

16S and 18S gene amplification was conducted using 5 Prime Hot MasterMix and a primer set utilizing the 515-Y forward primer and 926R reverse primer (Parada, Needham & Fuhrman, 2016). An adapted forward primer was utilized with the addition of the M13 sequence to allow sample barcoding during PCR (Caporaso et al., 2012; Stamps et al., 2016). Resulting amplicons were purified and normalized to equimolar concentrations. The samples were then concentrated using the 30K ultra centrifugal Amicon filters. The final concentrations were determined using the Qubit 2.0 fluorometric quantitation. The library was sent to the Duke Center for Genomic and Computational Biology for Illumina MiSeq sequencing using V2 PE250 chemistry. Raw sequence data has been deposited in the NCBI SRA database under SRA accession number PRJNA605259.

2.3.7 Sequence processing

The resulting raw reads from sequencing were joined, quality filtered, clustered and had chimeras removed using the DADA2 package (Callahan et al., 2016). Sequences were trimmed to excise forward and reverse primer sequences in methods utilized by (Honeyman, Day & Spear, 2018) in which the first 40 nucleotides of the forward primer sequences and the last 20 of the reverse primer were removed and then

trimmed using a quality score of 2. 18S sequences were analyzed separately from 16S where the forward and reverse reads were combined by concatenating the reads directly, despite the absence of an overhanging region of 18S gene sequenced by both the forward and reverse reads. Both 18S and 16S taxonomic assignments were created using Silva v128 (Pruesse, Peplies & Glöckner, 2012). Sequences for each sample were filtered into either Eukaryota (18S) or Bacteria (16S). Chloroplasts and Mitochondria were removed from all 16S sequences. After processing and quality filtering for the 16S genes, 796,847 sequences were obtained across 69 samples with sequence depth ranging from 18 -19,498 sequences per sample and were rarefied to 5,638 sequences. The yield for 18S sequences with these primers was lower at 34,009 across 69 samples. Sequence depth ranged from 0 to 2,950 sequences per sample and were rarefied to 200 sequences. All subsequent analysis except differential abundance testing was based on the rarefied sequence tables.

All downstream analysis was conducted in R (v1.0.153), using the Phyloseq (v1.26.1) (McMurdie & Holmes, 2013), vegan (Oksanen et al., 2019), DESeq2 (Love, Huber & Anders, 2014), and ampvis2 (Andersen et al., 2018) packages.

2.3.8 Statistical Analyses

Geochemical parameters between sample types were statistically differentiated using the Friedman test followed by the post hoc Wilcoxon test. Due to low sample sizes ($n=3$) no significant values were returned with these methods for the various geochemical tests conducted. Spearman and Pearson tests were used to assess nonlinear and linear correlations between gas flux and soil parameters. For the DNA sequence results, beta diversity was assessed using both weighted and unweighted UniFrac distance matrices and significant clustering was determined using the Adonis test. Differential abundance comparisons were conducted using DESeq2. In all statistical tests, P values less than 0.05 were considered significant.

2.4 Results

2.4.1 Chemical composition of harvested and decomposed needles

The initial chemical differences between needle types were determined with the harvested needles prior to deployment (Figure 2.1A, B). Clear differences in needle chemistry were apparent. Harvested naturally senesced lodgepole needles contained the highest percentages of total C and N in contrast to both spruce needle types. The harvested impacted (beetle-killed) spruce needles were higher in total C and N than the healthy (naturally senesced) spruce needles (Table A.1). Impacted spruce exhibited a higher carbon to nitrogen (C:N) ratio in contrast to the lower ratios between the lodgepole and healthy spruce needles (Figure 2.1A). After two years of decomposition, the differences between needle types were amplified with transitions as a function of tree species (Figure 2.1A, C). The average total carbon content decreased for all

decomposed needle types, however with high variability in the healthy spruce ($39\% \pm 15$) (Table A.1). Interestingly, the average total nitrogen content increased in both impacted and healthy spruce needles while lodgepole decreased in total nitrogen, again with high variability in healthy spruce ($0.9\% \pm 0.3$) (Table A.1). Overall, there was a decrease in the average C:N ratio for both impacted and healthy spruce needles and an increase in lodgepole after two years of decomposition (Figure 2.1A).

FTIR analysis further confirmed compositional differences between initial harvested needle types based on several peak absorbances (Figure 2.1B). Notably, the spectra for the 2016 lodgepole needles exhibited clear differences from the spruce needles in litter quality based on integrated peak areas at ether linkages (1150 cm^{-1}), aromatics (1510 cm^{-1}), amides (1600 cm^{-1}), and carbonyls (1720 cm^{-1}). Previous studies have associated lignin content with the aromatics peak and cellulose content with ether linkages (Pandey & Pitman, 2003; Özgenç, Durmaz & Kuştaş, 2017). By applying the same inferences as these past studies, differences in the aromatic and ether linkage peak areas indicate lower recalcitrance, or lignin quantity and more labile carbon such as cellulose in lodgepole needles when compared to the spruce needles (Figure 2.2A, B).

Impacted versus healthy spruce FTIR spectra, while more similar, exhibited differences in aromatics, amides, and carbonyl peak areas. After two years of decomposition, integrated peak analysis revealed a shift between the 2016 harvested needles and the 2018 decomposed needles (Figure 2.2). The observed differences between lodgepole and spruce needles in the four major functional groups were maintained two years after decomposition. Specifically, lodgepole needles maintained a more labile and less recalcitrant litter quality in contrast to both impacted and healthy spruce needles. (Figure 2.2A, B). Further, the ratio of ether linkages to aromatics ($1150:1510\text{ cm}^{-1}$) over two years of decomposition revealed a significant increase in lodgepole compared to the lesser increases in the healthy and impacted spruce needles (Figure 2.2E, Table A.2).

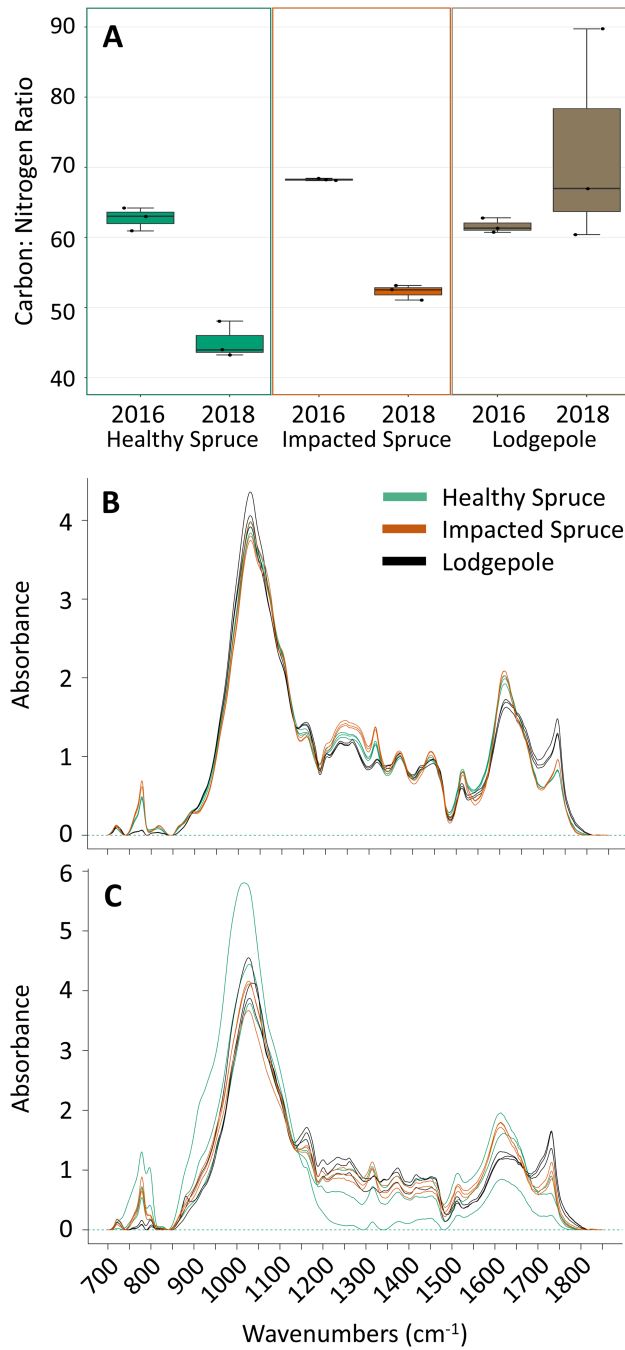


Figure 2.1 Differences in needle carbon, nitrogen, and organics composition. (A) Percentages of carbon and nitrogen needle composition represented as the carbon: nitrogen ratio ($n = 3$) for the harvested needles (2016) and after 2 years of decomposition (2018). Points on the plot represent actual values. (B) FTIR spectra for the initial needles and (C) after 2 years of decomposition. Peak heights at specific wavenumbers reveal differences in the spectra as a function of needle species. Total carbon and nitrogen percentages can be found in Table A.1.

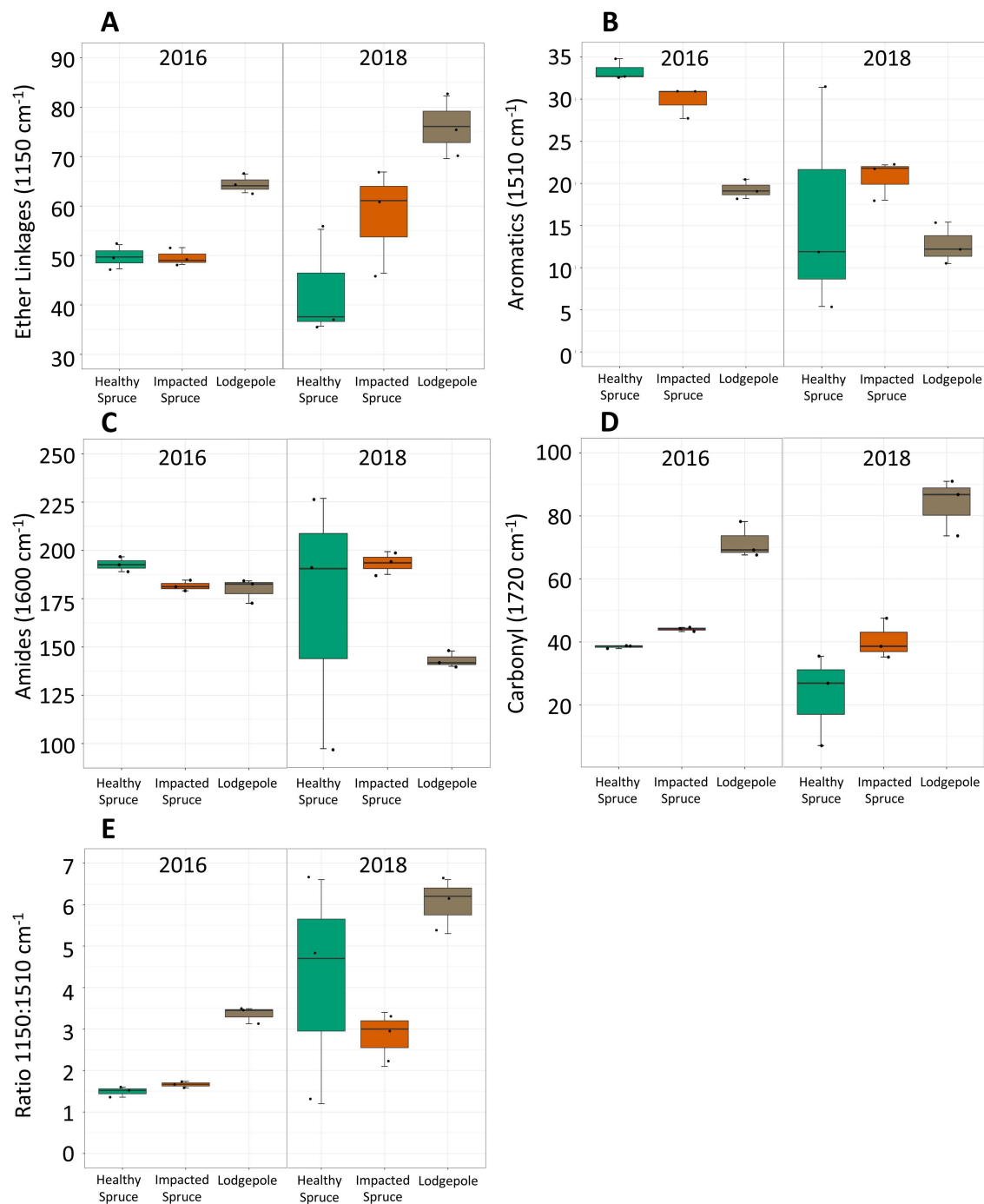


Figure 2.2 Differences in integrated FTIR spectra peak areas. Integrated areas of peak absorbance at wavenumbers with clear differences for the initial needles (2016) and after 2 years of decomposition (2018). Peak areas suggest differences in the chemical composition between needles, notably (A) ether linkages ($1,150 \text{ cm}^{-1}$), (B) aromatics ($1,510 \text{ cm}^{-1}$), (C) amides ($1,600 \text{ cm}^{-1}$), and (D) carbonyl signatures ($1,720 \text{ cm}^{-1}$). (E) The ratio of ether linkages to aromatics is also shown. Tabulated values of each area can be found in Table A.2.

DI water extractions with the harvested needles revealed water extractable constituents differed between all needle types in which impacted spruce needles released the highest extractable carbon in contrast to lower values for lodgepole and healthy spruce needles. The total extractable nitrogen was highest in association with spruce needles in contrast to lodgepole with similar values between impacted and healthy spruce. As determined by specific UV absorbance, more aromatic organic carbon was extracted from the healthy spruce and lodgepole in contrast to the impacted spruce (Table 2.1).

Table 2.1 Deionized water extractable nutrients as a function of needle type for harvested needles.

	DOC (mg-C/g dry litter)	TN (mg-N/g dry litter)	SUVA (g dry litter/mg-m)
Impacted Spruce	3.79 (± 0.34)	0.049 (± 0.003)	84 (± 2)
Healthy Spruce	2.29 (± 0.15)	0.041 (± 0.001)	130 (± 6)
Lodgepole	2.16 (± 0.48)	0.021 (± 0.002)	146 (± 28)

Notes:

Results are normalized based on an air-dried mass basis.

Each parameter averaged across triplicates (n=3).

Standard deviation shown in parenthesis.

2.4.2 Gas flux from needle and control deployments

Needle presence and seasonality impacted soil gas flux over the period of the study (Figure 2.3A, B). The presence of decomposing needles increased CO₂ production in contrast to the controls. Throughout all sampling events, the needle-free controls produced the lowest average CO₂ flux while lodgepole needle decomposition released the most CO₂. The shaded control added in 2018 produced CO₂ magnitudes that aligned or were less than the exposed control, confirming shading effects from the needles did not drive the differences observed in the needle samples (Figure 2.3A, B).

Seasonal trends were also observed during sampling events. Gas flux measurements were influenced by soil moisture as evidenced by peaks in CO₂ production that followed peaks in soil moisture (Figure 2.3). This observed relationship between CO₂ production and soil moisture resulted in a positive Pearson correlation in 2018 for all sample types (average $P=0.01$, $R^2=0.95$); however, no significant correlation was determined for the year 2017. In August 2017, peaks in CO₂ production for the lodgepole ($5,600 \pm 1,100$ mg-C⁻² d⁻¹) and healthy spruce needles ($5,400 \pm 500$ mg-C m⁻² d⁻¹) were almost two-fold higher than the needle-free control 2017 ($3,200 \pm 500$ mg-C m⁻² d⁻¹). This peak in production was followed by a return to baseline conditions by the next sampling event (Figure 2.3A). An analogous CO₂ peak was observed in

2018 after a moisture event in late June despite overall drier annual soil conditions. Interestingly, during the two high moisture time points, the decomposition of healthy spruce needles released more CO₂ than impacted spruce in 2017 ($5,400 \pm 500$ mg-C m⁻² d⁻¹ versus $4,100 \pm 500$ mg-C m⁻² d⁻¹ respectively); however, this trend was muted and potentially reversed in 2018 ($2,200 \pm 400$ mg-C m⁻² d⁻¹ versus 2800 ± 200 mg-C m⁻² d⁻¹, respectively). Sampling throughout 2017 and 2018 provided interesting insights related to seasonal parameters due to contrasting seasonal temperature and moisture conditions between years. The hourly soil conditions collected during each gas flux measurement averaged 14°C, 16% moisture in 2017 and 21°C, 10% moisture in 2018 (Figure 2.3C, D). These observed differences in soil temperature and moisture coincide with lower CO₂ peaks for all sample types during the drier 2018 monsoon season.

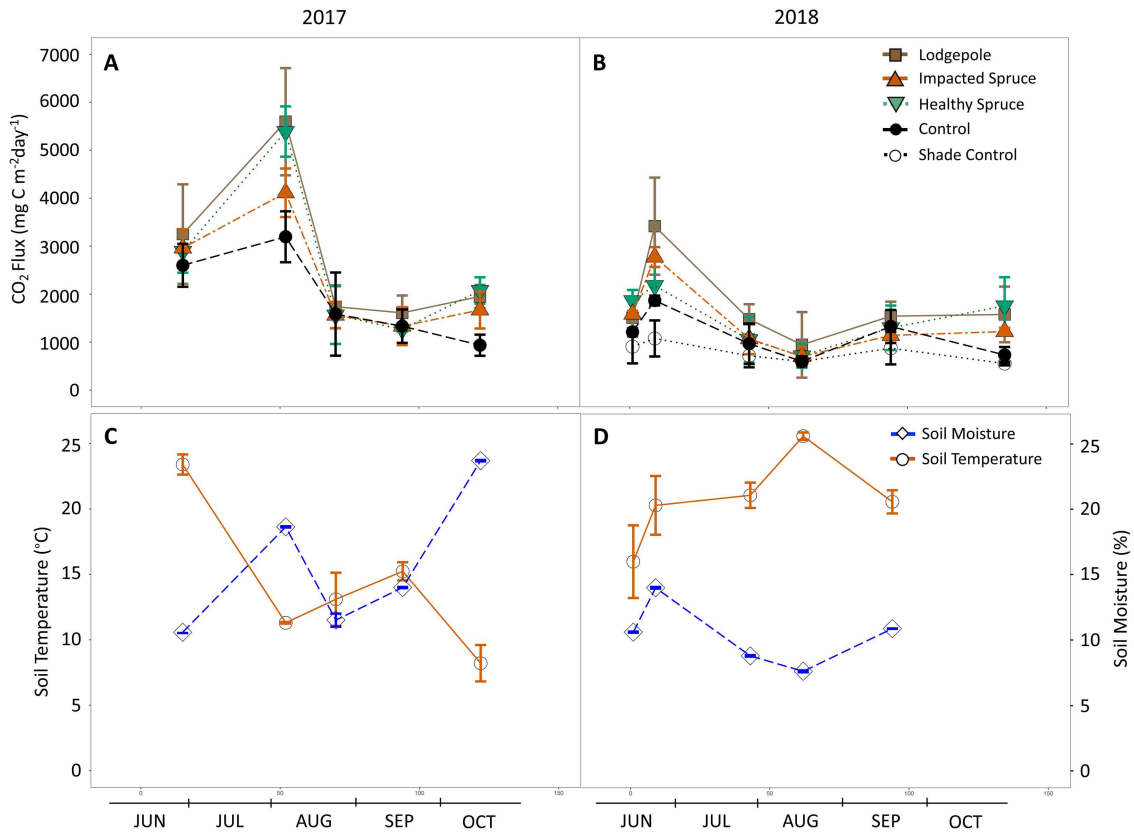


Figure 2.3 Gas flux as a function of season and needle decomposition. (A, B) Measured carbon dioxide above needle collars and (C, D) on-site soil moisture and temperature during the sampling events of 2017 (A, C) and 2018 (B, D). The gas flux error bars indicate plus or minus one standard deviation ($n = 3$ or 4). The soil temperature and moisture error bars indicate plus or minus one standard deviation of the hourly values collected during each sampling period. Soil temperature and moisture values are not available for the final date in October 2018.

Methane flux averaged a removal rate of -1 mg C m⁻² day⁻¹ above needle and control samples with no clear differences in association with needle presence in contrast to the control. Seasonal trends in CH₄

behaved similarly to the observed CO₂ fluxes in which the data points appear to correspond with soil moisture (Figure A.2). Nitrous flux was near ambient conditions with no clear trends apparent as a function of uptake or release. Ammonia was also monitored; however, no clear trends were apparent as a function of uptake or release among field triplicates.

2.4.3 Soil and porewater signatures

The 2017 and 2018 porewater collections revealed over time that the healthy spruce and lodgepole needles exported higher DOC concentrations within the aqueous near-surface in contrast to the control and impacted spruce samples. No clear trends were determined in total nitrogen concentrations during June 2017 due to high variability in lodgepole (0.38 ± 0.14 mg/L), healthy spruce (0.67 ± 0.29 mg/L) and impacted spruce (0.49 ± 0.19 mg/L). May 2018 sampling returned values below detection for all samples except for lodgepole (0.32 ± 0.30 mg/L). Late season measurements in October 2017 revealed similar concentrations between healthy spruce (0.79 ± 0.29 mg/L) and lodgepole (0.63 ± 0.24 mg/L) and lower concentrations for impacted spruce needles (0.38 ± 0.12 mg/L). Late season 2018 sampling returned similar concentrations between healthy spruce (0.92 ± 0.29 mg/L) and lodgepole (0.76 ± 0.10 mg/L) and lower concentrations released by impacted spruce needles (0.57 mg/L) however no standard deviation could be calculated due to low porewater yields. There were no clear differences in aromatic signatures (SUVA) as a function of needle presence (Table 2.2).

Table 2.2 Porewater results from four sampling events in 2017–2018.

	June 2017			May 2018		
	DOC (mg/L)	TN (mg/L)	SUVA (L/mg-m)	DOC (mg/L)	TN (mg/L)	SUVA (L/mg-m)
Impacted Spruce	5.3 (± 1.4)	0.49 (± 0.19)	2.2 (± 0.8)	6.6 (± 2.1)	BDL	3.0 (± 0.8)
Healthy Spruce	9.3 (± 5.1)	0.67 (± 0.29)	2.4 (± 1.0)	9.4 (± 3.1)	BDL	4.8 (± 4.4)
Lodgepole	4.3 (± 1.1)	0.38 (± 0.14)	2.1 (± 0.9)	10.3 (± 3.3)	0.32 (± 0.30)	3.7 (± 0.8)
Control	4.9 (± 2.1)	0.50 (± 0.21)	2.5 (± 1.2)	6.3 (± 1.8)	BDL	3.8 (± 0.6)
	October 2017			October 2018		
	DOC (mg/L)	TN (mg/L)	SUVA (L/mg-m)	DOC (mg/L)	TN (mg/L)	SUVA (L/mg-m)
Impacted Spruce	6.7 (± 2.3)	0.38 (± 0.12)	1.9 (± 0.9)	9.4 -	0.57 -	2.1 -
Healthy Spruce	14.9 (± 6.2)	0.79 (± 0.29)	2.1 (± 1.0)	14.1 (± 3.0)	0.92 (± 0.29)	2.4 (± 0.6)
Lodgepole	12.4 (± 3.4)	0.63 (± 0.24)	1.8 (± 0.5)	16.8 (± 5.9)	0.76 (± 0.10)	4.3 -
Control	11.8 (± 2.0)	0.68 (± 0.04)	1.8 (± 0.6)	13.0 -	0.96 -	-

Notes:

Each parameter was averaged across two consecutive days of sampling to increase sample size.

Standard deviation shown in parenthesis.

BDL = below detection limits.

Soil extractions in October 2017 agree with the porewater results of higher soil DOC concentrations in association with needle decomposition. Interestingly, in contrast to the harvested needle water extraction analysis, soil porewater under lodgepole needles contained the highest extractable DOC compared to the spruce needles, reaching more than a two-fold higher concentration (Table 2.3). Overall, the total extractable nitrogen in soil under all needle samples were lower than the control; however, differences in nitrogen speciation as a function of needle presence are apparent. The inorganic nitrogen species are low in association with needle decomposition while organic nitrogen comprised the highest percentage of the total nitrogen in soil associated with needle decomposition in contrast to the control. Specifically, organic nitrogen comprised an average of 86% of the total nitrogen in all three soil types associated with needle decomposition, while a lower percentage (50%) was observed in the needle-free control (Table 2.3). In addition, the needles resulted in lower soil pH values (average of 6.1 ± 0.2 across needle samples) than that of the control (pH= 6.6).

Table 2.3 Soil extractions from sacrificed single collars in October 2017.

	DOC (mg/kg)	TN (mg/kg)	NO ₂ -N (mg/kg)	NO ₃ -N (mg/kg)	NH ₄ -N (mg/kg)	TON-N (mg/kg)	SUVA (kg/mg-m)	pH
Impacted Spruce	63.0	7.63	0.10	2.41	0.10	5.02	0.52	6.04
Healthy Spruce	57.5	4.08	0.06	0.07	BDL	3.96	-	6.37
Lodgepole	167.2	5.86	0.08	0.18	BDL	5.60	0.66	6.02
Control	41.7	8.81	0.10	4.19	0.12	4.40	0.43	6.61

Notes:

Results are normalized based on an air-dried mass basis.

Soil samples were collected from a single collar for each sample type (n = 1).

2.4.4 Soil microbial communities in association with needle decomposition

Beta diversity differed as a function of sample type for both fungal and bacterial soil communities. Similar to gas flux, the most significant clustering determined by principal coordinate analysis was observed during or after high moisture events sampled in August 2017 and July 2018 (weighted UniFrac Adonis test: bacterial: $P < 0.05$ and fungal: $P < 0.01$; Figure 2.4). Consistent with the geochemical analyses, soil communities associated with needle samples clustered separately from controls. To a lesser extent, communities under lodgepole decomposition were distinct from those under spruce needles. In contrast, communities under the impacted spruce needles were indistinguishable from healthy spruce. Despite significant community clustering, alpha diversity metrics were not significantly impacted by the

decomposition of different needle types in comparison to the control (Figure A.3).

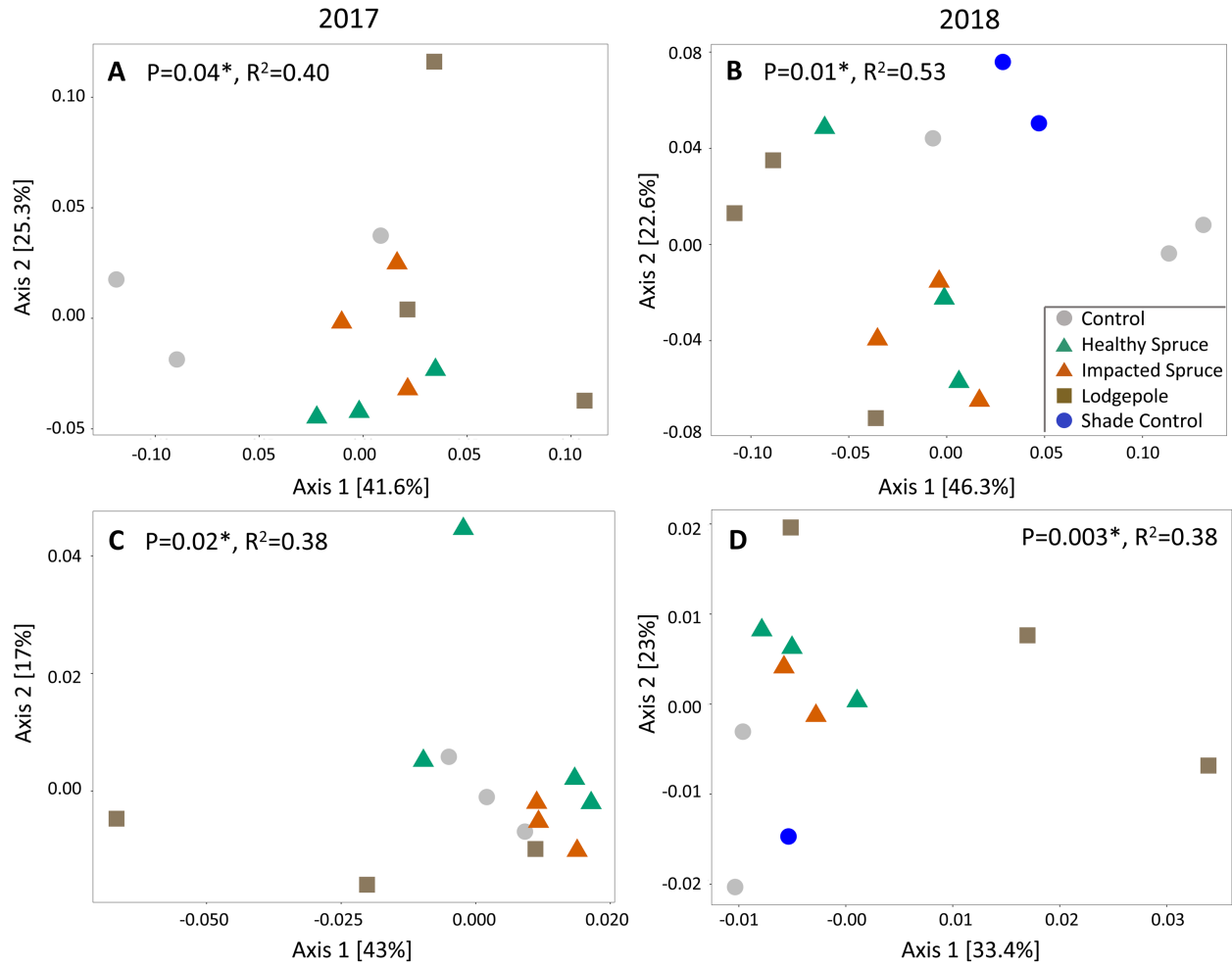


Figure 2.4 Needle decomposition shifts bacterial and fungal communities during wet seasons. (A, B) Bacterial and (C, D) fungal communities significantly cluster based on needle type during the high moisture event of August 2017 (A, C), and after a high moisture event in July 2018 (B, D) using the weighted UniFrac distance metric. Fungal communities under the spruce samples were grouped together in the July 2018 analysis due to low sample count. P and R^2 values for each figure are shown.

To further investigate differences in microbial communities in soil under the needle samples, differential abundance tests were conducted on the top 15 most abundant genera during the 2017 and 2018 high moisture sampling events to determine differentiation in relative abundances at the genus level (Figure 2.5). There were no significant differences between the shaded and exposed needle-free control samples or for healthy and impacted spruce. Communities under the lodgepole needles were most different from those found under the control with increasing significance in 2018 (Figures 2.5, A.4). Specifically, lodgepole significantly differed from the control in higher abundances of potential nitrogen fixers of the genera *Bradyrhizobium* and *Variibacter*. In addition, by comparing lodgepole and the controls, lower abundances

of chemoorganotrophs *Segetibacter*, *Flavisolibacter*, *Ferruginibacter* and a potential nitrogen fixer *Massilia* were observed in the lodgepole samples. Further, comparing lodgepole to the binned spruce samples, lodgepole is significantly lower in abundance of *Ferruginibacter*, *Segetibacter* and *Massilia*. Binned impacted and healthy spruce samples significantly differed from the controls with higher abundance of *Chthoniobacter* and lower abundances of *Segetibacter* and *Flavisolibacter* (Figure 2.5).

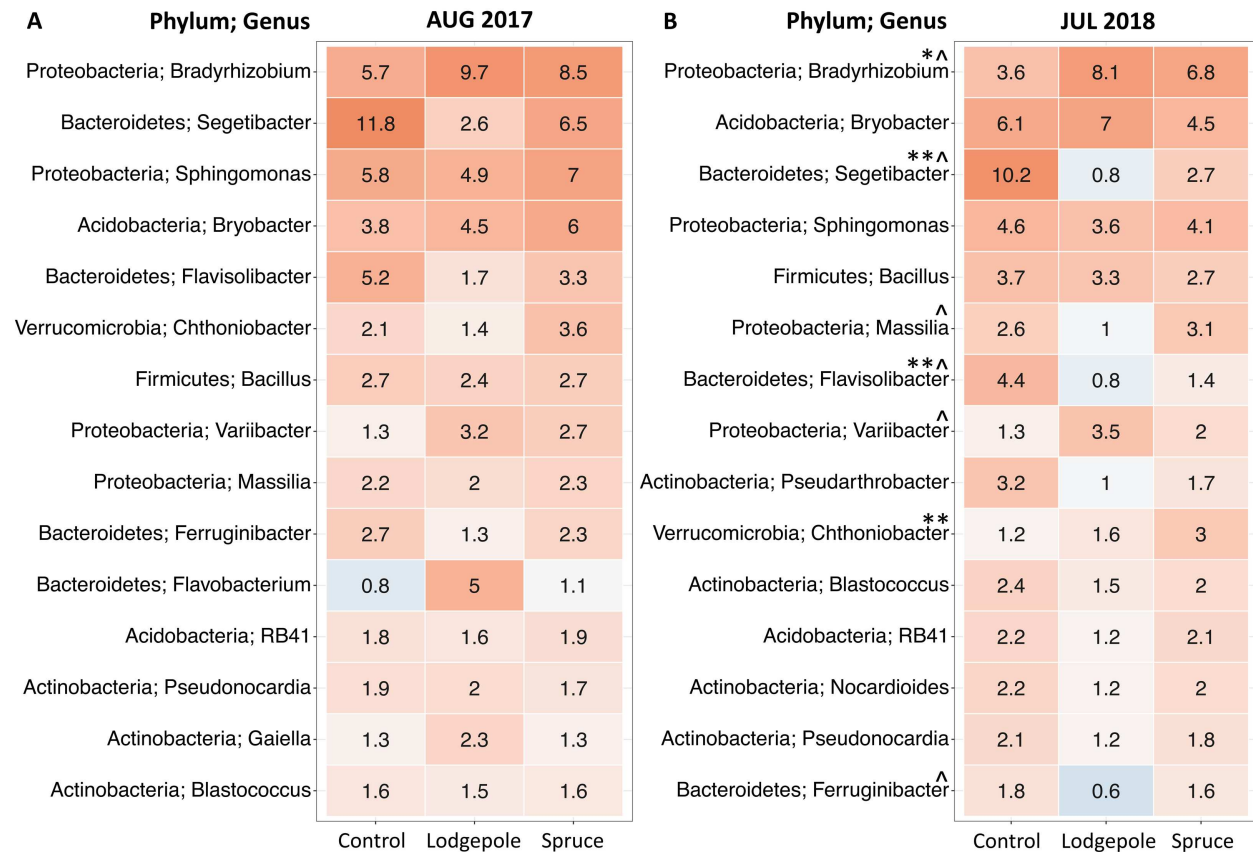


Figure 2.5 Top 15 most abundant bacterial genera differ as a function of sample type in (A) August 2017 and (B) July 2018. Numeric values within each box represent the percent read abundance. Spruce values are binned healthy and impacted spruce (n = 6). Control in July 2018 is binned shaded and non-shaded (n = 5). Mean abundance values are shown within each heatmap box with color representing numerical ranges visually with low values in blue and high values in red. Symbols indicate significant differences determined by differential abundance analysis as follows: ^ Significant difference between lodgepole and controls, *Significant difference between impacted spruce and controls, **Significant difference between the binned impacted and healthy spruce from the controls.

Turning to Eukarya, larger variations in relative abundance were observed and taxonomic rank was not completed at the Genus or Family level. Few significant differences were determined with differential abundance tests; however, basic comparisons between samples were possible. Fungal communities followed a similar trend to bacteria in which the communities under impacted and healthy spruce needles are similar, while there are differences between lodgepole and spruce (Figure A.5). Further, based on mean

relative abundances the Phylum Ascomycota comprised 50% mean abundance under the control, 52% under healthy spruce, 40% under impacted spruce, and 25% under lodgepole samples (Figure A.6). In contrast Basidiomycota represented 36% of the mean abundance under lodgepole and 10-19% under the spruce and control samples (Figure A.6).

2.5 Discussion

We gained a better understanding of the effects of needle decomposition on terrestrial soil biogeochemical processes by studying the compositional differences of senesced coniferous needles between two species and as a function of bark beetle impact. Additionally, by isolating the needle litter from the complexity of the tree canopy and rhizosphere, we were able to investigate the selective pressures derived from litter presence and decomposition over time under the same environmental conditions. By extension, this enhanced our understanding of biogeochemical cycling in natural and disrupted ecosystems. However, it is noted that conclusions with respect to bark beetle impacts are limited in our dataset to spruce trees and the study was further limited to two conifer species. Three key findings emerged from this study: 1) Under the same conditions and location, tree species exerts a larger role in needle decomposition dynamics than bark beetle impact, 2) conifer litter decomposition enhances soil organic carbon and nitrogen cycling, heterotrophic respiration, and exerts pressures on resident microbial ecology, and 3) biogeochemical signatures are enhanced during high moisture conditions.

2.5.1 Needle litter chemistry

The behavior of plant litter as it decomposes can be attributed to the quality of organic matter available for microbial decomposition. The amount of bioavailable nutrients and organic matter within the needles depends on the plant phenology, which determines the physiology of carbon and nitrogen storage throughout the tree (Millard & Grelet, 2010). Differences in initial litter quality can affect soil chemistry and microbial communities with the potential for more microbial activity associated with higher quality litter, which can in turn affect soil C and N retention within the first year of decomposition (Šantrůčková, Křišťůvková, and Vaněk, 2006; Fraterrigo, Ream, and Knoepp, 2018). As a result, discrete differences in organic content and nutrients as a function of needle type can shed light on the expected behavior of plant litter decomposition in the environment. To this end, high quality litter is understood to have higher nitrogen concentrations (low C:N ratios) and low recalcitrance, which can be estimated by lignin content. Following this understanding, the results of total C, N and FTIR analysis revealed that the harvested naturally senesced lodgepole needles are a higher quality substrate than naturally senesced spruce, while the beetle impacted spruce needles are of the lowest quality. A similar study comparing senesced conifer

litter decomposition rates across forests within the Rocky Mountains agree with the conclusion that lodgepole contains the lowest lignin levels, but contrasts with our observation of lodgepole containing the highest C:N ratio (Stump and Binkley, 1993). After two years of decomposition, the same results by Stump and Binkley in 1993 agree with the trend described in this study of a decrease in C:N for the spruce needles and an increase in lodgepole. The total carbon losses in this study after two years of decomposition revealed a modest decrease in lodgepole compared to the impacted spruce needles and a large standard deviation in the healthy spruce (Table A.1). While a lack of mass balance quantification limits interpretation, possible explanations include decomposer biomass formation as well as different mass loss rates associated with hydrolysis and oxidation due to the physical differences in needle shape and size between tree species (Ono et al., 2011).

FTIR analysis revealed lower quantities of lignin and higher quantities of cellulose in the lodgepole needles compared to both spruce needle types throughout two years of decomposition (Figure 2.2). These observations indicate functional differences in the decomposition processes of lodgepole and spruce needles. This observation agrees with a litter decomposition study by He et al. in 2019 that reported periodic lignin losses associated with increases in cellulose. Lignin and cellulose decomposition processes can be controlled by climate, litter quality, and the decomposers present (He et al., 2019). With the variability of climate and native soil microorganisms removed in this study, the needle chemistry is likely the main driver of the differences observed in decomposition across needle type. Lignin and cellulose decomposition appear to be linked, which can be attributed to the organization of polymers within plant cell walls. A greater distribution of lignin can prevent decomposers from accessing more labile organics within the cell (Berg & McClaugherty, 2014). As a result, litter types with higher levels of lignin may exhibit reduced decomposition rates until lignin degradation occurs. This explains why the lodgepole needles behave differently from the spruce needles which contain higher lignin levels.

2.5.2 Carbon & nitrogen cycling

During the process of decomposition, soil carbon and nitrogen speciation can shift as a result of abiotic and biotic factors. Specifically, chemical attributes of soils can influence decomposition processes differently across tree species (Vesterdal, 1999; Berg, 2000). By importing conifer needles to the same location, this study limited these effects to focus on the variable of needle litter chemistry. With this in mind, studies have reported similar observations across a variety of study locations which include increased inorganic soil nitrogen in association with bark beetle impact (Clow et al., 2011; Brouillard et al., 2017). Interestingly, while the harvested impacted spruce needle extractions returned the highest total nitrogen concentrations, inconsistent and contrasting trends in porewater total nitrogen and organic carbon were observed.

Differences between the initial needle extractions and porewater composition over two years can be influenced by changes in nutrient distribution and quantity within the needles when decomposition begins (Berg & McClaugherty, 2014).

A study by Kopáček et al. in 2018 observed terrestrial nitrogen speciation significantly responded to Norway spruce bark beetle tree death with increased NH_4^+ in the soil adjacent to tree dieback due to mineralization of organic N in the decomposing plant matter followed by nitrification. In contrast, this study revealed lower concentrations of inorganic nitrogen in soil under all needle samples when compared to the needle-free control. As a result, the fraction of organic nitrogen comprising the total extractable nitrogen in soil after one year of decomposition was the highest under all needle samples (Table 2.3). This observation of high organic nitrogen and relatively low inorganic nitrogen could be due to the dependency of nitrogen distribution on the litter C:N ratio. According to a study by Šantrůčková, Křišťůvková & Vaněk in 2006, a litter C:N threshold value of 32 is the point in which mineralized soil nitrogen concentrations begin to release in measurable concentrations. This threshold ratio value varies between studies, as it is dependent on the site location, litter age, and species (Eldhuset, Kjønnaas & Lange, 2017). The C:N ratios in the needles specific to this study were above 45 during two years of decomposition. In addition, due to the dominance of organic nitrogen in soils associated with needle decomposition in contrast to the control, the needle chemistry appears to enhance microbial N accumulation while little N mineralization is occurring. This agrees with other results in which nitrogen mineralization was low for lodgepole and moderate for spruce during decomposition (Stump & Binkley, 1993).

In addition to nitrogen shifts in association with needle decomposition, the study by Kopáček et al., 2018 revealed increased soil DOC concentrations within the first years of beetle impacted Norway spruce tree death due to organic carbon production from dead biomass. Our results exhibit similar results of increased near-surface carbon cycling in association with seasonal CO_2 production and higher magnitudes of extractable DOC in soil underlying needle decomposition. The largest magnitude of CO_2 production was observed in association with lodgepole needles, especially during high moisture events in which respiration was almost two-fold higher than the needle-free controls. Heightened decomposition of higher quality litter leads to nutrient utilization and ultimately terrestrial microbial respiration, which in turn is expected to decompose more quickly initially (Berg, 2000; Fraterrigo, Ream & Knoepp, 2018). As a result, lodgepole forests may contribute a larger flux of CO_2 to the atmosphere during needle drop compared to spruce forests, especially during warmer snow-free seasons with higher soil moisture. Looking closer at healthy versus impacted spruce needles, clear differences were not determined in CO_2 flux magnitudes between these needle types despite chemical differences, which suggests that beetle impacted spruce needle drop alone will not shift forest soil respiration rates from that of naturally senesced spruce needles. Past studies

comparing impacted forest plots to non-impacted have shown variations in results, but with a consensus of respiration values that are lower or unchanged in soil under impacted trees in comparison to soils under healthy trees for both spruce (Speckman et al., 2015) and lodgepole (Brouillard et al., 2017).

2.5.3 Soil microbial selection

In conjunction with geochemical shifts, microbial community analysis provided further insights into the collective impacts of needle decomposition. Past studies have documented shifts in soil bacterial (Mikkelsen, Lozupone & Sharp, 2016; Mikkelsen et al., 2017) and fungal communities (Štursová et al., 2014) after beetle infestation. However, these observed shifts are related to variables that include litter decomposition and the cessation of rhizodeposition among other variables such the changing water and energy budgets that occur during tree mortality (Mikkelsen et al., 2011). As significance was most pronounced near high moisture events, our results suggest that litter decomposition plays a more significant role in microbial community selection during the summer wet season when top down infiltration is occurring.

In comparing the bacterial communities, the presence of higher quality litter also exerted the strongest selective pressures on microbial communities. Interestingly, despite initial chemical differences between naturally senesced and beetle impacted spruce needles, no significant differences in microbial differential abundance were observed. This suggests that inherent complex organics associated with the tree species exert a stronger impact on biogeochemical cycling, while the impact of beetle infestation on the tree has little to no impact on the decomposition processes of spruce litter. This agrees with litter quality decomposition studies that have concluded complex organics, as well as other elements are the driving force of litter decomposition over C:N ratios (Fogel & Cromack Jr., 1977; Hobbie, 2015).

The increased relative abundance of putative nitrogen fixers (*Bradyrhizobium* and *Variibacter*) associated with rhizospheric processes suggests that the conditions necessary for nitrogen fixation are present within the needle decomposition horizon. However, due to the lack of observed mineralization in the soil chemistry, and no predominant abundance of ammonification, nitrifying, or denitrifying bacteria, the results reveal how crucial the rhizosphere is for the complete cycling of nitrogen and further why larger impacts to the nitrogen cycle are not observed in this study. This suggests that while the needle litter decomposition contributed to carbon cycling in this study, nitrogen cycling and especially inorganic nitrogen is likely linked to other mechanisms at the tree-scale, such as belowground nutrient contributions associated with rhizodeposits.

2.6 Conclusion

The results of this study shed light on the complex behavior of needle decomposition as a function of abiotic and biotic factors that are associated with temporal decomposition. Over a study period of two years, we have gained further insight into the expected impacts during the first stages of litterfall for healthy and beetle disrupted ecosystems. In a situation where needles fall to the forest floor following tree mortality it is likely that observable impacts will consist of increased CO₂ production from heterotrophic activity while enhanced inorganic nitrogen cycling will occur depending on the C:N ratio of the litter and rhizospheric processes. As more noticeable impacts on soil flux and microbial communities were observed during high moisture events, the biogeochemical signatures will be most pronounced in high moisture environments. This has been observed in past studies, in which moisture is correlated with higher coniferous decomposition rates (Pandey & Singh, 1982; Gunadi, Verhoef & Bedaux, 1998).

The expected outcomes of litter decomposition on soil biogeochemistry will differ as a function of tree species. It can be expected that lodgepole forest soil microbial communities will be different from those of a spruce forest within the first two years of decomposition after needle fall. During bark beetle infestation, needle decomposition is likely to exert a comparatively modest selective pressure on soil biogeochemistry when contrasted with other forest relevant processes such as evapotranspiration, canopy interception, energy fluxes, as well as rhizodeposition and the cessation of such processes.

CHAPTER 3

EFFECT OF ELEVATION, SEASON, AND ACCELERATED SNOWMELT ON BIOGEOCHEMICAL PROCESSES DURING ISOLATED CONIFER NEEDLE LITTER DECOMPOSITION

A paper accepted for publication in *PeerJ*¹

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3.1 Abstract

Increased drought and temperatures associated with climate change have implications for ecosystem stress with risk for enhanced carbon release in sensitive biomes. Litter decomposition is a key component of biogeochemical cycling in terrestrial ecosystems, but questions remain regarding the local response of decomposition processes to climate change. This is particularly complex in mountain ecosystems where the variable nature of the slope, aspect, soil type, and snowmelt dynamics play a role. Hence, the goal of this study was to determine the role of elevation, soil type, seasonal shifts in soil moisture, and snowmelt timing on litter decomposition processes. Experimental plots containing replicate deployments of harvested lodgepole and spruce needle litter alongside needle-free controls were established in open meadows at three elevations ranging from 2,800-3,500 meters in Crested Butte, Colorado. Soil biogeochemistry variables including gas flux, porewater chemistry, and microbial ecology were monitored over three climatically variable years that shifted from high monsoon rains to drought. Results indicated that elevation and soil type influenced baseline soil biogeochemical indicators; however, needle mass loss and chemical composition were consistent across the 700 m elevation gradient. Rates of gas flux were analogously consistent across a 300 m elevation gradient. The additional variable of early snowmelt by 2-3 weeks had little impact on needle chemistry, microbial composition, and gas flux; however, it did result in increased dissolved organic carbon in lodgepole porewater collections suggesting a potential for aqueous export. In contrast to elevation, needle presence and seasonal variability of soil moisture and temperature both played significant roles in soil carbon fluxes. During a pronounced period of lower moisture and higher temperatures,

bacterial community diversity increased across elevation with new members supplanting more dominant taxa. Microbial ecological resilience was demonstrated with a return to pre-drought structure and abundance after snowmelt rewetting the following year. These results show similar decomposition processes across a 700 m elevation gradient and reveal the sensitivity but resilience of soil microbial ecology to low moisture conditions.

3.2 Introduction

Coniferous forests are at high risk of ecosystem stress as climate disturbances amplify globally (Seidl, Schelhaas & Lexer, 2011). Drought-induced stress reduces tree canopy cover, net productivity, and rhizospheric processes (Schlesinger et al., 2016) with potentially lasting implications for terrestrial organic matter cycling and associated microbial activity (Hyde et al., 2016; Negrón & Cain, 2018). Hydrologic shifts associated with warming trends in snow-dominated mountain environments, such as shorter periods of snow cover, have further implications for downstream water availability (Barnett, Adam & Lettenmaier, 2005). Litter decomposition plays an important role in carbon and nitrogen cycling and can also be affected by drought conditions (Santonja et al., 2015). As a result, projected increases in global temperatures and shorter periods of snow cover could exacerbate poor soil health and ecology in mountain ecoregions (de Vries et al., 2018).

The North American Rocky Mountains largely consist of coniferous forests and the region has observed a variety of climate change impacts. Rocky Mountain National Park reported a 1.9°C rise in average annual temperature in the last century with snow now melting 2-3 weeks earlier (Rocky Mountain National Park, 2018). Collectively these climate shifts have been associated with increased conifer tree mortality along with hydrologic implications for the Colorado River watershed. Moreover, a recent study identified biomes that harbored a heightened risk for organic carbon release from soils in association with climate change (Kramer & Chadwick, 2018). Within the Rocky Mountains, these biomes include polar wet tundra and boreal wet forests that encompass montane and subalpine ecoregions of Colorado (GeoNetwork, 2007). Classifying sensitivity broadly by biome alone is limiting however due to the heterogeneity of local mountain terrain (Wainwright et al., 2020). Specifically, sensitivity to drought varies across locations with slope and aspect influencing energy balances and soil moisture (Körner, 2007). Soil type also can impact soil moisture and geochemical signatures (Lawrence & Hornberger, 2007; Wilson et al., 2008) as well as the variability of snowpack dynamics across elevation that can play an additional role in drought responses across ecoregions (Tennant et al., 2017).

Soil microbial community responses to environmental stress additionally can influence decomposition and biogeochemical cycling processes. Within this study, we focus on different species of tree litter as the

type of forest litterfall can affect associated microbial decomposition processes. Specifically, high quality litter typically defined by lower C:N ratios and higher bioavailability impact community composition by stimulating soil microorganisms and increasing decomposition rates (Šantrůčková, Křišťůvková & Vaněk, 2006; Fraterrigo, Ream & Knoepp, 2018). Our past work determined that lodgepole litter was more bioavailable than spruce as evidenced by lower lignin-based aromatics and higher CO₂ respiration rates after soil rewetting (Leonard et al., 2020a). Beyond the litter decomposition processes, soil conditions are known to affect microbial communities with reduced activity associated with low moisture conditions and in turn increased CO₂ rates after rewetting of these soils (Birch, 1958; Schimel, 2018). The cause of this effect as it relates to microbial mechanisms and whether resilience to drought drives these observations is not fully understood. Recent studies have shown microbial resilience to drought after rewetting cycles in a variety of environments (Nijs et al., 2019; Zhou et al., 2019) in which increased species richness contributes to functional redundancy with implications for carbon storage (Allison & Martiny, 2008).

The goal of this study was to 1) determine the role of soil type, elevation, and early snowmelt on biogeochemical processes during litter decomposition, 2) evaluate the effects of litter presence, type, and seasonal shifts in soil moisture on microbial decomposition processes, and 3) understand the resilience of soil microbial ecology to low moisture conditions. Within our study domain, we hypothesized that soil respiration rates and microbial community richness would be greatest during summer rewetting events. In addition to these biogeochemical indicators, needle decomposition and organic carbon export would be most pronounced at the mid-elevation where moisture and temperature were less limiting. By extension, we expected enhanced needle decomposition and organic carbon export at the mid-elevation with early snowmelt further enhancing decomposition and microbial respiration rates.

To address these research questions, we isolated lodgepole and spruce needles from the hydrobiogeochemical complexity of the forest. Harvested needles were deployed in similar slope and aspect open meadows representative of three ecoregions within Colorado. This allowed insight into the specific roles of tree litter on local soil biogeochemistry. Measurements including gas flux, porewater chemistry, microbial community structure, and changes in needle mass and chemistry were monitored over three years (2017-2019) and across three elevations ranging from 2,800-3,500 meters. Snowmelt was accelerated annually at the middle elevation adjacent to a control plot that underwent natural snowmelt rates to enable further investigation of snowmelt timing. The experiments encompassed large differences in snowpack and precipitation during a historically warm period, further enriching the observed impacts of climate change on local biogeochemical cycling (Colorado State Forest Service, 2020).

3.3 Materials & Methods

3.3.1 Needle collection and deployment

Conifer needles were collected from regions of Colorado identified in our prior work and transported to established field plots (Brouillard et al., 2017, Leonard et al., 2020a). This enabled us to study isolated decomposition processes in response to climate-associated variables in the field. Lodgepole pine (*Pinus contorta*) needle litter was harvested from the White River National Forest (39.54° N, 106.15° W, elevation 3,000 meters) while Engelmann spruce (*Picea engelmannii*) needles were harvested from Monarch Pass (38.50° N, 106.33° W, elevation 3,400 meters). The needles were harvested beneath respective tree stands in August of 2016 and air-dried at room temperature in a dark location. The dried needles were sieved with a Tyler Equivalent 10 mesh (1.7 mm) to separate debris and homogenize samples. Aliquots of 250 grams for each needle type were weighed and separated for deployment in October 2016.

Experimental plots were established at three elevations. An additional plot at the middle elevation was subjected to accelerated snowmelt for a total of four distinct plots. In order to focus on litter decomposition with minimal interference from flora, native foliage was raked to remove aboveground plant matter and belowground roots within the footprint of deployed 25 cm diameter and 18 cm height polyvinyl chloride (PVC) rings. The rings were pounded into the ground leaving approximately 10 cm of the ring aboveground. Lysimeters (Soil Moisture 1905L06 15 cm) were installed in the middle of each ring for future porewater collections (Figure 3.1A). A total of 48 rings were deployed across the three elevations with a duplicate plot at the middle elevation consisting of four replicates of the spruce, lodgepole, and needle-free controls as depicted in Figure 3.1B. The controls were bare soil within the rings while the spruce and lodgepole rings were filled with 250 grams of the respective needles. Randomization was ensured using R Studio version 3.5.2 to produce 4 x 4 matrices with sample assignments as depicted in Figure 3.1B. The experimental plots were monitored during the snow-free months of May through October during the years 2017, 2018, and 2019. In October of 2017, one of each sample quadruplicate was sacrificed to inform additional studies published in Leonard et al., 2020a. This reduced sample replicates to three thereafter. The needle deployments limited native plant growth within the rings, however any observed emergent plant growth within the sample rings was manually pulled including the roots monthly to minimize native foliage interferences. Temporal soil biogeochemical analyses including soil extractions, gas flux, porewater, and microbial DNA were conducted with sampling frequency and totals dependent on each method and media type sampled (Figure 3.2).

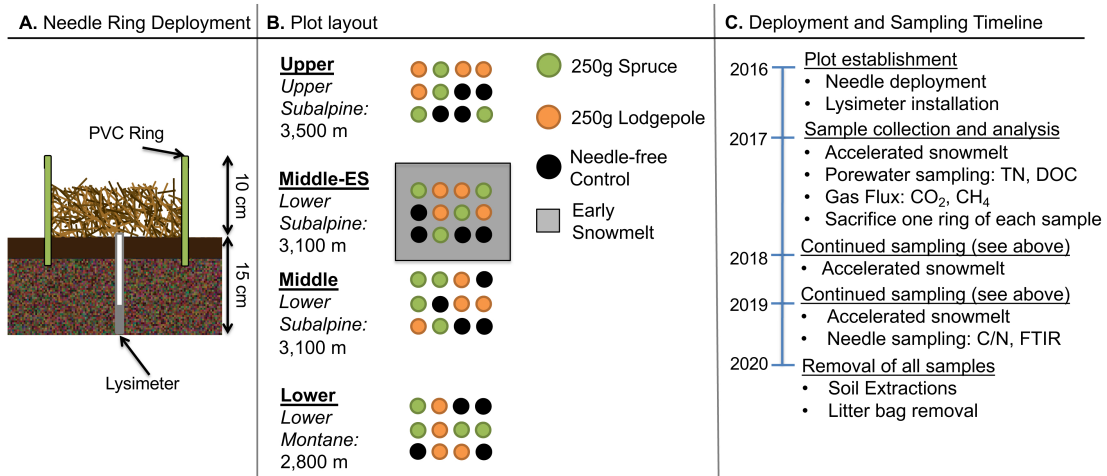


Figure 3.1 Experimental design, plot, and schedule. (A) The schematic of the ring deployment, (B) plot layout, and (C) sampling timeline are provided. Four replicate collars for each sample type were deployed in Fall 2016. In October of 2017, one of each sample quadruplicate was sacrificed to inform additional studies published elsewhere (Leonard et al., 2020a).

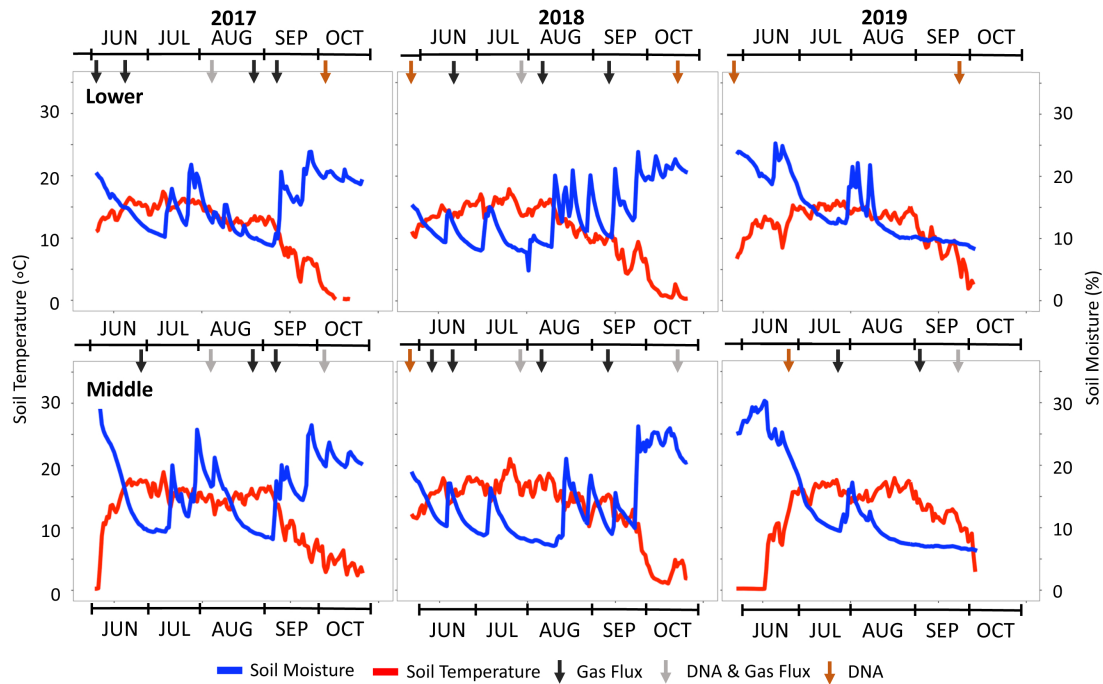


Figure 3.2 Seasonal soil temperature and moisture data across the three study years. Climate data are shown for the three study years with soil moisture and temperature data from the Lower (2,800 m) and Middle (3,100 m) locations. The Upper plot data are not shown but provided in Figure B.2. Arrows indicate specific sampling events conducted as indicated in black (gas flux), grey (DNA & gas flux), and orange (DNA).

In August of 2017, needle litter bags were deployed at each elevation to more effectively quantify mass loss associated with decomposition. The needle bags were constructed by wrapping 2 grams each of archived lodgepole and spruce needles in a single layer of white nylon mesh fabric (23 x 23 openings per 2.54 cm) that was further wrapped in one layer of window screen (17 x 13 openings per 2.54 cm). This contained the needles in a 5 x 5 cm flat surface area. Quadruplicates of the litter bags were placed level to the ground inside newly emplaced PVC rings. The PVC rings were deployed according to the same methods as the sample rings by removing native foliage and maintaining bare soil. Mass balance from each elevation after three years of needle decomposition (August 2017-August 2020) was calculated by the differences in weight from the initial 2 grams and the final 2020 needles in each litter bag.

3.3.2 Experimental plot descriptions

The experimental field plots were located in Crested Butte, Colorado within the Washington Gulch drainage of the East River watershed in designated areas approved by the Rocky Mountain Biological Laboratory. Field plots were established at three ecoregions: Lower Montane (38.92° N, 106.95° W, elevation 2,800 meters), Lower Subalpine (38.96° N, 107.03° W, elevation 3,100 meters), and Upper Subalpine (38.97° N, 107.04° W, 3,500 meters) (Figure 3.1B). These ecoregions are within the identified boreal wet forest and polar wet tundra Holdridge life zones (GeoNetwork, 2007). The study plots were established within northeast/ east facing aspect meadows with an average slope of 7-12° and will be referred to as the “Lower”, “Middle”, and “Upper” plots throughout the paper. The soil types of each location were unique with Tilton sandy loam at the Lower plot, a mixture of Tilton sandy loam, Cryaquolls and Histosols at the Middle, and Bucklon silt loam at the Upper plot (United States Department of Agriculture, 2019). The dominant bedrock material of Crested Butte in the study location is Sedimentary Clastic Mancos Shale with a mixture of Middle Tertiary Intrusive Igneous (U.S. Geological Survey, 2005).

Snowmelt timing increased with elevation and the earliest snow-free dates occurred at the Lower plot (Table B.1). Duplicate plots at the Middle elevation were established to further contrast the effects of snowmelt timing (Figure 3.1B). Early snowmelt manipulations were implemented once isometric snowpack conditions were established in the middle of April (April 10-25) from 2017-2019. During deployment, a permeable 50% black mesh fabric was carefully secured on top of the snow over the duplicate Middle plot for approximately one month as described in Leonard et al., 2020b. The tarp was removed shortly after the plot had fully melted and while snowpack within the control plot remained. These two Middle plots will be referred throughout the paper as the “Middle-ES” for the early snowmelt plot and “Middle” for the control snowmelt plot.

A snow telemetry (SNOTEL) station near the study locations on Mt. Crested Butte (38.89° N, 106.95° W, elevation 3,100 m) provided climate records for local weather data (Figure B.1). SNOTEL data for daily maximum air temperature, maximum soil moisture percentages at 20 cm depth, and snow water equivalent (SWE) precipitation data were tabulated for each study water year and averaged to determine climate normals for comparison. Water years were determined by the twelve months starting on October 1st and ending on September 30th of the following year. Averaged 20-year normals were calculated from 1990-2010 for all climate variables except soil moisture, which only had reliable data sufficient for a ten-year average from 2006-2016. In addition to SNOTEL data, unshielded microclimate sensors buried at roughly 12 cm were established at each of the four experimental plots to monitor hourly soil moisture and temperature that were used to determine daily average values. Microclimate measurements were recorded from 2017- 2019 at the Lower and Middle plots and 2017-2018 at the Upper plot (Figure B.2).

3.3.3 Needle, soil, and porewater compositional analyses

A chemical analysis of the 2016 harvested and 2019 decomposed needles focused on temporal shifts in carbon, nitrogen, and organic functional groups. These analyses were conducted with small subsamples collected over a three-year window of less than 5% of the total needles. The collected needles were air-dried, homogenized, and ground to a powder (Leonard et al., 2020a). Samples were submitted for total C and N measurements using a LECO TruSpec CN analyzer (LECO Corporation). A subset was submitted for Fourier Transform Infrared (FTIR) analysis at Lawrence Berkeley National Laboratory. Per the methods of Leonard et al., 2020a, the FTIR absorption spectra were baseline corrected in R Studio version 3.5.2 using R package hyperSpec (Beleites & Valter, 2018) and peak-by-peak integration analysis was conducted to quantify organic functional groups of interest. Specifically, biological-related functional groups (Naumann, 2000) observed in litter and bark studies (Pandey & Pitman, 2003; Özgenç, Durmaz & Kuştaş, 2017; Yang et al., 2019) were targeted for sample comparisons. The prominent peaks identified within this study were polysaccharides (900-1200 cm⁻¹), cellulose-based ether linkages (1157-1159 cm⁻¹), lignin-based aromatics (1508-1510 cm⁻¹), amide I (1600-1700 cm⁻¹), and carbonyl typically at 1738 cm⁻¹.

Soil extractions were conducted in 2020 in association with plot deconstruction. Soil was collected from the upper 2-3 cm soil horizon in contact with needle decomposition, homogenized on-site, and frozen at -20°C until analysis. Deionized (DI) water extractions of soils followed the methods from Leonard et al., 2020a. In brief, samples were sieved at 2 mm and a 2:9 solid mass to liquid weight ratio using 40 mL liquid volume for extraction in 50mL centrifuge tubes. The tubes were placed on a shake plate for one hour, then centrifuged and filtered at 0.45 µm. Aliquots of the filtrate were acidified with hydrochloric acid and analyzed for total nitrogen (TN) and dissolved organic carbon (DOC) using a Shimadzu TOC-550A Total

Organic Carbon Analyzer. The remaining filtrate was analyzed by ion chromatography for nitrate and nitrite, by colorimetric assays for ammonia, and by specific UV absorbance (SUVA) with a DU 800 Spectrophotometer. Extraction results were normalized to dried soil weights.

Porewater yields were sufficient during the shoulder months of spring and fall. All porewater collections were conducted on the same date when full snowmelt was established at each plot. Soil porewater was collected from the lysimeters in each sample ring over 12 hours with 50mL Soil Moisture locking vacuum syringes to ensure sufficient water yields. The syringes were covered in canvas cloth to prevent sample interference from solar radiation. The porewater samples were filtered at 0.45 μm and frozen the same day of collection for storage at -20°C until analysis. The porewater was analyzed for TN, DOC, and SUVA following the same methods for the filtrate from the soil DI water extractions. Porewater sampling was not possible at the Lower plot in 2018 due to dry conditions.

3.3.4 Soil gas flux

Gas flux measurements were conducted in 2017-2019 at the Middle plots (14 sampling events) and during 2017-2018 at the Lower plot (9 sampling events) (Figure 3.2). Equipment access was not feasible at the Upper plot. Gas flux measurements were conducted using a Picarro G2508 cavity ring-down spectroscopy analyzer capable of measuring CO_2 , CH_4 , N_2O , and NH_3 . A closed system was established within the headspace of each sample ring by placing an airtight PVC chamber over the deployed rings. Norprene tubing was used to circulate cavity headspace from the PVC chamber to the analyzer inlet and outlets. Flux data collection typically occurred midday. Sample collection began when steady state was determined by linear trends of production or removal, initiating a two-minute data collection period for each sample. Fluxes were calculated using the Hutchingson & Mosier method provided in the Picarro software using the calculated headspace volume. The sampling sequence was randomized during each measuring event to minimize temporal biases associated with hourly fluctuations in temperature. Further, the two plots at the Middle elevation were measured simultaneously during measurements, alternating between the Middle and Middle-ES plots for each sample.

3.3.5 Soil microbial DNA extraction & rRNA gene sequencing

Soil for DNA extractions was collected from surficial soils (<2-3 cm depth) after gently pushing aside existing needles. Within the area of the sample ring, no less than 10 grab samples were collected with autoclaved spatulas to fill a 2 mL sample collection tube up to 1 g for each sample ring. The grab samples were randomized throughout the ring's surface area to minimize biases. DNA extraction and amplification were conducted for each sample in singlet following the methods of Leonard et al., 2020a. In brief, soil

DNA was extracted using the ZymoBIOMICS DNA Miniprep kit and 0.25 g of soil were weighed out from the 2 mL sample collection tube after homogenizing the total sample mass. The 5 PRIME Hot MasterMix and a primer set using the 515-Y forward and 926R reverse primers were used to amplify the 16S and 18S ribosomal RNA genes (Parada, Needham & Fuhrman, 2016). Sample barcoding with the amplicons was completed using an adapted forward primer with the M13 sequence (Caporaso et al., 2012; Stamps et al., 2016). Final DNA concentrations were determined using the Qubit 2.0 fluorometer. Libraries were sequenced at the Duke Center for Genomic and Computational Biology using an Illumina MiSeq instrument and V2 PE250 chemistry. The raw sequencing reads were processed as described in Leonard et al., 2020a using the DADA2 package (Callahan et al., 2016) and taxonomic assignments with Silva v128 (Pruesse, Peplies & Glöckner, 2012). After quality filtering the 16S genes a total of 2,500,000 sequences were obtained with a sequence depth range of 9765 ± 4270 . Normalization was performed by rarefaction at 4,299 which retained 224 out of 254 samples. Similarly, 102,000 sequences were obtained after filtering the 18S genes with a sequence depth of 400 ± 420 . Due to the high variance in depth, rarefaction was performed at 200, retaining 143 out of 254 samples. Raw sequence data have been deposited in NCBI SRA databases under access number PRJNA 605259 for the Middle site 2017-2018 samples previously published in Leonard et al., 2020a and PRJNA 715914 with the rest of the samples. Rarified samples were further processed in R Studio version 3.5.2 using the Phyloseq (v1.26.1) R package for data sorting, analysis, and visualization (McMurdie Holmes, 2013) in addition to statistical analyses and visualization packages described in the next section

3.3.6 Statistical analyses

Due to low sample sizes ($n=3$), Friedman and post hoc Wilcoxon tests were not used in this study for statistical determinations. As a result, mean and associated standard deviation values for environmental triplicates were used as alternatives for geochemical parameters including needle composition, porewater, soil extractions, and gas flux. Possible significance between samples was indicated by non-overlapping error bars. Gas flux data were binned by year to assess linear Pearson correlations between annual flux and soil parameters.

DNA sequencing data were analyzed for beta and alpha diversity. Beta diversity of samples binned by elevation (Lower, Middle, and Upper) was determined using the Adonis function as an analogous multivariate analysis of variance using weighted and unweighted UniFrac distance matrices in the community ecology R package vegan (Oksanen et al., 2019). The Adonis test was followed by principal coordinate analyses (PCoA) performed on the weighted and unweighted UniFrac distances. In addition, the dual Middle plots (Middle and Middle-ES) were compared separately from the full dataset to

determine if significant differences were present as a function of snowmelt. Next, the same methods of the Adonis function followed by PCoA were conducted across all samples as a function of sample date for each elevation to determine temporal differences in beta diversity. Alpha diversity richness was determined for each sample by calculating observed mean richness and standard deviations with sample replicates followed by visual plots of the results as a time series for each sample date (Berry, 2016).

Further, canonical correspondence analysis (CCA) was conducted using the weighted UniFrac distance matrices in statistical software Past 4.01 (Hammer et al., 2001). Environmental conditions of daily average soil moisture and soil temperature from the microclimate sensors at each plot and monthly total precipitation data from the SNOTEL database were included in the CCA to understand the influences of soil conditions on community clustering. Due to a lack of microclimate data at the Upper plot, a CCA was not generated for this location. Finally, mean relative abundances were determined by phylum and family bacterial assignments with the 15 most abundant taxa ranked for binned samples (control, spruce, and lodgepole) across date and location using the ampvis2 package (Andersen et al., 2018). Differences in mean relative abundances across sampling dates were visually observed, but also statistically confirmed using differential abundance comparisons in R package DESeq2 (Love, Huber & Anders, 2014). A *P* value less than 0.05 was considered significant in all statistical tests.

3.4 Results

3.4.1 Variation of season, elevation, and accelerated snowmelt

The three-year study encompassed a wide range of weather patterns that impacted local soil temperature and moisture (Figure 3.2). Based on the SNOTEL database and microclimate sensor data from each plot, an active monsoon in the summer of 2017 was followed by a dry year with below-average snow water equivalent (SWE) precipitation in 2018 (Table 3.1). In 2019, SWE rebounded to almost double the prior year. Precipitation averaged 77 ± 25 cm (SWE) over the study period with a higher snow fraction that ranged from 60 - 70% of total precipitation. SNOTEL soil moisture data were consistent with these trends with above-average dry soil days in 2018.

In addition to climate variability observed in 2017-2019, early snowmelt deployments allowed us to experimentally manipulate the variable of snowmelt timing. Over the three study years, the permeable tarp deployment at the Middle-ES plot accelerated snowmelt by 15 ± 7 days in contrast to the control Middle plot. This was evidenced by the microclimate soil temperature profiles and visual time-lapse capture of plant growth (Figure B.2). Early snowmelt advanced spring plant greening by as much as 22 days in 2017 and both snowmelt and plant growth timing visually mimicked that of the Lower plot (-300 meters). The Middle-ES snowmelt plot became indistinguishable from the parallel Middle plot in visual

appearance as well as soil moisture and temperature values as the summer progressed.

Table 3.1 Climate variable shifts across each study water year.

Water Year	Dry Soil*	Hot Days**	Total Precipitation (cm SWE)	Snow (cm SWE)
2017	143	73	94	61
2018	163	88	48	31
2019	44	69	88	63
AVG (1990-2010)	132 $\pm$ 90	70 $\pm$ 27	80 $\pm$ 15	48 $\pm$ 14

Notes:

Data from the Butte 380 USDA SNOTEL station.

*Total days max soil water <10% at 20 cm depth. Data was available starting in 2006, thus the average is 2006-2016

**Total days with TMax >20° C

3.4.2 Needle decomposition

Spruce and lodgepole needle compositional changes were determined by comparing initial harvested needle carbon and nitrogen totals to the decomposed 2019 samples across experimental variables of elevation and snowmelt. While triplicate deployments limited statistical confirmation, no clear differences in average litter bag mass losses were observed as a function of elevation or snowmelt due to overlapping standard deviations of decomposed samples (Table B.2). When needle litter bag mass losses were binned across elevation (Lower, Middle, Upper), this returned a total mass loss after three years of 21 $\pm$ 4% for lodgepole and 16 $\pm$ 3% for spruce (Table B.2). In agreement with mass loss, no pronounced differences in average C:N ratios for lodgepole needles were observed across elevation after decomposition (Figure 3.3). This was similarly observed in the spruce needles between the Lower and Upper plots, but interestingly the Middle plot spruce ratio was comparatively lower. No pronounced differences in average C:N ratios were observed across snowmelt manipulations. Overall there was a temporal decrease in C:N ratios of approximately 32 $\pm$ 4% in spruce and 22 $\pm$ 3% in lodgepole after three years of decomposition (Figure 3.3). As a result, total carbon decreased in the lodgepole needles by approximately 9 $\pm$ 3% and 3 $\pm$ 10% for spruce, but with a large variance in spruce from an increase in total carbon at the Upper plot. Total nitrogen increased in both spruce and lodgepole needles by 44 $\pm$ 13% and 17 $\pm$ 7%, respectively (Table B.3).

Needle organic composition was analyzed to further understand decomposition processes. The FTIR spectra for the needle samples after three years of decomposition were similar across elevation and snowmelt manipulations (Figure B.3). Peak integrations were conducted for select organic groups to explore shifts in organic composition over time (Table B.4). This revealed a decreased presence of polysaccharides and increased relative presence of amides and aromatics after three years of decomposition.

Lodgepole needles contained more labile ether linkages in contrast to a higher presence of recalcitrant lignin-type aromatics in the spruce needles throughout the three years. The ratio of the polysaccharide to amide I peaks served as an estimate of organic C:N ratios. Consistent with total C:N values (Figure 3.3), no differences were found across elevation for spruce and lodgepole litter; however, a temporal decrease in this ratio was observed for both sample types (Table B.4).

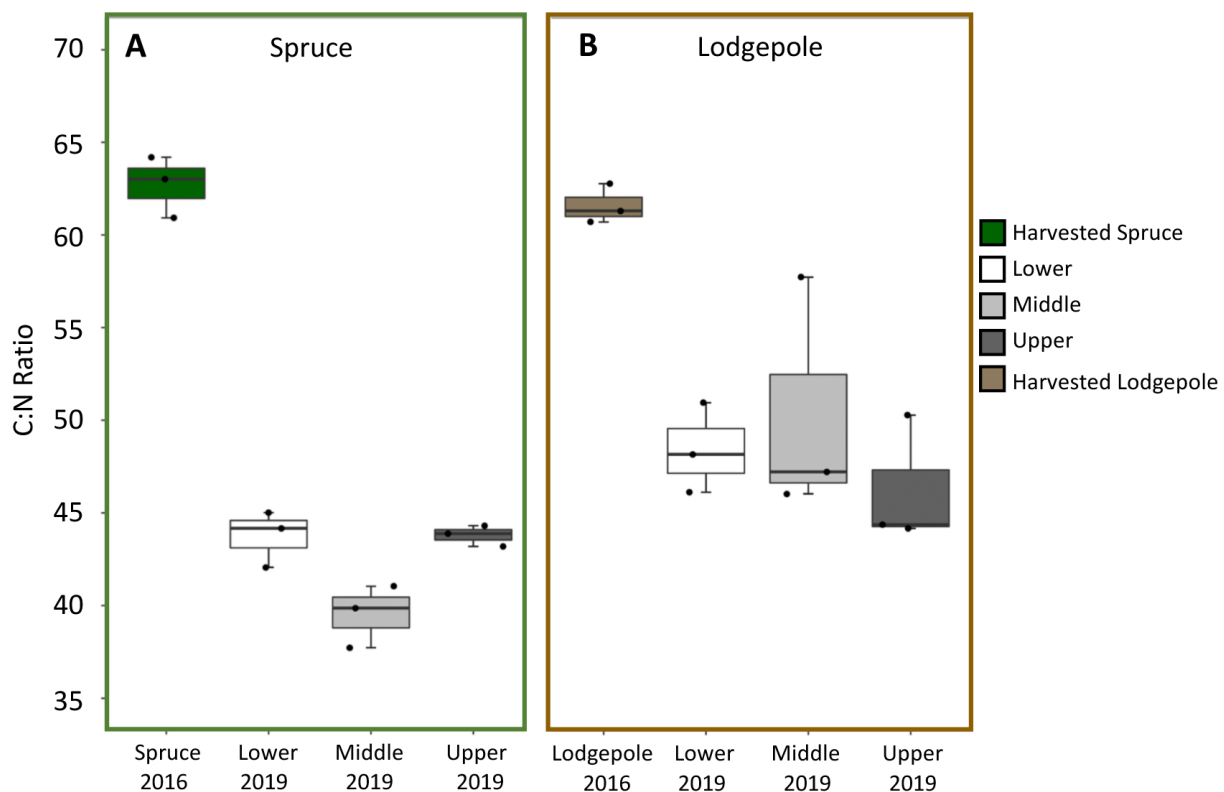


Figure 3.3 C:N ratios decreased over the three-year study period across elevations. Carbon:Nitrogen ratios (C:N) are shown across each elevation for (A) Spruce and (B) Lodgepole needle decomposition. The harvested 2016 needle values are shown first, followed by the 2019 decomposed Lower, Middle, and Upper.

3.4.3 Porewater & soil extractions

Soil porewater samples were collected to quantify dissolved organic carbon (DOC) and total dissolved nitrogen (TN) concentrations across elevations and study years. Differences in average values were observed across elevation based on standard deviation values with consistently higher concentrations of control sample porewater DOC and TN at the Lower plot (15.1 ± 1.7 mg/L DOC, 2.3 ± 1.2 mg/L TN) in contrast to the Middle (7.2 ± 3.4 mg/L DOC, 0.4 ± 0.3 mg/L TN) and Upper (2.8 ± 1.1 mg/L DOC, 0.3 ± 0.1 mg/L TN) (Figure 3.4). As a result, concentrations of DOC and TN decreased with rising elevation. No consistent trends were determined as a function of needle presence or type at each study plot. However,

there was a suggestive trend with respect to accelerated snowmelt where the Middle-ES plot exhibited higher DOC concentrations than the Middle control plot. This trend was observed across samples with the largest average increases observed under lodgepole needles in the spring sampling events (Figure 3.5). While the plots were adjacent but separate, variables beyond snowmelt timing cannot be fully ruled out as a possible explanation. The porewater TN concentrations followed a similar trend but standard deviation values overlapped (Figure B.4).

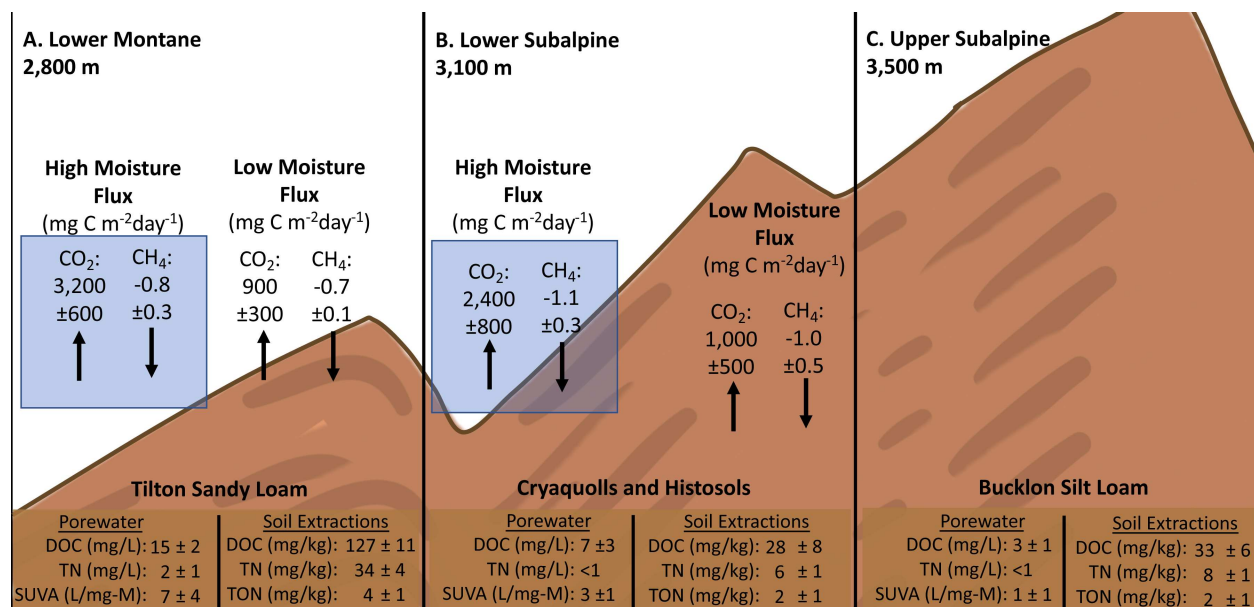


Figure 3.4 Compilation of gas flux, porewater, and soil extractions across experimental elevation plots. Averages of soil porewater and gas flux measurements for each elevation (n=3). Only control values are shown to remove needle decomposition biases.

Analogous to porewater results, sacrificial soil extractions at the end of the experiment exhibited differences across elevation with the highest DOC and TN concentrations at the Lower site. Control soil extractable concentrations were approximately five times higher at the Lower plot when contrasted with the Middle and Upper plots (Table 3.2). The higher TN concentrations at the Lower site were associated with greater levels of extractable nitrate in contrast to the other plots. Dissolved total organic nitrogen (TON) and nitrate constituted >90% of the nitrogen species within the soil. Accelerated snowmelt did not appear to impact average soil DOC and TN between the Middle and Middle-ES plots (Table 3.2). In contrast to porewater, the presence of needles resulted in higher extractable soil DOC across elevations when compared to the needle-free control rings (Table 3.2). Higher extractable DOC was associated with lodgepole needles relative to spruce.

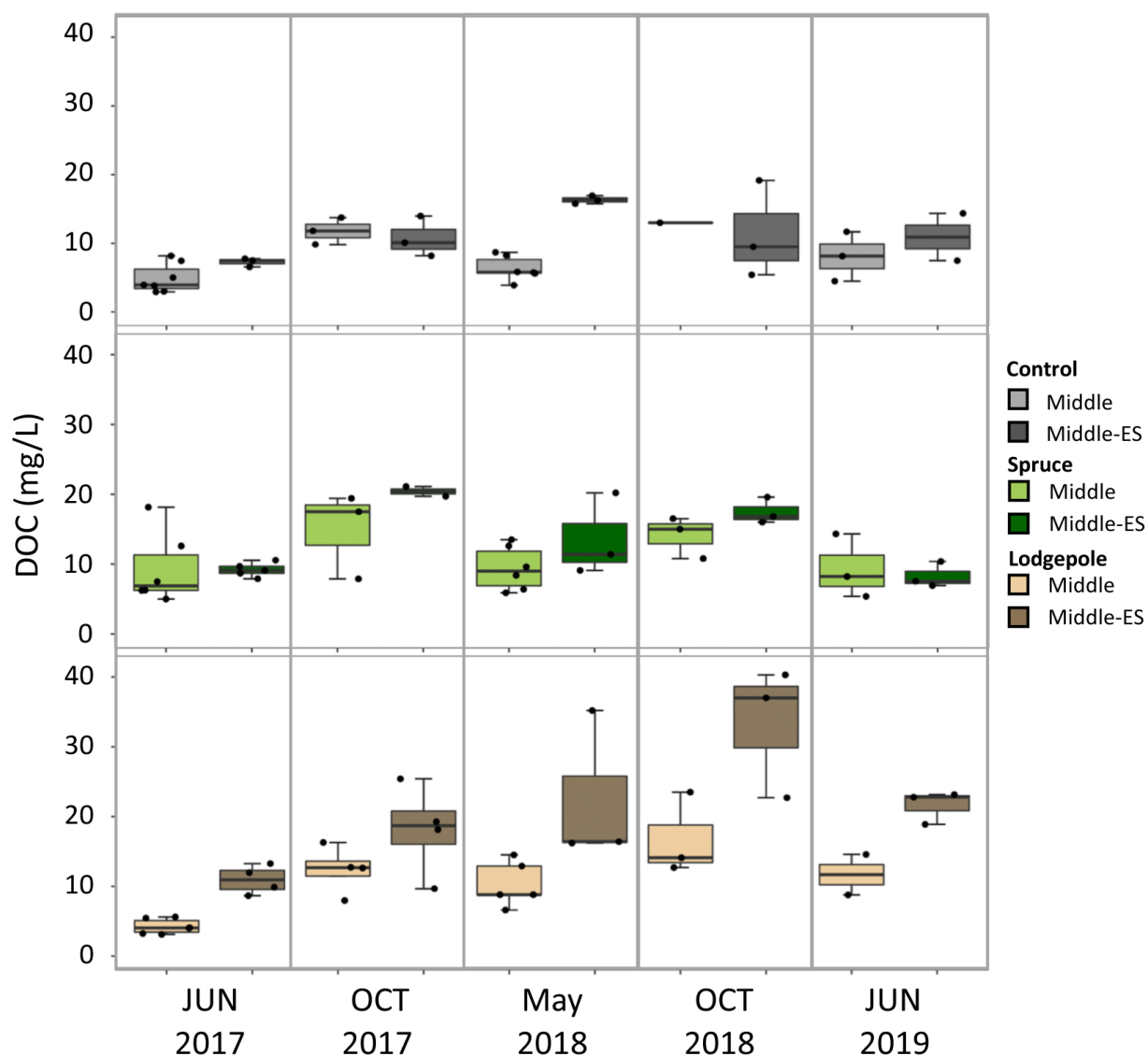


Figure 3.5 Higher organic carbon porewater concentrations are associated with the early snowmelt plot in contrast to the control snowmelt. 2017-2019 porewater DOC concentrations were collected for both the Lower Subalpine control (Middle) and early snowmelt (Middle-ES) plots for the Controls, Spruce, and Lodgepole. Error bars indicate plus or minus one standard deviation. Samples were collected during two consecutive days from both plots in 2017-2018 to increase the sample size ($n=3-6$).

Table 3.2 Soil extractions: Concentrations of DOC (dissolved organic carbon), TN (total dissolved nitrogen), and TON (total dissolved organic nitrogen) across elevations in August 2020 (n=3).

	Lower			Middle		
	Control	Spruce	Lodge	Control	Spruce	Lodge
DOC (mg/kg)	127 (± 11)	183 (± 39)*	171 (± 18)	28 (± 8)	68 (± 34)	115 (± 41)
TN (mg/kg)	34 (± 4)	33 (± 19)*	24 (± 7)	6 (± 1)	11 (± 9)	7 (± 3)
TON (mg/kg)	4 (± 1)	4 (± 2)*	5 (± 2)	2 (± 1)	4 (± 3)	5 (± 2)
	Middle-ES			Upper		
DOC (mg/kg)	32 (± 3)	54 (± 7)	94 (± 11)	33 (± 6)	56 (± 16)	92 (± 21)
TN (mg/kg)	6 (± 1)	5 (± 1)	4 (± 1)	8 (± 1)	9 (± 3)	16 (± 16)
TON (mg/kg)	2 (± 0)	2 (± 1)	3 (± 1)	2 (± 1)	1 (± 2)	1 (± 1)

Notes:

* n=2

3.4.4 Gas flux

To further understand the influence of needle decomposition in conjunction with climate variables on terrestrial carbon cycling, gaseous carbon flux was measured at both the Lower and Middle plots. Gas flux did not differ between the Lower and Middle plots or as a function of snowmelt manipulation based on overlapping standard deviation values (Figure 3.6). Peaks in average CO₂ production were observed across elevation in association with moisture events which returned flux values 2-3 times higher than rates during low moisture periods (Figures 3.2, 3.4, and 3.6). The seasonal CO₂ flux was further heightened by the presence and type of needle litter (Figures 3.6 and B.5). This held true when contrasting the comparatively wet summer of 2017 with drier years in 2018 and 2019 (Figures 3.6 and B.6). Pearson correlations returned inconsistent correlations between total annual flux measurements and soil moisture and temperature (Table B.5). Observed average CH₄ fluxes were not appreciably different as a function of elevation, snowmelt manipulation, needle presence, or type (Figure B.7). Average control CH₄ consumption was -0.7 ± 0.1 mg C m⁻² d⁻¹ for the Lower plot and -1.0 ± 0.5 mg C m⁻² d⁻¹ at the Middle plot in 2017-2018 (Figure 3.4). Additionally, NH₃ and N₂O fluxes exhibited high variability and no clear trends of production or removal.

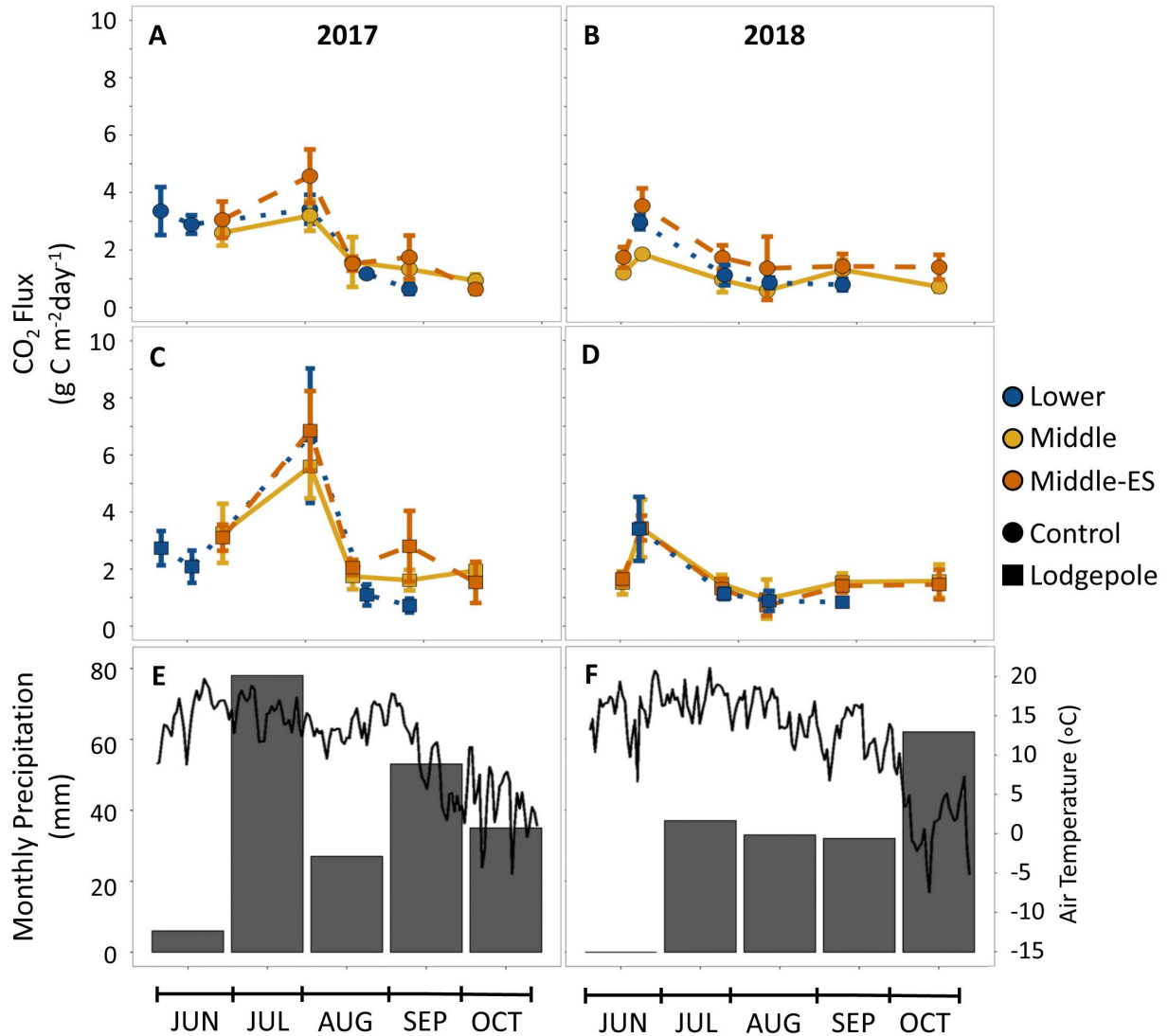


Figure 3.6 CO₂ production is influenced by seasonal shifts in temperature and moisture, but not elevation. CO₂ flux measurements over the snow-free months of 2017-2018 for the (A, B) control and (C, D) lodgepole samples across elevation and snowmelt manipulations. The 2019 full data and spruce samples are not shown but can be found in Figures B.4 and B.5. (E, F) Air temperature (black lines) and total precipitation (columns) are shown from the Butte 380 SNOTEL (3,100 m) database. Error bars indicate plus or minus one standard deviation.

3.4.5 Microbial ecological responses

Microbial community clustering was significant across locations as indicated by the Adonis multivariate analysis of variance using weighted and unweighted UniFrac distance matrices (Figure B.8). These differences were observed across elevation for both 16S bacterial and 18S eukaryotic genes ($P < 0.05$). Further comparisons between the two Middle plots revealed that accelerated snowmelt did not significantly

impact community clustering. Needle presence and type however influenced community clustering across elevations with a reliance on both date and location. Specifically, when Adonis analyses were conducted for each sample type across each year, significant differences in community beta diversity were observed in 2018 at the Lower and Middle plots. Due to low sample counts, this was not observed at the Upper plot (Table B.6).

The alpha diversity time series (Figure 3.7) further highlighted the impact of seasonal shifts on bacterial community structure and diversity in 2018 at the Lower and Middle plots with a relative increase in species richness in July 2018 for all sample types. Analogous trends were observed in the alpha diversity time series at the Upper plots (Figure B.9), but there was high variance in standard deviations. Mean relative familial abundance analyses further detailed community shifts in specific taxa at the Lower and Middle plots by contrasting early-season May 2018 against a drier July and October 2018 (Figure B.10). Upon visual inspection of the heatmaps and statistical confirmation using differential abundance analysis, clear changes occurred in 2018 for specific taxa. As a result, relative abundances of the early-season taxa of primarily *Chitinophagaceae* among others fell in July or October of 2018 at each elevation with fewer dominant families not observed previously increased in relative abundance. This corresponded to an average increase in community richness that was most pronounced at the Middle elevation (Figure 3.7) in 2018 as well as significant differences in variance in 2018 at both the Lower and Middle elevations (Table B.6). In contrast to the bacterial communities, this trend was less clear in eukaryotic 18S richness (Figure B.9 and B.11). Visually, shifts in relative abundances of specific phyla were observed (Figure B.12); however, due to low sample counts during rarefaction, the changes were not statistically confirmed with differential abundance comparisons. Few consistent trends in specific bacteria were found at the genus level except for drought-resistant *Mycobacterium* and *Isosphaera* which were present in multiple samples at the Middle and Lower plots during the dry phase in July 2018. The observed trends at the Upper plot were less clear due to high variance; however, it is recognized that fewer total samples were collected at this location with incomplete soil moisture and temperature data (Figures B.9 and B.11).

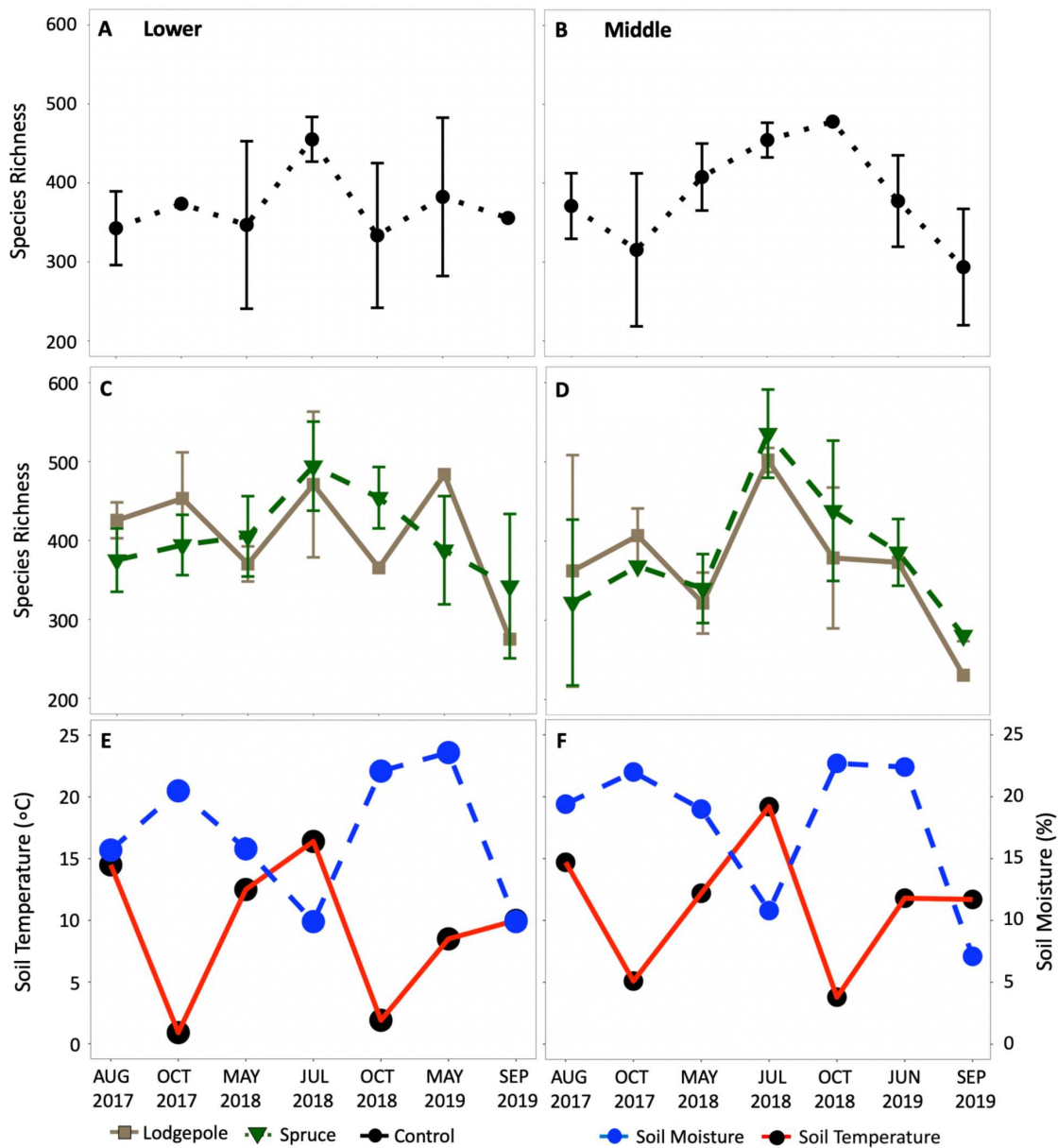


Figure 3.7 Alpha diversity richness peaks in the summer dry period of 2018. Species richness changes over time at the (A) Lower and (B) Middle plots. The average daily soil temperature and moisture are shown in red and blue for the (C) Lower and (D) Middle plots on each sample date. The Middle-ES and Upper plots are not shown. Error bars indicate plus or minus one standard deviation ($n=3,4$). Note a lack of error bars for a sample point is due to $n<3$ caused by sample removal during rarefaction.

The CCA plots highlighted the association of precipitation, soil moisture, and soil temperature with the observed shifts in bacterial community structure in 2018 (Figure 3.8). Specifically, at the Lower plot, the 2018 samples clustered by date with May and July 2018 samples associated with lower precipitation (32 ± 5 mm) and soil moisture ($14 \pm 3\%$) whereas October 2018 clustered in association with higher precipitation (58 ± 22 mm) and soil moisture ($22 \pm 1\%$). Interestingly, the September 2019 microbial samples also

clustered in proximity with high soil moisture and sampling dates, despite low precipitation (6 mm) and moisture (10%) conditions. The July 2018 samples at the Middle plot clustered tightly in association with lower precipitation (37 mm) and soil moisture (11%). The Middle-ES plot returned similar associations with the 2018 samples clustered separately from the 2017 and 2019 samples. Due to low sample counts after rarefaction, the 18S CCA plots returned high variability, which limits interpretation (Figure B.13).

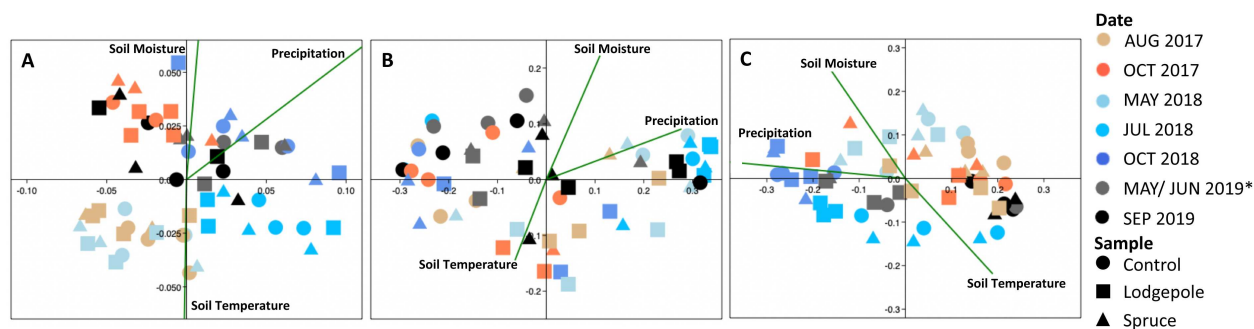


Figure 3.8 Bacterial communities cluster significantly as a function of date and environmental variables. Canonical correspondence analysis of environmental variables driving beta diversity community clustering as a function of soil temperature and moisture over time at the (A) Lower, (B) Middle, and (C) Middle-ES plots. Soil moisture and temperature are values unique to each plot from the microclimate sensors on the day of collection. Precipitation represents SNOTEL monthly totals.

*The Lower samples were collected in May 2019, and Middle in June 2019.

3.5 Discussion

This three-year experiment was designed to monitor needle decomposition and associated biogeochemical responses across the climate variables of elevation, snowmelt, and season in simplified soil plots. Our subsequent monitoring window encompassed a wide range of annual variability in snowpack and precipitation that included abnormally dry conditions. As such our results provide insight into carbon, nitrogen, and microbial shifts in montane and subalpine ecoregions during dry soil conditions and early snowmelt that are projected to become more frequent under climate change (Lukas et al., 2014). Additionally, by contrasting lodgepole and spruce litter against bare soil, we were able to discern contributions from conifer needle litter to biogeochemical processes.

3.5.1 The roles of soil type, elevation, and accelerated snowmelt on soil biogeochemistry

The experimental 700-meter elevation gradient across montane and subalpine ecoregions harbored clear differences in the baseline soil properties of porewater, extractable organic carbon and nitrogen, and microbial community structure (Figure 3.4). While soil characteristics drive bioavailable carbon, nitrogen (Delgado-Baquerizo et al., 2015), resident microbial communities (Thomson et al., 2015), and soil moisture

(Lawrence & Hornberger, 2007), trends as a function of elevation are not consistent. Past studies across a 1,000-meter gradient determined inconsistent trends in soil organic carbon across elevation (Djukic et al., 2010), while a larger gradient of 3,000 meters established positive and negative correlations in soil carbon and nitrogen content with altitude and temperature, respectively (Tashi et al., 2016). In our present study, the elevation gradient of 300 meters between the Lower and Middle plots did not appreciably impact gas flux (Figure 3.6), while mass losses from deployed conifer needles were similar across the full 700-meter gradient (Table B.2). This was surprising considering the differences in snowmelt timing across elevation (Table B.1); however, these results agree with other studies that have shown minimal impact on microbial structure with no lasting effects on microbial biomass and nutrient cycling processes after experimental snowmelt manipulations (Conner, Gill & Harvey, 2017; Darrouzet-Nardi et al., 2019). A plausible explanation is that ecological resilience overcomes the effects of accelerated snowmelt without additional climate variables of higher temperatures and lower precipitation (Darrouzet-Nardi et al., 2019).

Snowmelt timing has been shown to influence litter decomposition rates associated with freeze-thaw cycles by releasing more nutrients to bacteria that become active during thawing (Zhu, Yang & He, 2013). While the decomposition rates over three years of our study were not considerably different between the Middle plots, soil porewater DOC consistently trended higher under accelerated snowmelt in the spring. These trends continued in the fall, but with higher variability in significance (Figure 3.5). While the Middle-ES plot could have locational bias, the two plots (Middle and Middle-ES) were in proximity, of the same soil type, and the greatest differences were observed only under the lodgepole needles. Carbon losses can be enhanced with spring timing, early-season frost events, and late-season drought (Arnold, Ghezzehei & Berhe, 2014), all of which are location-dependent. Collectively, these observations could be explained by a climate-related phenomenon associated with more labile litter decomposition. Conifer litter decomposition depends on the quality, or bioavailability of organic matter and nutrients, resulting in different biogeochemical responses as a function of tree needle type (Leonard et al., 2020a). As a result, lower quality needles can limit bioavailable organics, while soil type is more influential in potential decomposer limitations under higher quality litter (Vesterdal, 1999). Collectively, this suggests that accelerated snowmelt could increase access to nutrients under high quality litter during the spring thaw cycle for microbes becoming more active under early snowmelt. Interestingly, this is in contrast to the findings of Conner, Gill & Harvey, 2017 in which no changes were observed in soil DOC over three years of early snowmelt in a subalpine aspen forest. These conflicting results reveal the limitations in this study from the exclusion of native foliage, as the observed effects of early snowmelt on carbon cycling can be mediated by plant growth (Wipf, Rixen & Mulder, 2006; Conner, Gill & Harvey, 2017).

3.5.2 The importance of needle litter and seasonality

While differences in soil temperature and moisture across a 700-meter gradient exerted little influence, needle presence and type increased soil DOC and CO₂ fluxes across the elevation transect. Despite overlapping in significance, there was a trend toward higher mass losses and higher rates of heterotrophic respiration in association with lodgepole needles across elevations (Figure 3.6). This agrees with other studies at lower forest elevations (Fujii et al., 2018), subarctic tundra (Sundqvist, Giesler & Wardle, 2011), and a subalpine elevation gradient (Zhu, Yang & He, 2013) that collectively concluded that plant litter type affects decomposition rates more than abiotic influences found across climate regimes. In support of higher mass loss rates, needle decomposition also influenced heterotrophic respiration with as much as twice the CO₂ production under lodgepole samples in contrast to needle-free controls. This is consistent with earlier findings confined to the Middle plot (Leonard et al., 2020a) where lodgepole needles provided a more labile carbon source than spruce. Further, as expected from previous findings, peaks in CO₂ production were associated with wetter soils from snowmelt and precipitation events (Figure 3.6) while CH₄ consumption remained relatively consistent. This supports prior findings where CO₂ production is more dependent on climate whereas CH₄ consumption relies more strongly on soil characteristics (Gulledge & Schimel, 2000).

Heterotrophic respiration depends on both water availability (Liu, Zhang & Wan, 2009) and temperature (Schindlbacher et al., 2011). As a result, both variables likely influence respiration rates as driven by soil saturation thresholds. This was observed in a prior boreal forest study where CO₂ production in dry soil conditions, defined as below soil half-saturation, was more sensitive to moisture, while wetter conditions were more dependent on temperature (Gulledge & Schimel, 2000). This phenomenon could explain our observation of conflicting correlations across plots. Alternatively, or in tandem, this could be explained by interchanges between abiotic and microbial activity-related influences on CO₂ rates where high moisture release is driven more by microbial respiration and low moisture release by abiotic mechanisms. This is consistent with the observed slowing of microbial activity during dry periods in previous studies (Davidson, Belk & Boone, 1998; Wu et al., 2011; Carbone et al., 2011). As brought to question by Schimel, 2018 the mechanisms which increase CO₂ during rewetting events are unclear. However, our study suggests increased biotic mechanisms under needle decomposition by demonstrating that areas with more labile litter are associated with higher carbon fluxes during moisture events. It has been projected that seasonal shifts in soil moisture will increase overall soil respiration in montane and subalpine ecoregions such as those analyzed here (Kramer & Chadwick, 2018). In our study, dry soil conditions were associated with low baseline fluxes and repressed heterotrophic activity. This could become more frequent with projected increases in temperature if precipitation remains the same or

decreases. By extension, there could be decoupling from temperature where moisture would be the main determinant of microbial activity and CO₂ respiration rates (Gulledge & Schimel, 2000). While drought could repress carbon release, this could be associated with more pronounced export during rewetting (Meisner et al., 2017).

3.5.3 Microbial resilience and response to drought

A temporal analysis of microbial community structure across deployments provided further insights. Consistent with heterotrophic respiration rates, resident bacterial communities were influenced by needle presence, type, and seasonal shifts in soil moisture. This had previously been explored at the Middle plot in Leonard et al., 2020a. Our present study furthered insights across elevation with significant community variability depending on the sample date (Table B.6). The CCA plots (Figure 3.8) and alpha diversity time series (Figure 3.7) emphasized the importance of reduced moisture and higher temperatures in bacterial ecology, which is supported by experimental simulations of drought (Rein et al., 2016) and a field study (Acosta-Martínez et al., 2014). Clear differences in bacterial communities were observed after the drier conditions of July 2018 as evidenced by significant beta diversity variance and increased alpha diversity species richness (Table B.6, Figure 3.7). There are inconsistencies in the literature where drought has been documented to both increase and decrease overall microbial richness (Peñuelas et al., 2012; Ochoa-Hueso et al., 2018). Specifically, at the Middle and Lower plots, drought-resistant *Mycobacterium* of Actinobacteria (Torvinen et al., 2006) and *Isosphaera* of Planctomycetes (Dai et al., 2019) ranked as the top 15 most abundant taxa only in July 2018. Interestingly, during this period the bacterial family *Chitinophagaceae* experienced its most pronounced decrease. Analogous findings were observed for fungal communities where the relative abundance of Ascomycota *Dothideomycetes* and Basidiomycota *Tremellomycetes* increased in July 2018 and maintained higher abundances through 2019 at the Middle and Upper elevations under the needle samples. This supports prior field drought-related findings of Acosta-Martínez et al., 2014.

More broadly, at the Phylum level, the July 2018 shift in community structure translated to a decreased abundance of Bacteroides across elevation and samples, agreeing with observed decreases in Bacteroides under elevated temperatures in other studies (Oliverio, Bradford & Fierer, 2017). Interestingly, this contrasts with a drought cycle study on agricultural land that reported increased Bacteroides (Acosta-Martínez et al., 2014). That same study however highlighted Acidobacteria as sensitive to temperature changes, which was consistent with observed increases in our system in 2018. These conflicting results highlight the importance of soil type and location as well as limitations in interpreting and predicting taxonomic shifts associated with a changing climate where heterogeneous mountain terrain is an example. Despite the changes associated with July 2018, a return of the previously dominant

pre-drought taxa and abundances in 2019 provided evidence for microbial resilience across our elevation gradient. This agrees with previous findings in which diverse communities are more resilient partially due to rare taxa that can continue functional processes and increase in abundance in response to disturbances (Shade et al., 2012). As a result, microbial communities have an inherent resilience to disturbances (Allison & Martiny, 2008) and greater tolerance than local flora and fauna (Schimel, 2018). However, the dynamics of plant and microbial interactions, though not explored in our current study, could impact these findings (de Vries et al., 2018).

3.6 Conclusions

This study highlights the sensitivity, but also the resilience of biogeochemical decomposition processes to elevation, season, and snowmelt. An elevation gradient of 700 m or less will likely have a minimal effect on the biogeochemical responses to drought conditions. Earlier snowmelt in the timescale of weeks is not expected to significantly affect local biogeochemistry beyond an increased potential for DOC release in organic horizons with more labile litter influences, whereas climate plays a more prominent role in soil baseline conditions. While drier soil conditions reduced heterotrophic respiration, carbon release could be magnified when moisture rewetting returns. This is especially significant in high sensitivity biomes that include the studied montane and subalpine ecoregions in Colorado. As a result, with higher dependency on moisture events, increased carbon release in tighter timeframes could occur under litter decomposition. The microbial communities are likely resilient to drought during short-term disturbances if soil saturation returns, but the impacts of long-term drought need to be further understood. If increased drought stress from climate change continues in Colorado, this could reinforce the lower respiration rates and microbial community shifts observed in the 2018 dry season of this study.

CHAPTER 4

A PRELIMINARY INVESTIGATION INTO THE ASSOCIATION OF CLIMATE WITH INCREASED
DISINFECTION BYPRODUCT FORMATION DURING DRINKING WATER TREATMENT IN
COLORADO WATERSHEDS

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4.1 Abstract

Climate change projections suggest ecosystems including conifer forested regions could be highly sensitive to temperature and moisture shifts in the future. In addition, previous studies have forecasted increased organic carbon export as temperature and moisture shifts continue in sensitive biomes. Alterations to geochemical cycles associated with environmental stressors could lead to unexpected consequences downstream during municipal water treatment. Disinfection byproducts (DBPs) for example, are a constituent of concern when organics react with chlorine. More specifically, past work has shown increased DBP formation in surface waters sourced from watersheds impacted by large-scale bark beetle infestation and resulting increased organic inputs. This study used long-term drinking water quality and climate data in the state of Colorado to conduct a historical analysis of DBPs across surface water sourced municipalities. The goal of this study was to conduct preliminary work that explored temporal trends and correlations between DBP concentrations and climate variables. This was achieved using linear regression analyses to determine significant temporal trends as well as linear correlations. Interestingly, several Colorado public water systems revealed increasing trends over the past 10-20 years of the two regulated DBP groups haloacetic acids and trihalomethanes. The observed trends varied across watershed and location, but with commonalities in declining forest health associated with the climate change variables. These preliminary results reveal a correlation between ecosystem perturbations and DBPs, confirming their known impacts on fundamental biogeochemical processes can lead to broader water quality issues. This exploratory work lays the groundwork for future studies with more time and resources while providing recommendations to move this study forward.

4.2 Introduction

Watershed health is a crucial component of water quality for downstream communities including the 80% of Colorado’s citizens who depend on forested watersheds for drinking water (CSFS, 2021). Several of these watersheds are experiencing increased water and temperature stress that could result in unexpected consequences downstream during municipal water treatment. This is of significance for disinfection byproducts (DBPs) that form when aqueous organic matter reacts with chlorine during water disinfection. Two major groups of DBPs are regulated in the United States and are grouped under five haloacetic acids (HAA5) and total trihalomethanes (TTHM). Past work has observed increased TTHM and HAA5 formation in surface waters sourced from watersheds impacted by large-scale bark beetle infestation, suggesting an association between climate-associated tree stress and public health (Mikkelsen et al., 2013b). More recent work has highlighted the importance of not only total organic carbon concentrations in surface waters but the structural attributes such as aromaticity that is additionally altered in association with large-scale tree mortality (Brouillard et al., 2016). While preliminary, the goal of this inquiry was to compare drinking water trends in DBP concentrations to climate variables across Colorado watersheds. Our guiding hypothesis was that long-term trends in annual air temperature and precipitation would significantly correlate with TTHM and HAA5 formation. Further, we expected the most correlations would be observed in Southwest Colorado, which has experienced substantial climate change impacts and is forecasted to continue to have pronounced trends toward higher temperatures and lower precipitation in the future (Lukas et al., 2014).

To test our hypothesis, we present an analysis of archived drinking water reports and climate data in the state of Colorado to characterize trends across surface water-sourced public water systems. We focused on surface water due to the direct impacts to nutrient content and suspended solids that can rapidly change by hydrologic snowmelt patterns and natural disturbances (Mikkelsen et al., 2013a; Rodríguez-Jeangros, Hering & McCray, 2018). These objectives were achieved using publicly available drinking water reports from the State of Colorado as well as the snow telemetry (SNOTEL) station database from the United States Department of Agriculture. These databases were used to determine annual climate indices for air temperatures and precipitation as well as annual DBP averages across watershed delineations. This work revealed that certain municipalities in Colorado are experiencing alarming increased trends in DBP concentrations. Specifically, the Crested Butte area returned especially interesting trends that motivated our final work explored in Chapter 5 for a watershed-specific study that resolves the limitations acknowledged herein.

4.3 Materials & Methods

4.3.1 Analysis of long-term trends in DPB formation during drinking water treatment

With the Disinfectants and Disinfection Byproducts (DBPs) Rules enacted by the Environmental Protection Agency (EPA) in 1998 and 2006, community water systems adding disinfectant during treatment must comply with the established maximum contaminant levels (MCL) of 80 µg/L for four identified total trihalomethanes (TTHM) and 60 µg/L for five total haloacetic acids (HAA5). The Colorado Department of Public Health and Environment (CDPHE) provides publicly available drinking water quality reports from the year 2000-present that track regulatory compliance. The data was accessed online and processed in R Studio version 3.5.2. Initial data sorting removed public water systems (PWS) that were not classified under surface water (SW) or purchased surface water (SWP) sourced facilities. The sorted data were further processed to retain recorded analytes HAA5, TTHM, and total organic carbon (TOC). This returned 318 PWS classified under the SW and SWP category that have reported on the analytes of interest. The sorted data was then loaded in Tableau version 2020.4.1 to compare temporal trends in each analyte and determine linear trend line fits and significance. These results were sorted based on the line P and slope values with significance determined by $P < 0.05$. Municipalities that returned line fit significance of $P > 0.05$ were recorded to have no significant change. The filtered datasets with significant P values and positive slopes were separated for comparisons with climate trends using annual averages of the TTHM and HAA5 concentrations from 2004-2020. Per drinking water compliance mandated by the running annual average, we deemed annual averages as the best method for initial trend comparisons (EPA, 2010). It is noted, however, that this method does not capture the seasonal variability in concentrations each year. As a result, the temporal data was analyzed by separated quarterly reports in watersheds of interest.

Several inconsistencies were determined across the PWS drinking water reports in sampling frequency, time frames, and analytes tested. From 2016-2021, 113 of the 318 total SW and SWP municipalities reported TOC concentrations. As a result, not all locations of interest allowed for comparative analysis of reported DBP and organic carbon. In addition, the DBP monitoring start dates were inconsistent across locations. Between 2000 and 2003 only 45 municipalities began to report HAA5 and TTHM concentrations. By 2004, the total was at 2011. Beyond the discrepancies in data start dates, the sampling frequency for each PWS depends on the population size served (EPA, 2010). As a result, sampling frequency was typically conducted quarterly; however certain locations reported DBP results less regularly.

4.3.2 Climate & watershed health characterization

The US Geological Survey (USGS) and US Department of Agriculture (USDA) National Watershed Boundary Dataset (WBD) accessed through the National Hydrography High-Resolution Dataset (NHD)

was used to attain watershed boundaries as well as point, line, and polygon data within the watersheds. WBD and NHD data were downloaded through the National Map Viewer. The dataset provided watershed delineations in ArcMap based on the hydrologic unit code (HUC) that ranges from 2-12. For this study, the WBD HUC values used to define basins were at the WBD6 unit (basin delineations at an average size of 27,000 km²), and WBD8 level (subbasin delineations at an average size of 1,800 km²). City assignments specific to each WBD level were used to map cities within watersheds of interest. This preliminary work alternated between WBD8 and WBD6 when appropriate. For example, many Southwest watersheds had fewer total PWS and climate monitoring locations, in which case the larger WBD6 basin delineations were used for statistical analysis. Northern Colorado watersheds contained higher resolution data, and in that case the WBD8 subbasin delineations were used.

Climate data was accessed from the USDA Natural Resources Conservation Service database which is populated by snow telemetry stations (SNOTEL) distributed across the Rocky Mountains. All available SNOTEL stations were mapped within the watershed boundaries to compile climate data associated with each watershed of interest. Climate trends were characterized using daily temperature and precipitation to calculate annual climate indices following similar methods to Vincent et al., 2018. The climate indices chosen for this preliminary work included total annual frost, ice, hot, summer, and precipitation days (Table 4.1). Soil moisture collection was not frequent across stations and was not included in this work. The total frost, ice, hot, summer, and precipitation days were calculated in R Studio version 3.5.2 using the daily datasets for each respective SNOTEL and the boundary limits shown in Table 4.1. The starting year for each climate index was based on the first complete year data was populated in full from January 1st-December 31st.

Table 4.1 Descriptions of each climate index calculated for each SNOTEL station.

Climate Index	Description
Frost days	Number of days with maximum air temperature <0 degrees Celsius
Ice days	Number of days with minimum air temperature <0 degrees Celsius
Hot days	Number of days with maximum air temperature >25 degrees Celsius
Summer days	Number of days with maximum air temperature >20 degrees Celsius
Precipitation Days	Number of days with rainfall >1 mm
Snow Total	Sum of snow water equivalent snowfall increment (mm)

Watershed health as a whole was characterized using averaged climate index values from all SNOTEL stations within each watershed delineation. The goal of the averaged values across watersheds was to remove error and outliers in each specific SNOTEL dataset, however, future work should aim to remove SNOTEL stations from the analysis that have missing or low-resolution data. Averaged climate indices for

each watershed were uploaded in Tableau version 2020.4.1 for linear trend line fits and significance outputs. Again, significance was determined by $P < 0.05$. Few significant trends were observed in the total hot days and precipitation days, thus these indices were not used for correlative comparisons. Correlations between the DBP concentrations and selected climate indices for each PWS location were calculated using Linear Pearson correlation tests in R Studio version 3.5.2 as a final step of this exploratory analysis using the annual averages of TTHM, HAA5, and climate indices.

4.3.3 Bark beetle infestation calculations

Bark beetle infestation was a final aspect of watershed health characterized at each basin delineation. Lodgepole forests were chosen for analysis due to the relevance from previous work in our research group (Mikkelsen et al., 2013b; Brouillard et al., 2016) and the prevalence of mountain pine beetle (MPB) in Colorado. MPB infestation has affected 80% of lodgepole and ponderosa forests in the state since the 1990s. More recently, the spruce beetle has been the most damaging forest insect affecting 40% of Colorado's high-elevation spruce forests (CSFS, 2020). As a result, spruce forest infestation was also quantified. Percent impacted areas were calculated for each watershed delineation using total forest and impacted areas specific to each tree species.

Lodgepole forest layers were created in ArcMap using the National Gap Analysis Program (GAP) that has delineated tree species types available from remote sensing and land survey data. Lodgepole pine species are denoted by the GAP species chart as a value of 149 which were then isolated from the GAP species raster dataset. All matching values were selected and extracted into a new raster file using the 'Export data' feature. The created layer was clipped to a Colorado boundary shapefile loaded in ArcMap and converted to a shapefile using the 'Raster to Polygon' tool. The raster was converted because all other datasets used in the analysis are in the form of shapefiles.

Beetle infestation data layers were then determined according to the following steps for both spruce beetle and spruce trees as well as mountain pine beetle and lodgepole trees as described. Mountain pine beetle impact from 2000-2018 was found from the United States Forest Service (USFS) Aerial Detection Survey (ADS). ADS delineates tree mortality, insect infestations, and disease by year for US forests. Areas with MPB as their primary damage causal agent (DCA coded as 11006) and lodgepole pine species as their host were used. These identifiers were separated in the 'select by attributes function' to select only qualifying data. Selected data were then exported to a new layer. The new MPB shapefile contained data for all years, it was first clipped to the Lodgepole forest layer that was created from GAP data. This ensured MPB infestations only occurred on lodgepole trees. The 'split' tool was used to extract each year into a new shapefile, 20 new shapefiles were created, denoted by 'MPB Year' (MPB 2000) to represent

MPB infestation for that year.

To ensure every shapefile created for each year had only new areas of beetle infestation, overlapping areas were deleted. This required several steps. The ‘erase’ tool was used to erase overlying areas of shapefiles. The first step merged the separate years gradually into a series of new shapefiles. MPB 2000 and MPB 2001 were merged and the resulting shapefile was then merged with 2002 to obtain a composition of data from 2000-2003. This process was repeated until a merge of 2000-2018 was created. The ‘merge’ tool was used to perform this function, which effectively merges data from multiple shapefiles into one. All processes were done in ‘batch mode’ to streamline the process. The ‘erase’ tool was also used to remove areas of overlapping polygons to allow for only new areas of infestation per year to be present. The first step was to erase the MPB 2000 overlapping polygons from the MPB 2001 data. The new shapefile only contained data for areas not present in the MPB 2000 layer. This cycle was completed using the merged shapefiles, followed by the merged MPB data from 2000-2001 that was used to get only new MPB data present in 2003. The cycle was completed with merged data from 2000 to 2018. These new layers are what are used in final areal infestation calculations.

4.4 Results & Discussion

4.4.1 Long-term trends in municipal drinking water

Colorado public water systems using surface water sources were first analyzed for long-term trends in HAA5 and TTHM concentrations. Out of the 300 municipalities analyzed, 104 returned significant linear increasing trends of TTHM concentrations. Similarly, 80 total public water systems returned significant linear increases in HAA5. Spatial mapping of the TTHM and HAA5 *P* values across Colorado did not reveal any clear trends within each watershed delineation due to high variability with either increasing, decreasing, or no changes at the larger watershed delineations (Figure 4.1). Beyond the increasing trends, many PWS locations reached alarmingly high concentrations of DBPs annually with 122 total public water systems exceeding the MCLs at some point in time and 43 at five or greater points in time during the past 15 years. These observations are concerning, however, regulatory compliance is based on mean annual concentrations. By using the running annual averages for compliance, this regulatory goal is achieved by most PWS locations due to the temporal fluctuations in the DBP concentrations each year. Specifically, concentrations were generally lower during the winter sampling periods than in the spring and summer.

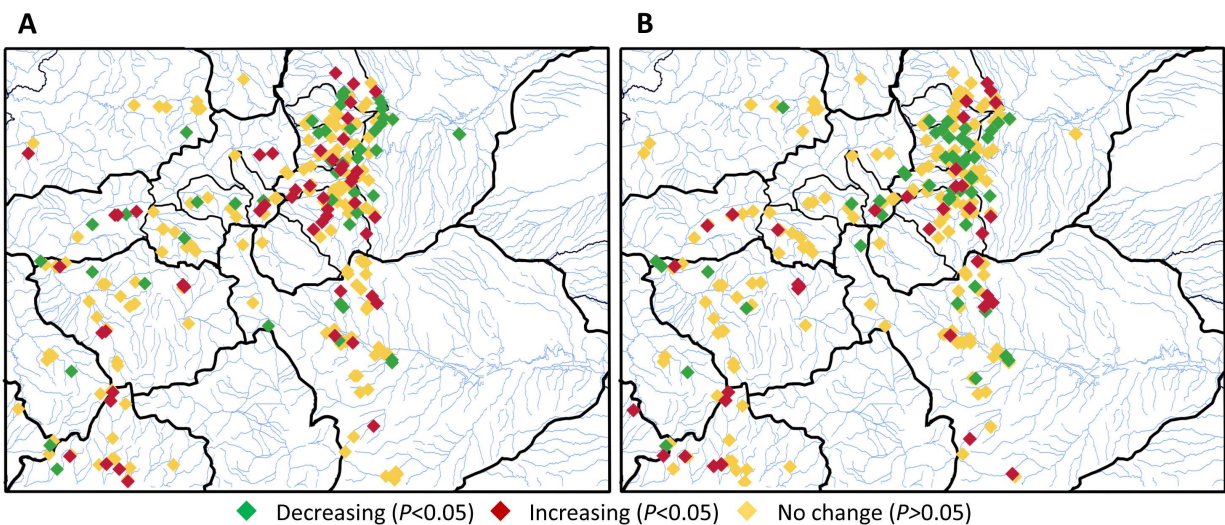


Figure 4.1 Visual inspection of long-term DBP trends across Colorado watershed delineations reveals no clear spatial trends as a function of watershed delineation. DBP trends are shown for (A) TTHM and (B) HAA5 across Colorado with linear trendline fit P values represented in color as decreasing, increasing, or no significant change.

Closer inspection of the temporal trends was conducted at WBD6 and WBD8 watershed delineations where appropriate. This approach allowed for a more targeted analysis in watersheds of interest with significant positive DBP trends. The first area of interest was the WDB6 Colorado River basin. This basin was further delineated into WBD8 subbasins that are called the Colorado Headwaters, Blue, Eagle, Roaring Fork, and Plateau as shown in Figure 4.2. Alarming positive trends in TTHM concentrations were observed at the PWS locations shown within each subbasin except for Carbondale and Battlement Mesa which returned significant positive trends in HAA5 concentrations (Figure C.1). Beyond the long-term decadal trends, seasonal fluctuations were observed with a consistent moment of peak concentrations during the second and third quarter (Q2, Q3) sampling events with the most significant linear trends associated with TTHM (Figure 4.3, Table C.1). These peak moments have exceeded the EPA MCLs recently during the past five years in Frisco, Granby, Hot Sulphur Springs, Rifle, and Clifton and are likely associated with seasonal increases in total organic carbon (TOC) during snowmelt. Few significant long-term trends were observed in TOC concentrations, but the seasonal increases align with the peak DBP concentrations (Figure C.2).

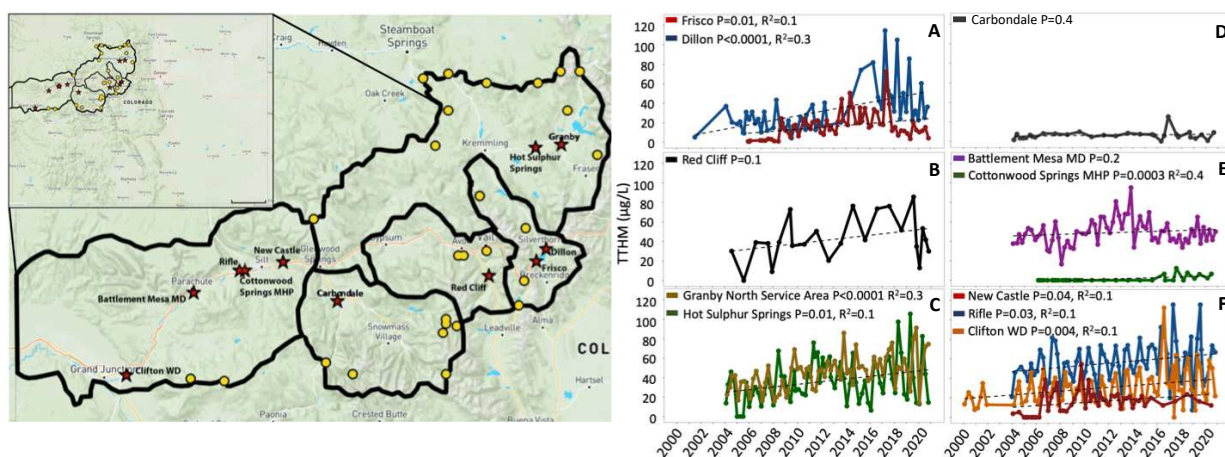


Figure 4.2 Long-term increased DBP trends were observed across the Colorado Headwater subbasins. The trends in TTHM concentrations are shown for surface water sourced municipalities within the (A) Blue, (B) Eagle, (C) Colorado Headwaters, (D) Roaring Fork, and (E, F) Plateau subbasin delineations. Public water systems of interest are shown in red stars on the map and all SNOTEL stations used for watershed characterization are shown in yellow circles. The map was created in Tableau using a Mapbox base map.

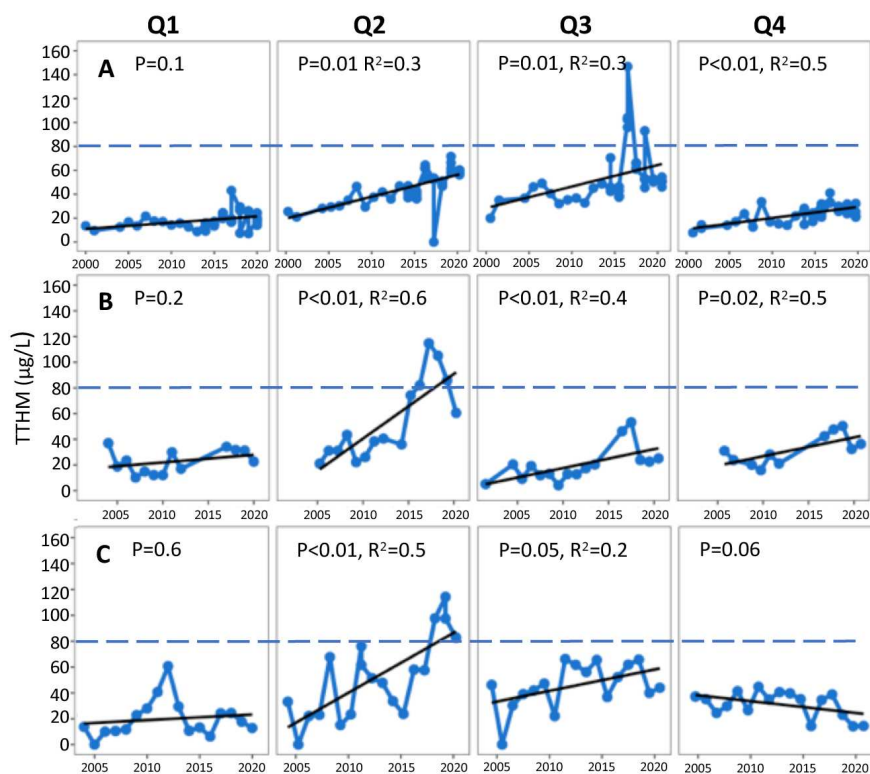


Figure 4.3 Quarterly trends in specific municipalities reveal peak concentrations in Q2 and Q3. The trends in TTHM concentrations for select subbasins (A) Clifton, (B) Dillon, and (C) Hot Sulphur springs. Linear trend lines are shown with P and R^2 values as well as the TTHM MCL at 80 ug/L.

Long-term positive trends of TTHM and HAA5 concentrations in the Colorado Headwaters have been previously described in past work focused on the associated impacts of mountain pine beetle-induced tree mortality on water quality (Mikkelsen et al., 2013b; Brouillard et al., 2016). In the study by Brouillard et al., 2016, increased DBP concentrations correlated with surface waters sourced from watersheds containing greater than 50% areal MPB impact. Since 2016, Colorado has continued to experience extensive bark beetle infestation. Specifically, the Colorado Headwater, Eagle, and Blue subbasins have experienced >50% lodgepole impact from MPB with lower relative contributions from spruce beetle (Table 4.2). Bark beetle-induced tree mortality has been associated with increased DOC and aromatic-like signals downstream; however, this perturbation is not likely the only factor associated with the increased DBP trends. The Plateau subbasin, for example, has experienced little bark beetle impact but returned significant long-term increases and peak concentrations well above the MCLs more recently. In addition, the total forested area is a key variable that could influence the observed trends (Table 4.2).

Table 4.2 Estimated percent forest impact by bark beetle infestation across watersheds of interest.

WBD6 Basin	WBD8 Subbasin	Spruce Forest Area (%)	Spruce Impact Area (%)	Lodgepole Forest Area (%)	Lodgepole Impact Area (%)
Colorado Headwaters	CO Headwaters	15	8	13	75
	Eagle	26	1	7	66
	Plateau	6	1	<1	<1
	Roaring Fork	22	<1	2	11
Gunnison	Blue	38	<1	16	78
	Upper Gunnison	27	49	<1	<1
	Lower Gunnison	7	1	<1	<1
	North Fork	28	<1	<1	<1
	East Taylor	42	17	15	1

Our query into specific watersheds of interest extended to the Gunnison basin located in Southwest Colorado that contains forests dominated by spruce trees (Table 4.2). With fewer total SNOTEL and PWS locations in the region, the WBD6 basin delineation was used to understand trends in the Gunnison watershed as a whole. In addition, TOC concentrations were not available for the PWS locations in the Gunnison Basin. Despite differences in forest composition from the Colorado Headwaters, significant positive trends were observed for certain PWS locations in the Gunnison basin as well. Interestingly, there was high variability in long-term DBPs trends and magnitudes across the basin (Figure 4.4). This was particularly relevant in the Crested Butte region of Colorado. Despite a separation of only 7 km between the town of Crested Butte and Mount Crested Butte, the magnitudes of each DBP varied considerably between the two municipalities with the town exceeding MCLs frequently in contrast to Mt. Crested Butte

which has maintained relatively lower concentrations. In turn, however, Mt. Crested Butte returned more significant trends in HAA5 and TTHM (Figures 4.4 and C.3). These results reveal the variability in reactive organics present in source waters that are within larger watershed basin delineations, indicating there are likely subbasin-specific differences in organic quantity and structure. This variability called for further characterization of watershed health beyond bark beetle infestation.

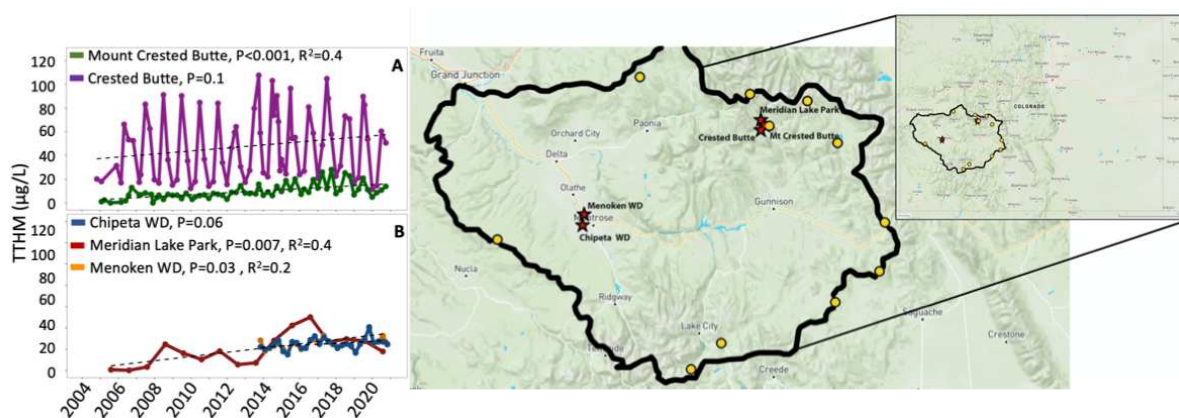


Figure 4.4 Variable DBP trends in the Gunnison basin. The trends in TTHM concentrations are shown for surface water sourced municipalities within the Gunnison basin for the (A) Crested Butte area and (B) the other three surface water locations at Meridian Lake Park, Chipeta Water District, and Menoken Water District. Public water systems of interest are shown in red stars on the map and all SNOTEL stations used for watershed characterization are shown in yellow circles. The map was created in Tableau using a Mapbox base map.

4.4.2 Long-term climate trends in watersheds of interest

Additional ecological stressors associated with drought and early snowmelt can affect surface water health. Specifically, forest health as a whole is affected by temperatures, moisture, and snowmelt dynamics which can contribute increased organic carbon to watershed streams (Arnold, Ghezzehei & Berhe, 2014; Conner, Gill & Harvey, 2017; Kramer & Chadwick, 2018). As a result, climate shifts have been associated with increased DOC export in Colorado streams (Rodríguez-Jeangros, Hering & McCray, 2018), as well as globally across various biomes (Kramer & Chadwick, 2018). To explore these variables, we determined the climate index assignments for each watershed of interest. The most significant climate trends observed across Colorado from 2000-2020 were decreased total annual frost and ice days representing the total days per year the minimum and maximum daily air temperatures were less than 0°C. The total snow depths were telling with significant decreasing trends; however, total precipitation days returned few significant linear trends across Colorado (Figure 4.5). Interestingly, the summer and hot days representing the total days with maximum air temperatures greater than 20°C and 25°C were less consistent across Colorado.

The significant climate trends observed in the Colorado Headwaters and Gunnison watersheds are displayed in Table 4.3.

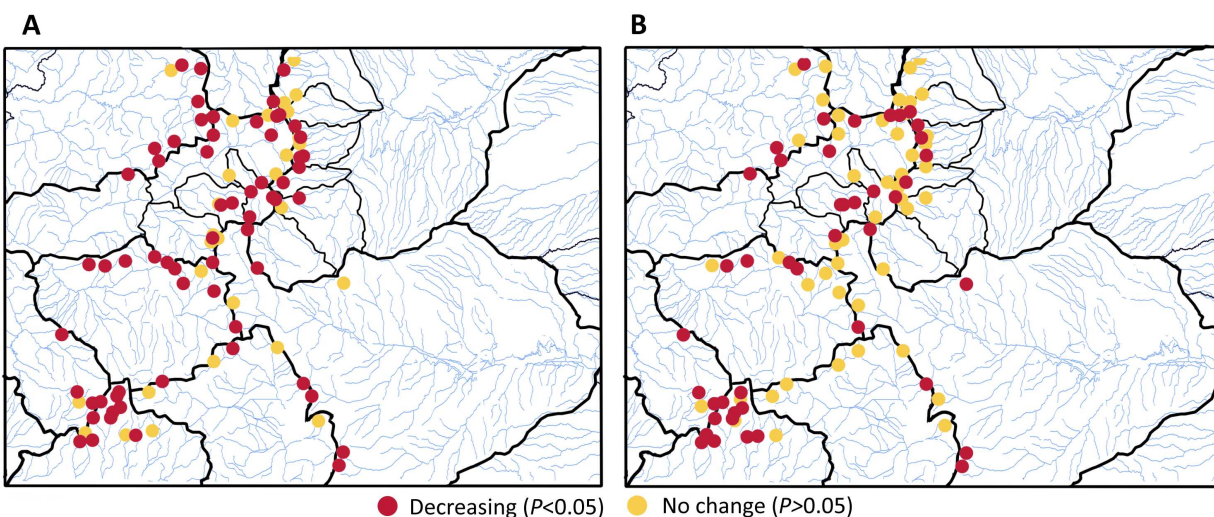


Figure 4.5 Long-term climate index trends reveal the most significant trends for decreasing total frost days and more variable trends for precipitation. No significant increases were determined. Trends in (A) total frost and (B) snow totals were plotted spatially using Tableau.

Table 4.3 Climate trends for the Colorado Headwaters and Gunnison basins reveal significant decreases or no change in total frost, ice, summer, and snow days.

Basin	Subbasin	Frost Days		Ice Days		Summer Days		Snow Total	
		P Value	R ²	P Value	R ²	P Value	R ²	P Value	R ²
Colorado Headwaters	CO Headwaters	0.001	0.4	0.0008	0.5	0.02	0.2	0.03	0.2
	Eagle	<0.0001	0.7	0.2	-	0.005	0.3	0.1	-
	Plateau	0.0001	0.4	0.0008	0.5	0.03	0.2	0.03	0.2
	Roaring Fork	0.1	-	0.4	-	0.01	0.3	0.9	-
	Blue	<0.0001	0.6	0.2	-	0.5	-	0.09	-
Gunnison	-	<0.0001	0.7	0.01	0.4	0.02	0.2	0.01	0.3

The observed trends in climate indices agree with the findings in a report prepared for the Colorado Water Conservation Board in 2014 using 30-year climate observations. The authors determined high variability in precipitation observations across the state. In addition, the authors noted that while statewide temperatures have increased by 1.1 degrees Celsius in the past 30 years, daily minimum temperatures increased more than the daily maximums. The report also highlighted the Southwest region of Colorado, which based on the previous 30-year trends is likely to be most impacted in the future by climate change due to the highest observed increases in air temperature and decreases in precipitation (Lukas et al., 2014). These results in addition to the observed decreases in total frost and ice days could be

indicative of shorter spring transition periods with higher than normal temperatures during the typical snowmelt periods of Colorado. This conclusion has been observed in other studies concluding the timing of snowmelt and peak runoff have shifted earlier by 1- 4 weeks in Colorado (Lukas et al., 2014; Clow, Williams & Schuster, 2016; CSFS, 2021).

4.4.3 Correlative findings between DBP and climate trends

The final aspect of this preliminary study was to determine any correlations that may exist between the climate indices and DBP concentrations for each public water system. The most significant correlations across all PWS locations were associated with decreased total ice and frost days and increased TTHM concentrations (Tables C.2 and C.3). This is an interesting observation that underscores the importance of the snow season in downstream carbon quantity and structure. A previous study explored the relationships between organic carbon fluxes and climate change variables in Colorado and associated temperature and forest disturbances with increased stream DOC concentrations (Rodríguez-Jeangros, Hering & McCray, 2018). More specific studies have found strong correlations in DOC release and frost-related conditions highlighting the importance of monitoring changes in snowmelt dynamics. Per the findings of Matzner & Borken, 2008, high carbon losses have been observed following mild winters with fewer freeze-thaw cycles. Further, a study by Haei et al., 2011 concluded frost intensity negatively affected DOC concentrations while the combined factors of frost duration and intensity significantly influenced DOC structure and aromaticity. In addition, a study by Eskelinen et al., 2016 concluded that sites without frost allowed for snowmelt to infiltrate belowground soil and flush out older water, increasing humic substances and suspended solids in surface water runoff. Not only snowmelt periods are of interest, however, as late-season drought has also been observed to induce carbon losses in high elevation meadows (Arnold, Ghezzehei & Berhe, 2014).

The Colorado Headwaters and Gunnison watershed basins returned the most significant correlations (Table 4.4) with TTHM trends in contrast to HAA5. This observation could indicate different ecological driving forces contributing to the two different groups of DBPs. In a previous study, higher haloacetic acid formation was observed from reacted lignin precursors, while trihalomethane yields were associated more broadly with humic and fulvic acids (Hua, Kim & Reckhow, 2014). As a result, reactive organic quantity and structure likely differ across watershed land areas depending on land cover. Fire severity and size for example can greatly impact organic composition and fate within watersheds (Smith et al., 2011; Olivares et al., 2021). Another reason for the variation across the watersheds specific to this study is that more local shifts in climate variables were not captured in our WBD6 and WBD8 delineations and broader SNOTEL averages across the large basin areas. Hence, closer inspections at the WBD10 watershed delineation (average area of 600 km²) could provide more specific characterizations of local climate trends. This goal

would require other climate datasets however since SNOTEL stations are sparse within the smaller watershed delineations. Further, elevation, soil type, and land cover are recognized as key variables associated with climate that can affect local organic export, as discussed in Chapter 3, and would also be better characterized at WBD10 delineations. These variables were not included in this current investigation but could be key influencing factors for aqueous organic carbon transport and downstream DBP formation after disinfection.

Table 4.4 Correlation P values between average annual DBP concentrations and climate indices in watersheds of interest.

Basin	Subbasin	PWS	Summer Days		Ice Days		Frost Days		Snow Totals	
			HAA5	TTHM	HAA5	TTHM	HAA5	TTHM	HAA5	TTHM
Colorado Headwaters	Plateau	Battlement Mesa	0.38	0.40	0.13	0.43	0.03	0.89	0.33	0.55
		Clifton	0.50	0.79	0.95	0.23	0.62	0.02	0.49	0.13
		Cottonwood Springs	0.37	0.29	0.01	0.16	0.19	0.55	0.84	0.14
		Rifle	0.27	0.26	0.56	0.06	0.22	0.06	0.85	0.59
	Blue	Dillon	0.31	0.18	0.02	0.01	0.88	<0.01	0.87	0.18
		Frisco	0.63	0.76	0.15	0.09	0.02	<0.01	0.77	0.61
	Colorado Headwaters	Granby	0.27	0.03	0.97	0.02	0.63	0.04	0.41	0.70
		Hot Sulphur Springs	0.62	0.53	0.58	0.04	0.41	0.49	0.88	0.78
	Eagle	Red Cliff	0.01	0.01	0.50	0.20	0.09	0.03	0.71	0.86
Gunnison	-	Roaring Fork	0.1	0.4	0.5	0.6	1.0	0.6	0.2	0.7
		Crested Butte	0.09	0.16	0.36	0.12	0.14	0.03	0.33	0.76
		Mt. Crested Butte	0.60	0.28	0.17	<0.01	0.11	<0.01	0.03	0.13
		Meridian Lake Park	0.22	0.05	0.10	0.06	0.03	0.01	0.44	0.54
		Menoken WD	0.39	0.97	0.39	0.83	0.39	0.55	0.22	0.30
		Chipeta WD	0.27	0.34	0.56	0.43	0.57	0.41	0.06	0.39

Beyond direct impacts to DOC concentrations and structure, more recent studies have attempted to understand the direct relationships between climate and DBP concentrations as well. This includes a study in Scotland that reported direct relationships between DBPs and temperature, with the strongest correlations in TTHM (Valdivia-Garcia et al., 2019). The complexity of these systems however is acknowledged, as studies have emphasized the importance of the treatment process in DBP formation (Doederer et al., 2014). Smaller treatment facilities with less complex treatment trains may be at the highest risk in the future and ill-equipped to respond to changing water quality (Delpla & Rodriguez, 2016). This is because sedimentation, flocculation, and activated carbon filtration can achieve higher TOC removal. Interestingly, the PWS locations of interest in this study implement a variety of treatment strategies that range from relatively simple (screening, filtration, disinfection) to more complex (settling,

coagulation, media filtration, reverse osmosis) (Table C.4). This highlights a critical variable pertinent to this work that was not included in the correlative analyses. Specifically, concentrations of organic precursors, temperature, pH, and residence time all are significant factors that influence DBP formation during treatment that should be included in future studies of this data (Doederer et al., 2014; Valdivia-Garcia et al., 2016).

4.5 Study Limitations & Future Work

These findings are based on preliminary work and only explore the correlations between DBPs and climate variables. With this in mind, however, the exploratory work herein revealed a subset of Colorado water treatment facilities that harbored increasing trends of HAA5 and TTHM concentrations over the past 10-20 years. The observed trends varied across watershed and location, but with commonalities in declining forest health associated with decreasing total frost and ice days as well as endemic bark beetle infestation in some locations. These results reveal the variability in reactive organics across streams that fall within the same larger watershed basins, indicating subbasin-specific differences in organic quantity and structure. As a result, this chapter inspired continued work in Chapter 5 that focused on the source waters for the town of Crested Butte at the WBD10 watershed delineation.

There were several limitations to this study that require more resources and time to continue this broader work across Colorado. The following recommendations are suggested for future studies:

- More accurate climate index values can be calculated by water year definitions. While calendar year calculations in this work still captured long-term climate trends, this distinction is important for a more accurate representation of annual snowfall.
- Linear DBP trends should be reevaluated based on quarterly separations. This method may return more PWS locations of interest that have significant trends during the Q2 and Q3 peak moments discussed.
- A control watershed should be included for further statistical analyses to determine which forest health variables can be significantly associated with DBP trends. This would likely require more specific watershed delineations at the WBD10 level.
- Watershed health should be characterized with more detailed datasets that include variables such as the US drought monitor as well as fire impact and intensity across watershed delineations.
- Field data could complement the historical analysis in watersheds of interest to pinpoint ecological sources of reactive organic carbon.

To address the major limitations of the study associated with incomplete climate variables, a useful resource from the Aspen Center for Environmental Studies (ACES) called the Forest Health Index is recommended as a resource in future work. This online dataset provides climate indices generated by ACES to characterize watershed health in Colorado. Collaboration with ACES could provide access to their extensive datasets and calculations for key climate variables that were excluded in this work (Aspen Center for Environmental Studies, 2020). An example of how this data could be used is demonstrated in Figure 4.6 to pinpoint watersheds of interest.

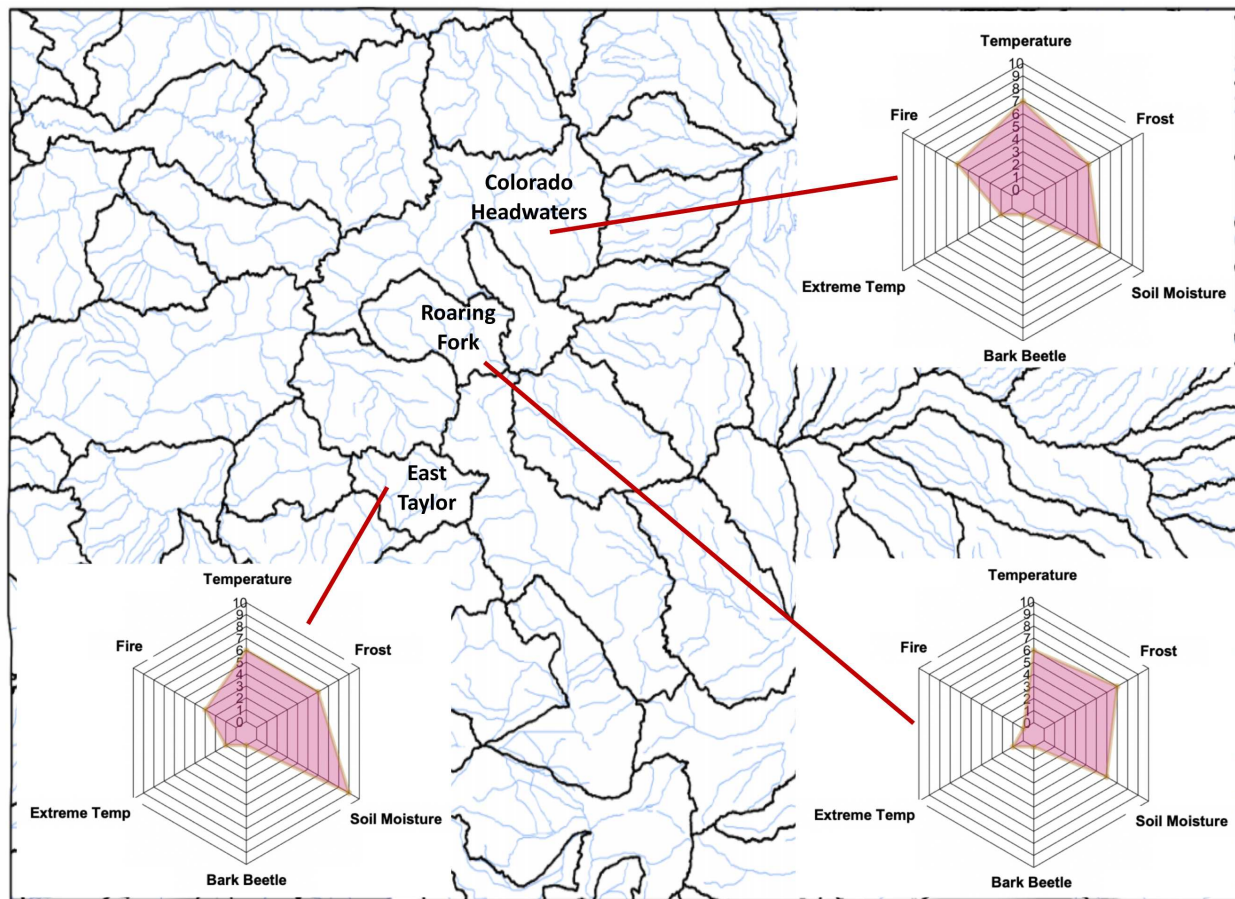


Figure 4.6 Watershed health classifications using radar plots and climate index values from the Forest Health Index. Each radar plot summarizes the forest health indices for 2018. The scale uses determined index values with 0 representing normal conditions and 10 as a deviation from the normal.

CHAPTER 5

DISINFECTION BYPRODUCTS FORMED DURING DRINKING WATER TREATMENT REVEAL
AN ECOSYSTEM CONTROL POINT FOR REACTIVE ORGANIC CARBON EXPORT IN A
SUBALPINE HEADWATER STREAM

In preparation for *Environmental Science & Technology Water*

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*Laura Leonard and Jonathan Sharp were primary authors in the writing of this manuscript. Gary Vanzin and Laura Leonard analyzed the data and prepared figures and tables. Kenneth Williams conducted field sampling, James Stegen and Vanessa A. Garayburu-Caruso conducted FTICR analyses, and William Mitch and Stephanie Lau conducted DBP analyses. All authors conceived and designed the experiments.

5.1 Abstract

Watershed health is crucial to maintain water quality for downstream communities; however, forested ecosystems such as those in Southwest Colorado are under increased stress associated with climate change. In this study, we focus on the Coal Creek watershed to discern an ecosystem control point for organic carbon export. Dissolved organic carbon can react with chlorine during disinfection treatment to form carcinogenic disinfection byproducts (DBPs). Hence, we analyzed decadal and seasonal trends over the past fifteen years in DBP formation from the Crested Butte water treatment facility that sources water from Coal Creek. Dissolved organic carbon (DOC) and streamflow data from the past five years were used to define correlations between organic carbon release and downstream DBP concentrations. Synoptic sampling along a 10 km reach of the water body focused on DOC and UV₂₅₄ absorbance. Chlorinated DBP formation potential tests were performed on select samples to compare the creek and treatment facility byproduct formation. Qualitative analyses of the chlorinated samples determined the specific organic classes contributing to DBP formation. From this methodology, the treatment facility water quality reports revealed a long-term increase in the formation of two major classes of DBPs: haloacetic acids (HAA5) and trihalomethanes (TTHM). Seasonal trends in TTHM and HAA5 consistently exceeded maximum contaminant levels during the spring snowmelt and early summer months, revealing a hot moment associated with increased organic carbon release during peak discharge. Synoptic sampling

revealed a confluence draining a northern aspect evergreen forest and downstream of a riparian wetland to have the highest concentrations of organic carbon and estimated aromaticity. As a result, this reach was identified as a biogeochemical hot spot for DOC export and the formation of carcinogenic DBPs.

Formation potential tests yielded similar dominant DBP species to those formed at the treatment facility, suggesting the treatment process is not effectively mitigating reactive DOC. Qualitative analyses of pre and post chlorinated samples indicated lignin, tannins, and condensed hydrocarbons were the major reactive organic classes with chlorine oxidation. These findings inform water management and treatment strategies and suggest that national drinking water quality archives could be used as a forensic tool to identify and better understand ecosystem control points for organic carbon export in analogous watersheds.

5.2 Introduction

Recent concerns have developed regarding the impacts of climate change on watershed biogeochemical processes and the resulting downstream impacts. Specifically, climate-related disruptions including increased drought, insect infestation, and large-scale tree death have been associated with increased organic inputs to surface water sources. These disruptions can affect ecosystem services associated with local changes to soil and stream water chemistry (Mikkelsen et al., 2013a; Kramer & Chadwick, 2018). The mechanisms associated with these observations are complex and depend on a variety of conditions. Specifically, warmer temperatures can increase decomposition rates of available soil organic matter (OM) (Freeman et al., 2001). In addition, soil freeze-thaw cycles have been associated with increased release by disrupting OM aggregates (Haei et al., 2010), plant roots (Cleavitt et al., 2008), and lysing microbial cells (Yanai, Toyota & Okazaki, 2004). Finally, drought can increase soil carbon through soil aeration that promotes increased decomposition rates (Sippel et al., 2018). Larger stocks of OM from these variables can then lead to mobilization at higher concentrations after precipitation events (Ågren et al., 2010). With an estimated 74% of water used in the United States sourced from surface water, biogeochemical shifts in watersheds from climate change could have large-scale impacts on downstream communities (Dieter et al., 2018). The terms biogeochemical “hot spots” and “hot moments” have been used in association with disproportionately high reaction rates over short periods of time to understand the episodic release of mobilized OM (McClain et al., 2003). These concepts have evolved recently to more succinctly describe the spatiotemporal release of organics as “export control points” in which high rates of reactants are exported during hydrologic events into receiving streams (Bernhardt et al., 2017). Exploring these control points is key in understanding watershed health, but also because mobilized OM can impact downstream water quality.

Moreover, drinking water treatment facilities are faced with the task of adapting treatment processes as source water chemistry changes. Specifically, disinfection used during water treatment is an essential method to prevent waterborne diseases in the United States. Chemical disinfection is the predominant method used at 70% of municipal treatment plants within the United States (AWWA, 2018). The process oxidizes water constituents with typically chlorine-based chemicals. However, a side effect of aggressive oxidation to inactivate pathogens is OM present within the water is also oxidized and forms reacted organics known as disinfection byproducts (DBPs). Identified as potential carcinogens and a health risk (Villanueva et al., 2015; Richardson & Plewa, 2020), certain DBPs are regulated by the Environmental Protection Agency (EPA) with an accepted tradeoff in risk of DBP formation to prevent pathogen outbreaks. While more than 700 types of DBPs have been identified, eleven total (trihalomethanes, haloacetic acids, bromate, and chlorite) are currently regulated in the United States (Richardson & Plewa, 2020). With the Disinfectants and Disinfection Byproducts Rules enacted by the EPA in 1998 and 2006, community water systems adding disinfectant during treatment must comply with the established maximum contaminant levels (MCL). In addition, distribution systems serving a population of 500 or more conduct quarterly sampling for total trihalomethanes (TTHM) and haloacetic acids (HAA5) to achieve regulatory compliance (EPA, 2010).

DBP concentrations can be reduced by a variety of approaches during treatment, such as reducing dissolved organic carbon through enhanced coagulation, activated carbon, nanofiltration, or alternative disinfection strategies. However, DBPs present a persistent problem because the complete removal of organic material is not always practical or cost-effective. Organic matter is a complex constituent by nature, and removal is regulated and monitored broadly by total organic carbon (TOC) concentrations and oxygen demand as an indicator of bioavailable organics (EPA, 2005). Further, vast differences in DBP concentrations can be observed across municipalities. Prior studies have focused on DBP reduction within the treatment train by changing conventional unit processes (Andersson et al., 2020; Zhang et al., 2021; Postigo et al., 2021); however, advanced or updated treatment options are not always economical for smaller municipal treatment plants. Meanwhile, non-chlorine disinfection methods can form alternative less understood byproducts (Richardson & Plewa, 2020). As a result, DBP presence and mitigation is a prominent regulatory concern for municipal treatment plants (AWWA, 2020).

The goal of this study was to determine the temporal and spatial aspects of an ecosystem control point contributing to seasonal OM release forming DBPs in a small catchment. The Coal Creek Watershed in Crested Butte is located in the Southwest region of Colorado that has experienced pronounced recent climate disturbances associated with drought and insect infestation (Zhi et al., 2019; CSFS, 2021). We hypothesized that (1) DBP formation potential in the creek would produce similar byproducts to those

formed during treatment, with (2) an export control point sourced from a wetland within the creek transect contributing to increased seasonal DBP trends, (3) and lignin as the most reactive organic class during disinfection. We accessed publicly available water quality reports from the treatment facility to assess seasonal and long-term DBP trends over the past two decades. We then used a dataset from the past five years of regular dissolved organic carbon sampling and stream discharge to assess drivers of a hot moment. We identified a hot spot with synoptic sampling across Coal Creek in 2021 during peak discharge using UV₂₅₄ absorbance and DOC measurements. Finally, a more detailed characterization of the reactive dissolved organic carbon with respect to chlorine-based reactivity and functional groups was conducted before and after the determined hot spot.

5.3 Materials & Methods

5.3.1 Watershed characterization & sampling

The Coal Creek watershed is the primary water source for the town of Crested Butte serving a population of approximately 2,500 people. The basin is relatively small with total drainage of 53 km² hosting several tributaries that descend a variety of land covers and anthropogenic influences (Zhi et al., 2019). The total watershed land cover consists of 64% evergreen forest, 20% herbaceous vegetation, 9% deciduous forest, 4% barren land, 2% woody wetland, and 1% developed open space (NLCD, 2011). The southern section of the basin is heavily forested with conifer trees experiencing increased stress from bark beetle impact starting in 2015. The northern reach contains three abandoned mines and an iron fen associated with legacy metal export (Ryan et al., 2007). The climate of the area is characterized by a nearby snow telemetry (SNOTEL) station on Mt. Crested Butte (38.89° N, 106.95° W, elevation 3,100 m). The average total precipitation from 1990-2010 was 80 ± 15 cm as snow water equivalent (SWE) with more than half (48 ± 14 cm SWE) falling as snow (Table D.1). Snow cover is dominant typically November-June. The past five years have been variable with a drought in 2018 quantified by below-average total precipitation and above-average hot days followed by a return to more normal conditions in 2019.

The relatively short catchment beginning at Lake Irwin and ending in the town allowed for easy access with a parallel road along the creek (Figure 5.1). Synoptic sampling in June of 2021 was conducted during peak discharge for targeted identification of carbon aromaticity during peak flow using UV₂₅₄ absorbance and dissolved organic carbon (DOC) concentrations. Samples were collected every 600 m or less along the transect beginning at the Lake Irwin outlet and ending at the lower end of the transect. A select sampling event was also conducted to align with Q3 and Q4 sampling by the drinking water facility. These select sample collections were at the upstream CC-5 (38.859° N, 107.096° W, elevation 3,000 m), mid-stream Coal-20 (38.856° N, 107.060° W, elevation 2,900 m) and downstream Coal-11 (38.863° N, 107.034° W,

elevation 2,900 m) locations in July 2020 followed by October 2020 collections at the same locations with additional points at the Splains Gulch tributary (Splains, 38.855° N, 107.064° W, elevation 3,000 m) and after Elk Creek (Coal-15, 38.856° N, 107.059° W, elevation 2,900 m). All samples were collected and shipped on ice for processing at the Colorado School of Mines where they were filtered with 0.45 μm polyethersulfone filters and analyzed for UV absorbance at 254 nm, dissolved organic carbon (DOC), specific UV absorbance (SUVA), and excitation-emission matrix spectroscopy (EEM). The 2020 sample collections were additionally chlorinated for DBP-FP tests and FTICR analysis.

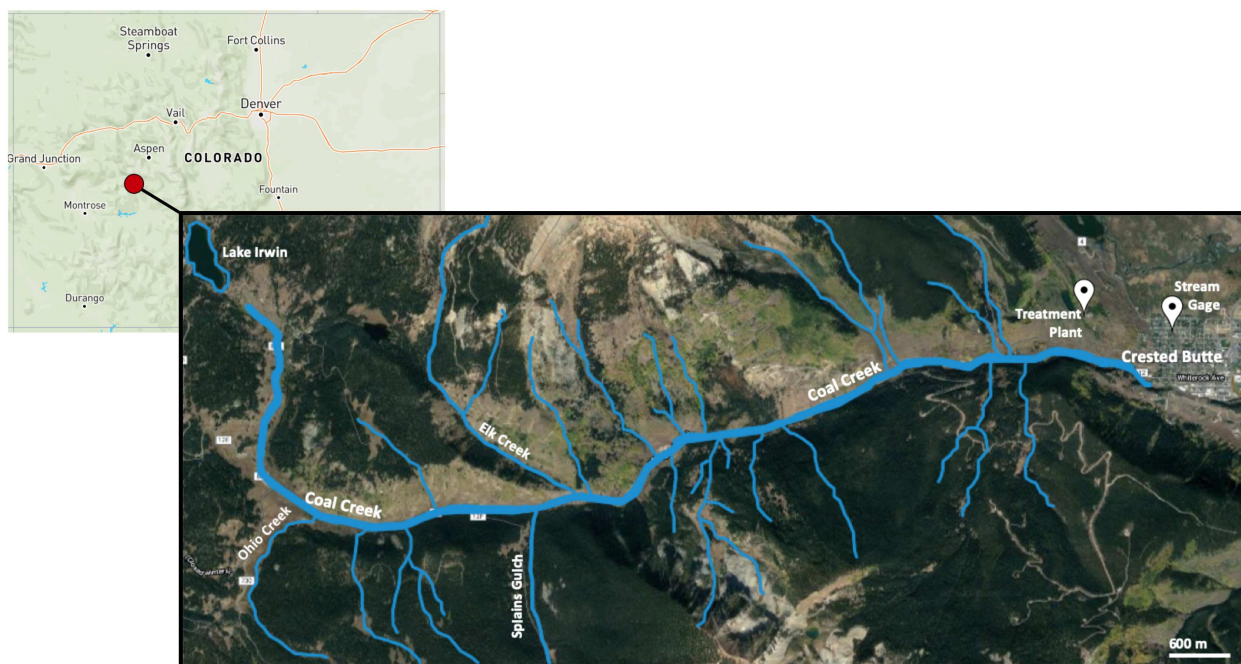


Figure 5.1 Coal Creek transect. The treatment facility and stream gage locations are also shown in white. The base map is provided by Google Maps.

5.3.2 Historical data collection & analysis

The Colorado Department of Public Health and Environment (CDPHE) drinking water compliance database is a public repository of water quality reports from public water systems (CDPHE, 2021a). Historical DBP data were collected from this repository and represents values reported by the town of Crested Butte drinking water treatment facility at quarterly intervals. Total trihalomethane (TTHM) and five haloacetic acid (HAA5) concentrations were reported in addition to the specific compounds (chloroform, bromodichloromethane, bromoform, dibromochloromethane, trichloroacetic acid, dichloroacetic acid, and dibromoacetic acid), but excluding chloroacetic acid, bromoacetic acid, and chloroacetic acid. Daily averages were determined for each analyte and plotted with linear trend fits in R

R Studio version 3.5.2. Coal Creek daily hydrograph data was obtained from the U.S. Geological Survey (USGS) database for site number 0911150: Coal Creek ABV McCormick Ditch (38.872 ° N, 106.985° W, elevation 2,700 m). Hydrograph data collection began in October 2014, allowing for comparative analyses from 2015-2020. Finally, historic DOC data were provided from previous work that frequently monitored DOC concentrations at a transect location near the drinking water intake (Coal-11) from 2016 – 2020 (Zhi et al., 2019). The samples were filtered to 0.45 µm and DOC concentrations were analyzed using a TOC-VCPH analyzer.

The time series using the available data from 2015-2020 for flow rates, and 2016-2020 for DOC concentrations were plotted in R Studio version 3.5.2. Correlations between the DBP, DOC, and flow data were determined using isolated dates associated with DBP measurements. The linear fits and associated Pearson values were determined in R Studio. A *P* value <0.05 was considered significant for all linear fits and correlations. Finally, daily DOC mass loadings were determined with the flow and DOC data by multiplying the DOC concentrations in mg/L by flow in m³/s with each associated date to return units of kg/day. These daily calculations were then used to determine monthly averages for each year of available data.

5.3.3 Organic carbon quantification methods

A portion of the 2020-2021 0.45 µm filtered stream samples was acidified with hydrochloric acid for dissolved organic carbon (DOC) analysis using a Shimadzu TOC-550A Total Organic Carbon Analyzer. The unacidified filtrate was analyzed for UV absorbance at 254 nm (UV₂₅₄) using a DU 800 Spectrophotometer. Specific UV absorbance (SUVA) was calculated by normalizing the UV₂₅₄ values with the respective DOC concentrations in mg/L following Environmental Protection Agency Method 415.3 and (Eq. 1) (Potter & Wimsatt, 2009):

$$SUVA(L/mg - m) = UVA(cm^{-1})/DOC(mg/L) * 100cm/m \quad (5.1)$$

The additional filtrate was used for fluorescence excitation-emission matrix spectra (EEM) analysis using a Horiba Aqualog 3D Fluorometer and the Aqualog 3.6 software. Experimental settings were conducted at an integration time of 1 second, excitation increments of 3 nm, and emission increments of 2.33 pixels. Samples were corrected for the inner-filter effect using the Aqualog software. Further, the samples were blank corrected and normalized to Raman units. Processed sample data was further analyzed using R Studio version 3.5.2 and R package eemR to determine the biological index (Bix), fluorescence index (Fi), and humification index (Hix) (Massicotte, 2019).

5.3.4 Chlorine formation potential tests

Disinfection byproduct formation potential tests were conducted using the remaining 0.45 μm filtrate according to standard method 5710 for chlorine formation potential (APHA, 2017). Reagent grade 4.00-4.99% sodium hypochlorite and deionized water were used to prepare a stock hypochlorite solution. A 1 M phosphate buffer (pH 7) stock solution was also prepared with 53 grams $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and 165 grams $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ in 1 liter of DI water. The quenching solution was prepared as a 25 g/L ascorbic acid solution. Amber vials were used for all reactions and pretreated with sodium hypochlorite stock solution filled headspace free for 24 hours (EPA, 2019).

Chlorine demand tests were first conducted to determine the necessary dose of sodium hypochlorite to achieve a residual chlorine concentration of 3-5 mg/L for the formation potential test. Based on the DOC results for each sample, chlorine volumes were calculated to achieve chlorine: DOC mass ratios of 1, 3, 5, 7, and 10. From these ratios the determined primary stock solution volume was dosed into 40 mL bottles and the sample was added to the neck of the bottle. Samples were buffered with the phosphate buffer to target 10mM strength and filled headspace free with sample and stored in the dark for seven days at room temperature. After seven days the residual chlorine concentration was measured using a Hach Chlorine Free & Total colorimetric test kit. A chlorine to DOC mass ratio of 5 achieved the target residual chlorine concentrations for all samples. Finally, the same protocol was performed using the chlorine dose specific to each sample. After seven days in the dark, the samples were quenched with the ascorbic acid solution to achieve a concentration of 33 mg/L. The samples were acidified with 5% v/v sulfuric acid to reach the required pH of 3.7 for DBP analysis. Samples were stored headspace free in the refrigerator until analysis for DBP concentrations. The samples were shipped overnight on ice to Stanford University, Stanford, CA for DBP measurements. A total of 33 known DBP compounds were analyzed including the complete set of TTHM (chloroform, bromodichloromethane, dibromochloromethane, and bromoform) and HAA5 (chloroacetic acid, trichloroacetic acid, bromoacetic acid, dichloroacetic acid, and dibromoacetic acid).

5.3.5 FTICR-MS

Fourier-transform ion cyclotron resonance mass spectrometry (FTICR) was conducted at the Pacific Northwest National Laboratory (PNNL) in Richland, WA to compare the initial sample collections and post-chlorination samples. The samples were shipped on ice overnight. Analysis was conducted following the methods of Garayburu-Caruso et al., 2020 at PNNL. In brief, a 12 Tesla Bruker Solarix Fourier transform ion cyclotron mass spectrometer was used to collect mass spectra of the pre and post-chlorinated transect samples. The FTICR-MS was equipped with a standard electrospray ionization (ESI) source. Further, FTICR data processing and analysis were conducted in R Studio version 3.5.2 following methods

of Garayburu-Caruso et al., 2020 using the R package *ftmsRanalysis* (Bramer et al., 2020) to remove peaks outside of the confidence range of 200-900 m/z and determine chemical class assignments for each sample. Further, average molecular properties were calculated and relative abundances were determined using all transect samples as a function of pre and post-chlorination for each sampling time point in R Studio.

5.4 Results & Discussion

5.4.1 Seasonal and decadal trends in disinfection byproducts during drinking water treatment

Our first goal was to understand seasonal and decadal trends in the formation of disinfection byproducts in association with drinking water treatment. Disinfection byproducts form when chlorine or other oxidants react with natural organic matter in the water. An understanding of these trends provides insight into carbon mobilization in source waters and the implications for community health (Mikkelsen et al., 2013b; Brouillard et al., 2016). The water quality reports from the treatment facility served to discern long-term trends of source water reactivity with chlorine-based disinfection. Specifically, the quarterly reports of HAA5 and TTHM concentrations revealed repeated annual peaks in DBP concentrations after spring snowmelt (Figure 5.2). Peak values exceeded the regulatory guidelines of 80 ug/L for TTHM and 60 ug/L for HAA5 frequently over the past fifteen years. Higher values were regularly associated with the third quarter of annual sampling (Q3) typically in July. Of the individual byproducts measured in the past five years, the regulated five haloacetic acids (HAA5) were dominated by dichloroacetic ($42 \pm 3\%$) and trichloroacetic ($55 \pm 4\%$) acids. The total trihalomethanes (TTHM) were dominated by chloroform ($85 \pm 9\%$) (Table D.2). While the temporal resolution of this dataset was confined to four samples per year, this data alludes to a seasonal hot moment after snowmelt contributing to DBP formation during drinking water treatment.

In addition to the annual cycle of peak DBP concentrations, our analysis revealed long-term trends in the formation of both HAA5 and TTHM from 2004-2020 (Figure 5.2A). Fitted linear trends returned significant increases ($P=0.03$, $R^2=0.1$) in reported HAA5 concentrations; however, analogous increases in TTHM lacked significance ($P=0.1$). Quarterly trends further revealed that Q3 (typically sampled in July) and to some degree Q4 (typically sampled in October) are driving the temporal increases (Figure 5.2B). The increased trends agree with previous studies in analogous forested ecosystems that experienced heightened tree stress and mortality (Mikkelsen et al., 2013b; Brouillard et al., 2016). TTHM and HAA5 formation have been associated with the ambient temperature of the environment and DOC concentrations (Valdivia-Garcia et al., 2016). With respect to a changing climate, a recent study concluded a 1.8-degree Celsius increase in mean summer temperatures could lead to a 39% increase in TTHMs (Valdivia-Garcia et

al., 2019). Further, a modeling-based study highlighted a close link between TTHM levels and raw water temperatures and predicted climate-related increases in both TTHM and HAA5 in the future due to higher reaction rates under increased temperatures (Valdivia-Garcia et al., 2016). The broader implications of that study suggested small water utilities applying disinfection with limited treatment processes (e.g., sedimentation, flocculation, or activated carbon filtration) could be at the highest risk for increased DBP formation under climate change scenarios (Delpla et al., 2016). While we were not able to associate DBP concentrations with direct measurements of dissolved organic carbon (DOC) or temperature at the treatment facility, the region has experienced recent climate shifts resulting in fewer total days with minimum air temperatures less than 0 degrees Celsius (Figure D.1) and more recently above average total maximum air temperatures greater than 20 degrees Celsius (Table D.1). From these results, it is apparent that Coal Creek hosts a seasonal and reproducible hot moment for DPB formation that is trending toward higher future concentrations in association with a warming climate.

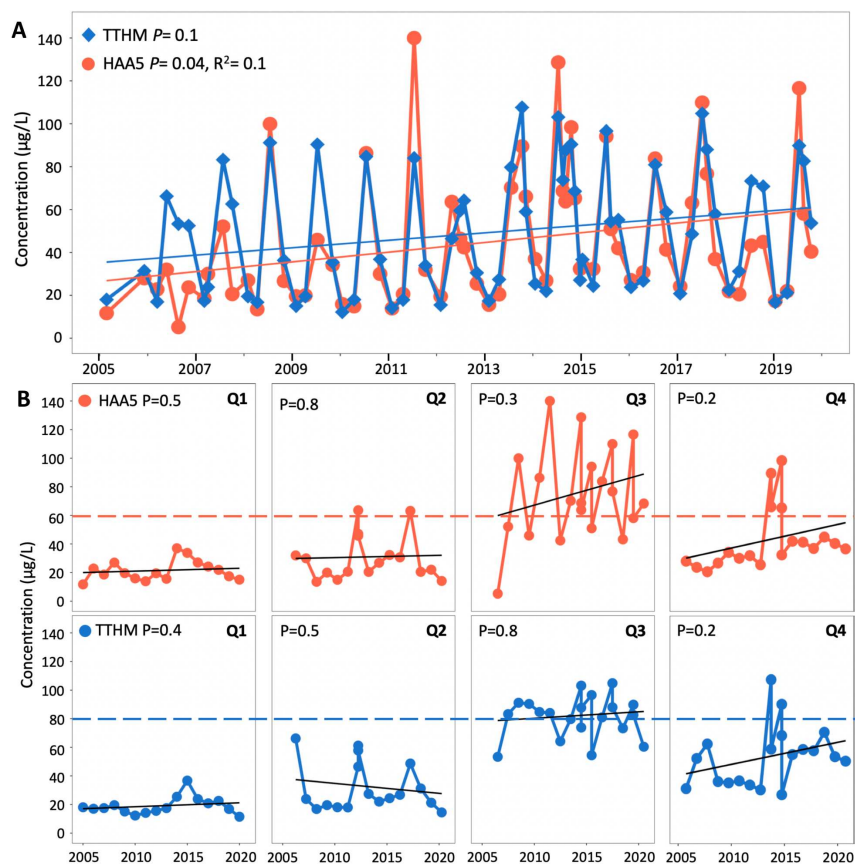


Figure 5.2 Long term trends of total trihalomethanes (TTHM) and haloacetic acids (HAA5) from the Crested Butte treatment facility over the past fifteen years. (A) TTHM and HAA5 water quality report data is shown from 2005-2020 and broken down by (B) quarterly trends with the respective maximum contaminant levels in dotted lines. Linear trendlines and the respective P values indicate linear fit significance.

5.4.2 Hydrochemical associations

Beyond the observed DBP concentrations at the treatment facility, the quantity and structure of natural organic matter present in source waters drive the relationship between hydrochemical variables and DBP formation after treatment. Hydrograph data and archived Coal-11 DOC concentrations that were taken near the drinking water diversion from Coal Creek (Zhi et al., 2019) were used for targeted analysis of these relationships. Over the past five years, the reported TTHM and HAA5 concentrations have consistently exceeded the EPA MCLs during Q3 sampling. While low in temporal resolution, seasonal peaks in DBPs occurred in concert with peak flow (Figure 5.3A) and DOC concentrations (Figure 5.3C). In the past five years peak flow has occurred on average near June 1st $\pm$ 13 days at 7 ± 3 m³/s while peak DOC has occurred near June 5th $\pm$ 16 days at 11 ± 7 mg/L. The reported HAA5 concentrations significantly correlated with stream DOC ($P = 0.001$) and flowrates ($P = 0.02$) while TTHM concentrations significantly correlated with DOC ($P = 0.03$) only (Figure D.2). Consequently, this hot moment for DBP formation extends to DOC mobilization and flow in association with increased seasonal temperatures and snowmelt.

This observed temporal window is relatively brief but responsible for the majority of DOC export from the watershed each year with an estimated average $78 \pm 10\%$ of DOC mobilized in May and June from 2015-2020. This aligns with a previous study across 30 forested watersheds wherein up to 86% of total DOC was exported during seasonal events associated with increased discharge and water temperatures (Raymond & Saiers, 2010). It is noted that the hydrograph data in this present study ends in October due to a lack of USGS discharge measurements during regularly frozen surface conditions from November-May (Table D.3). Despite this missing data, comparatively lower DOC concentrations were observed during the colder months of November-March of 2016-2020 (1.1 ± 0.9 mg/L) in contrast to the warmer months of April-October (4 ± 3 mg/L). By extension, we can assume comparatively lower DOC loading occurs during the winter months as a function of both lower flow and DOC concentrations. This assumption is supported by a recent study that concluded higher temperatures are a major driver of DOC concentrations with release driven by high flow during wet conditions (Wen et al., 2020).

The interplay between seasonal drivers of DOC export and temperature effects on reaction rates suggests that climate shifts could have a significant role in DBP formation. Specifically, enhanced DOC export has been associated with event-controlling snowmelt and storm events across a variety of watersheds including mountain and forested catchments (McKnight et al., 2001; Raymond & Saiers, 2010), high-latitude watersheds (Finlay et al., 2006), and agriculture dominated watersheds (Qiao et al., 2017). DOC concentrations and lability (organic sugar and protein structures easily consumed by microbes) have been shown to positively correlate with frost duration in upper soil horizons of riparian zones exposed to

frost (Haei et al., 2011). In addition, peatland studies have shown increased DOC and humic acid-like organics associated with more frost-free days (Eskelinen et al., 2016) and higher temperatures (Fenner et al., 2007). This provides a possible explanation for the linear increasing trend in DBP concentrations over the past fifteen years in association with the significant reduction in total ice and frost days in Crested Butte (Figure D.1). As a result, cumulative climate shifts likely impact downstream DOC concentrations and reactivity potential during drinking water treatment. However, DBP formation is also dependent on the treatment processes, which can change with time as water treatment facilities update for economic and regulatory reasons (Andersson et al., 2019).

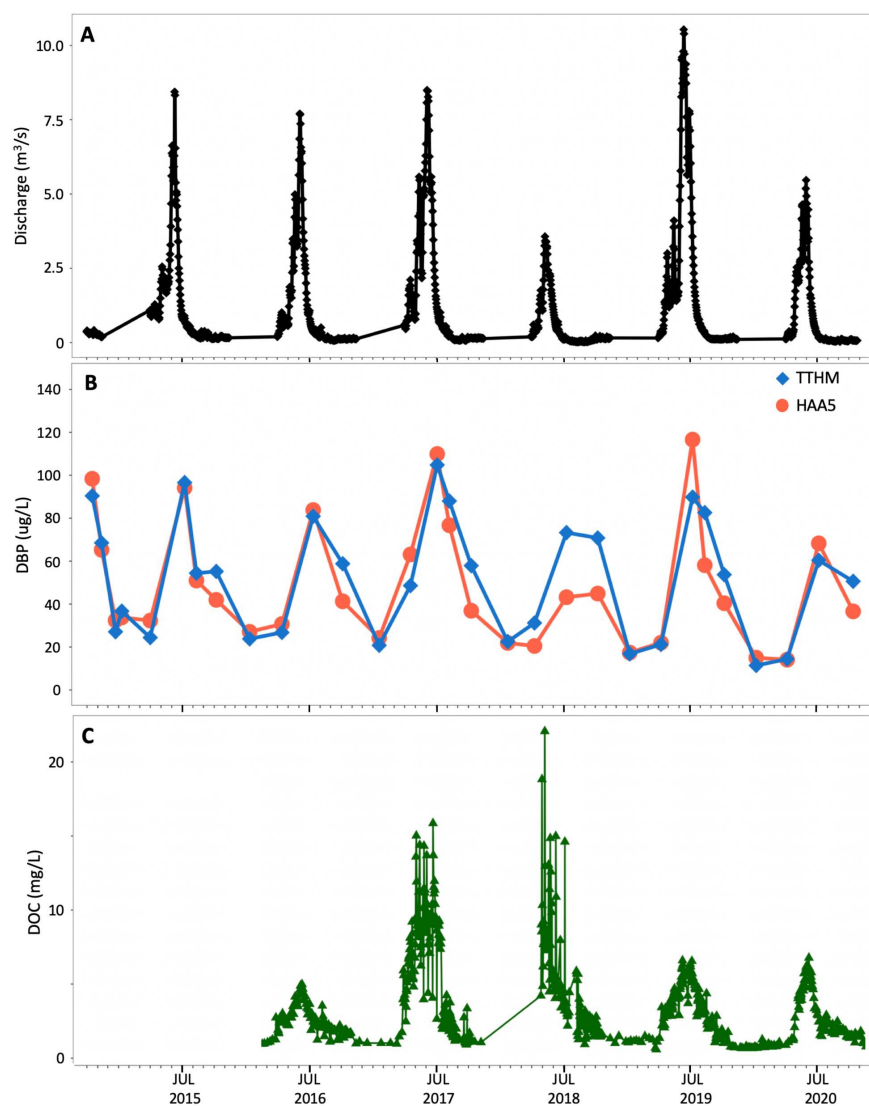


Figure 5.3 Seasonal trends in DBP concentrations after drinking water treatment are associated with peak flow and associated increases in DOC. (A) The Coal Creek hydrograph is plotted with respective (B) TTHM and HAA5 concentrations from the water treatment facility, and (C) upstream DOC concentrations.

Specific to this study, the Crested Butte water treatment facility involves a relatively simple system with few publicly reported modifications (CDPHE, 2021b). A holding pond provides storage for surface water flows. Coal Creek is the primary water source for the town and a secondary intake from Wildcat Creek is used for emergencies (Stantec, 2005). From the holding pond, incoming water is dosed with potassium permanganate to mitigate metals contamination. This pretreatment is followed by microfiltration and sodium hydroxide dosing for pH and alkalinity adjustments. Calcium hypochlorite is added for pathogen disinfection and flows through clear wells and storage tanks to achieve a targeted disinfection contact time. This is also the stage most directly involved in the formation of disinfection byproducts when chlorine reacts with the remaining DOC in the system. Broader studies have assessed a variety of water treatment facilities for associated processes that can more broadly impact DBP formation. Interestingly, despite differences in treatment, the ambient temperature of the environment was a primary determinant of TTHM and HAA5 across water distribution systems with DOC and chloride as significant secondary determinants (Valdivia-Garcia et al., 2016). Further, treatment-related variables of reaction time and pH have been significant influencing factors on DBP formation during treatment (Doederer et al., 2014). Water treatment potentially represents a confounding variable in observed DBP trends, therefore discrete sampling was conducted along the transect to remove treatment process variables.

5.4.3 Synoptic sampling to identify regions of enhanced export

The next step of our study determined the location of a potential export control point along the transect contributing to reactive DOC mobilization. The synoptic sampling campaign occurred two days after peak flow which occurred on June 6th of 2021. Synoptic sampling focused on UV₂₅₄ absorbance and DOC concentrations, which are strong indicators of HAA5 and TTHM formation potential in mountain streams (Brouillard et al., 2016; Golea et al., 2017). The beginning of the transect at Lake Irwin (IR-00) was comparatively low but values increased downstream with peak values derived from and near the Ohio Creek tributary. Ohio Creek meets Coal Creek near an extensive riparian wetland. The additional sampling location CC-7 downstream of the wetland also returned peak values. After this point concentrations of DOC and UV₂₅₄ absorbance decreased as the transect progressed further downstream towards the town (Figure 5.4). Further, all other major tributaries returned comparatively lower values of DOC and UV₂₅₄.

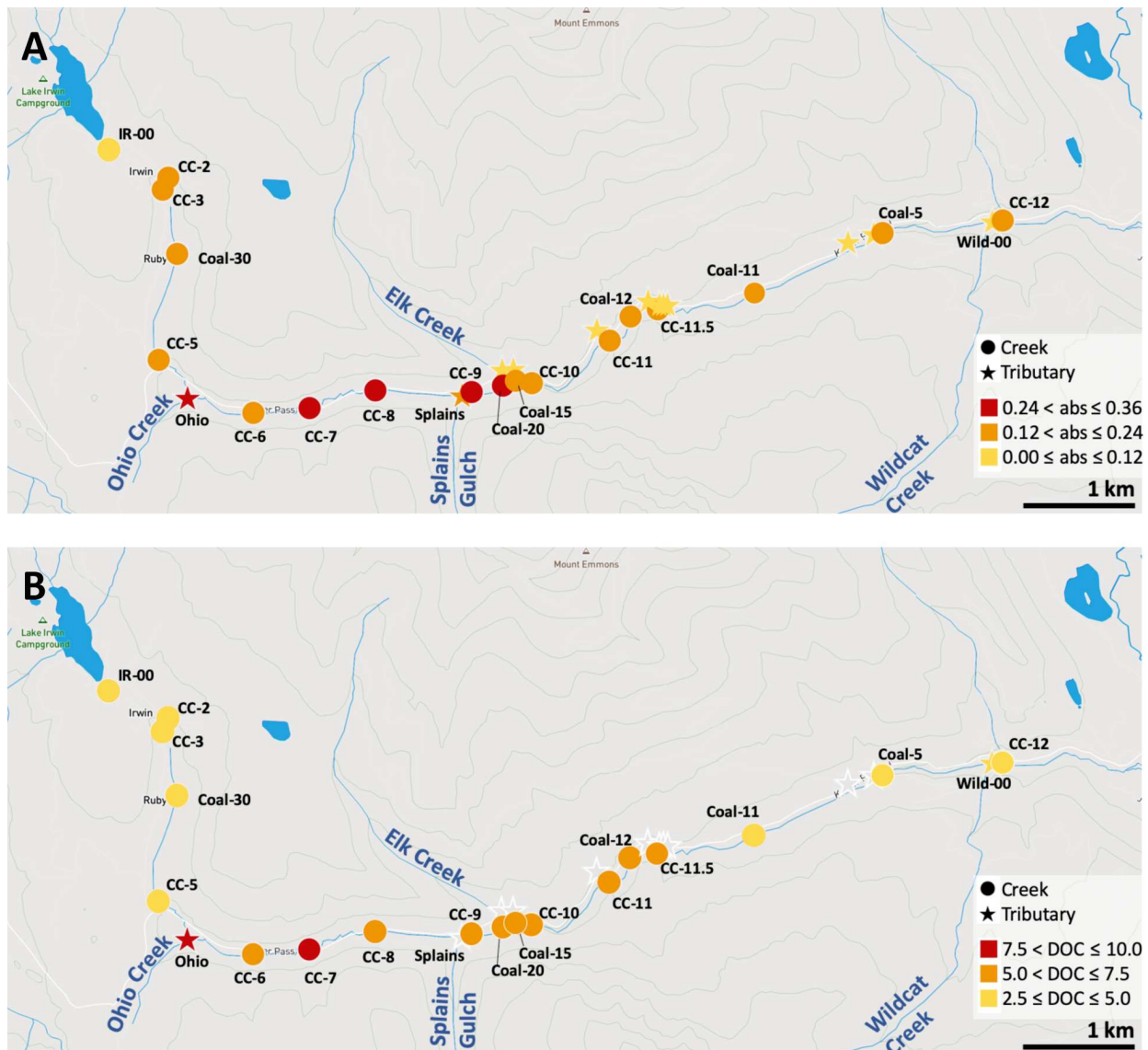


Figure 5.4 Synoptic sampling of the transect in 2021 revealed a hot region for DOC export in the middle reach of the creek. This region was downstream of CC-5 near the convergence with Ohio creek and upstream of CC-8. (A) UV₂₅₄ absorbance and (B) DOC (mg/L) ranges are shown. Actual values are provided in Table D.4 The base map was created in Mapbox with points plotted in Tableau.

The sampling resolution was not sufficient to assess the absolute roles of Ohio Creek versus other local processes in this hot spot region, which is recommended in future studies. The Ohio Creek confluence enters the riparian wetland that continues downstream until the stream narrows through the valley. Ohio Creek is sourced from the southwest section of the watershed, which is primarily composed of spruce-fir forests that have experienced a degree of spruce beetle impact since 2019 (Figure D.3). This is in contrast to the tributaries draining from the north side which are more dominated by sagebrush and grasslands (Alexander & Brown, 2009). Landcover is a distinct observation since aboveground litter can influence

DBP formation in which different plant litter leachates produce comparatively different DBP profiles (Franklin et al., 2021). In addition, the variation of snowmelt across elevation and slope is demonstrated in the area by longer snowfall storage on north-facing slopes in contrast to south-facing slopes that result in faster runoff and drier soils (Ryan et al., 2007). These differences in snow cover are important, as the timing of snowmelt across local climates has been shown to impact porewater DOC content under more labile litter as shown in Chapter 3. Forest health is an additional key variable that can impact stream chemistry. Specifically, natural perturbations including insect-induced tree mortality and fires have been associated with changes to soil DOC quality and quantity, leading to greater DBP formation downstream (Mikkelsen et al., 2013b; Brouillard et al., 2016; Uzun et al., 2020; Olivares et al., 2021). Beyond forest health, DOC export can be dependent on landscape context. Specific studies comparing catchments dominated by forest and the other wetlands determined higher stream DOC concentrations were most impacted by dry summer conditions, supporting the link between late-season drought and increased DOC concentrations (Tiwari, Sponseller & Laudon, 2019; Chen et al., 2020). These findings collectively highlight the importance of incorporating landcover with historical watershed data to pinpoint ecological sources of DOC during peak flow periods.

5.4.4 Formation potential of disinfection byproducts in Coal Creek

We expanded our inquiry to connect the ecosystem control point to DOC reactivity. Formation potential tests provided the absolute potential for reactivity with hypochlorite independent of treatment influences. By design, DBP formation potential (DBP-FP) determines all reactive components within a sample. Conversely, the treatment facility doses disinfectant to achieve two-log inactivation of pathogens. Comparisons between the historical dataset and reacted samples revealed the major byproducts that form from the source waters and during drinking water treatment are primarily chloroform, dichloroacetic acid, and trichloroacetic acid, suggesting that drinking water treatment has minimal impact in reducing the formation ratios specifically as well as general DOC reactivity (Figure D.3). Additional DBPs were observed at smaller concentrations that include chloral hydrate, an additional prevalent DBP in drinking water that is not currently regulated with an MCL (Dabrowska & Nawrocki, 2009).

Correlative analyses of the TTHM and HAA5 formed during potential tests revealed significant relationships with DOC and UV₂₅₄ values (Table 5.1 and Figure D.4) lending credence to the use of these comparatively simple and cost-effective analyses during synoptic sampling. Specific absorbance (SUVA) did not correlate significantly with DBP-FP. This is consistent with prior investigations in Colorado forested watersheds and treatment plants (Brouillard et al., 2016; Beauchamp et al., 2018). The DOC versus DBP and UV₂₅₄ linear slope values were within a range of published values summarized in (Golea et al., 2017)

that represent a variety of raw water types, as well as a study in bark beetle impacted regions of Colorado (Brouillard et al., 2016). The correlative equations were used to interpolate DBP-FP from the synoptic sampling campaign during peak flow. These estimates returned potential HAA5 ($642 \pm 162 \mu\text{g/L}$) and TTHM ($431 \pm 103 \mu\text{g/L}$) concentrations all well above the MCL recommendations. The values are over-estimates; however, they reveal the potential of the source waters during peak discharge, highlighting the precedent for increased monitoring and treatment during the hot moment. While the Crested Butte drinking water treatment facility is complying with mandated quarterly monitoring, the regular sampling events in July are after peak flow that usually occurs in June. Our synthesis of data linking DOC and flow to DBP formation suggests that this sampling routine is missing the true peak in DBP concentrations (Figure D.5).

Table 5.1 Detailed analysis of reactive components and their DBP formation potential for discrete samples along the creek transect.

Sample	Date	DOC (mg/L)	UV ₂₅₄	SUVA	TTHM	HAA5	Hix	Fi	Bix
CC-5	07/30/20	1.2	0.03	2.5	53	62	4.3	1.2	0.6
Coal-20	07/30/20	2.8	0.11	3.8	213	303	8.3	1.1	0.5
Coal-11	07/30/20	1.4	0.05	3.5	87	94	5.1	1.2	0.7
Coal-11	10/9/20	1.1	0.03	2.6	66	63	8.8	1.2	0.6
Coal-20	10/9/20	2.4	0.07	2.9	155	214	9.1	1.2	0.6

5.4.5 Reactivity of DOC functional groups during disinfection

The final aspect of this study involved qualitative analyses using EEM and FTICR methods to better understand the reactive components of Coal Creek with chlorine. EEM spectra revealed a prominent peak indicative of terrestrial fulvic acid-like organic inputs (Xue et al., 2017) (Figure 5.5). A recent study on drinking water sources in China concluded fulvic and humic components significantly correlated with DBP formation potential (Xu et al., 2021). Interestingly in our present study, the humification index did not correlate significantly with DBP formation potential ($P = 1$) in contrast to the fluorescence index, ($P = 0.03$) (Figure D.6). Moreover, FTICR results provided insight into the specific reactive organic classes within Coal Creek before and after chlorination. Comparisons of the relative abundances of organic classes present in pre and post-chlorinated samples revealed that lignin, tannins, and condensed hydrocarbons (HC) reacted with chlorine (Figure 5.6). Lignin comprised the largest relative abundance of organic classes before and after the hot spot of Coal Creek, while tannins and condensed hydrocarbons experienced the greatest losses in October (55% 64%, respectively, as opposed to 38% in lignin). Returning to the fractionated DBP compounds formed after chlorine addition the major byproducts can be linked to some

of the organic classes. A previous study associated higher trichloroacetic acid formation with lignin precursors, while chloroform yields were associated with humic and fulvic acids (Hua, Kim & Reckhow, 2014). A more recent study further corroborates these findings from drinking source waters in Spain in which lignin was deemed a major component of natural surface waters and most reactive after chlorination along with condensed hydrocarbons. The complexity of these reactions was also highlighted, as the authors observed increases in certain lignin signals (Sanchís et al., 2020). With the major DBPs formed in Coal Creek identified as chloroform, trichloroacetic acid, and dichloroacetic acid, it is likely the high abundance lignin structures are forming HAA5, while the tannins and condensed hydrocarbons could be contributing more so to TTHM formation. Further, given the differences in lignin content across conifer needles (Leonard et al., 2020a), the spruce forest landcover could be a contributing source of lignin, and landcover should be further studied to determine these sources.

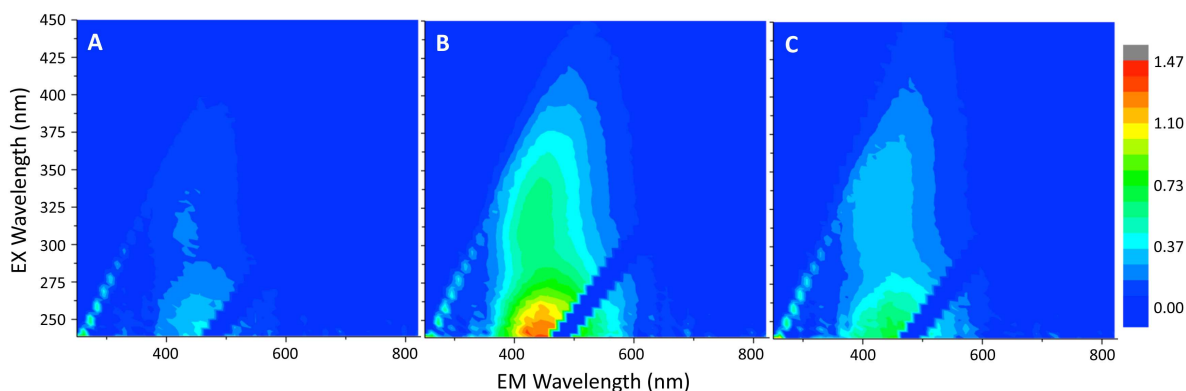


Figure 5.5 Excitation-emission matrix spectra reveal peak terrestrial organic matter inputs near the middle Coal Creek hotspot. (A) CC-5 , (B) Coal-20, and (C) Coal-11 transect sample results are shown.

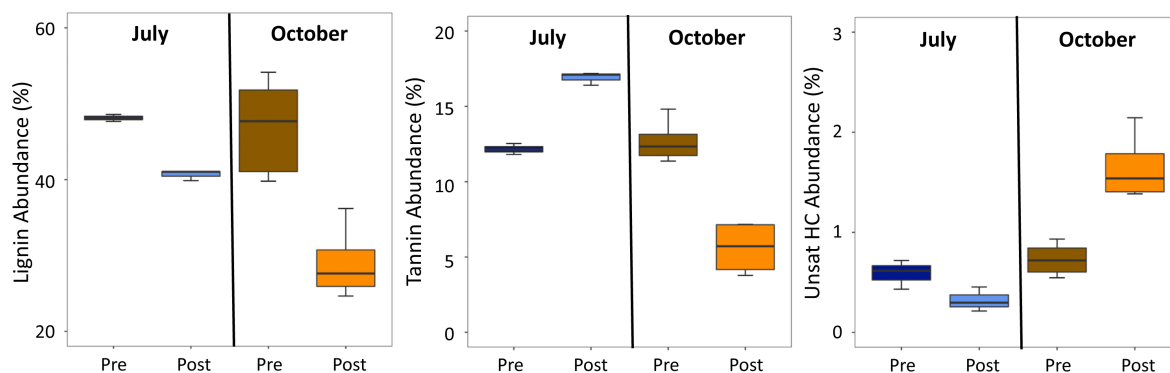


Figure 5.6 Average relative abundances of the three reactive organic classes indicate temporal differences. Pre-chlorination (Pre) and post-chlorination (Post) relative abundances are shown based on the average of all samples collected in 2020.

5.5 Conclusions & Implications

Watershed health and hydrochemical processes play an important role in water supply and quality. As these variables shift with climate change, certain hot moments could become “hotter” from ecological responses of increased organic release to surface waters from climate-induced stress (McDaniel, Kaye & Kaye, 2014). We present our approach of using historical DBP drinking water quality archives that can be used as a forensic tool to identify dynamic watersheds with heightened organic carbon export. When combined with higher resolution synoptic sampling, this method could better identify ecosystem control points within source watersheds that exhibit seasonal and temporal trends for increased natural organic matter export. This research highlights the necessity to plan for more effective water treatment processes that include comprehensive analyses from the source to tap. Due to the repeatability of the hot moments associated with hydrologic events each year, this recurrent DBP challenge can be proactively addressed. Possible solutions include targeted TOC treatment before chlorination with coagulation, activated carbon, biofiltration, or nanofiltration. In addition, DBPs can be removed after formation with membrane filtration, air stripping, or granular activated carbon (Zazouli & Kalankesh, 2017). However, these solutions are not always economical for smaller treatment facilities such as Crested Butte. Passive solutions, in that case, should be explored such as in Crested Butte where sourcing the secondary water supply of Wildcat Creek during the period of peak discharge could be an option. This research highlights the necessity to plan for more effective water treatment processes that include comprehensive analyses from the source to tap.

CHAPTER 6

CONCLUDING REMARKS

In this work, we addressed the tree-scale biogeochemical processes associated with conifer needle decomposition (Chapters 2 and 3). More broadly, however, we explored the watershed-scale implications of forest ecosystem disruptions on source waters and downstream treatment (Chapters 4 and 5). At the forest scale, Chapter 2 underscores the importance of tree species and resulting litter chemistry in decomposition processes and associated organic matter release. Bark beetle affected needles will have little impact on the soil biogeochemistry in contrast to the greater driving forces in decomposition processes related to the inherent tree chemistry. In addition to needle chemistry, seasonality and climate conditions as drivers of decomposition processes are important. These conditions highlighted how the cumulation of natural perturbations associated with increased temperatures, low moisture, and shifting snowmelt dynamics will affect future forest biogeochemical processes. More evidence is mounting that increased organic carbon is associated with shifts in forest health, which is exacerbated by higher temperatures and water scarcity. As a result, increased trends of organic carbon concentrations in streams have been observed in the Rocky Mountain region of Colorado (Rodríguez-Jeangros, Hering & McCray, 2018).

Furthermore, our historical analysis of Colorado complements these studies focused on organic carbon fluxes in watershed catchments and underscores the downstream consequences of climate change in which increased reactive organic inputs can interfere with treatment processes. Watershed health can decline from warmer spring conditions associated with fewer total snow depths as well as higher minimum air temperatures. As this happens, the increased trends of carcinogenic byproduct formation in treatment facilities could continue to put communities at risk. Chapter 5 serves as a unique study focused on a treatment facility that has experienced the heightened impacts of byproduct formation. This final chapter provides methods of source water sampling that could be easily employed by municipalities to monitor for ecosystem exporting control points of organic carbon. As a whole, this dissertation revealed the interconnected variables from the tree to the drinking water tap. Also, this dissertation underscores the importance of evaluating climate-related impacts to surface water on a watershed basis since landcover, soil type, elevation, and snowmelt dynamics can all collectively impact soil biogeochemical processes.

In a changing climate, municipalities should prepare for the local ecological shifts associated with declining tree health and water scarcity within local watersheds. If water treatment facilities do not respond to increased organic inputs to surface waters in certain watersheds, the trends observed in Chapters 4 and 5 could continue. Future work needs to understand these interactions between watershed

health and downstream water quality to ensure community health safety. An especially concerning trend not included in this thesis is increased fire incidence and severity. In 2020, the Cameron Peak, East Troublesome, and Pine Gulch fires collectively took the first, second, and third standing as the greatest fires on record in Colorado (CSFS, 2021). While fire plays an important role in maintaining forest health, the downstream impacts have the potential to additionally affect drinking water treatment, specifically with increased organic carbon export following intense storms (Writer, McCleskey & Murphy, 2012).

Recommended future work related to this thesis is proposed in three tasks. The greatest precedence for future studies is to continue the work in Chapter 4. Our preliminary exploration of historical climate and drinking water data laid the groundwork for continued research that can build off the rich dataset and associate all climate indices with disinfection byproduct concentrations at smaller watershed delineations. Additional variables should include drought indices, soil moisture and temperature, and areal fire impact with more statistical and mechanistic analyses. Second, continued work in Coal Creek is recommended to further assess the absolute roles of Ohio Creek versus other local processes in this hot spot region, which would require more sampling. Future researchers should explore proactive treatment options as well to reduce organic loading. These options should include not only the treatment processes, but resource management plans as well that include thinning dead tree stands to reduce wildfires and encourage regrowth. These management solutions could be cost-effective for smaller mountainous treatment facilities at most risk for increased disinfection byproduct concentrations in the future. Finally, the goal of this work is to highlight the importance for municipalities to monitor disinfection byproduct formation beyond the running annual average enforced by the Environmental Protection Agency. Disinfection byproduct precursors, including organic carbon and UV_{254} along with temperature, and pH concentrations, are raw water conditions that should be monitored frequently to understand susceptibility to byproduct formation. The impacts of climate change are upon us, and treatment facilities will need to react proactively in a manner that includes source water characteristics to protect community health.

CHAPTER 7

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APPENDIX A
CHAPTER 2 SUPPLEMENTARY INFORMATION

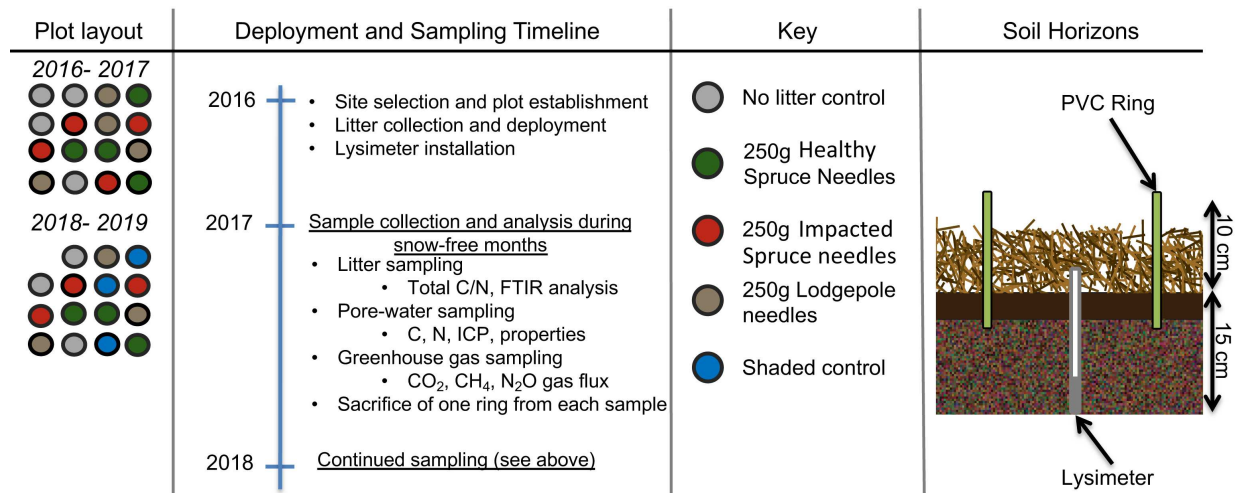


Figure A.1 Experimental design, plot, and schedule. Randomized placement of each sample collar is shown in the plot layout. In 2016-2017 there were quadruplicate collars for each sample type. This changed at the end of 2017 when one of each sample quadruplicate was sacrificed. This led to triplicates of each collar in 2018 and the addition of shade controls.

Table A.1 Percent carbon and nitrogen differs as a function of needle type.

	2016 Initial Needle Litter		
	Healthy Spruce	Impacted Spruce	Lodgepole
Carbon (%)	45.9 (± 0.2)	51.8 (± 0.1)	53.7 (± 0.1)
Nitrogen (%)	0.7 (± 0)	0.8 (± 0)	0.9 (± 0)
C:N	62.7 (± 1.7)	68.2 (± 0.2)	61.6 (± 1.1)
	2018 Decomposed Needle Litter		
	Healthy Spruce	Impacted Spruce	Lodgepole
Carbon (%)	38.9 (± 15.3)	48.6 (± 0.6)	51.9 (± 0.7)
Nitrogen (%)	0.9 (± 0.3)	0.9 (± 0)	0.7 (± 0.1)
C:N	45.1 (± 2.6)	52.2 (± 1.1)	72.4 (± 15.4)

Notes:

Averages plus or minus standard deviation in parenthesis (n=3).

Table A.2 FTIR peak area analyses differs as a function of needle type. Peak area integration values at known functional groups of interest.

	2016 Initial Needle Litter		
	Healthy Spruce	Impacted Spruce	Lodgepole
Ether Linkages (1150 cm^{-1})	50 (± 2)	50 (± 2)	64 (± 2)
Aromatics (1510 cm^{-1})	33 (± 1)	30 (± 2)	19 (± 1)
Amides (1600 cm^{-1})	193 (± 4)	182 (± 3)	180 (± 6)
Carbonyl (1720 cm^{-1})	38 (± 1)	44 (± 1)	72 (± 6)
Cellulose:Lignin	1 (± 0)	2 (± 0)	3 (± 0)
	2018 Decomposed Needle Litter		
	Healthy Spruce	Impacted Spruce	Lodgepole
Ether Linkages (1150 cm^{-1})	43 (± 11)	58 (± 11)	76 (± 6)
Aromatics (1510 cm^{-1})	16 (± 14)	21 (± 2)	13 (± 3)
Amides (1600 cm^{-1})	171 (± 67)	193 (± 6)	143 (± 4)
Carbonyl (1720 cm^{-1})	23 (± 15)	40 (± 6)	84 (± 9)
Cellulose:Lignin	4 (± 3)	3 (± 1)	6 (± 1)

Notes:

Averages plus or minus standard deviation in parenthesis (n=3).

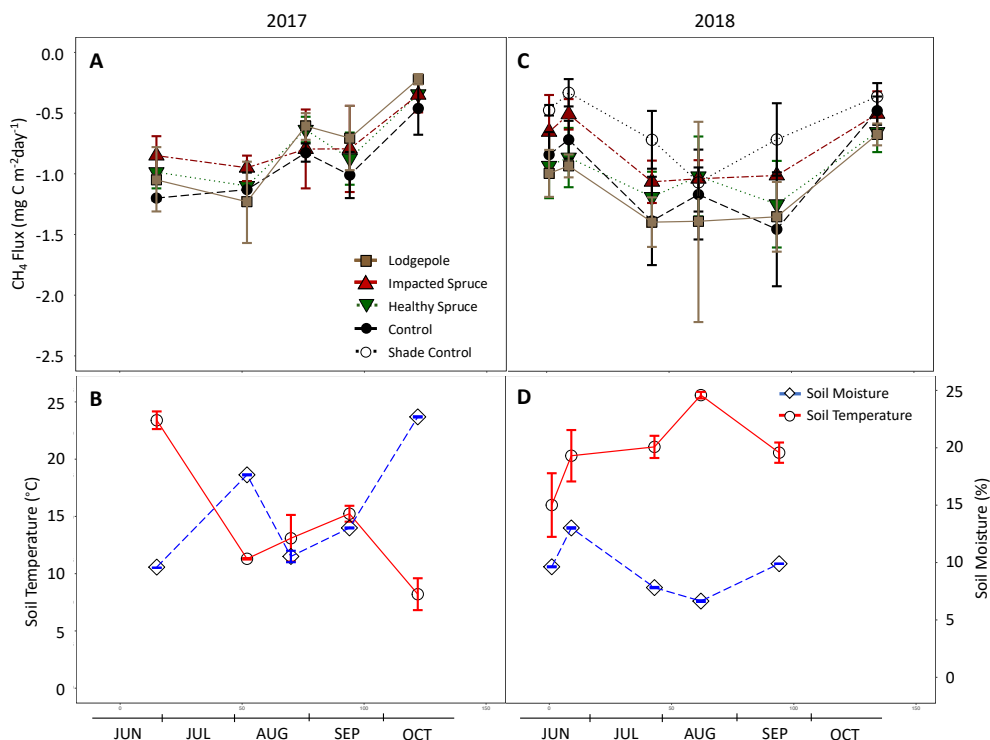


Figure A.2 Methane flux with seasonal variables. (A, C) Measured methane above decomposing needle collars and (B, D) seasonal variables of local soil at the experimental plot over the summer months of 2017 (A, B) and 2018 (C, D). Error bars indicate plus or minus one standard deviation (n = 3 or 4). The fifth point in September 2018 for impacted spruce is missing a third measurement, as result no error bars are present.

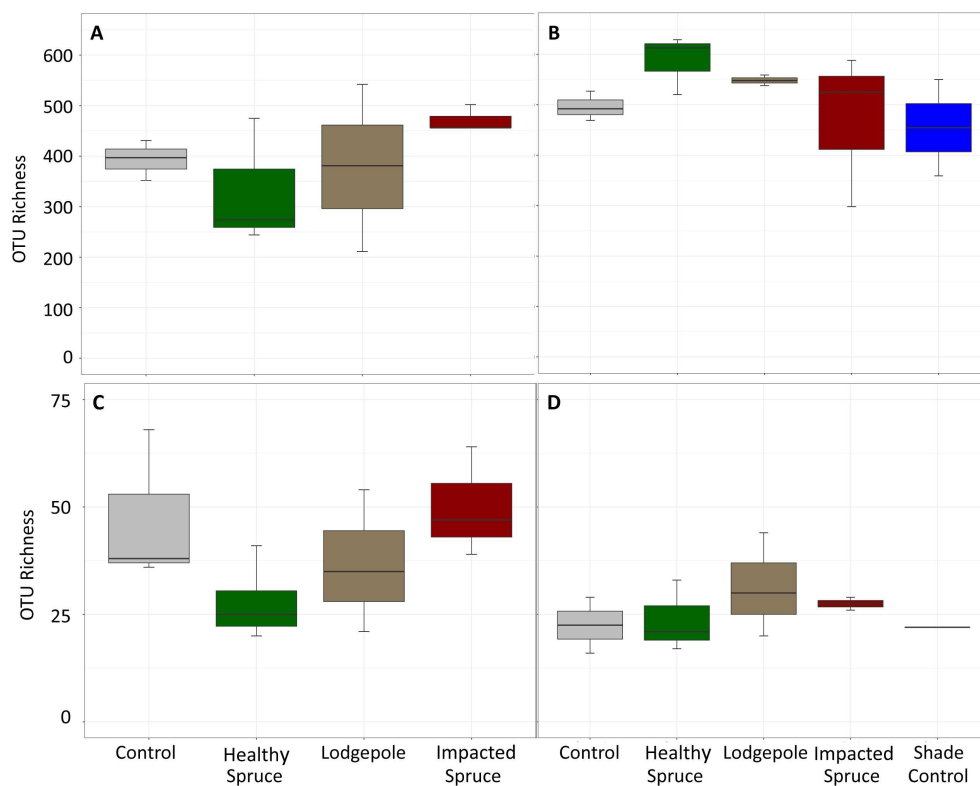


Figure A.3 Observed OTUs diversity plots. (A, B) bacterial, and (C, D) fungal communities in the Lower Subalpine plot for dates (A, C) August 2017 and (B, D) July 2018.

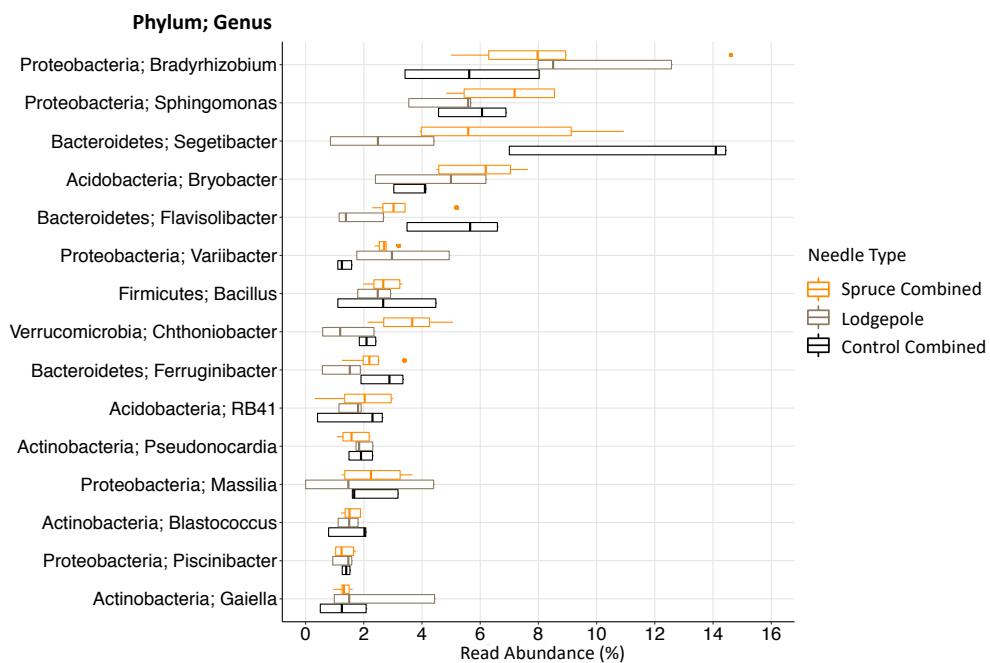


Figure A.4 Boxplots of mean relative abundance aggregated at the Genus level for 16S genes as a function of sample type for August 2017. Spruce is combined healthy and impacted (n=6).

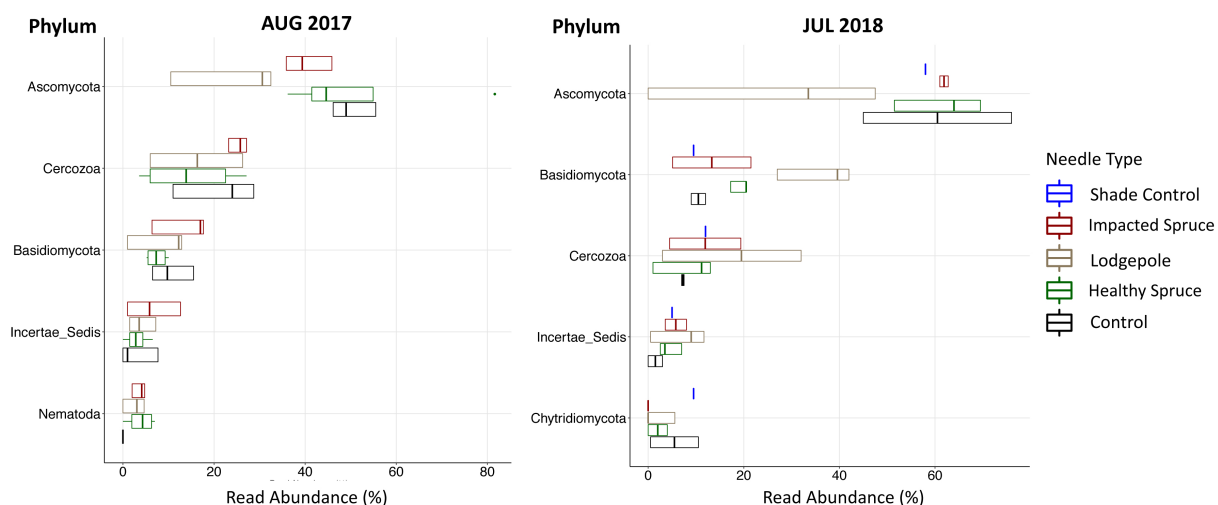


Figure A.5 Boxplots of mean relative abundance aggregated at the Phylum level for 18S genes as a function of sample type for August 2017 and July 2018.

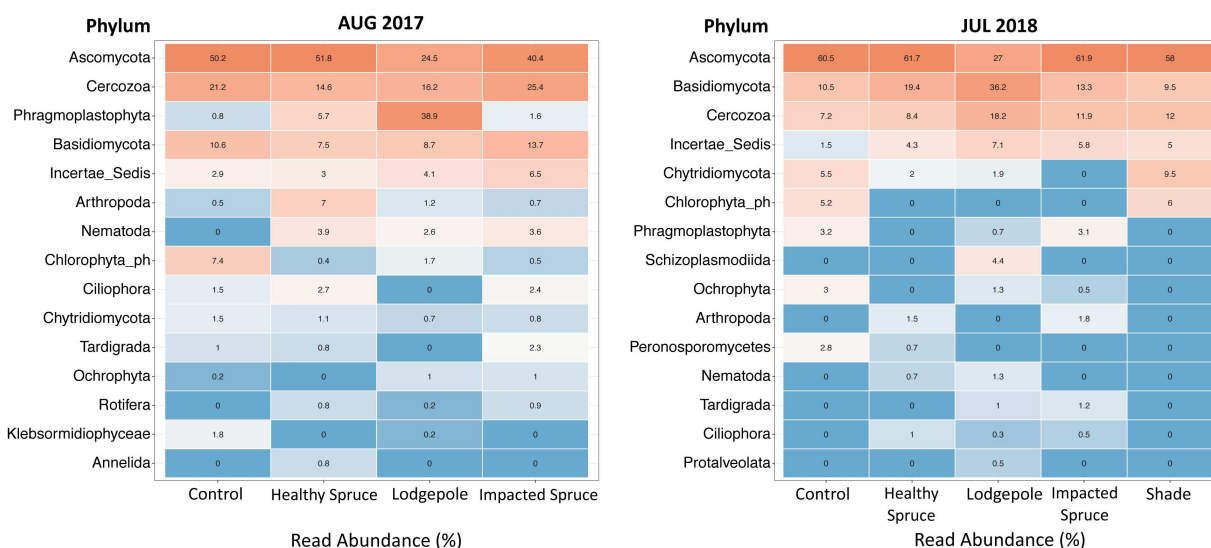


Figure A.6 Heatmaps as a function of Phylum for August 2017 and July 2018 aggregated by mean relative abundance 18S genes as a function of sample type. Samples were not combined for 18S due to more distinct differences between the impacted and healthy spruce.

APPENDIX B
CHAPTER 3 SUPPLEMENTARY INFORMATION

Table B.1 Snowmelt dates differ across elevation: Snow-free dates determined by the first spring date when soil microclimate temperatures were consistently >1 degree Celsius. These dates are estimates, as more specific dates would be determined using soil temperature data with time-lapse photos to determine when the complete plot area was snow-free.

Lower	Middle-ES	Middle	Upper
5/15/17	5/14/17	6/5/17	6/27/17
4/29/18	4/29/18	5/13/18	6/1/18
5/15/19	6/7/19	6/15/19	-

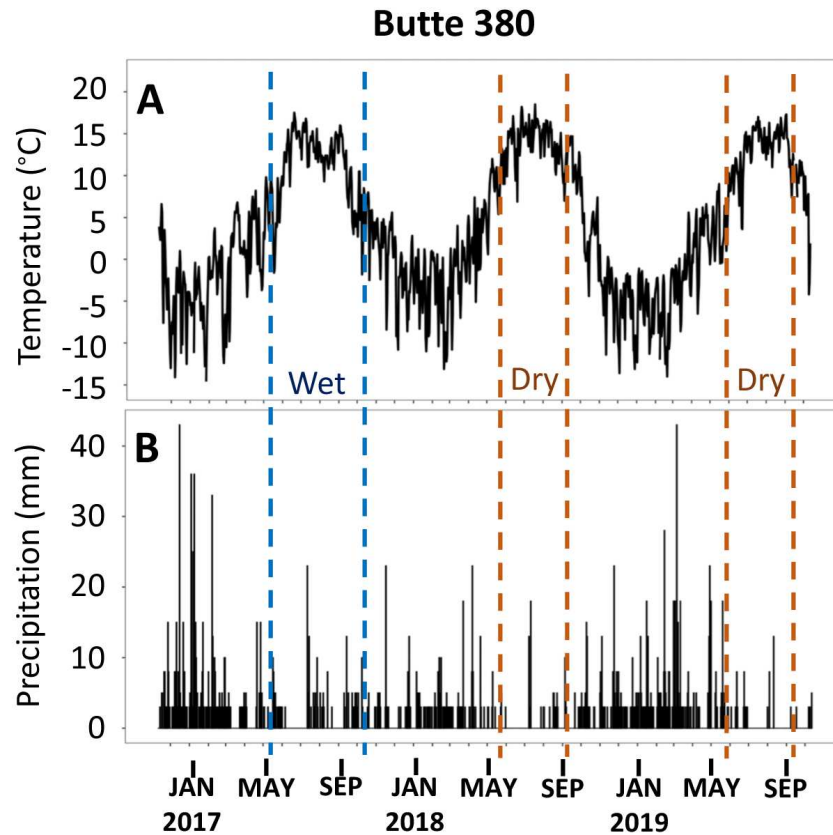


Figure B.1 Air temperature and snow water equivalent precipitation over the years of 2017-2019. Local air temperature and total precipitation are shown from the Butte 380 USDA SNOTEL station.

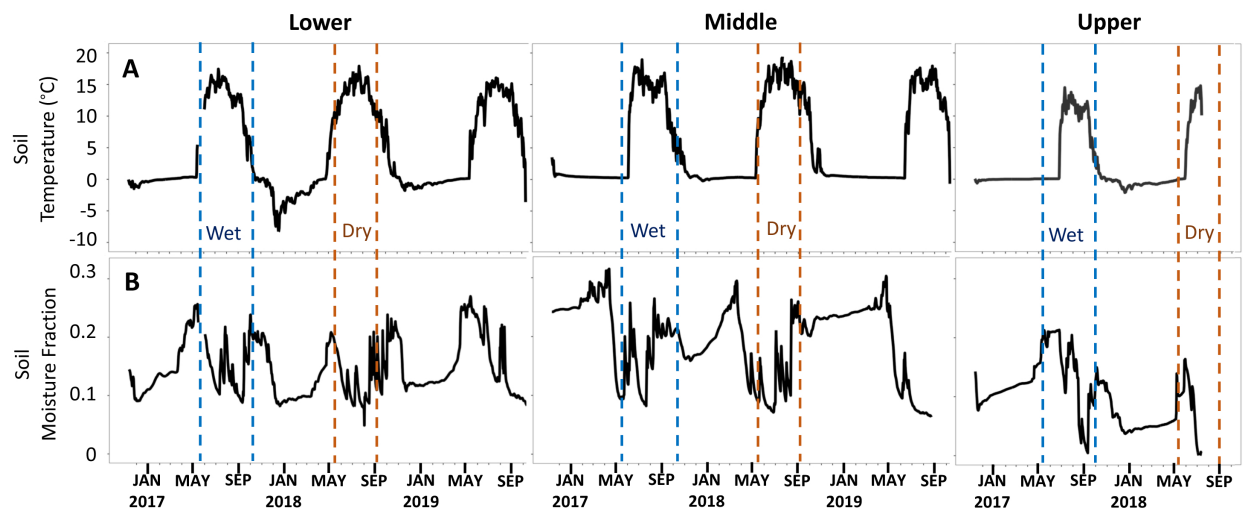


Figure B.2 Soil Moisture and temperature at each elevation over the years of 2017-2019. Each study elevation is shown at the Lower (2,800 m), Middle (3,100 m), and Upper (3,500 m) plots. Note microclimate data collection ceased in Fall 2018 at the Upper plot.

Table B.2 Litter decomposition losses after 3 years: Percent mass losses for the spruce and lodgepole needle litter bags. Note the lodgepole at the Lower site has no standard deviation due to $n=2$. Litter bags at the Upper site were displaced by cattle.

	Lower (%)	Middle-ES (%)	Middle (%)
Spruce	17 (± 1)	17 (± 3)	13 (± 4)
Lodgepole	21 -	22 (± 2)	21 (± 6)

Table B.3 Annual changes in total carbon and nitrogen for spruce and lodgepole (lodge) needles across elevations.

	2016 Litter		2019 Lower		2019 Middle		2019 Middle-ES		2019 Upper	
	Spruce	Lodge	Spruce	Lodge	Spruce	Lodge	Spruce	Lodge	Spruce	Lodge
C	45.9	53.7	41.6	50.7	42.4	47.4	44.5	50.2	50.0	49.3
(%)	(± 0.2)	(± 0.1)	(± 7.8)	(± 5.5)	(± 3.8)	(± 2.2)	(± 2.6)	(± 1.0)	(± 4.1)	(± 1.3)
N	0.7	0.9	0.9	1.0	1.1	0.9	1.1	1.0	1.1	1.1
(%)	($\pm 2E-2$)	($\pm 2E-2$)	(± 0.1)	(± 0.1)	(± 0.1)	(± 0.1)	($\pm 9E-3$)	($\pm 2E-2$)	(± 0.1)	(± 0.1)
C:N	62.7	61.6	43.7	48.4	39.5	50.3	40.4	51.9	43.8	46.3
	(± 1.7)	(± 1.1)	(± 1.5)	(± 2.4)	(± 1.7)	(± 6.4)	(± 2.7)	(± 1.0)	(± 0.6)	(± 3.5)

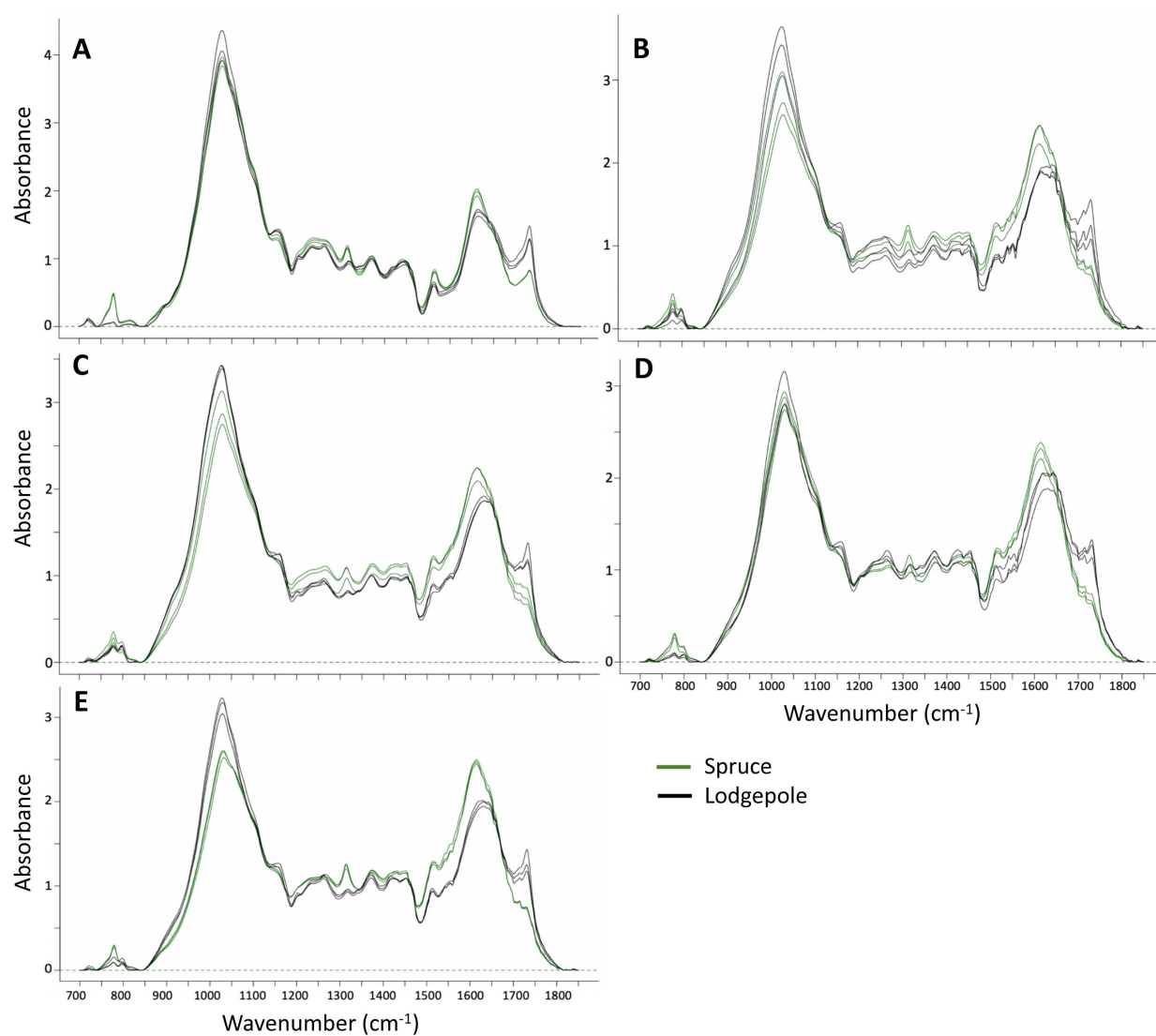


Figure B.3 FTIR spectra for harvested and decomposed needles. (A) The harvested needles FTIR spectra are shown against (B) Lower (2,800 m), (C) Middle (3,100 m), (D) Middle-ES (3,100 m), and (E) Upper (3,500 m) plots.

Table B.4 FTIR peak area integration values.

	Polysaccharides ¹ (900-1200cm ⁻¹)	Ether Linkage ² (1150 cm ⁻¹)	Carbonyl ² (1738 cm ⁻¹)	Amide I ² (1600-1700 cm ⁻¹)	Aromatics ¹ (1508-1510 cm ⁻¹)	Polysaccharide: Amide I Ratio
	Spruce 2016					
	529 (± 3)	50 (± 2)	38 (± 1)	193 (± 4)	33 (± 1)	2.7 (± 0.1)
	Spruce 2019					
Lower	425 (± 41)	51 (± 1)	41 (± 3)	302 (± 17)	51 (± 6)	1.4 (± 0.2)
Middle	440 (± 33)	54 (± 1)	43 (± 5)	290 (± 9)	47 (± 3)	1.5 (± 0.2)
Middle -ES	430 (± 20)	52 (± 1)	40 (± 3)	295 (± 11)	53 (± 1)	1.5 (± 0.1)
Upper	389 (± 5)	52 (± 1)	43 (± 1)	315 (± 7)	54 (± 3)	1.3 (± 0.0)
	Lodgepole 2016					
	535 (± 18)	64 (± 2)	72 (± 6)	180 (± 6)	19 (± 1)	3.0 (± 0.2)
	Lodgepole 2019					
Lower	487 (± 45)	59 (± 4)	66 (± 13)	258 (± 8)	34 (± 2)	1.9 (± 0.2)
Middle	470 (± 50)	57 (± 2)	63 (± 2)	271 (± 25)	36 (± 5)	1.8 (± 0.3)
Middle -ES	453 (± 49)	60 (± 3)	69 (± 1)	265 (± 17)	34 (± 7)	1.7 (± 0.3)
Upper	450 (± 12)	59 (± 3)	67 (± 6)	269 (± 4)	35 (± 2)	1.7 (± 0.1)

Notes:

¹Özgenç, Durmaz & Kuştaş, 2017²Naumann, 2000

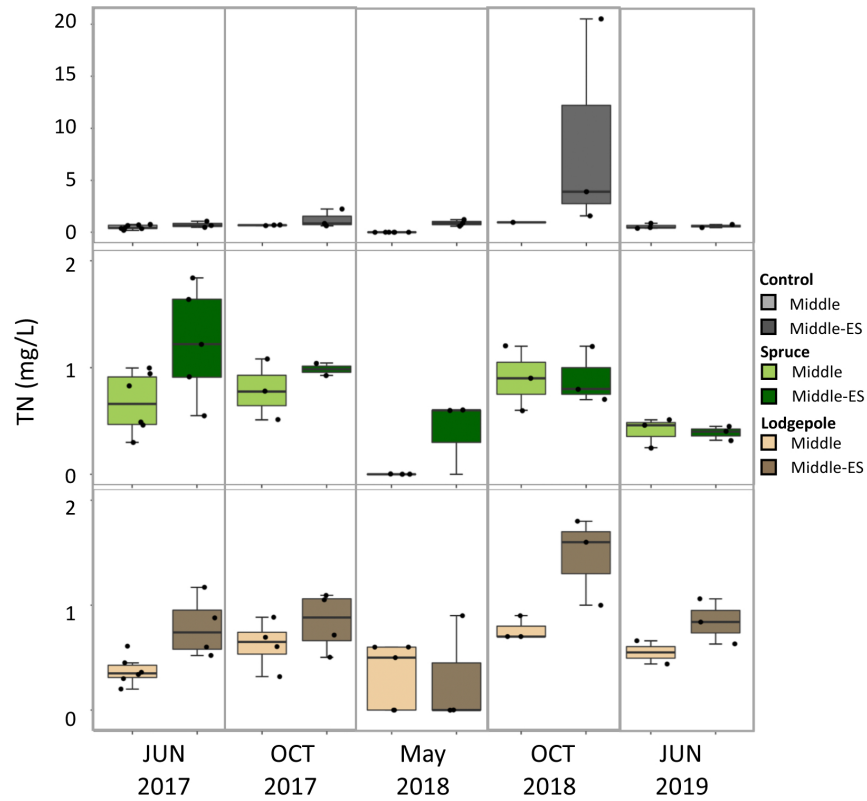


Figure B.4 Porewater TN concentrations at the dual Lower Subalpine plots. 2017-2019 concentrations at the Lower Subalpine control (Middle) and manipulated (Middle-ES) plots for the controls, spruce, and lodgepole samples. Error bars indicate plus or minus one standard deviation. Samples were collected during two consecutive days in 2017-2018 to increase the sample size ($n=3-6$). Individual points represent each sample measurement.

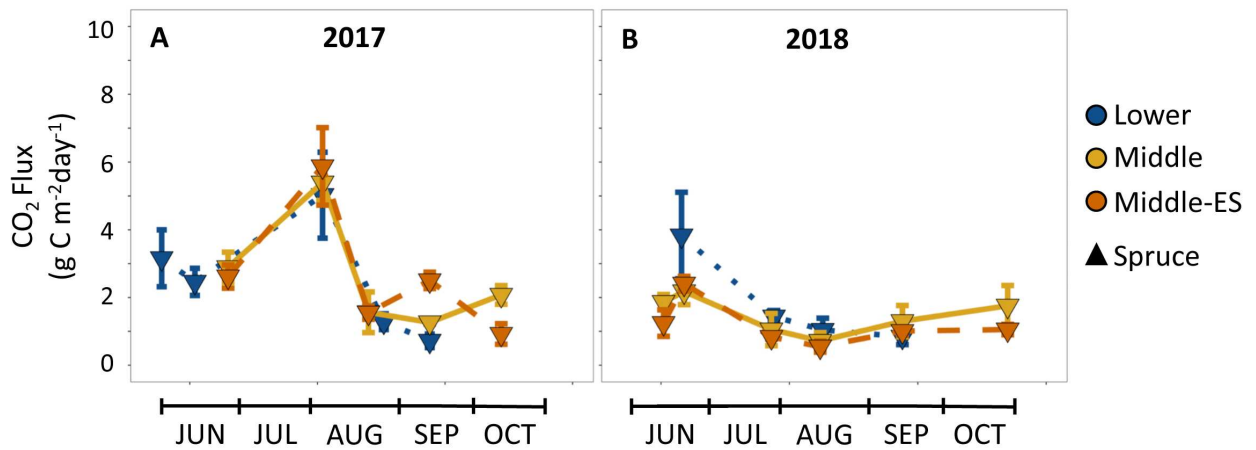


Figure B.5 CO_2 production is similar across snowmelt manipulations under spruce needles. CO_2 flux measurements over the snow-free months of (A, B) 2017 and 2018 under spruce needles across elevation and snowmelt manipulation.

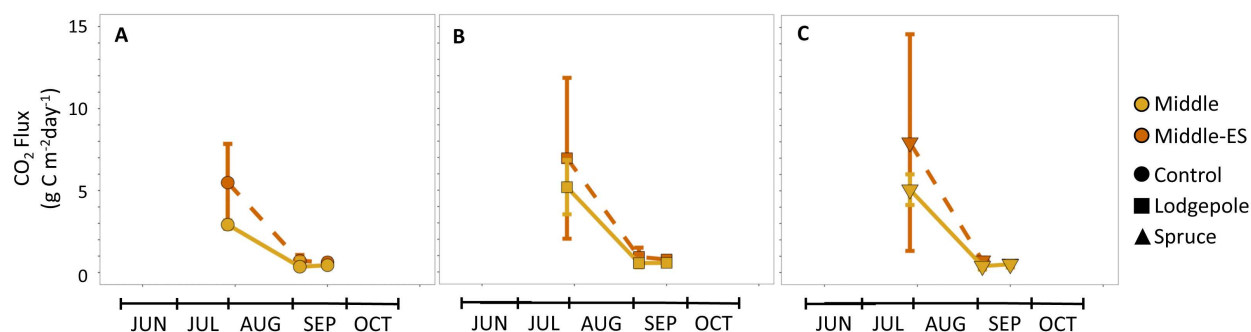


Figure B.6 CO₂ production is similar across snowmelt manipulations in 2019. CO₂ flux measurements over the snow-free months of 2019 under (A) control, (B) lodgepole, and (C) spruce samples across elevation and snowmelt manipulation. Lower measurements were not conducted in 2019.

Table B.5 Pearson *P* and R-value for correlations between CO₂ gas flux and soil temperature (T) and moisture (M).

	Lower							
	CO ₂ and T		CO ₂ and M		CH ₄ and T		CH ₄ and M	
	P Value	R Value	P Value	R Value	P Value	R Value	P Value	R Value
Control	0.00	0.59	0.00	0.82	0.32	-0.19	0.44	0.14
Spruce	0.00	0.62	0.00	0.62	0.35	-0.18	0.00	0.59
Lodgepole	0.00	0.64	0.00	0.49	0.36	0.17	0.00	0.61
	Middle							
	P Value	R Value	P Value	R Value	P Value	R Value	P Value	R Value
Control	0.21	0.19	0.47	0.11	0.00	-0.48	0.51	-0.10
Spruce	0.78	-0.04	0.25	0.18	0.00	-0.57	0.00	-0.46
Lodgepole	0.50	0.10	0.37	0.14	0.00	-0.54	0.00	-0.41
	Middle-ES							
	P Value	R Value	P Value	R Value	P Value	R Value	P Value	R Value
Control	0.04	0.31	0.13	0.23	0.00	-0.46	0.32	-0.15
Spruce	0.49	0.10	0.02	0.36	0.00	-0.41	0.00	-0.45
Lodgepole	0.91	0.02	0.09	0.25	0.00	-0.43	0.00	-0.43

Notes:

Bold indicates significant correlations with a *P* value less than 0.05.

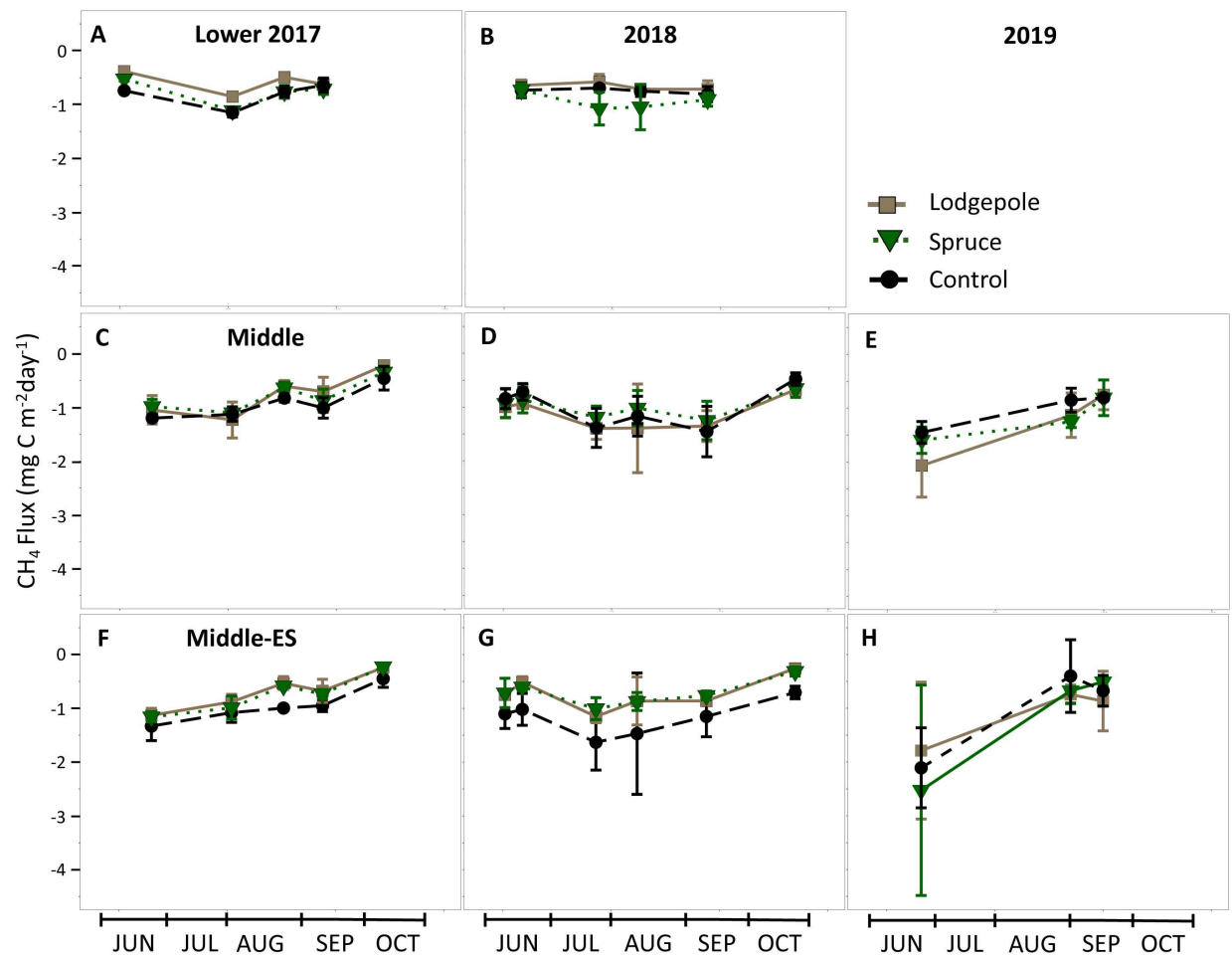


Figure B.7 CH_4 consumption across elevation and as a function of snowmelt. CH_4 flux measurements over the snow-free months of 2017-2019. Error bars indicate plus or minus one standard deviation.

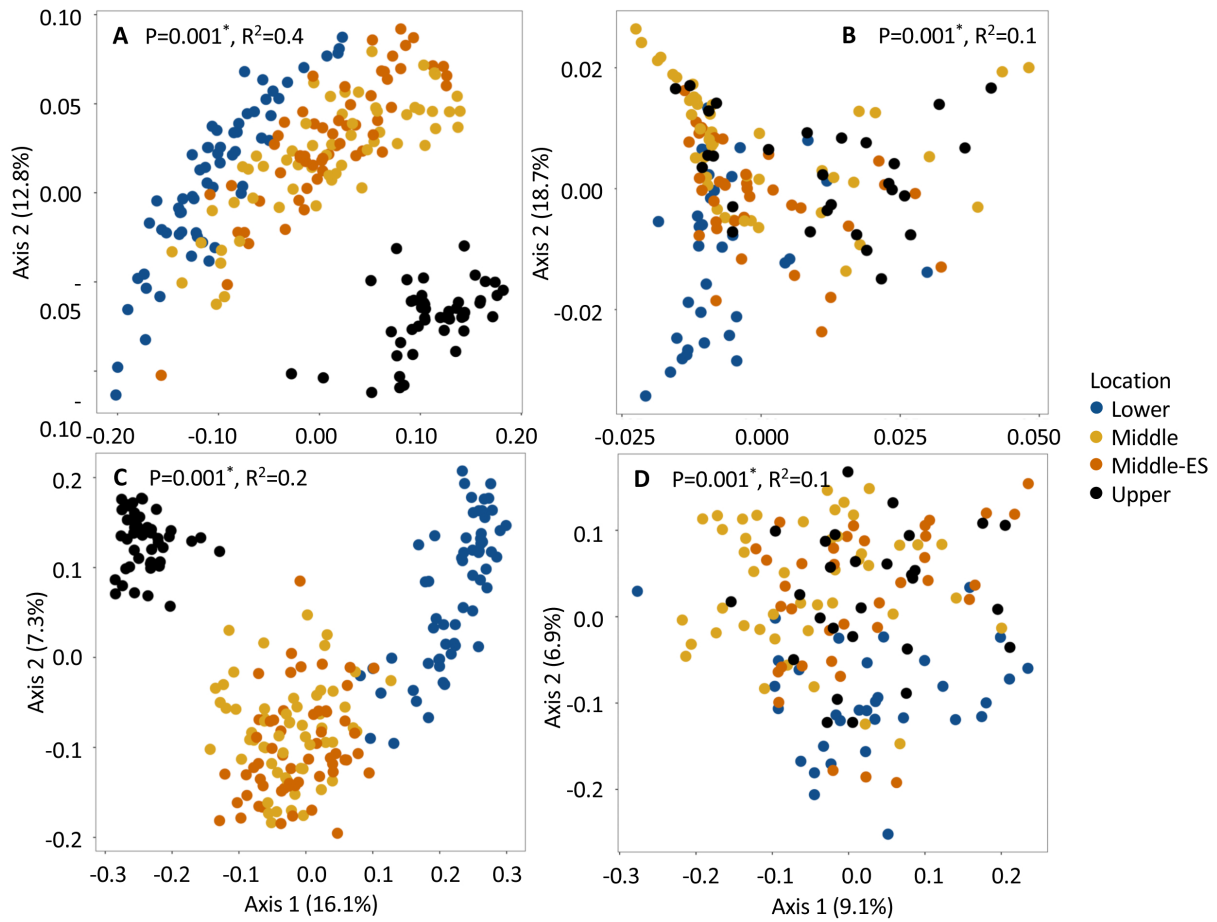


Figure B.8 Microbial communities significantly differ across elevation. All the samples collected over time are shown for (A, C) 16S and (B, D) 18S communities as (A, B) weighted and (C, D) unweighted. P and R -values represent ADONIS values aggregated by location.

Table B.6 Bacterial communities significantly differ over time in 2018 across sample types.

	Year	Control	Spruce	Lodgepole
Lower	2017	$R=0.2$ $P=0.2$	$R=0.2$ $P=0.6$	$R=0.2$ $P=0.06$
	2018	$R=0.5$ $P=0.001^*$	$R=0.7$ $P=0.03^*$	$R=0.7$ $P=0.03^*$
	2019	$R=0.2$ $P=0.1$	$R=0.1$ $P=0.8$	$R=0.3$ $P=0.7$
Middle	2017	$R=0.2$ $P=0.5$	$R=0.3$ $P=1$	$R=0.2$ $P=0.4$
	2018	$R=0.6$ $P=0.001^*$	$R=0.4$ $P=0.06$	$R=0.5$ $P=0.04^*$
	2019	$R=0.1$ $P=0.7$	$R=0.5$ $P=0.1$	$R=0.4$ $P=0.2$
Upper	2017	$R=0.3$ $P=0.6$	$R=0.2$ $P=0.6$	$R=0.2$ $P=1.0$
	2018	$R=0.2$ $P=0.2$	$R=0.5$ $P=0.1$	$R=0.2$ $P=0.4$
	2019	$R=0.3$ $P=0.8$	$R=0.2$ $P=0.7$	$R=0.2$ $P=0.4$

Notes:

P and R -values represent ADONIS values aggregated by date.

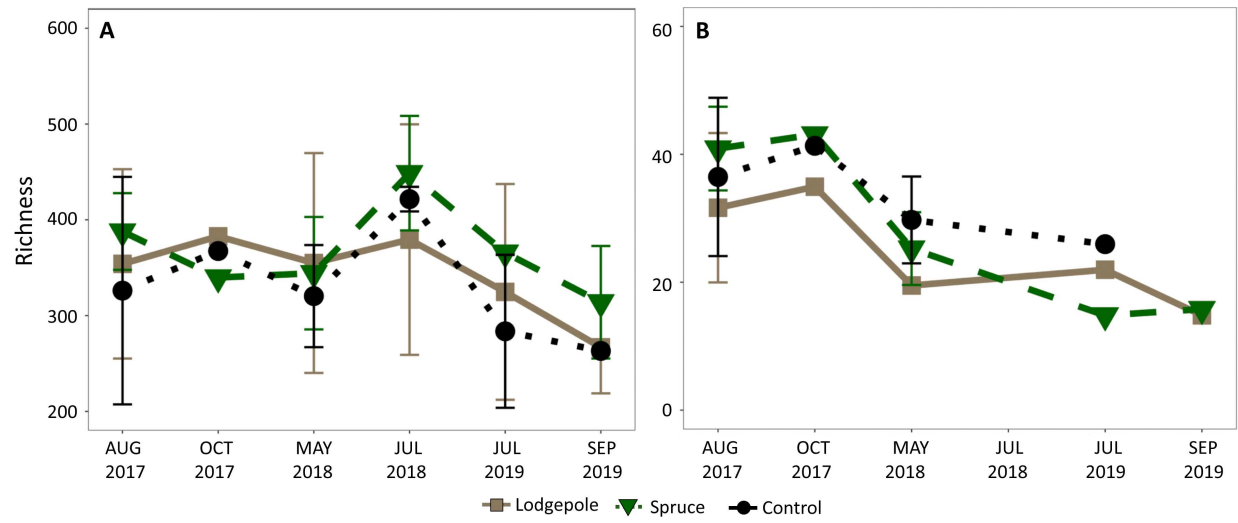


Figure B.9 Upper plot 16S and 18S alpha diversity richness over time. Species richness for the Upper (A) 16S and (B) 18S plots. Error bars indicate plus or minus one standard deviation (n=3, 4). Note a lack of error bars for a sample point is due to n<3 caused by sample reduction during rarefaction.

Lower		Phylum; Family							
	^{1, 2} Bacteroidetes; Chitinophagaceae	28.3	35.4	44.1	15.7	13.5	23.1	31.2	
	Verrucomicrobia; DA101_soil_group	3	2.1	5.2	6.9	9.8	6.4	5.9	
	Proteobacteria; Oxalobacteraceae	14	6.4	4.3	5.5	2.5	4.1	5.7	
	² Proteobacteria; Comamonadaceae	2.8	3.3	3.5	2.7	2.5	2.9	3.2	
	Proteobacteria; Sphingomonadaceae	2.2	4.2	2.6	2.2	3.3	3.6	2.5	
	^{1, 2} Actinobacteria; Nocardioideae	1.9	3.3	1.5	3.1	3.7	2.9	2.4	
	Proteobacteria; Methylobacteriaceae	2.8	3.6	2.5	2.7	1.8	2.9	3.7	
	Proteobacteria; Xanthobacteraceae	2.5	0.8	1.9	2.6	2.9	3.4	2.5	
	Planctomycetes; Planctomycetaceae	2.2	1.1	2.1	3.2	3.1	2.5	1.3	
	Proteobacteria; Bradyrhizobiaceae	2.6	1.3	1.1	2.4	2.3	3	3	
	^{1, 2} Actinobacteria; Geodermatophilaceae	1.4	1.5	0.9	2.7	2.4	2.5	3.2	
	Planctomycetes; Tepidisphaeraceae	1.6	2.4	1.7	3.2	2.3	2.1	1.7	
	Acidobacteria; Blastocatellaceae_(Subgroup_4)	1.3	1.2	2	2.5	2.9	1.5	2.1	
	Actinobacteria; Micrococcaceae	1.7	1.8	1.2	2.3	2.8	1.7	1.6	
	Firmicutes; Planococcaceae	1.4	1.2	1.9	2	2.3	1.8	2	
		AUG 2017	OCT 2017	MAY 2018	JUL 2018	OCT 2018	MAY 2019	SEP 2019	
Middle									
	² Bacteroidetes; Chitinophagaceae	14.9	17.9	17.2	9.5	4.7	13.4	17.3	
	Verrucomicrobia; DA101_soil_group	11.4	8.2	10.8	9.5	18.4	9.9	7.3	
	Proteobacteria; Bradyrhizobiaceae	4.3	3.1	3.4	3.9	4.2	4.7	4.4	
	Planctomycetes; Planctomycetaceae	3.9	3.1	4.2	3.7	5.7	4.2	2.6	
	Proteobacteria; Sphingomonadaceae	4.2	5.4	3.8	2.9	2.9	3.9	3.7	
	² Acidobacteria; Acidobacteriaceae_(Subgroup_1)	3.7	2.9	3.5	4.2	6.5	2.7	3.1	
	Acidobacteria; Solibacteraceae_(Subgroup_3)	3.6	3	3.2	4.1	4.1	3	3.6	
	Proteobacteria; Xanthobacteraceae	3.8	2.5	3.7	2.9	4.8	3.7	2.5	
	² Proteobacteria; Oxalobacteraceae	2.4	3.4	2.6	2.8	0.9	3.4	6.1	
	Planctomycetes; Tepidisphaeraceae	2.6	3	3	3	3.1	2.4	2.3	
	Proteobacteria; Acetobacteraceae	2.1	2.4	1.6	2.8	1.5	2.3	4.3	
	Firmicutes; Bacillaceae	1.7	1.9	1.6	2.2	1.9	2.1	2.4	
	² Proteobacteria; Comamonadaceae	1.8	2.2	2.1	1.9	1	2	2.3	
	² Proteobacteria; Methylobacteriaceae	1.7	2.2	1.8	1.9	0.8	2.1	2.9	
	Gemmatimonadetes; Gemmatimonadaceae	2.1	1	1.4	1.8	2.6	2.1	1.2	
		AUG 2017	OCT 2017	MAY 2018	JUL 2018	OCT 2018	JUN 2019	SEP 2019	
Upper									
	³ Acidobacteria; Acidobacteriaceae_(Subgroup_1)	9	8.9	11.4	12.9		8.7	7.8	
	¹ Bacteroidetes; Chitinophagaceae	9.2	7.8	11.3	5.7		9.8	12.5	
	³ Verrucomicrobia; DA101_soil_group	8.5	9.3	12.2	10.2		8.1	6.4	
	Acidobacteria; Solibacteraceae_(Subgroup_3)	5.2	4.8	5.8	6.5		5.3	6	
	Planctomycetes; Planctomycetaceae	4.2	4.6	6.8	6.5		4.5	4.2	
	Proteobacteria; Bradyrhizobiaceae	5.1	3.7	4.3	4.3		6.4	6.6	
	³ Proteobacteria; Acetobacteraceae	5.2	4.7	3.7	4.2		6.3	6.2	
	Proteobacteria; Xanthobacteraceae	3.4	3.1	3.5	3.3		3.6	4.1	
	Chloroflexi; HSB_OF53-F07	3.6	2.3	2.3	3.2		3.3	3	
	Chloroflexi; JG30a-KF-32	3.3	3	2.4	3.2		3.2	3	
	Planctomycetes; Tepidisphaeraceae	2.2	2.1	2.6	2.2		2.1	2.2	
	Proteobacteria; Sphingomonadaceae	2.4	3.8	2.1	1.8		1.7	1.7	
	Proteobacteria; Oxalobacteraceae	1.8	2.9	1.6	2		1.7	1.9	
	Firmicutes; ODP1230B8.23	2.2	1.9	1.4	1.6		2.3	1.9	
	Proteobacteria; Comamonadaceae	1.8	1.9	1.7	1.6		1.8	1.7	
		AUG 2017	OCT 2017	MAY 2018	JUL 2018	OCT 2018	JUL 2019	SEP 2019	

Figure B.10 The top 15 most abundant families differ across sampling dates and elevation. Numeric values in each box represent the percent read abundance with all samples binned by elevation (Lower, Middle, Upper) and sampling date. Colors represent numerical ranges with higher numbers in red and lower numbers in blue. Differences between dates were determined using differential abundance comparisons of bacterial families in DESeq2. Significant differences ($P < 0.05$) are highlighted in red with date comparisons indicated by the superscripts as follows: (1) May 2018 versus Jul 2018, (2) May 2018 versus Oct 2018, and (3) May 2018 versus May, Jun, or Jul 2019 depending on the elevation.

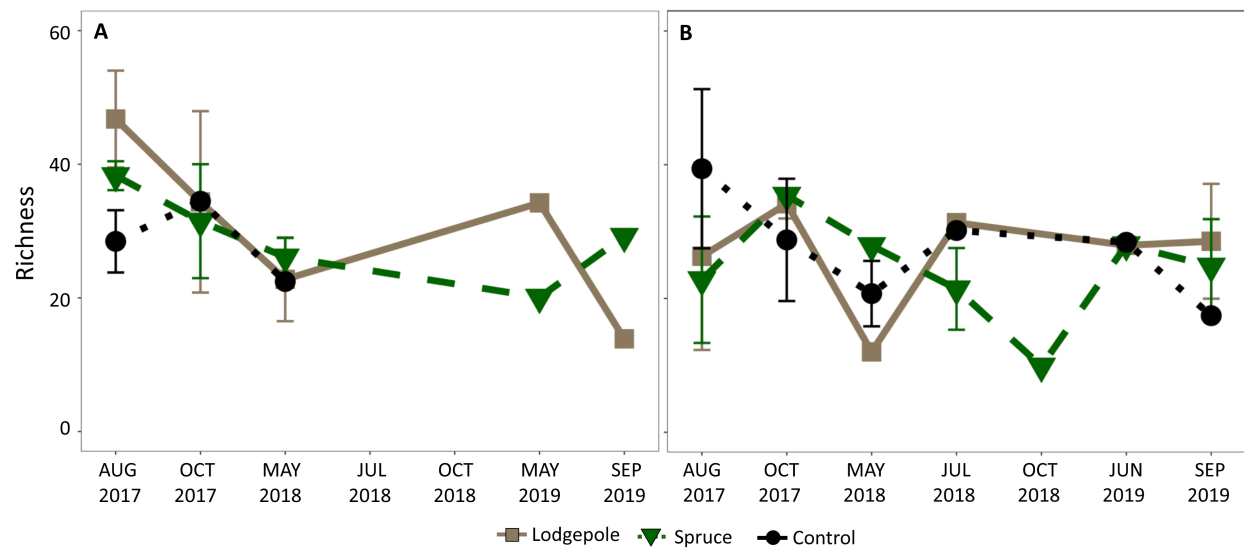


Figure B.11 18S Alpha diversity richness across elevation over time. Species richness at the Lower 18S (A) and Middle 18S plots (B). Error bars indicate plus or minus one standard deviation ($n=3, 4$). Note a lack of error bars for a sample point is due to $n<3$ caused by sample reduction during rarefaction.

Phylum									
Lower	Ascomycota	36.9	44.9	26.9	23.5		50.1	67.4	
	Cercozoa	25.8	27	39.2	48.5		21.5	3.7	
	Basidiomycota	13.4	6.4	15.5	15		10	25.3	
	Incertae_Sedis	7	5.6	6.6	4		3.4	2	
	Phragmoplastophyta	5.7	2.8	1.6	0		9.9	0	
	Apicomplexa	0.2	7.1	0.3	0		0	0	
	Chytridiomycota	2.2	2.2	1.4	4.5		0.3	0.3	
	Annelida	0	0.4	4.5	0		4.3	0	
	Peronosporomycetes	4.1	0.2	0.4	1		0	0	
	Ciliophora	1.1	1	0.1	3.5		0.2	0.5	
	Ochrophyta	0.3	0.6	0.9	0		0.3	0.5	
	Arthropoda	0.7	0.2	0.9	0		0	0.3	
	Tardigrada	0.4	0.4	0.8	0		0	0	
	Chlorophyta_ph	0.7	0.5	0	0		0	0	
	Microsporidia	0.1	0.2	0.8	0		0	0	
		AUG 2017	OCT 2017	MAY 2018	JUL 2018	OCT 2018	MAY 2019	SEP 2019	
Middle	Ascomycota	43.8	47.4	31.8	59	16.5	56.1	52	
	Basidiomycota	10.2	15.8	32.3	24.2	6.5	8.7	42.2	
	Cercozoa	14.4	13.5	15.4	6.8	0	13.8	3.1	
	Phragmoplastophyta	15.2	5	11.7	0	74	8.4	0	
	Incertae_Sedis	3.8	4.8	3.1	4.1	2	3.8	0.4	
	Chlorophyta_ph	1.6	4.4	1.8	0	0	3.3	0	
	Arthropoda	4.5	2.5	0.8	0.7	0	0.2	0.1	
	Nematoda	2.1	1.6	0.1	1.2	1	0.1	0.3	
	Chytridiomycota	0.9	0.4	0.2	1.2	0	3.5	0.1	
	Ciliophora	1.4	0.6	0.1	0.5	0	0.6	0.5	
	Tardigrada	0.6	1.1	0.4	0.5	0	0.3	0.9	
	Klebsormidiophyceae	0.7	0	2.2	0.2	0	0.3	0	
	Ochrophyta	0.3	1.4	0.2	0.6	0	0.1	0.1	
	Schizoplasmodiida	0	0.3	0	1.1	0	0.2	0.1	
	Hyphochytriumycetes	0.1	0.2	0	0	0	0.4	0	
		AUG 2017	OCT 2017	MAY 2018	JUL 2018	OCT 2018	JUN 2019	SEP 2019	
Upper	Ascomycota	29.5	48.2	31.9			43.3	33	
	Phragmoplastophyta	18.3	3.5	6.7			19.8	22.4	
	Cercozoa	14.2	6.4	20.7			7.9	15.4	
	Basidiomycota	11.3	30.6	12.8			6.3	3.8	
	Chlorophyta_ph	5.6	0.8	11.1			5.4	0	
	Incertae_Sedis	5.1	2.4	6.8			9.5	5.9	
	Arthropoda	2.5	4.8	0.6			2.4	9.3	
	Cryptomycota	3.3	0.3	1.4			1.8	0	
	Ciliophora	2.6	0.3	2			0.4	0	
	Chytridiomycota	1.1	0.8	2.3			0.9	0	
	Nematoda	0.9	0.5	1.1			0	7.7	
	Annelida	1.8	0	0.2			0	0	
	Tardigrada	0.3	0	0.1			2.2	2	
	Schizoplasmodiida	1.1	0.3	0			0	0	
	Klebsormidiophyceae	0.2	0.5	1.4			0	0	
		AUG 2017	OCT 2017	MAY 2018	JUL 2018	OCT 2018	JUL 2019	SEP 2019	

Figure B.12 The top 15 most abundant eukaryotic phyla. Numeric values in each box represent the percent read abundance with all samples binned by elevation (Lower, Middle, Upper) and sampling date. Colors represent numerical ranges with higher numbers in red and lower numbers in blue.

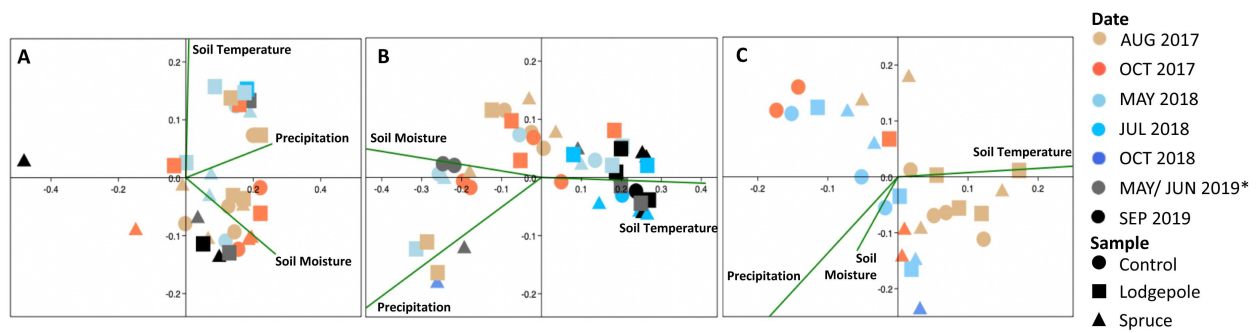


Figure B.13 Canonical correspondence analysis of environmental variables driving beta diversity community clustering as a function of soil temperature and moisture over time at the (A) Lower, (B) Middle, and (C) Middle-ES plots

*The early 2019 sampling dates were dependent on snowpack, with the Lower samples collected in May 2019, and Middle in June 2019.

APPENDIX C

CHAPTER 4 SUPPLEMENTARY INFORMATION

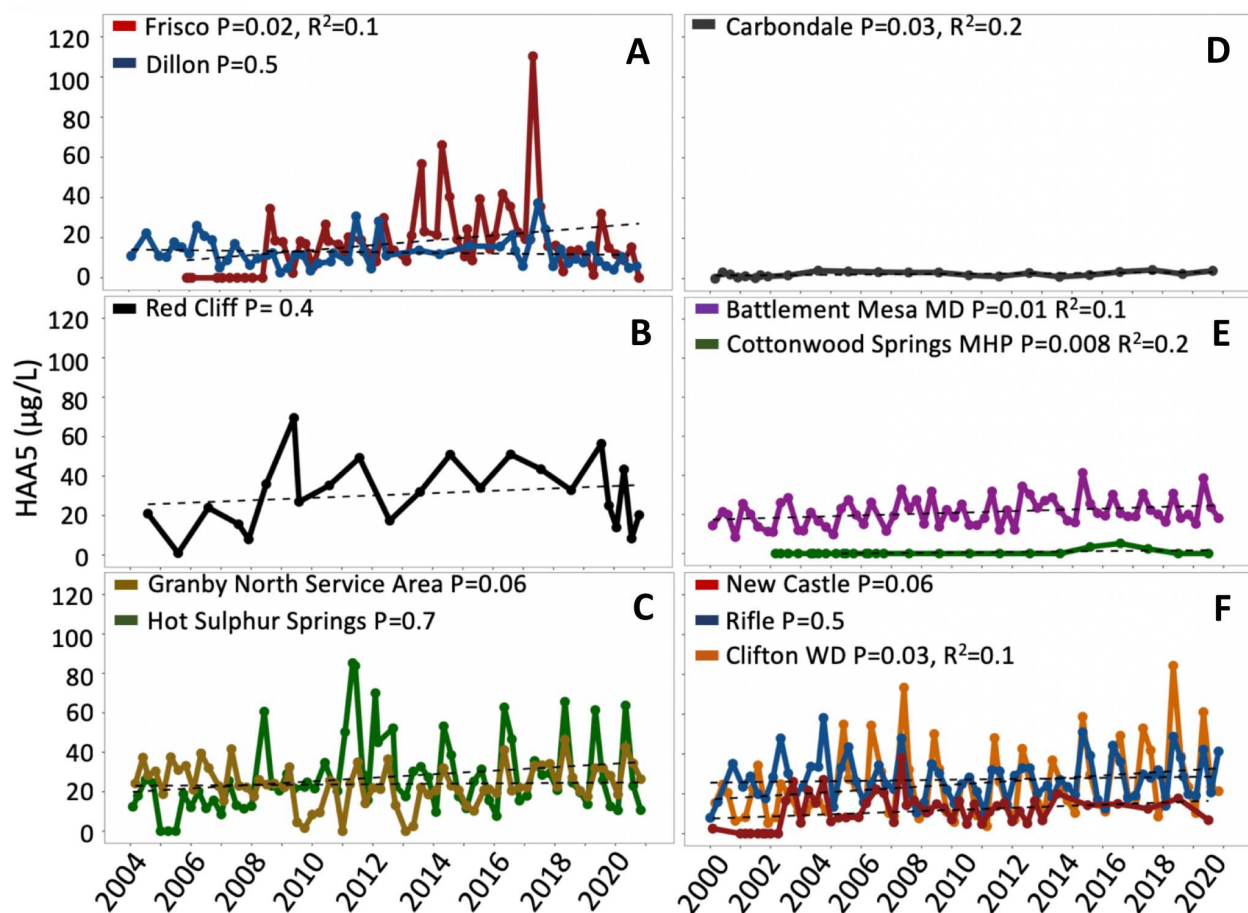


Figure C.1 Long-term increased DBP trends were observed across the Colorado Headwater subbasins. The trends in HAA5 concentrations are shown for surface water sourced municipalities within the (A) Blue, (B) Eagle, (C) Colorado Headwaters, (D) Roaring Fork, and (E, F) Plateau subbasin delineations.

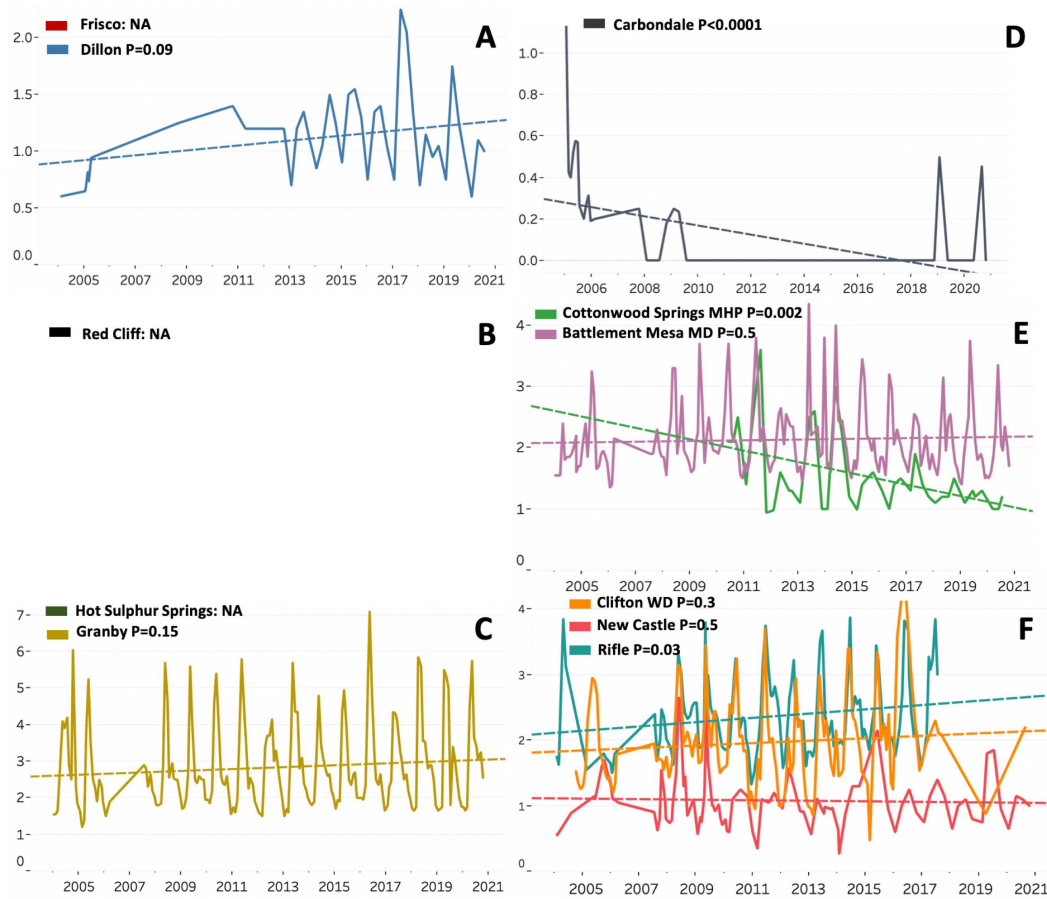


Figure C.2 Variable TOC trends across the Colorado Headwater subbasins. The trends in TOC concentrations are shown for the (A) Blue, (B) Eagle, (C) Colorado Headwaters, (D) Roaring Fork, and (E, F) Plateau subbasin delineations. A lack of data indicates no TOC concentrations were reported.

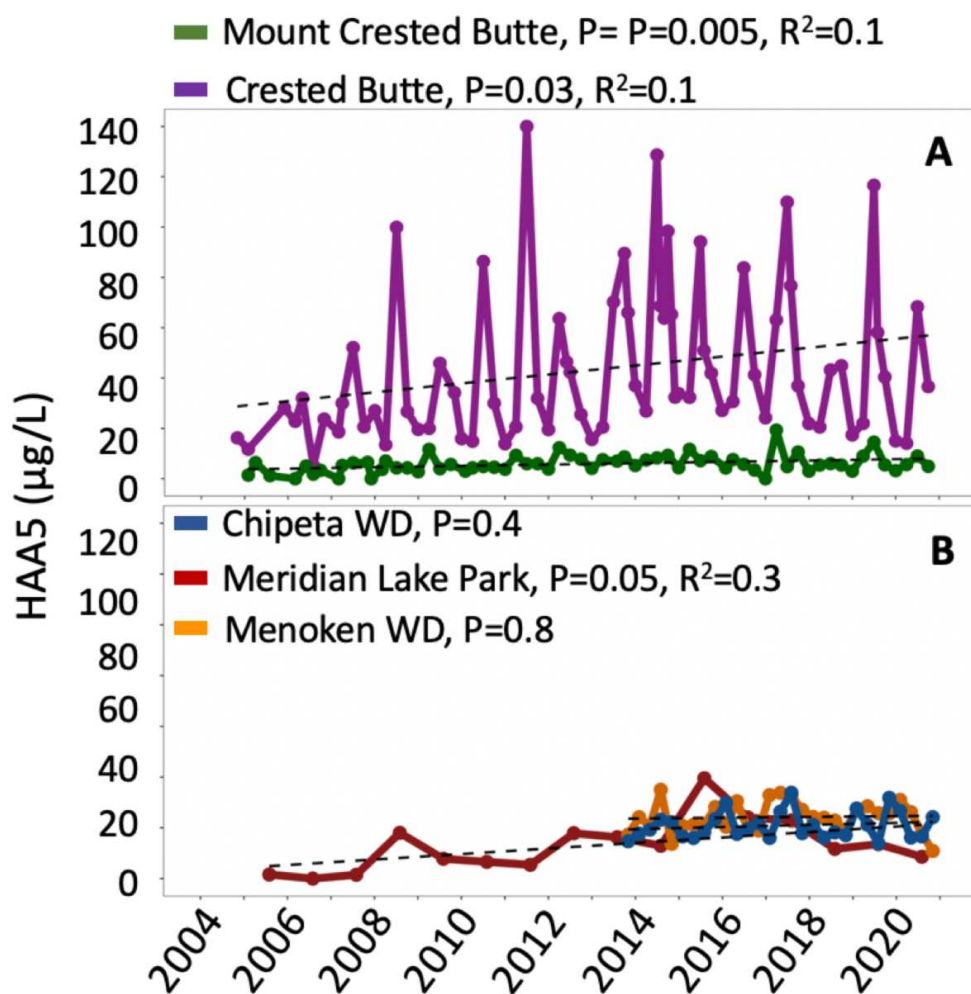


Figure C.3 Variable DBP trends in the Gunnison basin. The trends in HAA5 concentrations are shown for surface water sourced municipalities within the Gunnison basin for the (A) Crested Butte area and (B) the other three surface water locations at Meridian Lake Park, Chipeta Water District, and Menoken Water District.

Table C.1 Quarterly comparisons for quarter 1 (Q1), quarter 2 (Q2), quarter 3 (Q3), and quarter 4 (Q4) for each PWS of interest with P and R values from linear trend fits shown. No P value indicates data was not reported for that quarter.

Town	DBP	Q1	Q2	Q3	Q4
Dillon	TTHM HAA5	P=0.23 P=0.11	P<0.01, 0.6 P=0.61	P<0.01, 0.4 P=0.50	P=0.02, 0.5 P=0.24
Frisco	TTHM HAA5	P<0.01, 0.4 P<0.01, 0.5	P=0.35 P=0.40	P=0.41 P=0.39	P=0.26 P=0.21
Clifton	TTHM HAA5	P=0.12 P=0.83	P=0.01, 0.3 P=0.01, 0.3	P=0.01, 0.3 P=0.04, 0.3	P<0.01, 0.5 P=0.04, 0.3
New Castle	TTHM HAA5	P=0.01, 0.5 P=0.07	P=0.09 P=0.41	P=0.46 P=0.68	P=0.57 P=0.44
Rifle	TTHM HAA5	P=0.86 P=0.78	P=0.02, 0.4 P=0.22	P=0.08 P=0.43	P=0.53 P=0.17
Hot Sulphur Springs	TTHM HAA5	P=0.55 P=0.86	P<0.01, 0.5 P=0.06	P=0.05, 0.2 P=0.06	P=0.06 P=0.48
Granby	TTHM HAA5	P=0.10 P=0.98	P=0.02, 0.3 P=0.93	P=0.01, 0.4 P=0.55	P<0.01, 0.5 P=0.85
Battlement Mesa	TTHM HAA5	P=0.46 P=0.43	P=0.17 P<0.01, 0.6	P=0.20 P=0.66	P=0.13 P=0.02, 0.3
Carbondale	TTHM HAA5	- -	- -	P=0.8 P=0.3	P=0.5 -
Cottonwood Springs	TTHM HAA5	- -	- -	P<0.01, 0.5 0.1	- -
Crested Butte	TTHM HAA5	P=0.43 P=0.47	P=0.48 P=0.84	P=0.75 P=0.32	P=0.07 P=0.08
Chipeta WD	TTHM HAA5	P=0.41 P=0.21	P=0.14 P=0.98	P=0.46 P=0.43	P=0.46 P=0.20
Menoken WD	TTHM HAA5	P=0.39 P=0.15	P=0.22 P=0.39	P=0.01, 0.7 P=0.31	P=0.63 P=0.97
Meridian Lake	TTHM HAA5	- -	- -	P=0.01, 0.4 P=0.05, 0.3	- -
Mt. Crested Butte	TTHM HAA5	P=0.01, 0.4 P=0.16	P<0.01, 0.4 P=0.31	P<0.01, 0.6 P<0.01, 0.5	P<0.01, 0.5 P=0.15

Table C.2 Correlative Pearson P values between DBPs and each climate index for WBD8 subbasins within the South Platte watershed basin.

Basin	Subbasin	PWS	Summer Days		Ice Days		Frost Days		Total Snow	
			HAA5	TTHM	HAA5	TTHM	HAA5	TTHM	HAA5	TTHM
South Platte	Cache La Poudre	Platte River PA	0.96	0.23	0.94	0.05	0.88	0.06	0.96	0.04
	Big Thompson	Loveland	0.21	0.12	0.02	0.16	0.46	0.62	0.07	0.07
	St. Vrain	Broomfield	0.57	0.04	0.87	0.86	0.18	0.13	0.39	0.55
		Jamestown	0.19	0.18	0.27	0.90	0.08	0.06	0.52	0.21
		Louisville	0.21	0.02	0.40	0.34	0.07	0.02	0.50	0.64
		Superior MD	0.30	0.46	0.09	0.70	0.09	0.22	0.51	0.35
	Clear	Central City	0.30	0.15	0.06	0.92	0.92	0.35	0.42	0.45
		Denver	0.09	0.09	0.12	0.21	0.90	0.22	0.17	0.87
		Empire	0.24	0.29	0.08	0.42	0.41	0.28	0.73	0.76
		Silver Plume	0.16	0.12	0.86	0.75	0.07	0.03	0.20	0.11
		Thornton	0.68	0.91	0.83	0.21	0.65	0.79	0.88	0.24
		Westminster	0.67	0.44	0.36	0.03	0.33	0.73	0.07	0.23
	Upper South Platte	Antelope Hills	0.05	0.05	0.51	0.72	0.32	0.33	0.94	0.88
		Bailey WSD	0.33	0.12	0.11	0.42	0.84	0.74	0.78	0.77
		Castle Rock	0.94	0.64	0.69	0.82	0.55	0.61	0.22	0.71
		Conifer	0.05	0.18	0.58	0.60	0.08	0.96	0.85	0.49
		Elk Creek	0.22	0.41	0.42	0.33	0.01	0.95	0.24	0.64
		Homestead WC	0.53	0.58	0.15	0.99	0.35	0.30	0.42	0.96
		Lookout Mountain WD	0.49	0.47	0.35	0.24	0.06	0.69	0.18	0.61
		Pierce	0.29	0.72	0.19	0.12	0.07	0.15	0.37	0.02

Table C.3 Correlative Pearson P and coefficient values between DBPs and each climate index for WBD6 watershed delineations.

Basin	Subbasin	PWS	Summer Days		Ice Days		Frost Days		Total Snow	
			HAA5	TTHM	HAA5	TTHM	HAA5	TTHM	HAA5	TTHM
Arkansas	-	Cañon	0.25	0.24	0.15	0.05	0.03	0.00	0.76	0.49
		Colorado Springs	0.51	0.54	0.60	0.12	0.02	0.01	0.37	0.29
		Holcim	0.54	0.52	0.69	0.40	0.08	0.74	0.29	0.95
		La Veta	0.67	0.48	0.10	0.96	0.22	0.57	0.01	0.27
		Palmer Lake	0.30	1.00	0.01	0.53	0.15	0.63	0.62	0.95
		Rainbow Valley	0.31	0.23	0.96	0.17	0.12	0.97	0.31	0.98
		Rock Creek Mesa	0.45	0.13	0.05	0.49	0.33	0.38	0.44	0.76
		Rye	0.35	0.24	0.05	0.02	0.31	0.33	0.17	0.46
		Security WSD	0.07	0.14	0.17	0.01	0.24	0.01	0.25	0.11
		Walensburg	0.32	0.52	0.71	0.96	0.19	0.89	0.33	0.49
San Juan	-	Dove Creek	0.86	0.52	0.61	0.33	0.19	0.48	0.25	0.04
		Durango	0.65	0.28	<0.01	0.42	0.48	0.01	0.47	0.15
		El Rancho FI MD	0.75	0.88	0.13	0.11	0.16	0.19	0.64	0.93
		Lake Durango	0.14	0.46	0.06	0.32	0.30	0.89	0.55	0.35
		Mancos Rural WC	0.16	0.33	0.29	0.05	0.13	0.03	0.12	0.07
		Narrow Gage MHP	0.33	0.98	0.63	0.86	0.82	0.48	0.57	0.05
		Van Den Berg MD	0.86	0.81	0.85	0.90	0.62	0.14	0.79	0.01
Dolores	-	Naturita	0.43	0.79	0.76	0.74	0.02	0.03	0.36	0.10
		Ophir	0.71	0.43	0.70	0.21	0.47	0.50	0.03	0.41
		Telluride	0.43	0.79	0.76	0.74	0.02	0.03	0.36	0.10
Upper White	-	Rangely	0.05	0.24	0.04	0.59	0.49	0.16	0.14	0.22
		Steamboat Springs	0.57	0.42	0.59	0.85	0.92	0.84	0.18	0.32
		Trinidad	0.51	0.14	0.65	0.99	0.95	0.74	0.58	0.05

Table C.4 Treatment process overviews reported by each public water system to the Colorado Department of Public Health and Environment (CDPHE, 2021b).

Dillon	-
Frisco	Infiltration gallery with screening, coagulant addition/in-line static mixing, membrane filtration, gaseous chlorination for disinfection and chlorine contact time through a baffled contact tank, distribution system and three finished water storage tanks.
Clifton WD	Membrane filtration with gas chlorination for disinfection. Pretreatment with coagulation, flocculation and sedimentation. Reduction of total dissolved solids by blending reverse osmosis permeate into finished water.
New Castle	Direct filtration including coagulation, clarification, and multi-media filtration followed by disinfection via post gas chlorination with contact time provided in two baffled clearwells typically operated in series prior to EP sampling.
Rifle	Pretreatment consists of adding chlorine dioxide and Alum at a flash mix ahead of the flocculators. Flocculation includes two trains of 3-stage flocculation basins with mixers. The clarification step includes two trains of plate settlers. Clarification is followed by three microfiltration skids for compliance filtration. Sodium hypochlorite is added to the filtered water and disinfection contact time is achieved through a constant level disinfection contact basin.
Hot Sulphur Springs	Coagulation and membrane filtration with calcium hypochlorite disinfection
Granby	-
Battlement Mesa MD	PAC feed, coagulant and coagulant aid feeds, 2 clariflocculators, filter aid feed, 4 multimedia filters, gas chlorine disinfection, and contact time provided via clearwell, pipeline, and serpentine baffled chlorine contact chamber. House water contact time provide by clearwell and two Flexcon Mixmaster tanks in series.
Carbondale	Roaring Fork WTP includes two types of filtration with sodium hypochlorite disinfection. Disinfection includes with the same sodium hypochlorite disinfection treatment as the membrane trains. Sodium hypochlorite is added to raw water at the Roaring Fork Well House Pump Station for chlorine contact time in the pipeline prior to the filtration process. Chlorine is also added to filtered water prior to the WTP clearwell. Chlorine contact can be achieved via two disinfection segments: 1) prefiltration pipeline and 2) baffled clearwell at the WTP. Treated water is pumped from the clearwell into the distribution system.
Cottonwood Springs	-
Crested Butte	Raw water is pretreated with potassium permanganate and reaction time provided in the Reaction Tank. Water flows from the tank through membrane filtration, pH and alkalinity adjustment using sodium hydroxide, calcium hypochlorite disinfection, and through a clearwell and water storage tanks for disinfection contact time.
Chipeta WD	-
Menoken WD	-
Meridian Lake	Membrane filtration with sodium hypochlorite disinfection. Sodium hydroxide (caustic soda) is used to raise treated water pH for distribution system corrosion control. UV disinfection equipment is installed but is not used for surface water treatment disinfection compliance.
Mt. Crested Butte	Rapid Mix Nalco 8157 aluminum chloride, Flocculation, Upflow Clarifier, Filtration - Nalco 8170 polymer filter aid, Disinfection Contact Basin. Dedicated basin for disinfection, serpentine baffle configuration, calcium hypochlorite tablet chlorinator. Ultraviolet Radiation Finished Water Storage: Timberland Tank No. 1 Timberland Tank No. 2 Distribution System pumping

APPENDIX D
CHAPTER 5 SUPPLEMENTARY INFORMATION

Table D.1 Snow telemetry (SNOTEL) climate summaries for Crested Butte, elevation 3,100 m.

Water Year (Oct-Sep)	Dry Soil ¹	Hot Days ²	Ice Days ³	Frost Days ⁴	Total Precipitation (cm SWE)	Snow (cm SWE)
2015	0	49	52	183	79	38
2016	39	60	66	196	82	43
2017	143	73	59	171	94	61
2018	88	38	174	48	31	31
2019	44	69	73	205	88	63
2020	286	83	71	203	62	39
AVG (1990-2010)	132 ± 90	70 ± 27	75 ± 29	206 ± 31	80 ± 15	48 ± 14

Notes:

¹Total days max soil water <10% at 20 cm depth. Data was available starting in 2006, thus the average is 2006-2016

²Total days with TMax >20 ° C

³Total days with TMax <0 ° C

⁴Total days with TMin <0 ° C

Table D.2 DBP fractions of the regulated HAA5 and TTHM groups present in the historical data. The groups include trichloroacetic acid (TCAA), dichloroacetic acid (DCAA), chloroform (TCM), bromodichloromethane (BDCM), and dibromochloromethane (DBCM)

Date	HAA5		TTHM		
	TCAA	DCAA	TCM	BDCM	DBCM
1/9/15	56%	40%	87%	13%	0%
4/1/15	55%	41%	81%	19%	0%
7/9/15	58%	40%	96%	4%	0%
8/12/15	44%	50%	86%	14%	0%
10/8/15	55%	45%	87%	13%	0%
1/12/16	53%	47%	85%	15%	0%
10/6/16	54%	43%	82%	18%	0%
1/19/17	54%	39%	85%	15%	0%
4/19/17	50%	44%	80%	20%	0%
7/5/17	58%	40%	98%	2%	0%
8/9/17	53%	44%	94%	6%	0%
10/11/17	52%	44%	80%	2%	18%
1/24/18	52%	48%	84%	16%	0%
4/11/18	58%	42%	63%	30%	7%
7/12/18	51%	46%	86%	13%	1%
10/10/18	60%	40%	84%	15%	1%
1/10/19	61%	39%	77%	23%	0%
4/10/19	58%	42%	74%	22%	4%
7/10/19	60%	40%	98%	2%	0%
8/14/19	59%	41%	92%	8%	0%
10/9/19	58%	42%	92%	8%	0%
1/9/20	56%	44%	75%	25%	0%
4/7/20	45%	45%	75%	25%	0%
7/7/20	61%	39%	95%	5%	0%
10/14/20	59%	41%	89%	11%	0%

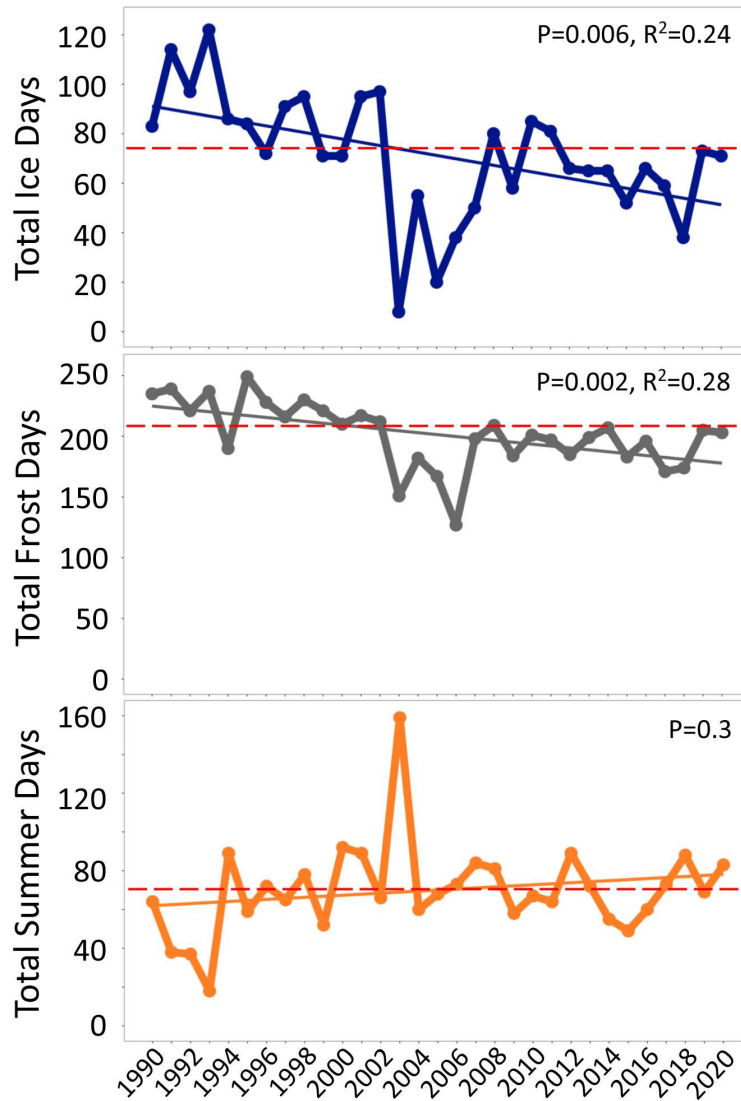


Figure D.1 Climate trends in Crested Butte for total ice, frost, and summer days. The 1990-2010 normal value is shown in the dotted red line.

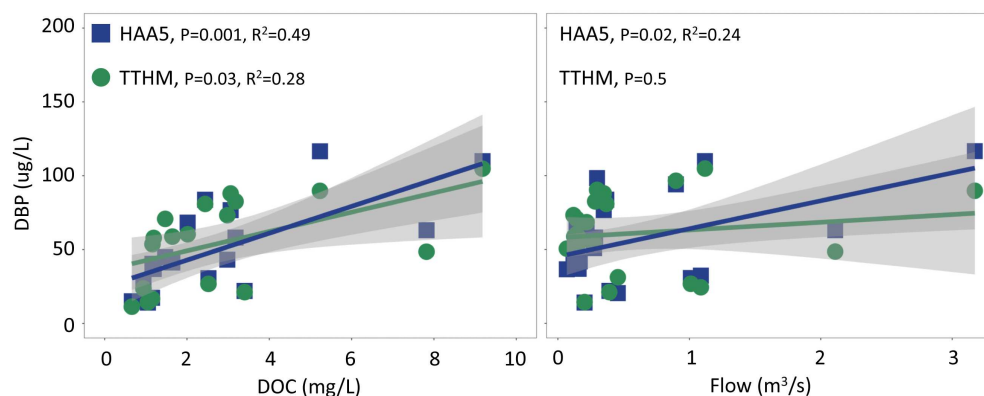


Figure D.2 Correlation plots between peak DBP concentrations and the same-day flow and DOC concentrations. Values represent the five-year analysis of daily Coal Creek DOC concentrations and flow from the USGS hydrograph associated with the quarterly reported DBP concentrations.

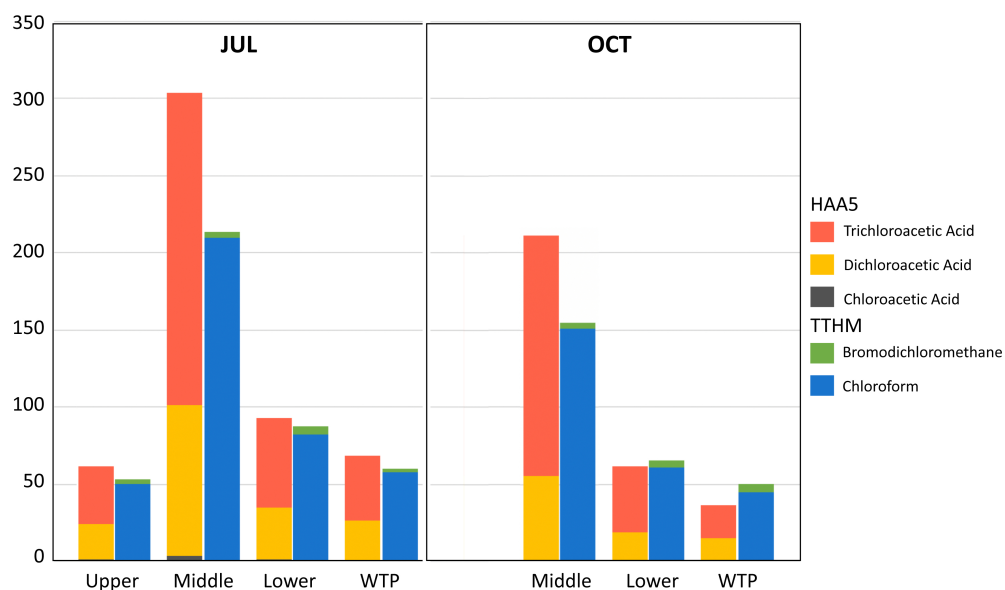


Figure D.3 Major DBP group comparisons across the 2020 sampling locations for July and October.

Table D.3 Average monthly DOC loading per year.

Month	Year	Average DOC (kg/day)
Apr	2016	125
	2017	624
	2018	1077
	2019	388
	2020	35
May	2016	787
	2017	2863
	2018	1907
	2019	653
	2020	1206
Jun	2016	1537
	2017	4407
	2018	296
	2019	3549
	2020	1391
Jul	2016	105
	2017	398
	2018	38
	2019	1239
	2020	34
Aug	2016	33
	2017	30
	2018	8
	2019	83
	2020	10
Sep	2016	14
	2017	11
	2018	10
	2019	17
	2020	10
Oct	2016	17
	2017	18
	2018	21
	2019	12
	2020	8

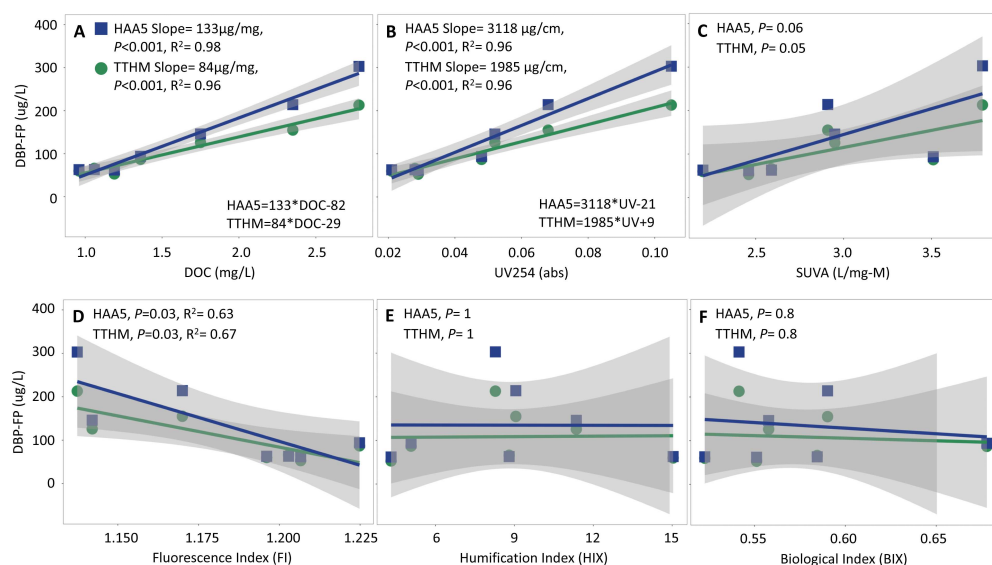


Figure D.4 Correlation plots between (A, B, C) quantitative metrics of DOC, UV₂₅₄, and SUVA as well as (D, E, F) qualitative EEM indices from the 2020 transect samples.

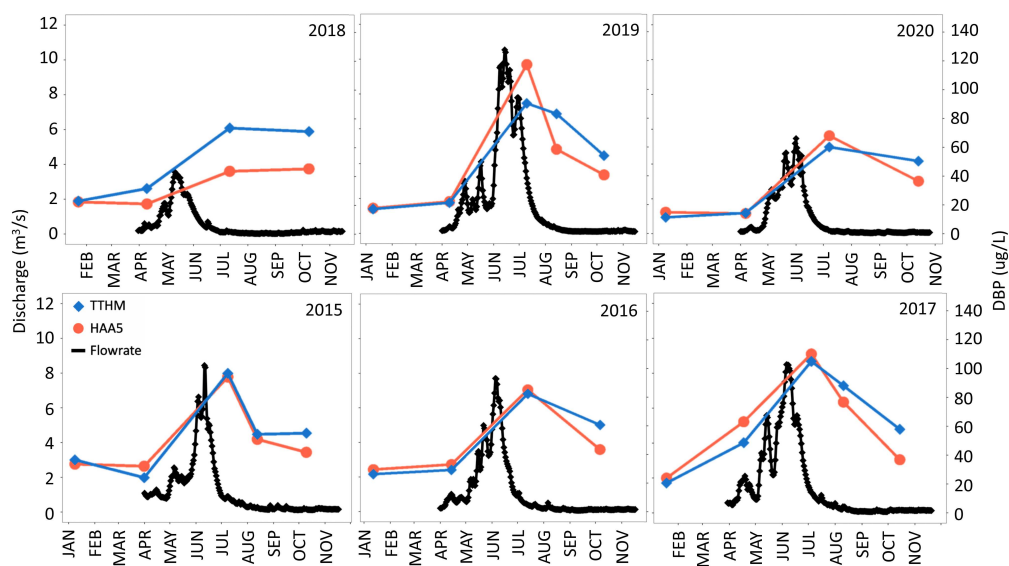


Figure D.5 Annual comparison of discharge and quarterly DBP concentrations.

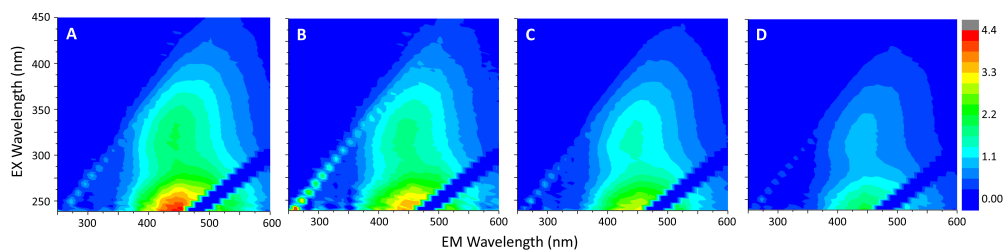


Figure D.6 EEM spectra for select samples in 2021 reveal Ohio Creek and CC-7 as hot spots for organic sources. (A) Ohio Creek, (B) CC-7, (C) Middle, and (D) Lower sample transects are shown.

Table D.4 UV₂₅₄ and DOC results from 2021 transect sampling with estimated DBP values based on linear correlation equations.

Sample	UV ₂₅₄ (abs)	Estimated HAA5 (ug/L)	Estimated TTHM (ug/L)	DOC (mg/L)	Estimated HAA5 (ug/L)	Estimated TTHM (ug/L)
IR-00	0.08	218	161	2.5	248	179
CC-2	0.17	496	338	4.7	548	369
CC-3	0.16	478	327	4.5	514	347
Coal-30	0.17	507	345	4.7	544	367
CC-5	0.17	524	356	4.9	563	378
Ohio	0.34	1044	687	8.8	1082	706
CC-6	0.24	717	479	5.7	679	451
CC-7	0.34	1024	675	8.4	1031	674
CC-8	0.27	834	553	7.0	842	555
Splains	0.23	693	464	-	-	-
CC-9	0.25	769	512	6.6	790	522
Elk-00	0.10	290	207	-	-	-
Elk-East	0.04	103	88	-	-	-
Coal-20	0.26	778	518	6.5	776	513
Coal-15	0.23	681	456	5.7	669	446
CC-10	0.22	656	440	5.5	653	436
Evans	0.04	97	84	-	-	-
CC-11	0.19	575	389	5.2	610	408
Coal-12	0.20	607	409	5.4	642	428
BOG-00	0.03	59	60	-	-	-
BOG-02	0.02	49	53	-	-	-
BOG-03	0.03	78	72	-	-	-
BOG-04	0.04	112	93	-	-	-
CC-11.5	0.20	599	404	5.2	614	410
Coal 11	0.19	564	382	4.9	567	381
Keystone	0.02	46	52	-	-	-
Coal-5	0.17	511	347	4.6	535	361
Red Lady	0.02	34	44	-	-	-
Wild-00	0.11	320	226	3.3	358	249
CC-12	0.16	464	318	4.4	501	339
Coal-2	0.16	481	328	4.5	519	351

APPENDIX E

ACCELERATED SNOWMELT PROTOCOL TO SIMULATE CLIMATE CHANGE INDUCED
IMPACTS ON SNOWPACK DEPENDENT ECOSYSTEMS

A publication in *Bio-protocol*¹

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E.1 Abstract

Field studies that simulate the effects of climate change are important for a predictive understanding of ecosystem responses to a changing environment. Among many concerns, regional warming can result in advanced timing of spring snowmelt in snowpack dependent ecosystems, which could lead to longer snow-free periods and drier summer soils. Past studies investigating these impacts of climate change have manipulated snowmelt with a variety of techniques that include manual snowpack alteration with a shovel, infrared radiation, black sand and fabric covers. Within these studies however, sufficient documentation of methods is limited, which can make experimental reproduction difficult. Here, we outline a detailed plot-scale protocol that utilizes a permeable black geotextile fabric deployed on top of an isothermal spring snowpack to induce advanced snowmelt. The method offers a reliable and cost-effective approach to induce snowmelt by passively increasing solar radiation absorption at the snow surface. In addition, control configurations with no snowpack manipulation are paired adjacent to the induced snowmelt plot for experimental comparison. Past and ongoing deployments in Colorado subalpine ecosystems indicate that this approach can accelerate snowmelt by 14-23 days, effectively mimicking snowmelt timing at lower elevations. This protocol can be applied to a variety of studies to understand the hydrological, ecological, and geochemical impacts of regional warming in snowpack dependent ecosystems.

E.2 Background

The intergovernmental panel on climate change reported in 2014 that over a period of 1976-2012 snow cover in the Northern Hemisphere has decreased by 11.7% per decade in the month of June (IPCC, 2014). Snowpack storage is crucial for headwater regions, and early water release could lead to water resource management and ecosystem function issues (Elias et al., 2015; Demaria et al., 2016). It has been predicted that snowmelt timing in the western U.S. could be enhanced by as much as two months by the end of this century (Rauscher et al., 2008), while a more recent study (Clow et al., 2016) reported advanced snowmelt offset by 1-2 weeks over the past two decades in Colorado, which is an essential headwater state. Shorter periods of snow cover and earlier water release have the potential to affect several aspects of ecosystem health in mountainous environments such as water availability and storage (Barnett et al., 2005), plant phenology and succession (Livensperger et al., 2016), and the soil microbial community structure (de Vries and Griffiths, 2018). With several aspects of the environment impacted by shifts in snowmelt timing, it is helpful to have a reliable and reproducible method that enables paired comparisons of enhanced and natural snowmelt across researchers and geographic regions.

A variety of methods have been used to manipulate snowmelt rates. Some have used a shovel to remove snow depth (Wipf et al., 2006), while others implemented more involved methods such as infrared heating sources (Harte et al., 1995; Bokhorst et al., 2008), or distributing black sand across the snow surface area (Steltzer et al., 2009; Blankinship et al., 2018). In contrast to these methods, the protocol described here is a noninvasive, low cost, and easily reproducible method that increases the absorption of solar radiation with a permeable black fabric deployed directly on the snow surface (Walsh et al., 2003; Steltzer et al., 2009). In addition, the method enables paired contrasts between accelerated and natural snowmelt processes in adjacent plots with conserved water equivalents. In contrast to black sand applications, the fabric is removed once the snow has fully melted and does not leave residual non-native material in associated soils. This refined protocol builds upon insights from past studies using permeable fabric covers (Steltzer et al., 2009) with a goal of increasing method utility, adoption and reproducibility. More recent efforts from 2017-2019 in Crested Butte, Colorado have documented snowmelt advancement of 14-23 days when contrasting manipulated and control plots at an elevation of 3,170 meters (Figure E.1). The protocol and equipment explained below allows others to establish, deploy and monitor several aspects of the snowmelt process and can be adapted for a variety of environmental studies including but not limited to plant litter decay processes, geochemical shifts in the hydrosphere, and local plant phenology and growth.



Figure E.1 Visual representation of tarp deployment and resulting accelerated snowmelt during spring 2019 manipulation. A. The geotextile fabric was deployed after an isothermal snowpack was established. B. Removal of the tarp reveals the clear influence of this protocol on enhanced melting in the target plot. C. Contrast of the accelerated snowmelt plot with the control snowmelt reveals localized, accelerated onset of plant growth in the manipulated plot. Note, white rings visible within the area of manipulation were used for a separate experiment and are not discussed in this protocol (B,C).

E.3 Materials and Reagents

A. Snow cover and anchors

1. Permeable black UV PE Knitted Shade Cloth 50%: 9.8 x 9.8 m with hemmed grommets spaced every 1.2 m (Agriculture Solutions, catalog number: KS50)
2. Type III 550 paracord 60 m spool (Paracord Planet)
3. 12 Pack aluminum alloy locking Carabiners (Bondream)
4. Four 1.1 cm x 15.2 cm wooden dowels (Hardware Store)
5. Four 5 cm x 10 cm x 12 cm wood blocks (Hardware Store)
6. Drill with a 6.35 mm bit (Hardware Store)

B. Snow stakes and plot markers

1. Four PVC pipes 2.5 cm x 3 m; Plain end PVC Schedule 40 Pressure Pipe (Hardware Store)
2. Multi-Colored Duct Tape (Hardware Store)
3. Four 1.3 cm x 1.2 m 4 Rebar (Hardware Store)
4. Small sledge hammer (Hardware Store)

E.4 Equipment

A. Deployment

1. Post hole digger or metal shovel (Hardware Store)
2. 35.5 cm Big Grip Garden Knife (Hardware Store)

B. Data analysis

1. Two Hobo H21 Data Loggers (Onset HOBO data logger, catalog number: H21-002)
2. Four Hobo moisture probes (Onset HOBO data logger, catalog number: S-SMD-M005)
3. Four Hobo temperature probes (Onset HOBO data logger, catalog number: S-TMB-M006)
4. Two Chemical resistant washdown enclosures 22.9 cm x 22.9 cm x 11.4 cm (McMaster-Carr, catalog number: 8261k27)
5. Ultra-abrasion resistant expandable sleeving stainless steel 1.3 cm ID, 3 m length (McMaster- Carr, catalog number: 1478T3)
6. 250 g Plumber's putty (Hardware Store)
7. TimelapseCam Pro camera (Wingscapes, catalog number: WCB-00121)
8. At least 6 Lysimeters (15.2 cm Soil Moisture lysimeters, catalog number: 1905L06)

E.5 Procedure

A. Plot Selection: During fall snow-free season

1. Find a location for experimentation with annual snowfall that allows for snowpack manipulation: Ensure the location is not heavily forested or shaded and has a slope aspect that receives direct sunlight radiation throughout the day. In addition, seasonal human and animal traffic should be considered. It is possible that snowmobilers and skiers may frequent the area and precautions may be necessary to prevent damage to the plot. In our case, the PVC snow stakes were successful in designating the area and prevented most human interference.

2. The control and manipulated plots should be positioned horizontally adjacent at the same elevation and slope aspect to allow for comparison while also preventing interference between the two plots.

B. Snow fabric preparation: During fall snow-free season

1. Choose a geotextile fabric surface area larger than the predetermined plot perimeter under investigation to allow for error in deployment placement as well as to minimize boundary effects. Here, we utilized a 9.8 x 9.8 m geotextile that was deployed over a 7.6 x 7.6 m experimental grid (Figure E.2).
2. Cut four 0.6 m lengths of paracord and thread each through the grommets of the four corners of the fabric and tie the ends to a carabiner. These ends will serve as attachments to the corner snow anchors described in Procedure C and as shown in Figure E.2.
3. Cut two lengths of paracord that will thread across the full length and width of the geotextile fabric as shown in Figure E.2. For our experimental area, two 13 m lengths of rope were cut. When choosing a length, ensure enough extra paracord (1-1.5 m) is allowed on each end of the tarp to anchor the fabric and allow tension during snowmelt for the fabric to fall flush with the melted snow surface.
4. Drill holes in the middle of the wooden dowels with a 6.35 mm or larger drill bit to ensure the paracord will thread through the hole.
5. Thread one stretch of the pre-cut paracord along the length and the other along the width of the fabric through the middle edge grommets and tie the ends of the paracord through the drilled holes of the wooden dowels (Figure E.2).
6. Determine the logistics of deployment and if it is feasible to leave the geotextile fabric folded nearby the plot under study to be recovered during deployment. If not, plan to travel with the tarp during deployment. In our case, we traveled with the snow fabric tied to a travel pack.

C. Snow stake deployment: During fall snow-free season

1. Prepare the snow stakes. In our case we used a 3 m PVC pipe length; however, if a higher snowpack is expected the PVC pipe may need to be longer than documented here. Mark each sequential height of one foot with multicolored duct tape on each pipe to allow for approximate snow depth measurements. Multicolored tape is suggested to ensure ease of measuring sequential heights from the time-lapse photos.

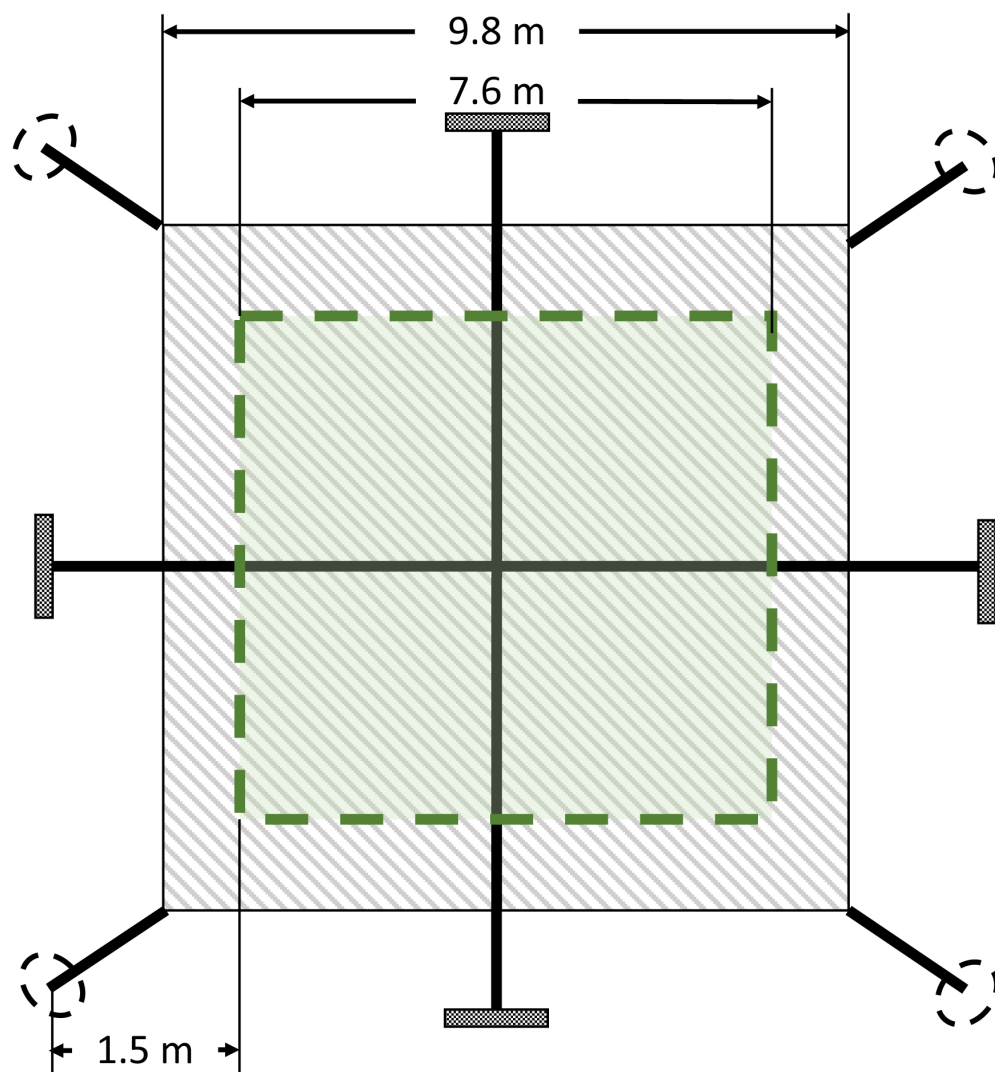


Figure E.2 Geotextile fabric schematic according to our plot dimensions. Perimeter sizing is easily customizable for different plot sizes. Here our plot dimensions are shown with a square 7.6 x 7.6 m plot (green dashed square), and a 9.8 x 9.8 m geotextile fabric (larger shaded square). The rope lengths should be long enough (at least 1.5 m from the plot) to maintain sufficient tension over the desired plot while allowing the fabric to stay flush against the snow surface area. Carabiners are shown at the fabric corners (dashed ovals) and dowels are shown tied to the ropes threaded across the middle length and width of the fabric (rectangles). Note dowels, rope and carabiner objects are enlarged for visual purposes and are not to scale.

2. Prepare snow anchors: drill holes in the middle of the wooden blocks with a 6.35 mm or larger drill bit to ensure the paracord will thread through.
3. Drill a hole at the bottom and top of the PVC pipes on the same side approximately 15 cm from the ends using a 6.35 mm or larger drill bit.
4. Thread the paracord through the drilled hole at the top of the PVC pipe, tying it securely at the top of the pipe. Thread the tied portion of paracord along the outside of the pipe and into the bottom drilled hole and out through the bottom of the pipe leaving 0.3-0.6 m of free paracord.
5. Tie the free paracord end to the wood block through the drilled hole and do this for all four PVC pipes (Figure E.3).
6. Determine the desired snowmelt area by providing at least 1.5 m extra distance from the perimeter of the plot under investigation for anchors to be placed to secure the fabric (Figure E.2). Hammer the rebar into the four corners of the determined anchor perimeter until half of the rebar is above the soil surface.
7. Place the PVC pipes over each rebar with the bottom of the pipe flush against the soil surface to stand in an upright position during the snow season. Ensure the wood block is located at the bottom of the PVC pipe.
8. Extend the wooden block at the bottom of the PVC pipe outward from the plot to serve as a snow anchor during snowfall.

D. Data logger assembly: During fall snow-free season

1. Prepare the soil moisture and temperature probes for the induced snowmelt and control plots following manufacturer instructions. You should have at least two of each probe placed in the center of both plots to ensure redundancy.
2. Avoid leaving loose wires open to the environment by threading the probe wires through the abrasion resistant steel covers for resistance against critters.
3. Use the garden knife to make a cut into the soil for the temperature (6 cm depth) and moisture probes (6-12 cm depth) and insert the sensors into each cut.

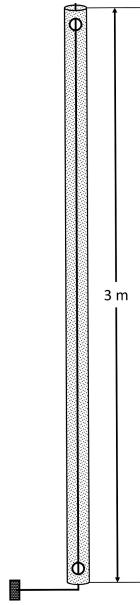


Figure E.3 Snow stake schematic. The anchoring design is shown with holes (circles) drilled in the PVC pipe with paracord (black line) threaded along the height of the pipe to the bottom. The drilled hole at the top of the pole enables quick access to the paracord when the snow has fallen, and the bottom hole is an attachment for the wood block to serve as a snow anchor (bottom rectangle).

4. Place the data logger in the respective waterproof enclosure for each plot to avoid weather damage.
You will need to drill a hole in one side of each box to allow the sensor wires to thread through into the box. Once the sensor wires are threaded through, use plumber's putty to seal the drilled hole.
5. Connect the sensors to the data logger station using manufacturer instructions and ensure your computer is connected correctly to the logger using the Hoboware software provided. Set the logger to begin logging hourly data acquisition.
6. Find a landmark such as a tree nearby to secure the time-lapse camera, or use a tripod to capture snowmelt progression directly above the plots. Ensure the camera is higher than the expected snowpack height to ensure photos are collected daily.
7. Set the camera to activate daily time series using manufacturer instructions. In our case, the time series was set for collection every 24 h in the afternoon.

E. Snow fabric deployment: During spring peak snowpack

1. Determine the best time frame for deployment: Ideally the snowpack will be isothermal and entering a net seasonal melting cycle. Our deployment was conducted between April 10-25 in 2017-2019 in

Colorado at 3,170 m.

2. It is best to determine a time when peak snowpack is reached, and no further large snow storms are forecasted. Over the years 2017-2019 snowpack during deployment ranged 1.2-1.5 m.
3. Access to the site can vary depending upon conditions and distance. Snowshoes or backcountry skis work well with minimal damage to the site; however, snowmobile access is fastest for longer deployment distances.
4. Once at the site with the pre-deployed snow stakes identified, lay down the prepared fabric with the threaded paracord facing upward and accessible.
5. Align the corners of the fabric with the snow stake anchor points. Untie the pre-deployed paracord from the top of the snow stakes and tie each tight to the carabiners that are attached to the corner grommets of the fabric (Figure E.4A). Note that the end of the untied paracord will be buried in the snow at the bottom of the snow stake.
6. Dig a trench of increasing depth in the snow from the corners of the geotextile along the length of the paracord to achieve roughly a 45-degree angle to ensure tension on the corners as the snow melts.
7. Extend the length of the paracord attached to the grommets at the middle length and width of the fabric to secure the four middle edges with the wooden dowels (Figure E.4B).
8. Dig a tapered trench of increasing depth in the snow under the paracord from the edge of the fabric to the end where the wooden dowels will be buried to maintain a 45-degree angle. Use the garden knife to lead the tapered trench and use the post hole digger to dig a hole at the deepest depth of the snow. For our deployments, a tapered trench that reached a 1.2 m deep hole 1-1.5 m from the fabric edge was sufficient.
9. Bury the dowels by placing the dowel at the bottom of each hole and fill the holes with snow.
10. Ensure the fabric is flush to the snow surface and secure at all four corners and middle lengths of the perimeter (Figure E.4C).

F. Snow fabric Removal: During spring snowmelt Remove the geotextile fabric. This should be when the manipulated snowmelt plot boundary is 60- 80% bare ground. Over the years of 2017-2019, the geotextile was removed within a date range of May 30-June 7 to achieve 80% bare ground.

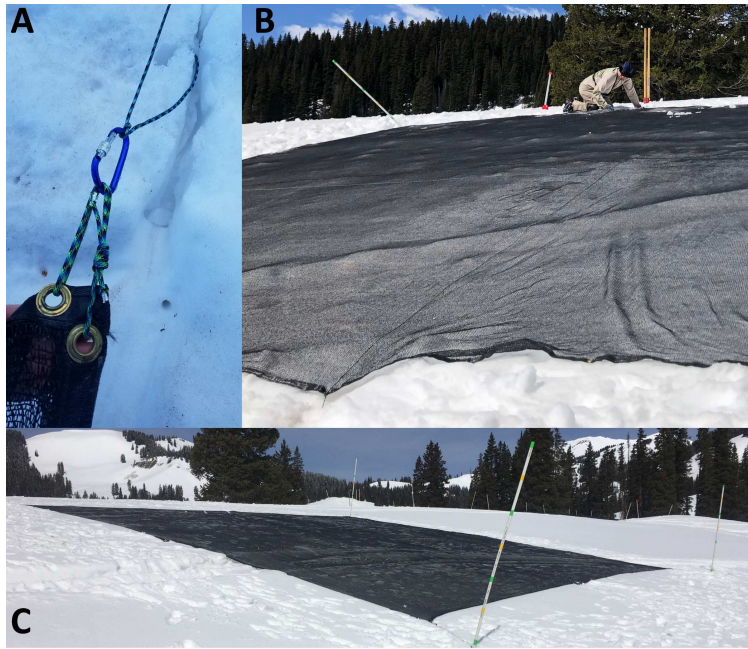


Figure E.4 Geotextile fabric after a successful deployment. A. The corners of the geotextile fabric are tied securely to a carabiner and the paracord from the top of the snow stakes. B. The paracord threaded across the length and width of the fabric is anchored by the wooden dowels buried 1.2 m deep in the snow 1.5 m away from the fabric edge at a 45-degree angle. C. The fabric is shown deployed flush to the snow surface.

E.6 Data Analysis

Visually over time, the induced snowmelt should be apparent from the time-lapse photos. The Onset Hoboware software downloadable on any computer will provide outputs of the hourly data during the experiment. It should be clear that the induced snowmelt plot has melted out before the paired control plot and it is possible to infer the time difference using the soil moisture and temperature results while visually validating dates with the time-lapse camera (Figure E.5).

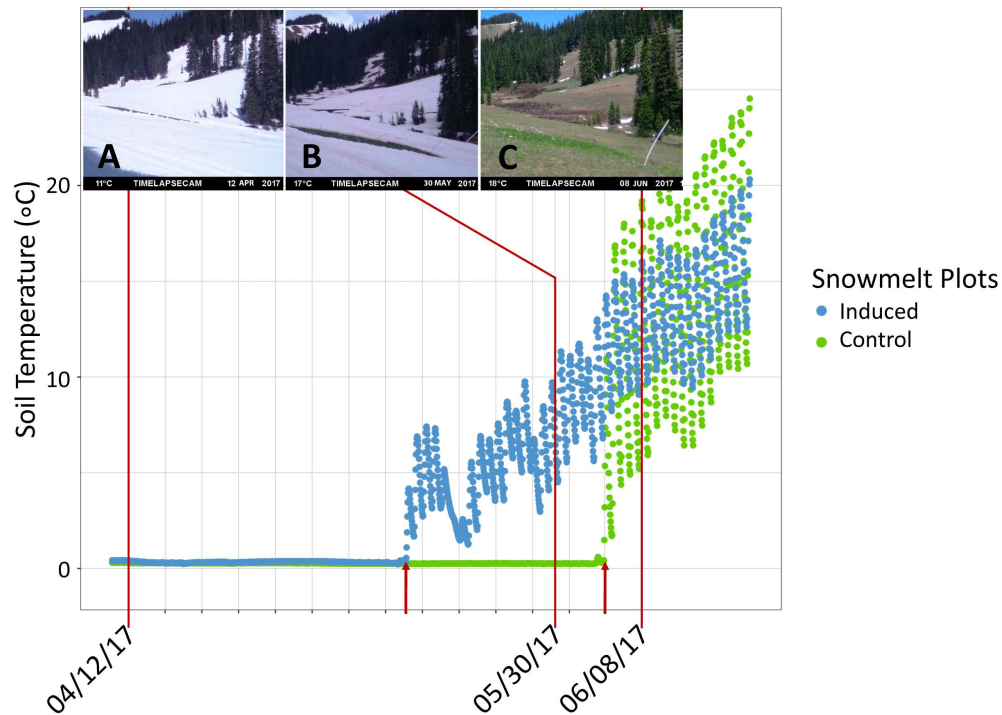


Figure E.5 Data analysis to determine advanced snowmelt timing in 2017. Use of the time-lapse camera with the soil moisture temperature data will help determine the dates of full melt-out. The fabric was deployed on April 12th (A), and melt-out of the induced plot appears to have occurred on May 13th (first arrow) based on the soil temperature increase; however, the fabric was removed on May 30th (B). New plant growth is apparent in the former location of the tarp demonstrating accelerated plant growth associated with snowmelt manipulation (C). Natural melt-out of the control plot began on June 4th (second arrow), and the full control plot melted completely on June 8th (C). It can be concluded from these observations that the induced snowmelt was advanced by three weeks.

Notes

1. This method allows a variety of hydrological and ecological aspects of a plot-scale field experiment to be tested for additional analysis. Porewater can be collected using lysimeters; for example, in our experiment 20 lysimeters were utilized with a grid of 4 x 5 across each transect of the square plots. This value however is dependent on the experimental design and should be determined within the scope of each experiment. A minimum of six is suggested assuming three for the manipulated plot and control each. Soil microbial DNA analysis can also be conducted to study the soil microbial communities along with a variety of other methods easily adapted to this paired plot protocol.
2. When determining the experimental implementation of this protocol, it is recommended to consider whether environmental replicates of manipulated snowmelt are necessary for statistical analysis. Note that an increase in replicates will alter the number of materials and equipment needed, as the

numbers provided here are for one manipulated plot and one control. An example of deployed fabric in triplicate is depicted in Figure E.6.



Figure E.6 Deployed fabric in triplicate. An increase in replicates utilized for ongoing projects is displayed as an example if environmental replicates are deemed necessary

3. Over the past three years using this method, the manipulated snowmelt plot has undergone advanced melt-out 14-23 days prior to the control. Use of the time-lapse camera allowed us to capture the behavior of the induced melting and determine when the complete melt-out of the manipulated versus the control plots occurred (Figure E.7).
4. When deciding the best location for our study, we normalized the paired plots by choosing a meadow landscape with an east-northeast aspect to allow for sufficient sun irradiation throughout the day. Sun exposure is necessary for success of this protocol, which could complicate utility in forested regions. In addition, it is best to research annual snowfall depths and variability in the region of investigation to tailor deployment. Ensure the snow stakes will not be covered by the snowpack when it is time to deploy the geotextile. In addition, the snow stakes can partially bend in association with snowpack movement and wind on sloped surfaces effectively shortening their overall height. Create a contingency plan if the snow stakes are covered by the snowpack. We suggest mapping out the location measurements of the stakes using a long measuring tape and compass with respect to a non-moving landmark, or obtain precise GPS coordinates of the anchors.
5. Snow avalanche awareness is necessary for safe and successful implementation of the protocol. It is required in our research group that members trained in avalanche awareness help with deployment. Keep an eye on the conditions of your site and use avalanche reporting stations if available to analyze

the risk. In addition, keep in mind that if the geotextile is deployed early, significant snowfall can upset the progress of the induced snowmelt by covering the fabric and returning sunlight absorption similar to that of the paired control. Advanced melting will not occur until the fabric is snow free, however in our experience subsequent snowfall has not strongly impacted the overall efficacy of this system with respect to net accelerated snowmelt.

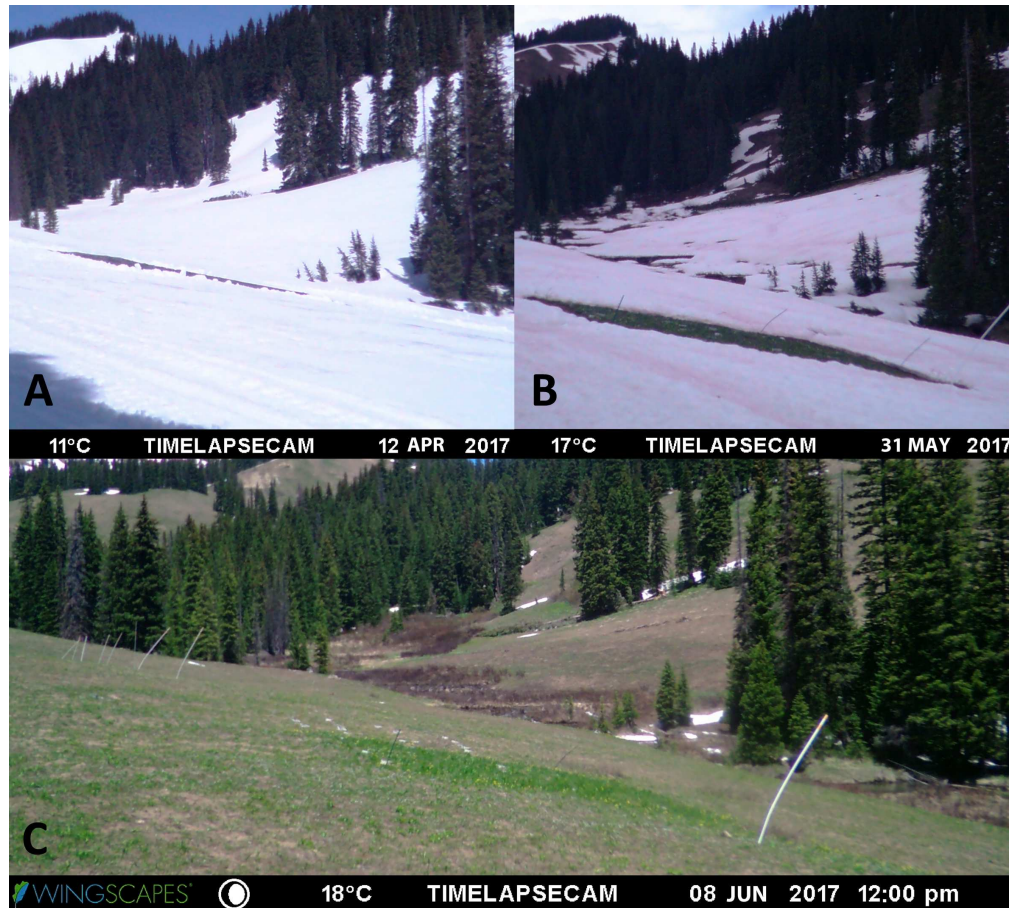


Figure E.7 Time-lapse photos captured during induced snowmelt in 2017. Geotextile deployment on April 12th (A), the fabric is removed on May 30th (B), and full melt-out of the two paired plots on June 8th (C).

E.7 References

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APPENDIX F

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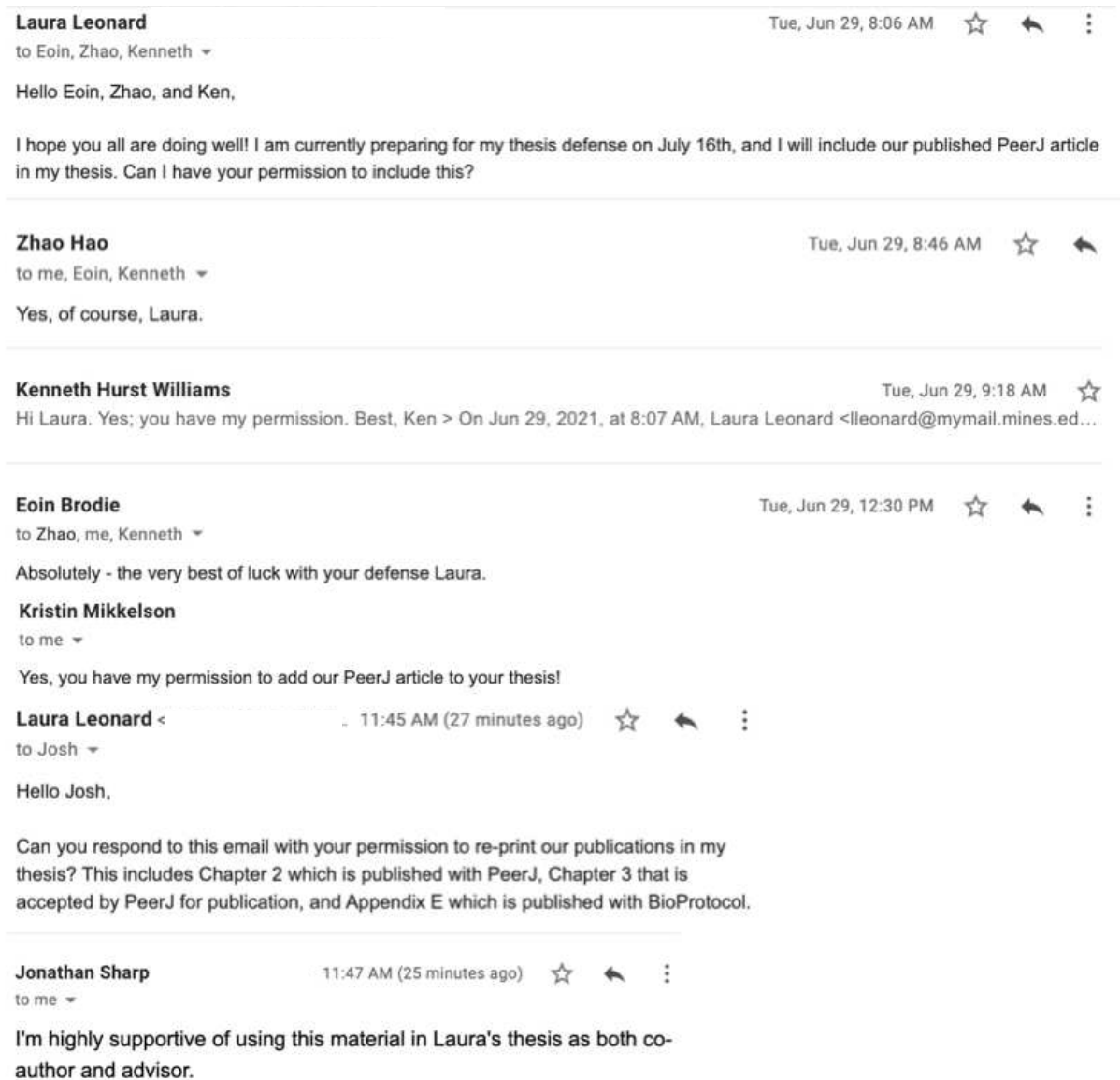


Figure F.1 Coauthor permissions for Chapter 2.

Laura Leonard

Mon, Jul 19, 6:00 PM (2 days ago)



to Eoin, Kenneth, Josh ▾

I am happy to inform you all that PeerJ has officially accepted our second manuscript. I am expecting proofs soon that I will share with you all for a final proofread of funding references, affiliations, and any other small changes that might be noted.

In addition, Eoin and Ken, if I could have you respond to this email with permission to include this publication in my written thesis I would appreciate it.

Kenneth Hurst Williams

Mon, Jul 19, 10:21 PM (2 days ago)

to me ▾

Hi Laura.

I have no problem with your including this published paper as part of your PhD dissertation.

Eoin Brodie

Tue, Jul 20, 12:01 AM (1 day ago)

to Josh, me, Kenneth ▾

Congrats Laura. I am very happy for you to publish this as part of your thesis.

Laura Leonard

11:45 AM (27 minutes ago)



to Josh ▾

Hello Josh,

Can you respond to this email with your permission to re-print our publications in my thesis? This includes Chapter 2 which is published with PeerJ, Chapter 3 that is accepted by PeerJ for publication, and Appendix E which is published with BioProtocol.

Jonathan Sharp

11:47 AM (25 minutes ago)



to me ▾

I'm highly supportive of using this material in Laura's thesis as both co-author and advisor.

Figure F.2 Coauthor permissions for Chapter 3.

Laura Leonard Tue, Jun 29, 8:00 AM ☆ ↩

to Chelsea, Heidi ▾

Hello Chelsea and Heidi,

I hope you both are doing well! I am currently preparing for my thesis defense on July 16th, and I will be including the BioProtocol journal in my thesis. Can I have your permission to include this article in my thesis?

Steltzer, Heidi Tue, Jun 29, 10:22 AM ☆ ↩ ⋮

to me, Chelsea ▾

Laura,
Yes, of course.

Heidi

...

Chelsea Wilmer Tue, Jun 29, 10:37 AM ☆ ↩

to Heidi, me ▾

Yes, Laura, that's fine!

...

Kenneth Hurst Williams 6:32 PM (23 minutes ago) ☆ ↩ ⋮

to me ▾

Hi Laura.

You have my permission to use the Bio-Protocol paper in your dissertation.

Laura Leonard 11:45 AM (27 minutes ago) ☆ ↩ ⋮

to Josh ▾

Hello Josh,

Can you respond to this email with your permission to re-print our publications in my thesis? This includes Chapter 2 which is published with PeerJ, Chapter 3 that is accepted by PeerJ for publication, and Appendix E which is published with BioProtocol.

Jonathan Sharp 11:47 AM (25 minutes ago) ☆ ↩ ⋮

to me ▾

I'm highly supportive of using this material in Laura's thesis as both co-author and advisor.

Figure F.3 Coauthor permissions for Appendix E.