Fire and harvesting impacts on forest floor and water extractable organic matter chemical composition in the Canadian Rocky Mountains

by

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Abstract

Forests globally store a significant portion of terrestrial organic carbon, where two-thirds of that carbon is stored as soil organic matter (SOM). The dissolved organic matter (DOM) in soils plays a crucial role in carbon and nutrient cycling. Understanding the dynamics of DOM and SOM in forest ecosystems, especially their chemical composition is crucial for managing water quality and ecosystem health, particularly in response to land disturbances. Water extractable organic matter (WEOM) represents a fraction of DOM obtained through laboratory extraction and has been proposed as an early and sensitive indicator of shifts in SOM dynamics. In this study, different analytical techniques were used to assess the chemical composition of WEOM in forest soils (forest floor and underlying mineral soil horizon) impacted by clear-cut harvesting and forest fire. The research took place in two pairs of watersheds within the southern Canadian Rocky Mountains. The analytical techniques employed were solid state magic-angle spinning ^{13}C nuclear magnetic resonance (NMR) in both direct polarization (DP) and cross polarization (CP) pulse sequence, specific ultraviolet (UV) absorbance at a wavelength of 254nm ($SUVA_{254}$), and several gas chromatography–mass spectrometry (GCMS) techniques. Each NMR spectrum was analyzed by either calculating the total signal intensity found within defined chemical shift regions or by using the distribution of signal intensity across the entire spectrum. Relative to the traditional use of spectral regions analyzing the whole spectrum allowed variations in the size of individual peaks and their chemical shift values to be incorporated into the analysis. DP and CP ^{13}C NMR analyses revealed differences in both the chemical shift regions and the spectra between forest floor OM and WEOM. Forest floor OM was relatively enriched in aromatic, phenolic, and di-O-alkyl regions, while WEOM showed relatively stronger signals in the carbonyl, alkyl, methoxyl, and O-alkyl regions. Harvesting had a negligible impact, with comparable spectra obtained for reference and harvested lodgepole (*Pinus Contorta*) samples. However, fire-affected samples displayed differences in aromatic and phenolic regions, consistent with increased presence of charred materials. Spectral differences were also observed between conifer and trembling aspen (*Populus Tremuloides*), with conifer samples enriched in alkyl and O-alkyl C compared to aspen samples. The ^{13}C NMR analysis was complemented by SUVA_{254}, where the SUVA_{254} values were consistent with the aromatic character that ^{13}C NMR measured. Different GCMS analytical techniques were evaluated, including thermal desorption and pyrolysis along with the use of tetramethylammonium hydroxide to identify the most effective approach for generating a comprehensive WEOM chemical fingerprint. Overall, no single GCMS technique outperformed any of the others, but method selection should depend on specific study objectives. The most sensitive method for distinguishing differences between forest floors and mineral soil horizons was thermal desorption phase gas chromatography–mass spectrometry (TDGCMS) with the addition of tetramethylammonium hydroxide (TMAH). For identifying differences among dominant canopy vegetation types, pyrolysis phase gas chromatography–mass spectrometry (PyGCMS) was the best suited analysis and double-shot PyGCMS was most effective in capturing chemical signatures related to fire impact. Forest floor WEOM compositions differed from those of underlying mineral horizons, with forest floor WEOM relatively enriched in carbohydrates and phenolic compounds, while mineral soil WEOM contained proportionally more nitrogen-containing, aromatic, and aliphatic compounds. Clear-cut harvesting had a negligible impact on WEOM, with the chemical signature of both forest floor and mineral soil under disturbance comparable to that of reference sites, suggesting the enduring influence of dominant canopy vegetation. However, fire disturbance impacted forest floor WEOM signatures more significantly than the mineral soil WEOM, where the samples were associated with higher aromaticity and black carbon biomarkers were detected.

Overall, the combination of ^{13}C NMR, $SUVA_{254}$ and GCMS has facilitated a thorough assessment of the chemistry of forest floor C and the associated WEOM.

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Chapter 1 - Background and research objectives

Soil is part of the interface between the lithosphere and the atmosphere, hydrosphere, and biosphere (Maynard et al., 2014; National Research Council, 2009; Yaalon, 2007) and helps support life on Earth. In Canada, climate change and anthropogenic disturbances are having an increasing impact on soil carbon dynamics. Globally, forests account for over half of the terrestrial organic carbon (C) (Pacala et al., 2001). This organic carbon is partially stored as soil organic matter (SOM), which consists of plant litter, microbial biomass, animal matter, humus, stabilized organic matter and anthropogenic inputs (Lichtfouse et al., 1998; Schulten, 1999; Seelen et al., 2006; Stevenson, 1994). SOM makes up 1-5% of the total soil mass (Hatten & Liles, 2019) and affects the biological, chemical, and physical properties of soil (Seelen et al., 2006). SOM can be further broken down into particulate organic matter (OM; >0.45 µm) and dissolved organic matter (DOM; <0.45µm). Dissolved organic carbon (DOC) plays a significant role in forested ecosystems due to its function in nutrient cycling (Michalzik & Matzner, 1999; Qualls & Haines, 1991), its role in the hydrological transport of carbon and nutrients between different pools in the ecosystem (Kolka et al., 2008), its ability to act as a major source of carbon to the mineral soil (Guggenberger et al., 1994; McDowell & Likens, 1988; Neff & Asner, 2001; Qualls & Haines, 1991) from the organic layer in the forest floor (Kolka et al., 2008), and its potential lability and provision of energy to decomposer organisms.

Laboratory studies such as those by Cronan (1985) and Vance & David (1991) have shown that between 25% to nearly 50% of the annual loss of carbon from forest floor layers may be due to production and leaching of DOC. Land disturbances within a watershed can change the chemical character and concentration of DOC (Aiken et al., 2011; Emelko & Sham, 2014; O'Donnell et al., 2010), as DOC is the main form by which carbon is transported within soil solution and streams (Kolka et al., 2008). Previous studies have indicated that increased DOC concentrations and increased water fluxes after harvesting (Sørensen et al., 2009) result in increased DOC export from watersheds, increased water treatment costs and potential human health implications.

The aromatic fraction of DOC has the ability to interact with disinfectants used in the treatment process to create compounds known as trihalomethanes (THMs) and disinfection by-products (DBHs), which have the potential to be harmful to human health(Reckhow et al., 1990). Without understanding the chemical signature of DOC, treatment options can be limited, expensive to implement and may lead to the creation of compounds that present a risk to human health. Understanding the impacts of fire and forestry management strategies on the quantity and quality of DOC can ensure that water resources within forested systems are protected and remain fit for human use.

In Alberta, the headwaters of the eastern slopes of the Canadian Rocky Mountains play an integral role in downstream water supplies(Emelko et al., 2011). These headwaters have been increasingly impacted by fire and forest harvesting in recent decades, which has significant implications for watershed management (Laudon et al., 2009; Silins et al., 2009). These disturbances can result in a change in the quantity and quality of runoff delivered to streams and, in turn, the organic compounds that are entering the surrounding waterways which can affect treatability of surface water for human consumption (Emelko et al., 2011). There is limited knowledge around the differences in SOM based on disturbance types and how disturbance impacts soil or DOM. There is also a gap in knowledge regarding how the DOM composition changes with depth within soil profiles. Due to DOM being labile within the terrestrial and aquatic environment, a good understanding of the water extractable chemical signature of these compounds and the relationship between forest floor and mineral soil is needed as water treatment options can be limited, expensive to implement and may even lead to the creation of compounds that present a risk to human health.

1.1 Methods of analyzing the impact of land disturbance on water extractable organic matter

There are numerous analytical techniques used to investigate the chemical composition of organic matter, which vary with molecular insight and level of complexity (McCallister et al., 2018). For the purpose of this research the main analytical techniques to review are nuclear magnetic resonance (NMR) and analytical pyrolysis. Both NMR and analytical pyrolysis are complex to implement and have increasing specificity regarding insight into the molecular structure of chemical compounds. Another technique that was also utilized was ultraviolet (UV) absorbance with the use of a UV-Visible spectrophotometer.

Analytical pyrolysis has been deemed as an efficient tool to characterize SOM and provide molecular markers (proxies for soil properties) as to evaluate the effects of various changes

(Derenne & Quénéa, 2015) that can occur. Its main benefit is the identification of individual chemical compounds obtained following pyrolysis, i.e., pyrolysates (Derenne & Quénéa, 2015). Specific chemical compounds can be further classified into carbon functional groups to provide a broader understanding of their carbon character. Such classification typically includes aliphatics (including alkanes, alkenes and alkynes), aromatics, carbohydrates, and phenolics (including lignin). Another analytical tool often used with analytical pyrolysis for thermochemolysis is tetramethylammonium hydroxide (TMAH). Thermochemolysis is an assisted chemolytic reaction that cleaves larger molecules into smaller molecules (Page et al., 2002). Once the molecules have been cleaved, they can then be identified using gas chromatography mass spectrometry (GCMS). The use of TMAH also results in the derivatization of carboxyl and hydroxyl groups to methyl ester and methoxy groups which again can be more easily identified (Page et al., 2002). The benefit of adding TMAH when using GCMS to analyze lignin-derived moieties, is well documented (Chefetz et al., 2000; Collard et al., 2015; Derenne & Quénéa, 2015; Grote et al., 2000; Jeanneau et al., 2014; Stewart, 2012). For instance, Stewart (2012) used PyGCMS with and without TMAH treatments to characterize SOM chemical composition and concluded that the two TMAH treatments provided complementary information.

Spectroscopy techniques such as NMR provide an insight into the chemical structure of soil organic molecules. NMR has been widely used to analyze OM and was instrumental in the early discoveries of understanding DOM structure (Benner et al., 1992). This research will focus on utilizing NMR to analyze WEOM and to compare SOM and its WEOM component. Analysis with NMR relies on the resonance frequency of atomic nuclei, which is influenced by surrounding electronic structures (McCallister et al., 2018). For this research, the atomic nuclei of ^{13}C were examined where the complete spectra along with separate chemical shift regions were investigated. The NMR chemical shift regions examined included alkyl, methoxyl, O-alkyl, di-O-alkyl, aromatic, phenolic, and carbonyl carbon.

UV absorbance is an analytical technique that measures the absorbance of a certain wavelength of light in the ultraviolet spectrum as it passes through a sample in comparison to a reference or a blank sample (Weishaar et al., 2003). Specific Ultraviolet Absorbance (SUVA) analysis can be used as an indicator of the degree of reactivity a source water will undergo when exposed to certain reagents (Weishaar et al., 2003) leading to the formation of chemicals that interact with natural organic matter (NOM) with the addition of disinfectants in water treatment. These chemicals include disinfection by-products (DBPs) including the four primary trihalomethanes (THM); chloroform, bromodichloromethane, dibromochloromethane and bromoform. These chemicals have the potential to impact human health. A SUVA calculated for the wavelength of 254nm positively associated with the aromatic fraction in OM (Weishaar et al., 2003), however this research was based on aquatic environments. In my research, $SUVA_{254}$ is calculated for the WEOM component of the forest floor and then compared to the ^{13}C aromatic shift region for the same samples to determine their potential correlation.

Overall analytical pyrolysis techniques provide an in-depth understanding of the molecular characterization of OM, especially with WEOM whereas NMR supports a broader interpretation of their chemical structure. UV absorbance, specifically $SUVA_{254}$ can only provide a very simple indication of the aromatic fraction of the OM. Using all three techniques separately, especially pyrolysis and NMR are limited in providing complete and in-depth information on OM (Kaal et al., 2007). However, using all techniques together may provide a more complete and complementary interpretation.

1.2 The forWater Network and the Canadian Rocky Mountains

The forWater Network is a multi-disciplinary, Canada-wide research program that is investigating forest management strategies and the effect they have on source water quality and treatability (Emelko & Silins, 2023). Part of theme 1 within the forWater Network, looked to characterize the impacts of three different forest harvesting strategies on source water quality and treatability within the Montane Cordillera Ecozone (Emelko & Silins, 2023). The DOC produced within the Montane Cordillera watershed could potentially affect the treatability and source water quality for downstream municipal use. For this study the relative effects of clear-cut forest harvesting and fire on the chemical signature of SOM and WEOM were examined. Although a range of analytical techniques exists to quantify the composition of SOM and WEOM, a standardized protocol for assessing the impact of forest harvesting practices on WEOM loads and composition has not been established for use in the forWater Network project as no consistent technique can be found within the literature. In my study, a combination of ^{13}C NMR, UV-Vis and GC-MS will be used to determine SOM and WEOM chemical composition.

1.3 Study Location; The eastern slopes of the Canadian Rocky Mountains

The Montane Cordillera has been impacted by fires as well as forest harvesting. Dissolved organic matter (DOM) produced within its watersheds can potentially affect the treatability and source water quality for downstream municipal use within Alberta, Saskatchewan and Manitoba. However, little is known on how fire and harvesting practices may be impacting DOM quantity and composition within this ecozone.

In this study, forest floor and mineral soils (0-10 cm) were sampled from two watersheds in both the Crowsnest Pass (a recently clear-cut harvested forest and a reference forest) and Waterton Lake National Park (a recently burnt forest and a reference forest). Samples were taken under differing dominant canopy vegetation and were characterized with an emphasis placed on quantifying the content and composition of WEOM to determine the potential linkage between soil organic matter (SOM) and DOM. The chemical composition of organic matter in these samples, as well as in corresponding water extracts, were analyzed using a few different methods: cross-polarization magic angle spinning ^{13}C nuclear magnetic resonance (CPMAS ^{13}C NMR), direct polarization magic angle spinning 13C nuclear magnetic resonance (DPMAS 13C NMR), specific UV absorbance at a wavelength of 254nm (SUVA254), and several gas chromatography–mass spectrometry (GCMS) techniques including double shot pyrolysis gas chromatography mass spectrometry (PyGCMS).

The watersheds in Crowsnest Pass and Waterton Park (Figure 1-1: Overview map of Alberta, the 2018 sampling locations and the four studied watersheds; Star Creek Watershed (burgundy), North York Watershed (green), Yarrow Creek Watershed (blue) and Cameron Creek Watershed (brown).

The Oldman watershed is one of Alberta's main water supplies where it starts in the Rocky Mountains and extends east through the foothills, agricultural plains and into the prairie grasslands. There are approximately two-hundred thousand residents who live in the Oldman watershed (Oldman Watershed Council, 2019). The Oldman watershed is part of the larger Saskatchewan River Basin watershed, which encompasses parts of Alberta, Saskatchewan and Manitoba. The Saskatchewan River Basin is home to approximately three million residents (Oldman Watershed Council, 2019).

1.3.1 Crowsnest Pass

Crowsnest Pass is a municipality made up of five towns occurring in southwestern Alberta within the front range of the Canadian Rocky Mountains. In 2003, the Lost Creek wildfire occurred within the Crowsnest Pass area, which resulted in the creation of Phase 1 of the Southern Rockies Watershed Project (SRWP). This initial phase was to study the impacts of the fire across burnt and reference watersheds through a broad network of climatic stations. Phase 2 of the project has evolved to research the impact of three different forest harvesting strategies on a range of climatic parameters. In 2015 a forest harvesting program was implemented within three sub-watersheds of the Star Creek watershed (49° 37’ N; 114° 40’ W); Star West, Star East and McLaren Creeks, with three different harvesting strategies. In Star West the strategy was clear-cut harvesting with retention, east to west alternating strip-shelterwood harvesting (35m strips of shelterwood) was implemented in Star East and partial cut harvesting occurring in McLaren. Another watershed, North York, remained harvest free to be included in studies as a reference watershed. Further disturbance within the study area includes ATV use, hiking trails and pastoral grazing. For the purpose of this study, Star West Creek (the clear-cut harvest) was the focus, where 68 ha of 463 ha of forested land (15%) were harvested.

As seen in Figure 1-1: Overview map of Alberta, the 2018 sampling locations and the four studied watersheds; Star Creek Watershed (burgundy), North York Watershed (green), Yarrow Creek Watershed (blue) and Cameron Creek Watershed (brown).

The mean elevation of Star Creek is 1851 m and the mean elevation of North York is 1931 m. The average precipitation of the study area for the past decade from 2007-2016 was 1100 mm of precipitation per year with 1147 mm occurring within North York and 814 mm occurring within Star Creek during the 2018 study year. Most of the precipitation occurs in June and the peak of snow melt occurs in spring. The average temperature in January is -10°C and the average temperature in June is 11°C.

Sampling occurred within the upper Montane regions of Star Creek and North York Creek where soils typically belong to the Eutric or Dystric Brunisolic great groups (Bladon et al., 2008). These soils have developed on glacial till parent material that is underlaid by shale and sandstone from the Cretaceous period. The dominant forest canopy vegetation in Star Creek includes lodgepole pine (*Pinus Contorta),* white spruce (*Picea glauca*) and trembling aspen (*Populus Tremuloides*). The dominant canopy vegetation in North York Creek includes lodgepole pine, white spruce and sub alpine fir (*Abies lasiocarpa*) at higher elevations.

1.3.2 Waterton Lakes National Park

Waterton Park is a hamlet in southwestern Alberta within Waterton Lakes National Park. In 2017 19,903 ha (38%) of the National Park was burnt in the Kenow wildfire, which prompted Phase 3 of the SRWP. Phase 3 was set up to study the impact of the Kenow wildfire on water quantity and quality within and beyond the boundary of Waterton National Park. Other disturbances within the study area include bituminized roads and hiking trails. Waterton receives the highest average annual precipitation in Alberta with 1072 mm (Government of Canada, 2023), however the precipitation varies greatly across the park. Approximately 433 mm occurred within the study year of 2018 (Government of Canada, 2023). The majority of precipitation occurs as snow and the peak of snowmelt occurs between April and June. The average temperature in January is -7°C and the average temperature in June is 12°C.

The underlying bedrock of Waterton Park includes some of the world's oldest exposed sediments from the Proterozoic Eon and is dominated by red and green argillite outcrops. There are currently no glaciers present within Waterton Park however, there are glacial features evident including moraines and out washed plains (Coen & Holland, 1976). The overlying soils are dominantly Regosolic (Canadian Society of Soil Science, 2020; Coen & Holland, 1976) with the common occurrence of Brunisols and Luvisols. Landforms within the park are heavily influenced by wind, due to extreme wind that occurs through the valley.

Sampling occurred within the Yarrow Creek and the Cameron Creek watersheds as seen in Figure 1-1: Overview map of Alberta, the 2018 sampling locations and the four studied watersheds; Star Creek Watershed (burgundy), North York Watershed (green), Yarrow Creek Watershed (blue) and Cameron Creek Watershed (brown).

The watershed of Yarrow Creek occurs on the perimeter edge of the Waterton Park boundary as is the reference watershed whereas Cameron Creek mostly occurs within the boundary and is the fire impacted watershed. The mean elevation of Yarrow Creek is 1400 m and the mean elevation of

Cameron Creek is 1550 m. In Cameron and Yarrow Creek, the dominant vegetation is mixed conifer with lodgepole pine and Engelmann spruce (*Picea engelmannii*).

1.4 Research Objectives

The overall objective of this research was to define the impact of wildfire and clear-cut forest harvesting on the chemical composition of WEOM and how this may impact water quality and drinking water treatability requirements. It may also be informative for current and future public health implications as well as support a change of management practices within the forestry industry. Specifically, research outlined in Chapter 2 focused on assessing the forest floor and WEOM carbon composition after the two disturbance regimes with solid state ^{13}C NMR spectroscopy. A correlation between the forest floor $SUVA_{254}$ and ^{13}C NMR spectroscopy, in particular the aromatic shift region, was also investigated. The specific research objectives were to determine how:

- vegetation type, fire or harvesting altered forest floor chemical composition, and
- differences in forest floor chemical composition translated to their associated WEOM

Research reported in Chapter 3 focused on evaluating different GCMS analytical techniques to produce a detailed WEOM chemical fingerprint of the forest floor and the underlying mineral soil following a forest fire event and clear-cut harvesting. The specific objectives were to:

- assess six different gas chromatography mass spectrometry analytical techniques to determine which one may be more advantageous in producing a detailed WEOM chemical fingerprint, and
- identify whether the signature of the WEOM in both the forest floor and mineral soil was similar under clear-cut harvesting and fire.

Chapter 4 integrates the results from Chapter 2 and Chapter 3. This chapter also details the scientific contribution of this research for watershed management.

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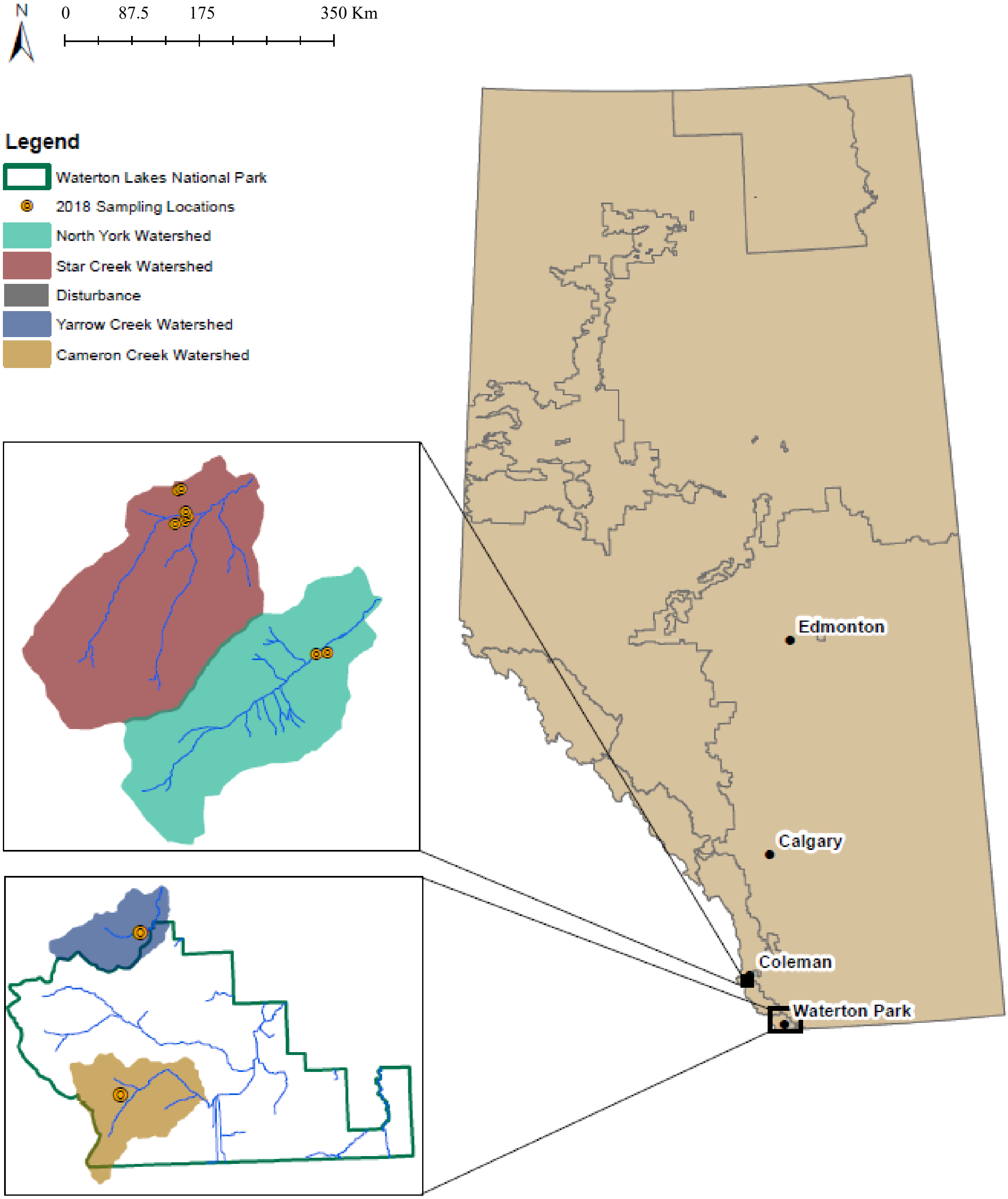

Figure 1-1: Overview map of Alberta, the 2018 sampling locations and the four studied watersheds; Star Creek Watershed (burgundy), North York Watershed (green), Yarrow Creek Watershed (blue) and Cameron Creek Watershed (brown).

Chapter 2 - The relationship between soil organic matter and its associated water extractable organic matter from Canadian Rocky Mountain forest soils using ^{13}C nuclear magnetic resonance.

2.1 Introduction

Organic matter (OM) is a significant component of soil. Soil organic matter (SOM) is the largest pool of terrestrial carbon, and it is a significant factor in global biogeochemical cycles involving the global carbon cycle. SOM impacts soil chemical, physical and biological properties (Baldock & Skjemstad, 1999) including cation exchange capacity, soil structure and nutrient availability (Derenne & Quénéa, 2015). SOM is comprised of a mixture of fresh organic residues, living organisms, partially decomposed and decomposing OM and stabilized OM existing in the form of carbon adsorbed on mineral surfaces as well as inside soil aggregates. The water-extractable component of OM (WEOM) can be differentiated into soluble OM (≤0.45µm) and colloidal OM (≥ 0.45µmaccounts for a small fraction of the total OM present in soils yet can greatly impact soil biological activity (Chantigny, 2003; Flessa et al., 2000; Paul & Beauchamp, 1989; Xu & Juma, 1993), influence the transport of metals and organic pollutants through the ecosystem (Chantigny, 2003; Chiou et al., 1986; Römkens & Dolfing, 1998; Schnitzer & Khan, 1972), play a role in mineral weathering (Chantigny, 2003; Pohlman & McColl, 1988; Raulund-Rasmussen et al., 1998), as well as in podzolization processes (Chantigny, 2003; Dawson et al., 1978; van Hees & Lundström, 2000).

SOM stocks are a function of the balance between OM inputs and losses and may be sensitive to changes in land use (Derenne & Quénéa, 2015). Labile fractions with comparatively rapid turnover of SOM such as soluble OM or water extractable OM (WEOM) can respond rapidly to changes in management practices (Harrison et al., 1993; Liang et al., 1997; von Lützow et al., 2002). As such, WEOM components have been suggested as early and sensitive indicators of the changes seen in SOM (Chilima et al., 2002; Ghani et al., 2003; Haynes, 2000).

The interactions between soil and vegetation are complex (Binkley, 1995; Girona-García et al., 2019). Litter deposition is usually the primary source of forest floor OM, which corresponds to the fresh and decomposed litter located above the mineral soil; therefore, changes in the amount and composition of surficial and belowground litter deposition will influence the complexity and stability of forest floor OM (Girona-García et al., 2019; Kögel-Knabner et al., 2008). Harvesting removes tree biomass and impacts SOM by altering both rates of litter deposition (Hughes & Fahey, 1994; Ussiri & Johnson, 2006) and decomposition of residual litter and SOM (Ussiri & Johnson, 2006). This also results in changes in a range of soil biogeochemical processes, such as mineralization or microbial immobilization of nutrients (Bormann et al., 1974; Boyle et al., 1973; Likens et al., 1970; Mann et al., 1988; Ussiri & Johnson, 2006). Other processes impacted by harvesting include a decrease in the interception of atmospheric moisture, a reduction in transpiration water loss from the soil, and an increase in soil temperature (Ussiri & Johnson, 2006)which in turns increases evaporation from the soil surface. The warmer soil conditions created can increase soil microbial activity and therefore the rate of OM decomposition (Bormann et al., 1974; Dahlgren & Driscoll, 1994; Johnson et al., 1995; Ussiri & Johnson, 2006). With the increase in OM decomposition after harvesting, it is likely that the amount, structure, and chemistry of OM would also change (Ussiri & Johnson, 2006) along with WEOM. Consequently, it is important to study how forest management such as harvesting, can influence the composition of the forest floor OM. Given the impact of vegetation on the quantity and nature of litter added to forest floors, forest management practices that alter the amount or diversity of vegetation have the potential to impact both OM and WEOM content and composition.

Disturbances such as fire, can also impact the composition of forest floor and SOM. The impact of fire on soil is variable due to factors such as fire intensity, soil physical properties and the type of material being burnt (González-Pérez et al., 2004). Fire typically results in a loss of OM and an alteration of SOM chemical composition (Thiffault et al., 2008). The impact of fire on SOM and its component fractions can result in an increase in aromaticity as well as a decrease of oxygen containing compounds (Almendros et al., 1990, 2003; Baldock & Smernik, 2002; Knicker et al., 2005; Vergnoux et al., 2009, 2011). Other than altered lignin (González-Vila et al., 2001), new aromatic structures are generated by the alteration of lipids and peptides (Almendros et al., 2003; Knicker et al., 1996; Vergnoux et al., 2011) and carbohydrates (Almendros et al., 1997; J. Baldock & Smernik, 2002; González-Vila et al., 2001; Vergnoux et al., 2011).

Solid state magic-angle spinning (MAS) ^{13}C nuclear magnetic resonance (NMR) has been used to investigate the chemical characteristics of many forms of OM in soils, including decomposing plant residues, forest floor layers, organic matter contained in soil mineral horizons, and organic amendments added to soils (Preston 2001, 2014). It can provide an insight into the molecular structures present. MAS experiments spin samples at an angle of 54.7° to the magnetic field (Quideau et al., 2001) and apply either a direct polarization (DP) or a cross polarization (CP) pulse sequence to assess the chemical nature of ^{13}C nuclei (Mitchell et al., 2013). Application of both CP and DP pulse sequences to a sample provides complementary information. The CP analysis incorporates a transfer of magnetization from the ^{1}H atoms to the less abundant ^{13}C atoms before acquisition, which enhances the ^{13}C signal intensity by up to four times (C. M. Preston, 2014). However, it is important to note that, although the CP analysis results in higher sensitivity and shorter pulse delays, the resultant distribution of signal intensity can be biased towards ^{13}C nuclei with a close proximity to ^{1}H. The ^{13}C nuclei remote from ^{1}H (e.g., ^{13}C separated from ^{1}H by more than 3-4 bonds as can occur in condensed aromatic rings associated with pyrogenic carbon) will not be observed with the same efficiency as ^{13}C with ^{1}H directly attached (e.g., ^{13}C contained in alkyl or O-alkyl structures) due to different rates of magnetization transfer. Such discriminations are not present in DP analyses, but the DP analysis requires additional analysis time to acquire a spectrum with a signal to noise ratio comparable to CP. The DP technique is also known as single-pulse excitation or Bloch decay (C. M. Preston, 2014), where there is direct irradiation at the observed frequency with ^{1}H decoupling. The resulting distribution of signal intensity in both the CP and DP spectra across chemical shift values can be attributed to general chemical shift regions or viewed as a complete spectrum, where signal intensity in each region corresponds to the % composition of alkyl, methoxyl, O-alkyl, di-O-alkyl, aromatic, phenolic, and carbonyl carbon components.

There have been few studies reporting on compositional differences between forest floor OM and WEOM, especially where the chemical composition of both was defined using ^{13}C NMR. However, Balaria et al. (2009) characterized hot WEOM (HWEOM) using ^{13}C NMR to compare different forest soil organic horizons (Balaria et al., 2009), while Dai et al. (2001)assessed the structural chemistry of both soil OM and DOM in response to clear-cut harvesting. The HWEOM is considered to be positively linked to microbial biomass (Balaria et al., 2009; Sparling et al., 1998) and bioavailable OM (Balaria et al., 2009; Chen et al., 2004; Ghani et al., 2003). For instance, Balaria (2009)showed that the HWEOM was enriched in carbohydrates and N-containing compounds compared with insoluble soil OM. Balaria (2009) further concluded that more labile and bioavailable carbon was present based on the enrichment in carbohydrates, and that the N-containing compounds could be attributed to a larger microbial biomass. Dai et al. (2001) concluded that after clear-cutting there was a restructuring of OM compounds and DOC but that overall, harvesting had a negligible effect on the structural chemistry of SOM however, there was an increase in DOC aromaticity. The increase in aromaticity after harvesting was also present in the study by Hannam et al (2005), particularly in the aspen forest floor.

The objectives of this research were: (1) to determine whether vegetation type, fire or harvesting altered forest floor chemical composition, and (2) to how differences in forest floor chemical composition translated to their associated WEOM. Chemical composition was assessed using DP and CP ^{13}C NMR spectroscopy applied to a range of forest floor OM samples and their corresponding WEOM fractions. The chemical shift region integrals as well as the whole spectra were processed using traditional and multivariate approaches to test for significant differences and to determine whether a correlation existed between the $SUVA_{254}$ and the ^{13}C NMR aromatic shift region.

2.2 Methods and Materials

2.2.1 Study Area and Sampling Strategy

This research was completed using samples collected from the Montane Cordillera Ecozone of south-western Alberta, Canada. The study area included four watersheds: Star Creek, North York Creek, Yarrow Creek and Cameron Creek. Star Creek and North York Creek (Figure 2 1) are located in Crowsnest Pass near Coleman, Alberta (49.64° N, 114.50° W) whereas Yarrow Creek and Cameron Creek (Figure 2 2) are situated near Waterton Park (49.05° N, 113.91° W). These watersheds are located on the eastern slopes of the southern Albertan Rocky Mountain range, and are within the headwaters of the Oldman watershed, which is one of southern Alberta's main water supplies. The watersheds are found in ecological zones encompassing the upper montane, sub-alpine, and alpine (Natural Regions Committee, 2006) at an elevation of 1200-2000m. The study area has an annual precipitation between 650 and 1900 mm, with almost half of it falling as snow. The average January temperature is -8.5°C, whereas the average June temperature is 11°C (Bladon et al., 2008; Coen & Holland, 1976). The main canopy vegetation found at lower elevations is lodgepole pine (*Pinus Contorta*), intermittent with stands of trembling aspen (*Populus Tremuloides*). The dominant canopy vegetation at the higher, subalpine elevations is lodgepole pine and subalpine fir (*Abies lasiocarpa*). White spruce (*Picea glauca*) occurs in the riparian areas.

The soils in the Crowsnest Pass area are typical of high elevation northern climates and are weakly developed (Bladon et al., 2008) and include Eutric and Dystric Brunisols (Bladon et al., 2008). The dominant soil types in Waterton Park are mainly Regosolic, with Brunisols and Luvisols also commonly found (Canadian Society of Soil Science, 2020; Coen & Holland, 1976).

Ten sampling locations were selected within the four watersheds using ArcGIS to span two different disturbances: harvesting and fire (Table 2-1). Sampling was conducted three years following harvesting and one year after the fire. The corresponding undisturbed reference sites were also sampled at the same time. At each sampling location, 3 cores of the forest floor were collected within a 3 m radius with a hand auger and combined to form a composite sample to represent that sampling location.

2.2.2 Laboratory Analyses

The forest floor samples were sieved to ≤ 4 mm (Chantigny et al., 2008) to remove rocks and vegetation debris and homogenize the samples. The sieved samples were then air dried and finely ground using a Retsch MM200 ball grinder. Once ground, the forest floor samples were either left as is for analysis or they underwent a water extraction process adapted from Zsolnay (1996); Kalbitz et al. (2003) and Chantigny et al. (2008) to obtain WEOM. With the water extraction process, ultra-pure water was added to the forest floor samples (5 g of forest floor and 25ml of water) and then mixed by vortexing to ensure a homogenized solution. This was followed by sonification using an ultrasonic bath (Branson 2800, Danbury, CT) for 15 minutes and centrifugation at 4000 rpm for one hour. The extraction process was repeated three times until ~75ml of extracted solution was collected resulting in a 1:15 soil:water ratio. The extracted solutions were then filtered through a 0.45 μm filter (Kolka et al., 2008), frozen for 12 hours at -20°C and then freeze-dried.

Forest floor samples and their corresponding freeze-dried WEOM extracts were analyzed by solid-state ^{13}C ramped cross polarization (CP) with magic angle spinning (MAS) and direct polarization (DP) MAS ^{13}C NMR analysis. Samples were analyzed using a Bruker Avance ($B0=9.4T$, νL (^{13}C) = 100.6MHz NMR spectrometer using a 4 mm double resonance MAS probe (Norris et al., 2018) and high power 1H decoupling. All ^{13}C NMR spectra were referenced to TMS ($\delta = 0$ ppm) by setting the high- frequency isotropic peak of solid adamantane to 38.56 ppm (Bryce et al., 2001; Earl & Vanderhart, 1982; Hannam et al., 2004) and for the Hartmann-Han matching condition, the carbonyl signal of glycine signal was used. All CP spectra were acquired using a 1H 90° pulse width of 4 μs, a pulse delay of 5.0 s, a contact time of 1.0 ms, an acquisition time of 17.1 ms, and a spinning frequency of 13 kHz. The corresponding DP spectra were acquired using a 30 second recycle delay, 1504 to 2800 transients were collected with the hahnecho pulse sequence ($\pi/2$ - τ - π - τ - ACQ) and a spinning frequency of 13 kHz. Bruker's TopSpin 3.6.1 software was used to process the resulting free induction decay (FID). Processing included: line broadening of 200 Hz, phasing, baseline correction and normalization of the signal intensity associated with each of the chemical shift values to a percentage of total signal intensity. All of these processing steps were completed using Top Spin 1.3 software. As well the signal intensity associated with each of the following chemical shift regions based on previous research (Kögel-Knabner, 2002; Simpson & Preston, 2008) were calculated: alkyl (0–47 ppm), methoxyl (47–59 ppm), O-alkyl (59–93 ppm), di-O-alkyl (93–121 ppm), aromatic (121–140 ppm), phenolic (140–163 ppm), and carbonyl (163–188 ppm) carbon.

Subsamples of the WEOM extracts (prior to freeze-drying) were submitted to the University of Alberta Biogeochemical Analytical Services Laboratory (BASL) to quantify their DOC concentration and UV-Vis absorbance. The DOC concentration was analyzed on a Shimazdu TOC-500A Total Organic Carbon Analyzer and a Varian Cary 50 Probe UV-Visible Spectrophotometer was used to analyze the UV-Vis absorbance. UV absorbance is an analytical technique that measures the wavelength of UV that is absorbed by or transmitted through a sample in comparison to a reference or a blank sample (Weishaar et al., 2003). Specific Ultraviolet Absorbance (SUVA) refers to the absorbance of a solution at a given wavelength which is then normalized by the DOC concentration. In this research, SUVA is calculated for the wavelength 254 nm which positively correlated to the aromatic fraction in organic matter (Weishaar et al., 2003). The $SUVA_{254}$ value was calculated by dividing the UV absorbance measured at λ =254nm by the DOC concentration – mgL^{-1}, with units being in liter per milligram carbon per meter – $Lmg\text{-}C^{-1}m^{-1}$ (Weishaar et al., 2003).

2.2.3 Data Analysis

All statistical analyses were completed using R Version 3.6.1 (2019-07-05 ucrt). Initially the mean allocation of the normalized NMR signal intensity (% of total signal intensity) for the seven chemical shift regions across all samples were calculated along with their standard deviations. The DP spectra acquired for the fire affected and the reference spruce forest floor OM as well as the CP spectrum of the fire affected forest floor OM were not included as part of the mean proportion, due to the presence of carbonate, to ensure that the spectra were only representative of the organic carbon present in samples. A Wilcoxon test was completed to assess the potential statistical differences between the forest floor OM and the WEOM means for each region. The Pearson's correlation coefficient (r-value) and p-value associated with the allocations of signal intensity to the chemical shift regions of the forest floor OM and WEOM were calculated to examine similarities in chemical composition.

The integrated CP and DP signal intensities associated with the seven chemical shift regions and the entire spectra (2501 chemical shift values) were analyzed using non-metric multidimensional scaling (NMDS) ordinations. Overall, four ordinations were completed with two for CP and two for DP. The NMDS were completed using the nmds function in the ecodist package (Goslee & Urban, 2007). The Manhattan distance was used in all NMDS analyses due to each data point being part of a continuous trace instead of being independent (Plant et al., 2013). The carbon region integrals were considered as potential correlation vectors ($p<0.05$) which were fitted to the ordinations using the envfit function as found in the vegan package in R (Oksanen et al., 2019).

In addition to the NMDS analyses, a principal component analysis (PCA) was completed using the prcomp function in the R statistical package (R Core Team, 2023) on the acquired CP and DP NMR spectra to view the loading spectra. With PCA, there is an assumption of normality which is not required with NMDS ordinations. Both NMDS and PCA were run to compare the analysis in terms of their ability to extract information from the NMR spectra. Unlike PCA analysis, a NMDS ordination is qualitative where an extra set of extra set of variables are input into the ordination to help explain the similarities and differences across the samples. In this study the vectors used corresponded to the signal intensity associated with each of the seven chemical shift regions and provided an indication of what was causing the separation in the ordination space. PCA analysis is more quantitative, and an examination of the loadings produced can define the chemical similarities and differences across the samples. Overall, both techniques can be employed collaboratively to gain a comprehensive understanding of the spectral data.

2.3 Results and Discussion

2.3.1 Chemical shift region integrals

Mean DP (averaged across 7 sites) and CP (averaged across 9 sites) NMR chemical shift regions differed between the forest floor OM and the WEOM (Table 2-2).

For the DP analyses, the di-O-alkyl, aromatic and phenolic region integrals were greater for the forest floor OM; however, the methoxyl, O-alkyl and carbonyl regions were greater for the WEOM. Similarly, the signal intensity associated with the di-O-alkyl, aromatic and phenolic regions of the CP analyses accounted for a greater proportion of total signal intensity in the forest floor OM; however, only the O-alkyl and carbonyl regions had a larger proportion of carbon in the WEOM. The increased contributions to the O-alkyl and carbonyl regions in the WEOM compared to the forest floor OM are consistent with carbon functional groups that contribute to increased solubility.

A regression analysis was performed to investigate the potential correlation between the forest floor OM and WEOM chemical regions (Table 2-3). For the DP analysis, the carbonyl regions were positively correlated with a significant p-value (<0.05); however, the average % of total signal intensity accounted for by the carbonyl region was only 11.3%. For the CP analysis, the methoxyl, di-O-alkyl and carbonyl regions were positively correlated with significant p-values (<0.05). The carbonyl region contributed 8.5% of the total signal intensity with the methoxyl and di-O-alkyl, respectively, contributing 6.7% and 12.9%. Taken together, the chemical shift regions showing correlation between WEOM and the forest floor OM accounted for less than 30% of the total signal intensity. The alkyl, O-alkyl, aromatic and phenolic carbon regions for both NMR analyses and the methoxyl and di-O-alkyl regions for DP were not correlated.

Both NMDS and PCA were used to analyze the chemical shift region integrals and whole spectra for both DP and CP analyses. The NDMS ordination was first applied to the seven chemical shift region integrals for the DP (Figure *2-3*a) and CP (Figure *2-3*b) spectra. Figure *2-3*a shows a two-dimensional representation of the NMDS analysis of the carbon region integrals associated with the DP spectra for the forest floor OM and WEOM samples where the final stress was 3% and the percentage of spectral variance explained for X1 was 76.5% and for X2 was 12.4%. A clustering of the reference and harvested samples and a separation of forest floor OM and WEOM indicated that the chemical character of the forest floor OM was comparable across samples but different to that of the WEOM. The character of the WEOM appeared more variable than that of the forest floor

OM as indicated by the greater range of X1 and X2 dimension values. There was also a separation of the fire sample (C3) from the rest of the samples for both forest floor OM and WEOM. The vectors presented in Figure *2-3*a and Figure *2-3*b showed that WEOM samples were more strongly associated with the carbonyl, alkyl, methoxyl and O-alkyl regions whereas the forest floor OM was associated with the aromatic, phenolic and di-O-alkyl regions. The fire affected forest floor OM and WEOM had more signal in the aromatic and phenolic regions, consistent with an increased presence of charred materials. The NDMS analysis completed for the carbon region integrals of the CP spectra (Figure *2-3*b) produced similar results to those found for the DP spectra. The final stress for the CP NDMS was 2% and the percentage of spectral variance explained was 75.3% for X1 and 16.1% for X2.

Figure 2-4a shows a scores plot including the labelled loadings obtained from the PCA of the chemical shift region integrals associated with the DP analysis of the forest floor OM and WEOM samples. Table 2-4 provides the proportion of variance explained by the full PCA model and by PC1 and PC2. The majority of the variance for the PCA analysis of the DP regions was explained by PC1 (82.5%), however, PC2 also contributed (11.6%). Similar to the NMDS analysis, the scores plot showed a clustering of samples by organic matter type (forest floor OM versus WEOM) but a separation between them and additionally a separation of the fire affected organic matter from their respective groups. The forest floor OM samples had negative PC1 scores and the WEOM samples, except for fire (C3), had positive PC1 scores. With PC2, most of the WEOM samples except for the harvest (L4) and a reference (L2) had positive scores and all of the forest floor OM samples had negative scores. The PC loadings (Figure 2-4a) indicated that the majority of the WEOM was enriched in alkyl, methoxyl and O-alkyl C and depleted in phenolic, aromatic and di-O-alkyl C relative to the forest floor OM. The fire WEOM sample (C3) was enriched in aromatic and phenolic C (PC1) and carbonyl C (PC2) in comparison to all other samples. Figure 2-4b and Table 2-4 provide the results obtained from the PCA applied to the chemical shift region integrals associated with the CP regions for the forest floor OM and WEOM samples. The PC loadings (Figure 2-4b) for the CP regions also showed that the WEOM was enriched in methoxyl and O-alkyl. Overall, the PCA produced similar WEOM results for both the DP and CP chemical shift regions.

2.3.2 Whole Spectra

The normalized DP and CP spectra were then plotted based on the disturbance type and vegetation type to assess differences in the chemical composition between the forest floor OM and the WEOM (Figure 2-5 and Figure 2-6, respectively).

For both DP (Figure 2-5a) and CP (Figure 2-6a) analyses, the impact of fire was visible with the presence of charcoal (Baldock et al. 2013), as evidenced by a broad peak across the aromatic and phenolic spectral region. This peak was not as large in the WEOM spectrum as it was in the forest floor OM spectrum. Preston et al. (2000) indicated that a broad resonance near 130 ppm could be derived from C-substituted aromatic carbons, which Thiffault et al (2008) suggested could arise from either black carbon and/or highly altered lignin. For the DP analysis the resonance at 165 ppm in both the WEOM and forest floor OM indicated the presence of carbonate (Moore et al., 2015), which was confirmed by an effervescence test conducted in the laboratory with 1 M HCl. Although confounded by the presence of carbonate in DP spectra, the enhanced signal intensity in the carbonyl and O-alkyl regions suggested a more oxidized structure for the WEOM than the forest floor OM.

The harvested and reference lodgepole pine samples produced similar CP and DP spectra, but within the two separate analyses differences existed between the WEOM and forest floor OM. The forest floor OM and WEOM (Figure 2-5b and Figure 2-6b) differed in the allocation of signal intensity to the alkyl region with two peaks present in the WEOM compared to one in the forest floor OM. Differences also existed for the di-O-alkyl, aromatic and phenolic regions. The forest floor OM had stronger signals consistent with lignin (Thiffault et al., 2008) than the WEOM samples. The WEOM samples had larger proportions of O-alkyl and carbonyl in both the CP and DP spectra than the forest floor OM. In regard to disturbance impact, harvesting had a negligible impact with comparable spectra obtained for the reference and harvested lodgepole pine forest floor OM and WEOM. A study completed by Hannam (2005)concluded that aspen and spruce clear-cut stands differed from uncut stands where the forest floor from the clear-cut stands had become enriched in aromatic C. Hannam (2005) noted that earlier research studies indicate a link between postharvest changes in forest-soil OM and changes in the patterns of nutrient cycling, though this is still being investigated. However, a study completed by Parfitt and Newman (2000) used ^{13}C NMR and chemical analysis to see if there was an impact of clear-cut harvesting on *Pinus radiata* D. Don. This study was done over a period of three years where the needles and underlying soil were analyzed. Parfitt and

Newman (2000) broke their spectra down into only four chemical shift ranges but concluded that the needles had close similarities in their overall appearance. Overall, with the Parfitt and Newman (2000) study there was an increase in alkyl C (0-50 ppm) and a decrease in O-alkyl C (50-110 ppm) over the three years since harvesting.

The ^{13}C NMR analysis revealed similar discrepancies between the DP and CP spectra in the aspen samples (Figure 2-5c and Figure 2-6c) as were observed in the lodgepole pine samples. The main difference in the DP spectra was a larger allocation to the alkyl region for the WEOM than the forest floor OM. With CP there was also a smaller allocation to O-alkyl in the WEOM. The WEOM samples had greater intensities associated with the alkyl, O-alkyl, and carbonyl regions. The forest floor OM, especially with CP showed signs of lignin and overall had a greater signal intensity in the di-O-alkyl, aromatic, phenolic and carbonyl regions.

The DP spectra derived from the mixed conifer and white spruce forest floor OM (Figure 2-5c) were similar except for the presence of a signal at 168 ppm for the spruce consistent with the presence of carbonate (Moore et al., 2015). The presence of carbonate was confirmed by a positive effervescence test using 1 M HCl. The carbonate peak was not present in the WEOM sample or in the CP analysis (Figure 2-6c). The main differences between the forest floor OM and WEOM appeared in the alkyl region where there were two peaks for the WEOM, but only one for the forest floor OM. Smaller differences were also observed in the di-O-alkyl, aromatic, and phenolic regions suggesting the presence of more lignin in the source OM (Thiffault et al., 2008) than in the WEOM.

While DP and CP spectra differed, general similarity of spectral patterns were evident within broadly similar vegetation groups for each of DP and CP analysis approaches (Figure 2-5b-d and Figure 2-6 b-d). These similarities included a peak in forest floor OM in the alkyl region at 30 ppm attributed to long chain insoluble aliphatic structures that are polymethylene in nature (Hannam et al., 2004; Keeler & Maciel, 2000; Thiffault et al., 2008; Zech et al., 1987). However, the larger contribution made by the peak at 20 ppm in the alkyl region for WEOM suggest the presence of short-chain alkyl material like acetate (Hannam et al., 2004). There was also a shoulder at 63ppm for all vegetation types that indicated the presence of C-6 carbon in carbohydrates (Hannam et al., 2004; Thiffault et al., 2008). The O-alkyl peak close to 70 ppm was characteristic of the C-2, C-3 and C-5 carbons of cellulose and hemicellulose (Thiffault et al., 2008) and at a peak near to 104ppm was attributed to anomeric carbons (Preston et al., 2000; Thiffault et al., 2008). A shoulder at 57ppm, characteristic of lignin (Thiffault et al., 2008) was present in all forest floor OM spectra except for the DP aspen sample (Figure 2-5c). Variations in intensity of the peak at 57 ppm were similar to other associated NMR signals associated with lignin (Hannam et al., 2004), e.g. signals at 130 ppm and 150 ppm. The peak at 105 ppm present in the forest floor OM samples for the CP analysis Figure 2-6b-c) would normally be indicative of tannins being present in the samples (Thiffault et al., 2008) however, the presence of significant O-alkyl C would suggest that the main component of the 105ppm signal was likely derived from the anomeric carbon found in carbohydrates. Most spectra for both DP and CP had a peak at approximately 173-175 ppm in the carbonyl region indicating the presence of carboxyl carbon (Thiffault et al., 2008; Zech et al., 1987), aliphatic esters (Zech et al., 1987) or amide carbon.

Overall, spectral differences among vegetation types occurred between the aspen and the conifer species (lodgepole pine, white spruce and mixed conifer) in both the DP and CP spectra. A study completed by Zech et al. (1987) that examined decomposed spruce and pine litter showed that with conifer species aromaticity decreases whereas the aliphatic substances and carboxyl groups increase with decomposition. The published spectra by Zech et al. (1987) showed little difference between the conifer species studied. With our study, even though we did not study decomposition like Zech et al. (1987), the DP (Figure 2-5) and CP (Figure 2-6) spectra show that the forest floor OM and WEOM from conifer samples were similar in composition and were enriched with alkyl C (30 ppm) and O-alkyl C (75 ppm) when compared to the aspen forest floor and WEOM. The aspen forest floor OM in the DP spectra (Figure 2-5) was enriched in aromatic and phenolic C and depleted O-alkyl C. With the CP spectra (Figure 2-6) the aspen WEOM sample was enriched in carbonyl and alkyl C and the aspen forest floor OM sample was slightly enriched in carbonyl and phenolic C when compared to the conifer species WEOM. Even though the Aspen samples were enriched in carbonyl C, the conifer samples did also show a peak with carbonyl C at approximately 175ppm in both the WEOM and the OM sample.

By completing a PCA on the whole spectrum a more in-depth examination of the spectral differences between forest floor OM and its associated WEOM was possible. In the previous section, applying PCA to the seven chemical shift regions allowed a broad assessment of the chemical differences between samples. However, such an analysis can mask spectral differences that occur within a region (e.g., whether a region contains a single or multiple well resolved peaks). In this section the results obtained by applying NMDS analysis and PCA to the distribution of signal intensity at each chemical shift value of the NMR spectra is presented.

The results obtained by applying an NDMS analysis to the whole DP and CP spectra are provided in Figure 2-7a and b, respectively. The final stress for the NDMS analysis to the whole DP spectra was 5% and the percentage of spectral variance explained was 72.2% for X1 and 12.6% for X2. The final stress for the NDMS analysis to the whole CP spectra was 3% and the percentage of spectral variance explained was 71.7% for X1 and 13.1% for X2. For both Figure 2-7a and Figure 2-7b there was a separation between the forest floor OM and the WEOM groups. The reference and harvested samples were clustered together, meaning that similarly to the chemical shift region integrals, these forest floor samples had a similar composition, but differed from the WEOM samples. The fire samples (C3) also separated from the rest. The correlation vectors indicated that the di-O-alkyl, phenolic and aromatic C signals were enhanced in the forest floor OM relative to the WEOM. The O-alkyl, methoxyl, alkyl and carbonyl were enhanced in the WEOM.

The results of applying a PCA to the whole DP and CP spectra are provided in Figure 2-8a and Figure 2-8b, respectively, and the variance explained can be found in Table 2-4. The PCA analysis of the DP spectra showed a separation between the forest floor OM and the WEOM, and a separation of the fire samples from their respective OM groups. In the DP scores plot (Figure 2-8a), the forest floor OM plotted along the negative PC1 axis and the WEOM samples, except for fire (C3), plotted along the positive PC1 axis. For PC2, all but three of the WEOM samples (S2, L2, L4) had positive scores and most of the forest floor OM samples except for two references (S1 and S2) had negative scores.

Expressing the PCA loadings at each chemical shift value allowed loading spectra to be generated and enabled a detailed assessment of the differences in chemical composition of the forest floor OM and WEOM within the samples. As seen in Figure 2-8c, positive PC1 values were indicative of a greater contribution of O-alkyl C, and small contributions from alkyl and carbonyl C. Negative PC1 values were indicative of a presence of a broad aromatic signal consistent with charcoal (Baldock et al., 2013; Thiffault et al., 2008) and a sharp carbonyl signal at 168ppm indicative of carbonate (Moore et al., 2015). Positive values along PC2 were associated with carbonyl C and some contribution from alkyl C. Negative PC2 values were indicative of enhanced contributions from phenolic and aromatic C having chemical shift values indicative of lignin (Thiffault et al., 2008).

The negative O-alkyl peak in PC2 is likely derived from the O-alkyl C associated with lignin side chains. The WEOM appeared to be enriched with carbohydrates (PC1 loadings) and two forms of carbonyl C, an organic carbonyl form and carbonate (PC2) loadings). Figure 2-8b shows the scores plot of the PCA applied to the CP spectra with the respective loadings shown in Figure 2-8d. Table 2-4 details the variance explained for this PCA. The scores obtained for the CP spectra for PC1 were similar to that obtained for the DP spectra. The loadings associated with PC1 of the CP spectra were also consistent with those observed for the DP spectra with the exception of an absence of any signal derived from carbonate C. The absence of a carbonate signal in the CP loadings is consistent with a reduced ability of the CP analysis to detect carbonate C. In the CP scores plot (Figure 2-8b), the forest floor OM plotted along the negative PC1 axis and the WEOM samples, except for fire (C3), plotted along the positive PC1 axis. For PC2, all of the WEOM samples except for a lodgepole reference (L2) had negative scores and all of the forest floor OM samples except for fire (C3) had positive scores. As seen in Figure 2-8d, positive PC1 values were indicative of a greater contribution of O-alkyl C, and small contributions from alkyl and carbonyl C. Negative PC1 values were indicative of a presence of a broad aromatic signal consistent with charcoal (Baldock et al., 2013; Thiffault et al., 2008) and phenolic components. Positive values along PC2 were associated with carbonyl C and some contribution from alkyl C. Positive PC2 values were indicative of alkyl, O-alkyl, di-O-alkyl and aromatic. The WEOM also appeared to be enriched with carbohydrates (PC1 loadings).

A further PCA was completed on the DP and CP spectra after removing the spectra for the samples affected by fire as well as those containing carbonate (spruce reference samples). The PCA analysis of the DP spectra showed a separation between the forest floor OM and the WEOM. In the DP scores plot (Figure 2-9a), the forest floor OM plotted along the negative PC1 axis and the WEOM samples plotted along the positive PC1 axis. For PC2, with the forest floor OM samples all but the aspen reference sample (A) had negative scores and with the WEOM samples all but two (L2, L4) had positive scores. The forest floor OM aspen samples were more enriched in carbonyl C when compared to the other forest floor OM samples. As seen in Figure 2-9c, the positive PC1 values were associated with a greater contribution of O-alkyl C and a small contribution from alkyl, di-O-alkyl and carbonyl C. Negative PC1 values were associated with higher contributions of phenolic and aromatic C along with a contribution of alkyl C. Positive values along PC2 were indicative of alkyl, methoxyl and carbonyl C with the negative values along PC2 associated with O-alkyl and phenolic and aromatic C indicating lignin (Thiffault et al., 2008). Figure 2-9b shows the scores plot of the PCA applied to the reduced CP spectra with the respective loadings shown in Figure 2-9d. Table 2-4 details the variance explained for this PCA. Despite the loadings looking similar between DP and CP for PC1, the CP PC2 scores are rotated 180° relative to DP PC2 scores. Once samples containing broad aromatic signals consistent with charcoal and carbonate signals were removed, the remaining CP and DP spectra were similar. This suggested that for this reduced set of samples the CP analysis provided a more quantitative assessment of the C chemistry than was obtained for samples containing charcoal and carbonate. It is also important to note that for the NMDS the similarities and dissimilarities in ordination values for the samples were consistent whether the whole spectrum or the chemical shift regions were used. Similarly, the score plots from the PCA results obtained for the chemical shift region integrals compared to the whole spectrum show similar results, however the loadings on the regions gave a less informative analysis than the loadings obtained from the whole spectrum. The seven chemical shift regions detail the gross composition whereas the spectra detail the nature of the C in the seven chemical shift regions.

2.3.3 SUVA$_{254}$ and % aromaticity ^{13}C NMR

A correlation analysis between the SUVA$_{254}$ values obtained for the forest floor OM and WEOM samples and their corresponding % aromaticity values derived from the DP spectra (Figure 2-10a) and CP spectra (Figure 2-10b) were completed. Both correlations were positive for the DP spectra with strong R^2 values of 0.8 (forest floor) and 0.9 (WEOM) with similar results observed with the CP with R^2 values of 0.8 (forest floor) and 0.8 (WEOM). It is important to note that the fire impacted site had a strong influence on the results. With fire excluded, the effect was reduced. However, these results support the positive relationship between SUVA$_{254}$ and ^{13}C NMR % aromaticity as determined by Weishaar et al (2003) but within a terrestrial environment rather than an aquatic environment. Overall, the results suggest that the aromatic character of the forest floor organic matter from which the WEOM materials were derived increased, the increase was reflected in the WEOM and could be detected by SUVA$_{254}$.

2.4 Conclusion

Results from the regression analysis and visual observations from the spectral plots showed some similarities between the forest floor OM and WEOM samples, but overall, significant differences in chemical composition existed. In addition, the absence of significant correlations in the proportion of carbon allocated to most of chemical shift regions between the forest floor OM and WEOM suggests that WEOM chemistry does not closely reflect the chemical composition of the forest floor OM. This was also visible in the PCA scores and loading plots. However, we do see a positive correlation between WEOM and forest floor OM with the use of $SUVA_{254}$ and the % aromatic fraction of ^{13}C NMR. This is due to a fraction of the aromatic C making it into the solution, and with the fire impacted site the value was higher due to the nature of the fire chemical signature. Overall, the WEOM for the DP and CP analysis was more oxidized than the forest floor OM with an enrichment of the functional groups that tend to make organic molecules soluble i.e. alkyl (short chain), methoxyl,O-alkyl C and carbonyl C. On the other hand, the forest floor OM was enriched in aromatic and phenolic C compared to the WEOM and showed the presence of charcoal and/or lignin which tend to be more water insoluble. The impact of fire was associated with a higher aromaticity in both the forest floor OM and WEOM; with the increase being larger in the forest floor OM which may account for the higher $SUVA_{54}$ value for this sample.

In regard to disturbance type, the fire impacted samples showed the presence of charcoal as well as carbonate. However, the impact of harvesting was negligible with similar chemical compositions being observed to the corresponding reference samples. With the dominant canopy vegetation, there were many similarities between tree species including the presence of carbohydrates as well as lignin and tannins. Nevertheless, there were small differences that occurred between the aspen and the rest of the conifer tree type species. As indicated from the DP analysis, the aspen spectrum was enriched in aromatic and phenolic C but depleted in O-alkyl C; the CP aspen spectrum was enriched in carbonyl and phenolic C.

What is important to note is the outcome of using the whole spectra over the chemical shift region integrals. By using the whole spectra, any spectral differences that otherwise may have been masked were instead identified clearly. Overall, the materials that were found in the WEOM were consistent with the solubility of OM and this is what was driving the differences between the composition of the forest floor OM and the associated WEOM samples. The fire impacted forest floor OM had higher aromatics which translated into the associated WEOM sample. Vegetation also impacted the WEOM composition, where there were differences between the aspen samples and the conifer samples. Conifer samples were enriched with alkyl and O-alkyl C.

Out of the analytical techniques used in this chapter, the DP analysis is recommended due to it being quantitative in nature and because it does not discriminate against aromatic C relative to the other forms of C. However, samples need to be pre-treated to remove carbonates and it does require a lot more time in order to get spectra with an equivalent signal to noise of the CP analysis.

Further research into why there were greater differences in WEOM composition among sites than those observed among forest floors OM samples would help with a more in-depth understanding of WEOM chemical composition.

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Table 2-1: Key site characteristics including dominant vegetation, location, and disturbance.

Sample ID	Dominant Vegetation	Watershed	Easting* (m)	Northing* (m)	Elevation (m)	Disturbance
L1	Lodgepole Pine	Star Creek	675375	5497971	1596	Reference
L2	Lodgepole Pine	Star Creek	675282	5497386	1589	Reference
L3	Lodgepole Pine	Star Creek	675325	5497943	1600	Harvest
L4	Lodgepole Pine	Star Creek	675444	5497451	1562	Harvest
A	Trembling Aspen	Star Creek	675453	5497584	1559	Reference
C1	Mixed Conifer	Star Creek	675494	5497532	1551	Reference
S1	White Spruce	North York Creek	677775	5495295	1622	Reference
S2	White Spruce	North York Creek	677595	5495265	1624	Reference
C2	Mixed Conifer	Yarrow Creek	719162	5453459	1554	Reference
C3	Mixed Conifer	Cameron Creek	717305	5438303	1840	Fire

* UTM zone 11 north and east

Table 2-2: Mean allocations of normalized NMR signal intensity (% of total signal intensity) to the alkyl, methoxyl, O-alkyl, di-O-alkyl, aromatic, phenolic and carbonyl chemical shift region integrals of ^{13}C DP and CP NMR spectra obtained from the forest floor OM layer and associated WEOM. For DP samples were collected from clear-cut harvested and reference stands (not including spruce) and with the CP samples were collected from clear-cut harvested and all reference stands. The standard deviation of the mean across sites is provided in the parentheses. Different letters after the parentheses indicate a significant (α ≤ 0.05 as defined by Wilcox test) difference between the forest floor OM and WEOM for the DP analyses or for the CP analyses.

Carbon Region Integrals	**DP (% of total signal intensity)**		**CP (% of total signal intensity)**	
	Forest floor OM	**WEOM**	**Forest floor OM**	**WEOM**
Alkyl (0–47 ppm)	19.2 (2.2) A	19.7 (2.6) A	19.2 (1.3) a	19.8 (2.8) a
Methoxyl (47–59 ppm)	6.3 (0.3) B	7.3 (0.4) A	6.4 (0.5) a	6.9 (0.8) a
O-alkyl (59–93 ppm)	27.0 (2.3) B	37.8 (5.5) A	35.0 (2.1) b	45.3 (4.9) a
di-O-alkyl (93–121 ppm)	15.8 (0.6) A	11.0 (0.8) B	15.1 (0.4) a	10.6 (1.0) b
Aromatic (121–140 ppm)	10.9 (1. 5) A	5.5 (0.9) B	8.9 (1.0) a	3.9 (0.7) b
Phenolic (140–163 ppm)	10.8 (0.9) A	6.1 (1.0) B	8.3 (0.6) a	3.7 (0.6) b
Carbonyl (163–188 ppm)	10.0 (1.9) B	12.5 (1.8) A	7.2 (0.7) a	9.8 (1.6) b

Table 2-3: Pearson's correlation coefficient and p-value associated with the allocation of signal intensity to each chemical shift region integrals for the forest floor OM and WEOM across all sites based on the normalized DP or CP NMR spectra. With DP fire and spruce samples were not included, with CP fire was not included.

Carbon Region Integrals	**DP WEOM: forest floor OM**		**CP WEOM: forest floor OM**	
	R Value	p-Value	R Value	p-Value
Alkyl (0–47 ppm)	0.36	0.42	0.01	0.85
Methoxyl (47–59 ppm)	0.26	0.57	0.66	0.05
O-alkyl (59–93 ppm)	0.39	0.39	0.01	0.82
di-O-alkyl (93–121 ppm)	0.53	0.22	0.71	0.03
Aromatic (121–140 ppm)	0.35	0.44	0.15	0.71
Phenolic (140–163 ppm)	-0.27	0.56	-0.48	0.19
Carbonyl (163–188 ppm)	0.91	<0.01	0.54	<0.01

Table 2-4: Number of principle components and variance explained for the normalized spectra and the integral regions of DP and CP NMR obtained from the forest floor OM soil layer and associated WEOM. The number of principle components were selected when the variance explained was greater than or equal to 98%. Samples were collected from fire, clear-cut harvested and reference stands. The reduced sample set analysis was completed without the fire and fire reference samples.

	DP				CP			
	Number of principle components	Variance explained			Number of principle components	Variance explained		
		Total	PC1 (%)	PC2		Total	PC1	PC2
Integral regions	3	98.9	82.5	11.6	3	99.2	87.8	8.7
Whole spectra full sample set	7	98.2	75.1	11.4	5	98.7	82.0	9.5
Integral regions reduced sample set	3	99.2	78.5	15.1	3	99.6	79.3	17.7
Whole spectra reduced sample set	6	98.2	75.4	15.2	5	98.6	81.5	12.7

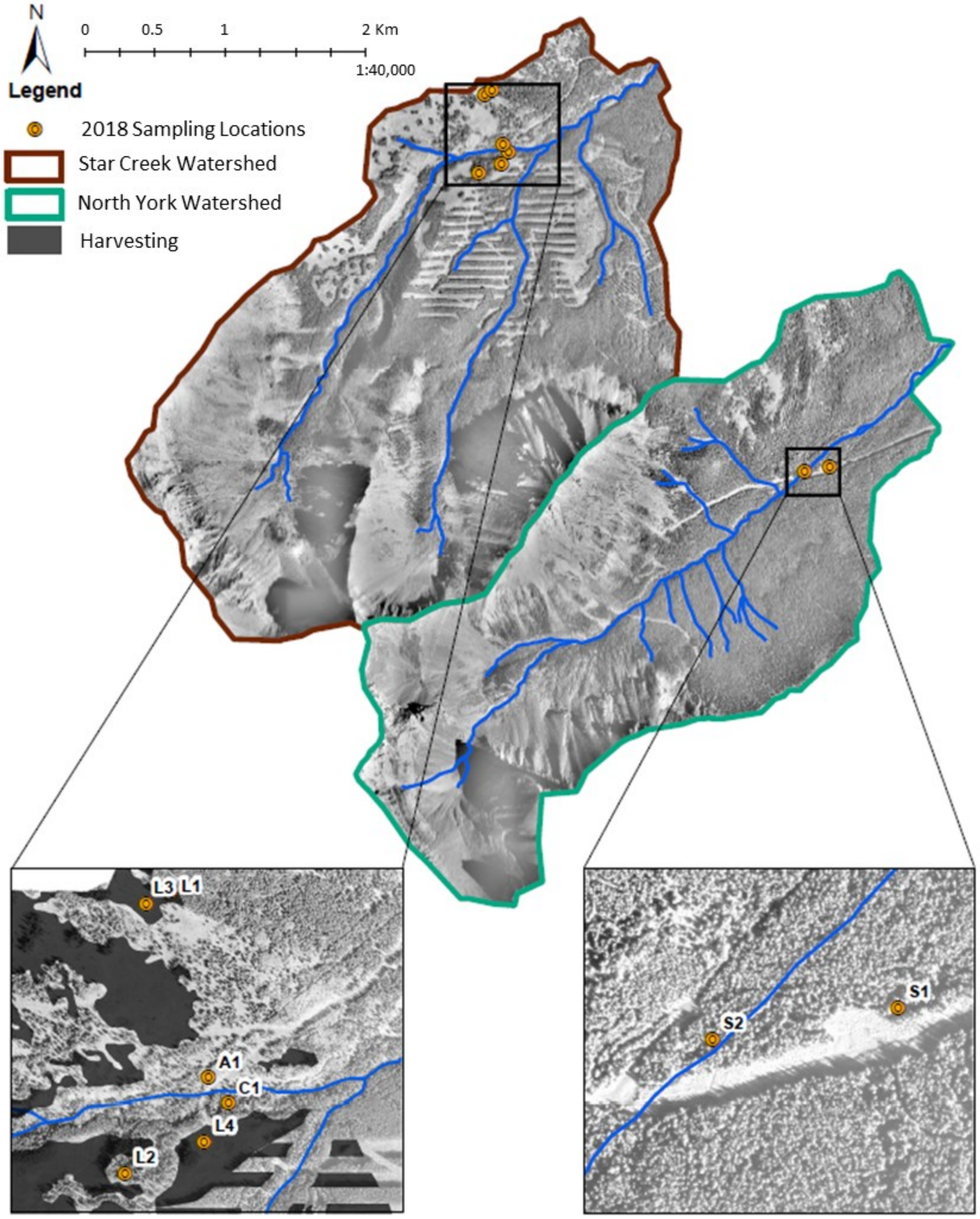

Figure 2-1: Map of the Crowsnest Pass study site. The Star Creek watershed has the brown outline and the left inset and the North York watershed has the green outline and the right inset. The sampling locations across both watersheds are in orange. The harvesting disturbance is in dark grey.

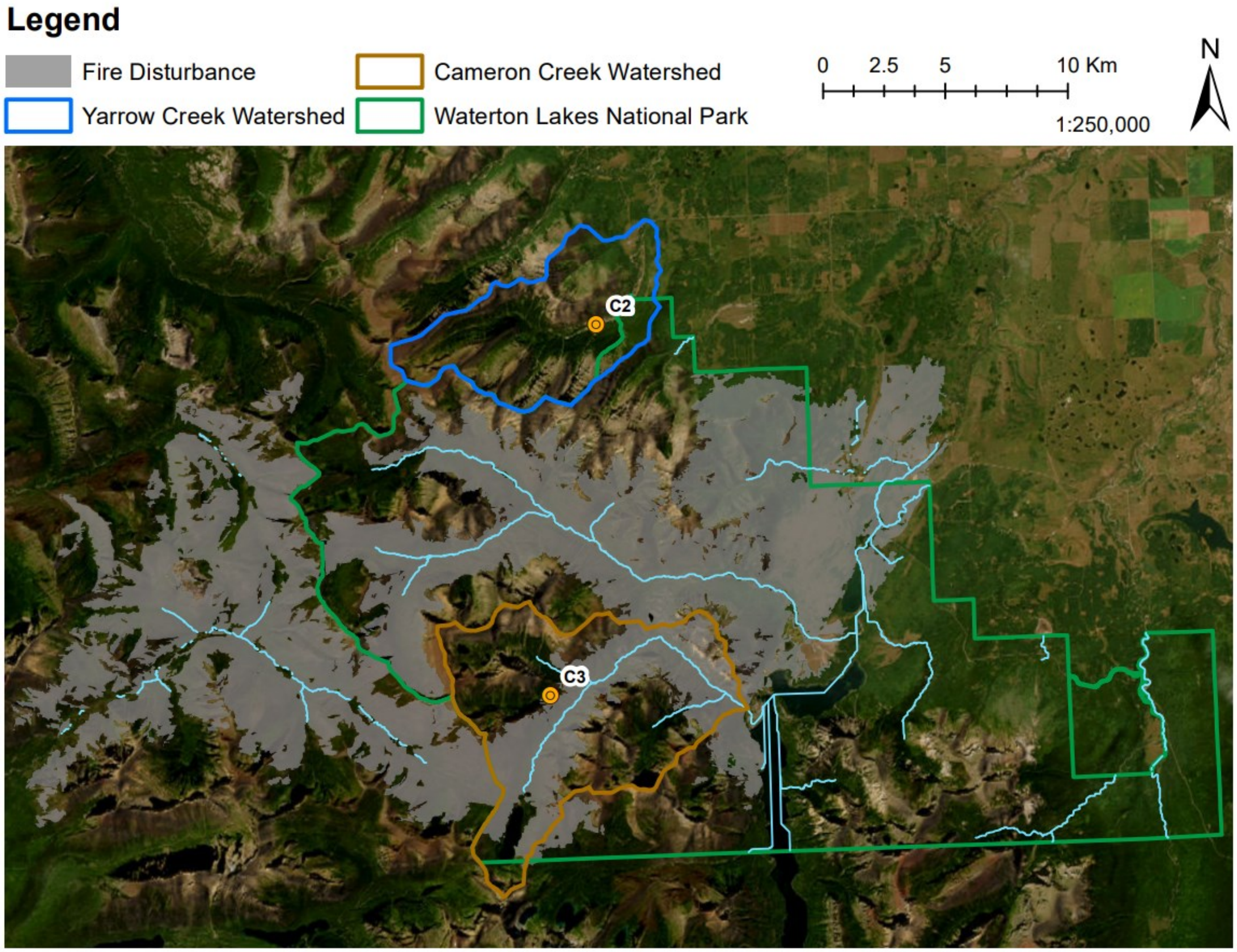

Figure 2-2: Map of the Waterton area study site. The Yarrow Creek watershed is outlined in blue, and the Cameron Creek watershed is outlined in brown. The sampling locations across both watersheds are in orange. The fire disturbance is in dark grey, and the Waterton Lake National Park is in green.

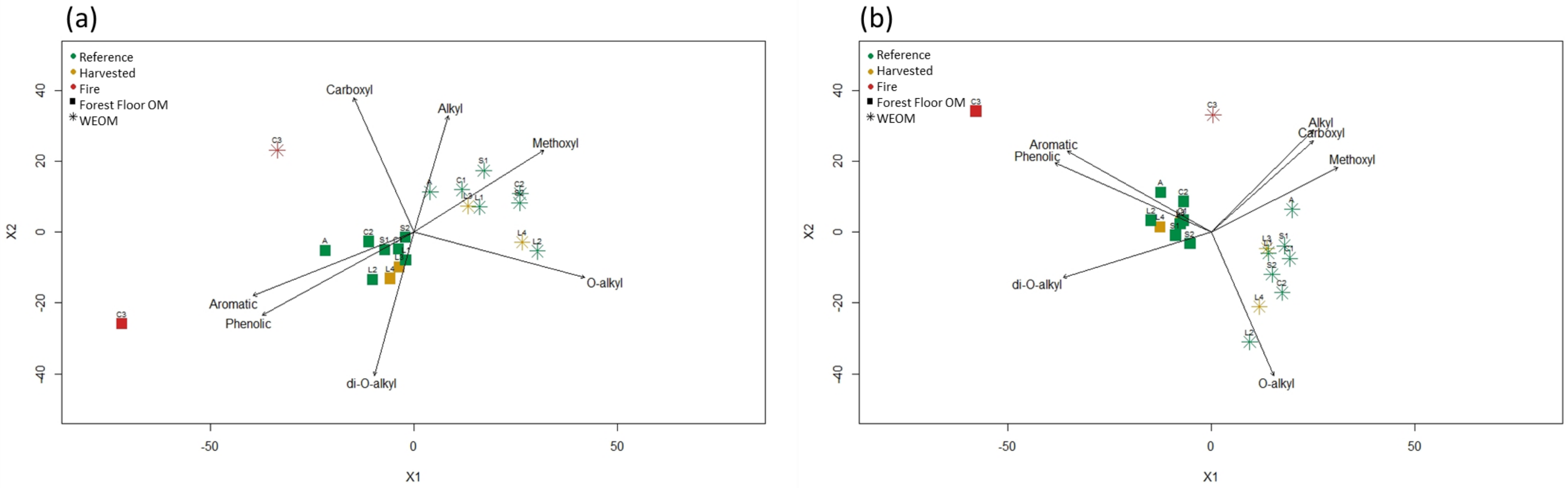

Figure 2-3: Two-dimensional NMDS ordination obtained from the chemical shift regions (% total signal intensity) of the (a) DP and (b) CP NMR spectra acquired for the forest floor OM and WEOM. Symbol labels are associated with site disturbance and vegetation type; L lodgepole pine, S white spruce, A trembling aspen and C mixed conifer. The vectors ($p<0.05$) correspond to chemical shift regions.

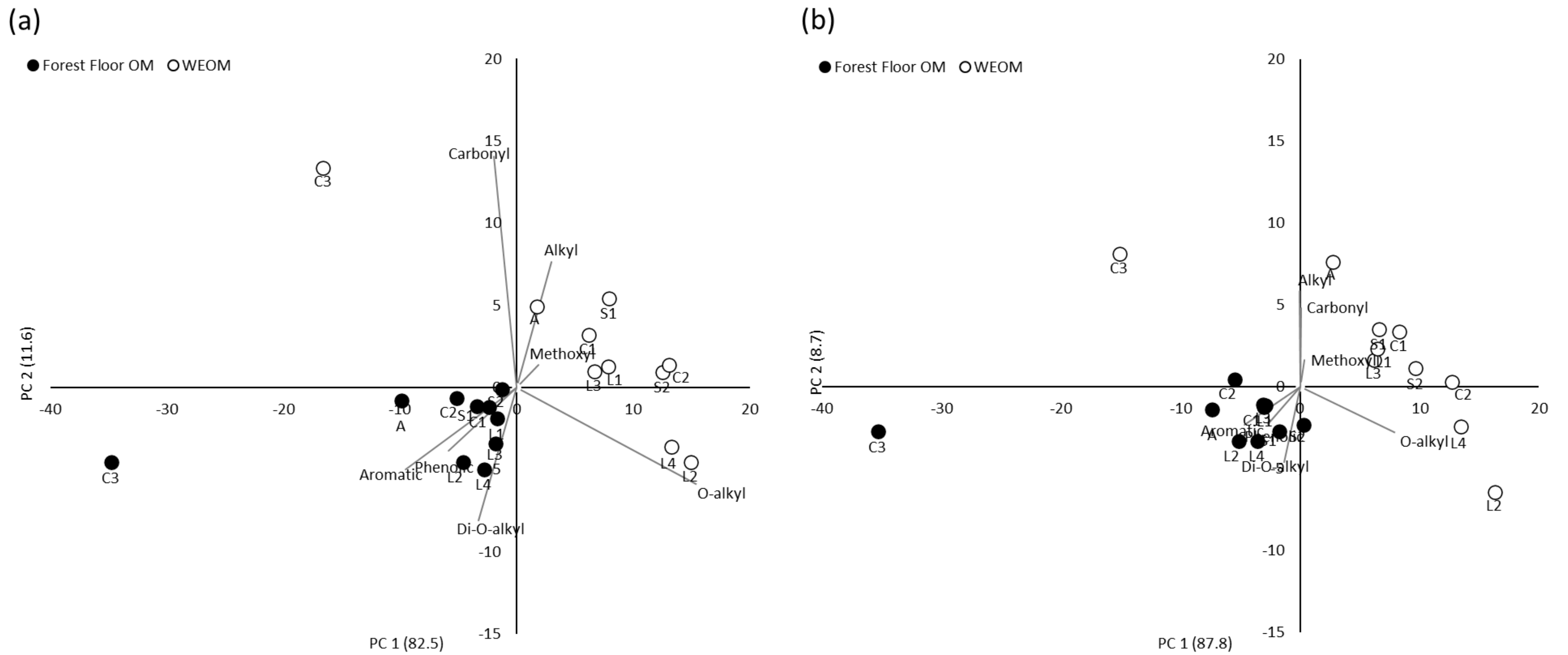

Figure 2-4: a) Scores plot obtained from a PCA of the percentage of total signal intensity found in each chemical shift region of the normalized DP NMR spectra acquired for the forest floor OM and associated WEOM. Samples taken from fire, clear-cut harvested and reference stands. b) Scores plot obtained from a PCA of the percentage of total signal intensity found in each chemical shift region of the normalized CP NMR spectra acquired for the forest floor OM and associated WEOM. Samples taken from fire, clear-cut harvested and reference stands.

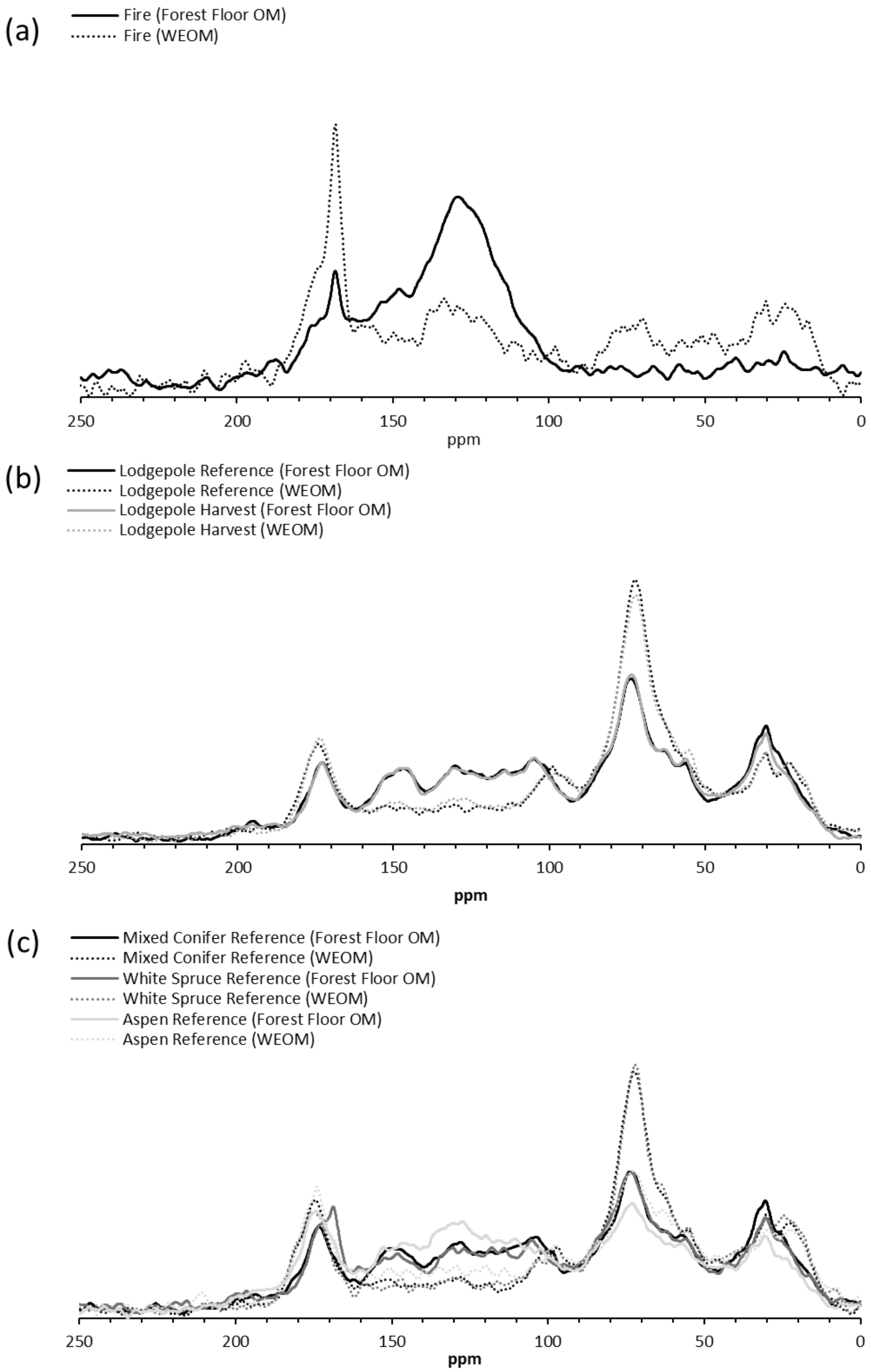

Figure 2-5: Normalized DP NMR spectra obtained for the forest floor OM and the associated WEOM. (a) mixed conifer stand affected by fire, (b) lodgepole pine harvest and reference sites, (c) spruce, mixed conifer and aspen reference sites. The spectra for lodge pole pine harvest, lodge pole pine reference, white spruce reference and mixed conifer reference have been averaged (n=2).

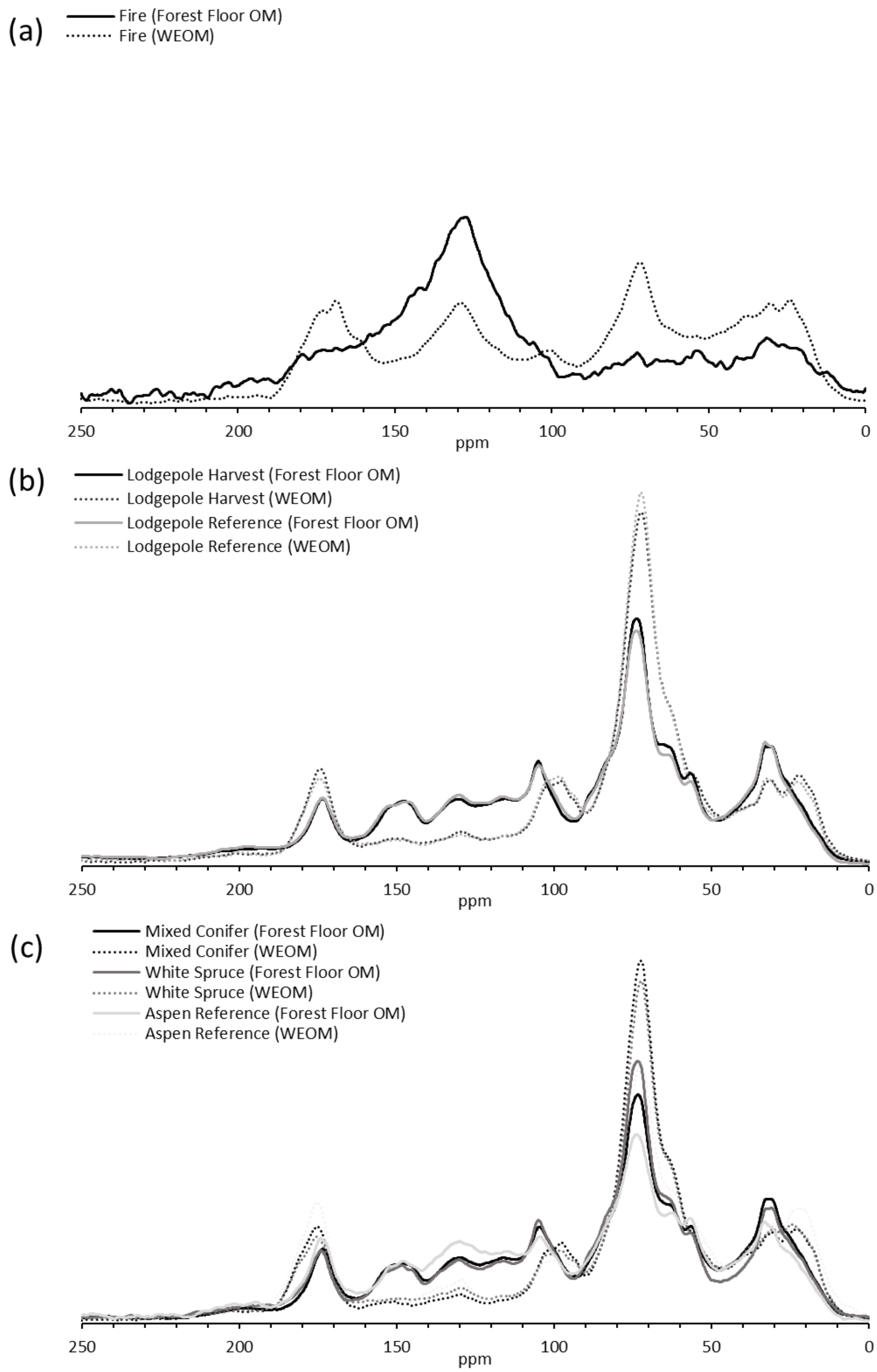

Figure 2-6: Normalized CP NMR spectra obtained for the forest floor OM and the associated WEOM. (a) mixed conifer stand affected by fire, (b) lodgepole pine harvest and reference sites, (c) spruce, mixed conifer and aspen reference sites. The spectra for lodge pole pine harvest, lodge pole pine reference, white spruce reference and mixed conifer reference have been averaged (n=2).

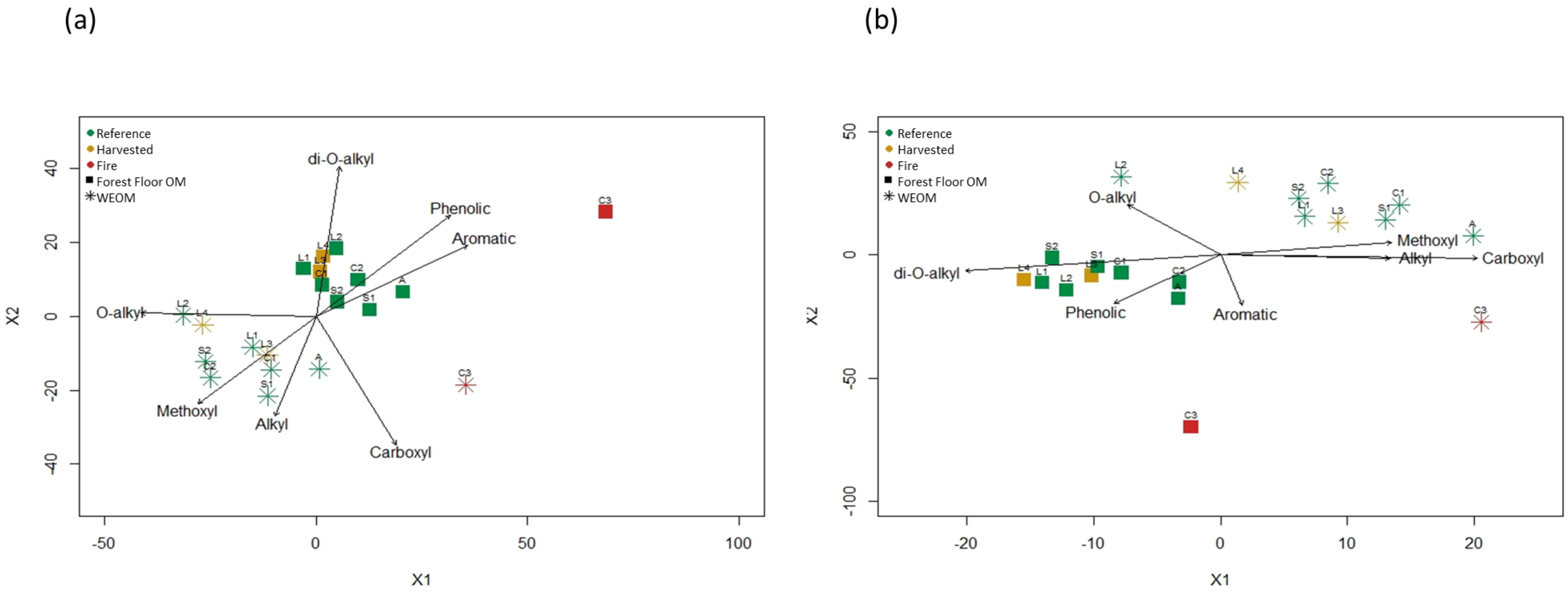

Figure 2-7: a) Two-dimensional NMDS ordination obtained for the trace of the normalized DP NMR spectra acquired for the forest floor OM and WEOM. Symbol labels are associated with site disturbance and vegetation type; L lodgepole pine, S white spruce, A trembling aspen and C mixed conifer. The vectors ($p<0.05$) are the chemical shift regions. b) Two-dimensional NMDS ordination obtained for the trace of the normalized CP NMR spectra acquired for the forest floor OM and WEOM. Symbol labels are associated with site management and vegetation type; L lodgepole pine, S white spruce, A trembling aspen and C mixed conifer. The vectors ($p<0.05$) are the chemical shift regions.

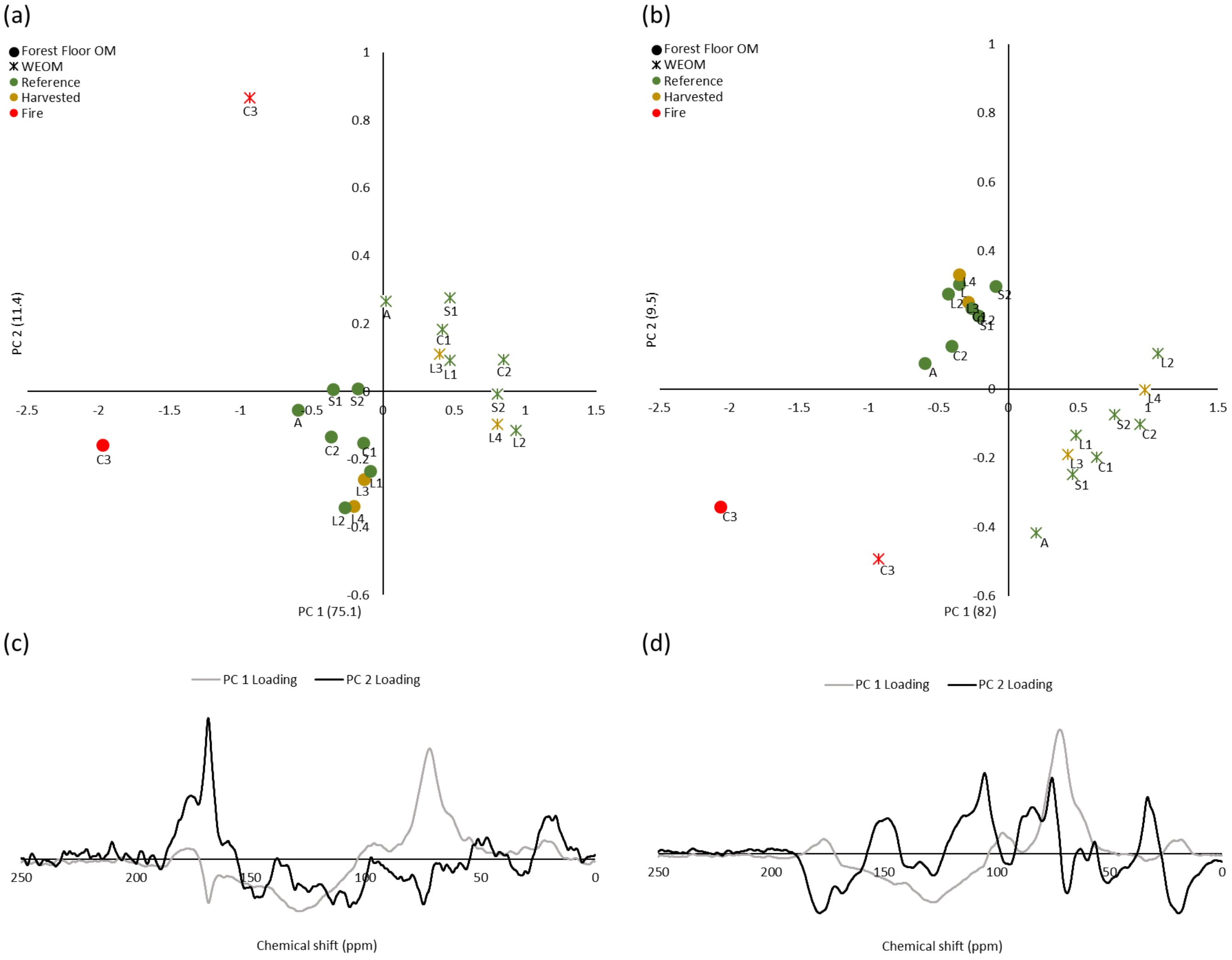

Figure 2-8: a) Scores plot obtained from a PCA of the trace of the normalized DP NMR spectra acquired for the forest floor OM and associated WEOM. Samples taken from fire, clear-cut harvested and reference stands. b) Scores plot obtained from a PCA of the trace of the normalized CP NMR spectra acquired for the forest floor OM and associated WEOM. Samples taken from clear-cut harvested and reference stands. c) Scatter plot detailing the loading spectra associated with the PCA of the DP signal intensity across of the trace. d) Scatter plot detailing the loading spectra associated with the PCA of the CP signal intensity across of the trace.

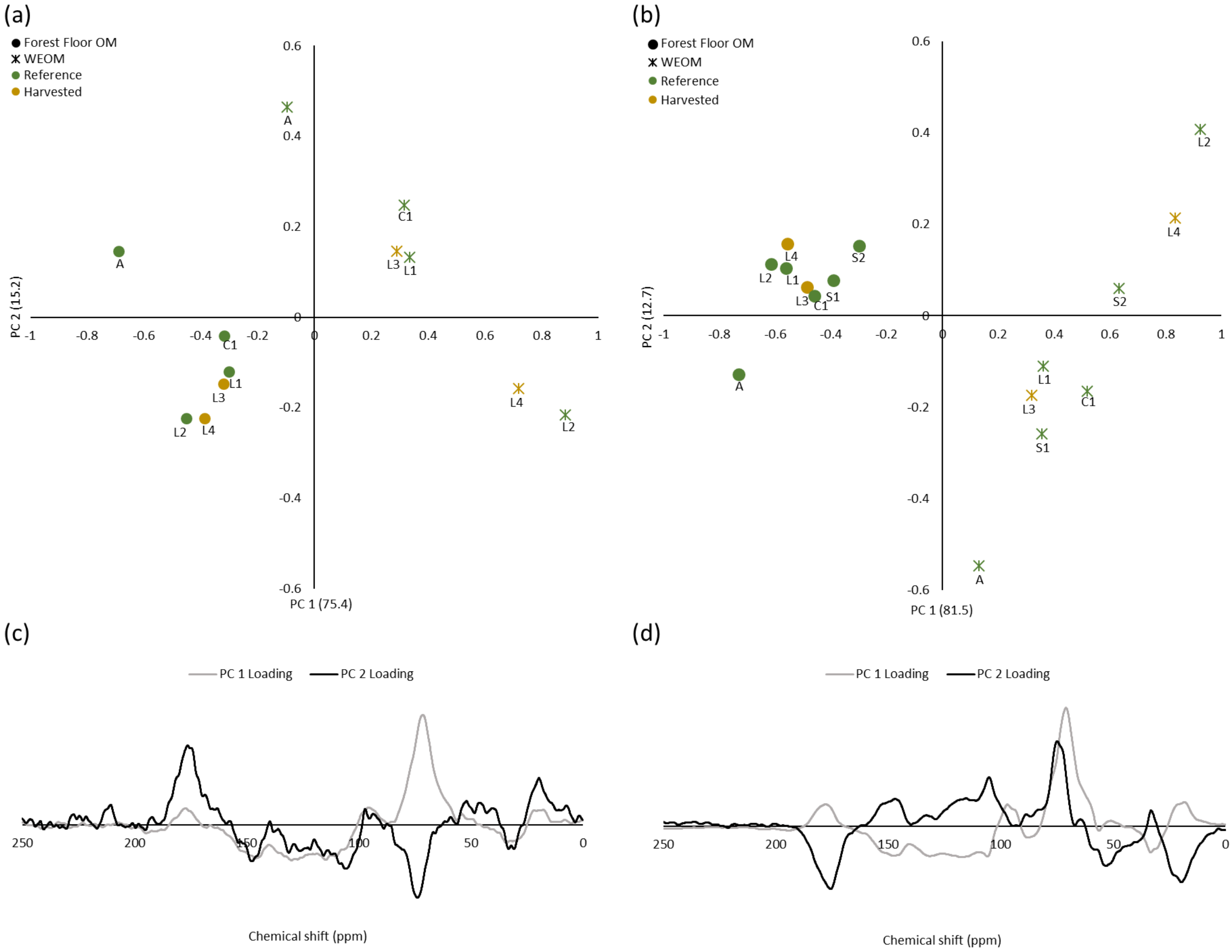

Figure 2-9: a) Scores plot obtained from a PCA of the trace of the normalized DP NMR spectra acquired for the forest floor OM and associated WEOM. Samples taken from clear-cut harvested and reference stands. b) Scores plot obtained from a PCA of the trace of the CP NMR spectra acquired for the normalized forest floor OM and associated WEOM. Samples taken from clear-cut harvested and reference stands. c) Scatter plot detailing the loading spectra associated with the PCA of the DP signal intensity across of the trace. d) Scatter plot detailing the loading spectra associated with the PCA of the CP signal intensity across of the trace.

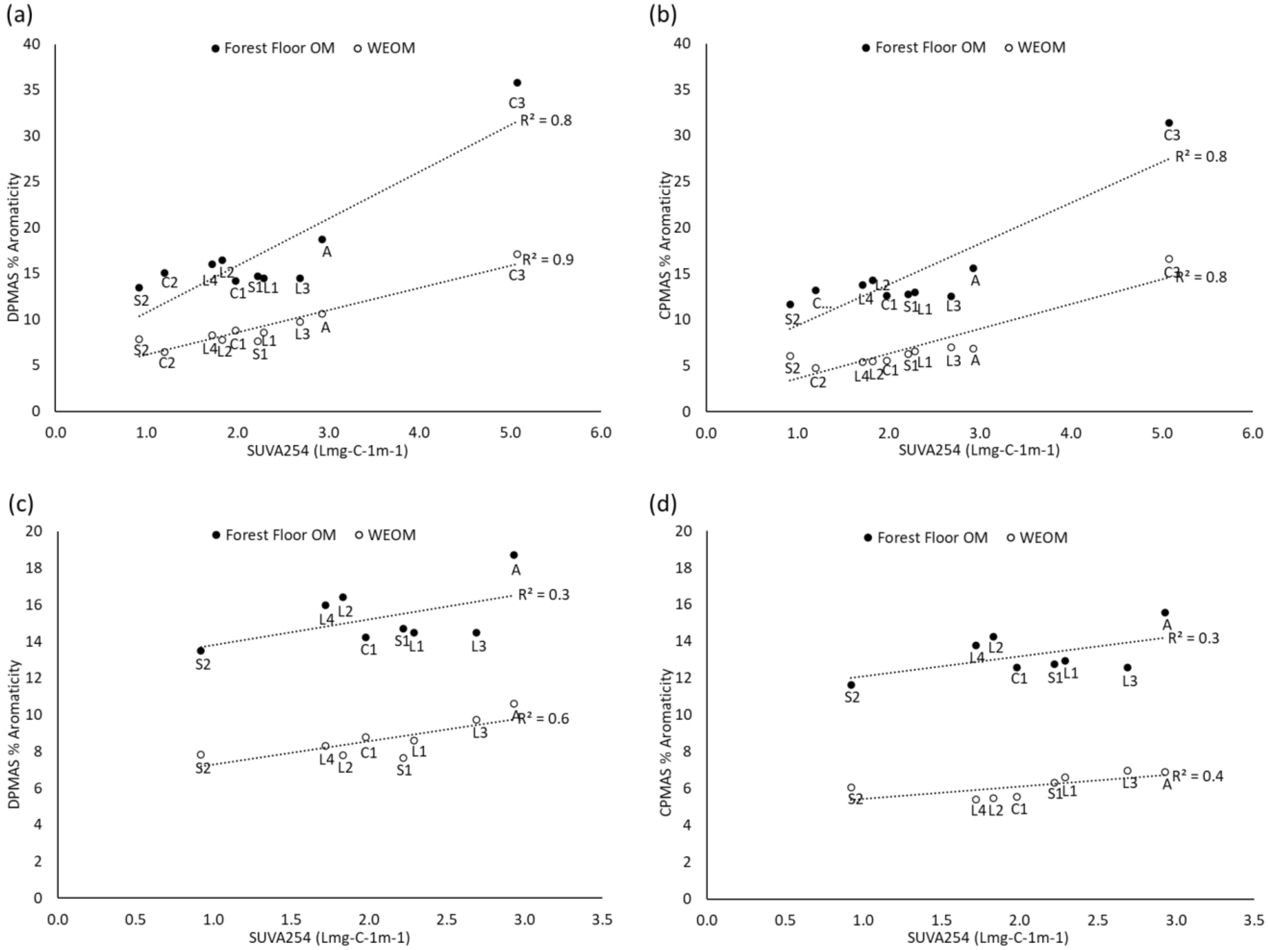

Figure 2-10: a) Scatterplot of DPMAS % Aromaticity for forest floor OM and WEOM against SUVA254 (Lmg-C-1m-1). Samples taken from fire, clear-cut harvested and reference stands. b) Scatterplot of CPMAS % Aromaticity for forest floor OM and WEOM against SUVA254 (Lmg-C-1m-1). Samples taken from fire, clear-cut harvested and reference stands. c) Scatterplot of DPMAS % Aromaticity for forest floor OM and WEOM against SUVA254 (Lmg-C-1m-1). Samples taken from clear-cut harvested and reference stands. d) Scatterplot of CPMAS % Aromaticity for forest floor OM and WEOM against SUVA254 (Lmg-C-1m-1). Samples taken from clear-cut harvested and reference stands.

Chapter 3 - Water-extractable organic matter from Canadian Rocky Mountain forest soils, and its analysis using pyrolysis-gas chromatography-mass spectrometry.

3.1 Introduction

Globally forests account for over half of the terrestrial organic carbon, the majority of which is stored belowground as soil organic matter (IPCC, 2004). Dissolved organic matter (DOM), while a small fraction of soil organic matter, plays a key ecological role in terrestrial ecosystems as their most labile soil carbon and nutrient pool (Kolka et al., 2008; Michalzik & Matzner, 1999; Qualls & Haines, 1991). It provides energy to decomposer organisms and acts as a major carbon influx to deeper soil horizons (Guggenberger et al., 1994; Mcdowell & Likens, 1988; Neff & Asner, 2001; Qualls & Haines, 1991). In forest soils, DOM leaching to mineral horizons accounts for 10 to 46% of the total amount of carbon annually lost from forest floors (Cronan, 1985; Vance & David, 1991). While some of the produced DOM is mineralized by microbes or adsorbed to the soil mineral matrix (Kalbitz et al., 2000; Nakhavali et al., 2020), the remainder is transferred to aquatic ecosystems, and constitutes an important yet often overlooked component of the terrestrial carbon balance (Nakhavali et al., 2020). The magnitude of terrestrial DOM export to surface waters greatly varies according to climatic conditions, soil type, landscape connectivity, and land use. A compilation of studies across forest types showed lower DOM concentrations in boreal compared to temperate soils, and lower concentrations under broadleaved compared to conifer forests (Camino-Serrano et al., 2014). However, there is much less clarity about the relative importance of these different environmental controls on DOM composition.

Land disturbances within forested watersheds can potentially change both the concentration and character of DOM (Aiken et al., 2011; Emelko & Sham, 2014; O'Donnell et al., 2010). Increased DOM concentrations and increased water fluxes following forest harvesting (Sørensen et al., 2009) result in increased DOM export from watersheds which subsequently impacts water quality for human uses including drinking water quality. The impact of fire on water quality and quantity from source watersheds can increase the export of sediments (Moody et al., 2008; Silins et al., 2009), nutrients (Bladon et al., 2008, 2014; Mast & Clow, 2008; Silins et al., 2014), and trace metals (Kelly et al., 2006). Also, elevated DOM levels can promote algae and bacterial growth resulting in taste and odor-causing issues with municipal drinking water (Emelko et al., 2011) and may have long-term implications with the potential production of carcinogenic disinfection by-products (DBP) during drinking water treatment (Ledesma et al., 2012; Siddiqui et al., 1997). Many of these factors may be altered in the future with a changing climate. A review of existing evidence highlighted how the projected increased frequency and intensity of extreme weather events may increase the seasonality and alter the chemical character of DOM; it also underlined the need for further research into the effects of vegetation and land management on DOM export and composition (Ritson et al., 2014).

Numerous analytical techniques have been used to investigate the chemical composition of soil organic matter and DOM, although they vary in terms of molecular insight and level of complexity (McCallister et al., 2018). One such technique is pyrolysis–gas chromatography–mass spectrometry (PyGCMS), which has been proven an efficient tool to characterize organic matter composition at the molecular level including identification of biomarkers of microbial and plant origin (Derenne & Quénéa, 2015). The addition of tetramethylammonium hydroxide (TMAH) to samples facilitates chemolytic reactions that cleave larger molecules into smaller molecules, which are then identifiable at lower, sub-pyrolysis temperatures (Page et al., 2002). The use of TMAH also results in the derivatization of carboxyl and hydroxyl groups to methyl ester and methoxyl groups, which improves detection of polar compounds such as lignins, tannins, and cutin and suberin markers (Chefetz et al., 2000; Collard et al., 2015; Grote et al., 2000; Jeanneau et al., 2014). The addition of TMAH is not without drawbacks, however, as it does not allow for differentiation between hydroxyl groups and pre-existing methoxyl groups and may result in an overestimation of lignin moieties (Derenne & Quénéa, 2015). Stewart (2012), who analyzed vegetation and soil samples from a tropical forest ecosystem using both TMAH-PyGCMS and PyGCMS, concluded that the two treatments provided complementary information and were most useful when used in combination. Similarly, using double-shot pyrolysis can provide additional information, as two sequential GC analyses are conducted on the same sample. Double-shot pyrolysis includes both the detection of the more volatile compounds obtained during the first, low temperature thermal desorption stage (80-350°C); and the fragmentation and detection of the higher molecular weight polymeric compounds present in the residual sample during the second, high temperature (500-800°C) pyrolysis stage of the analysis (Quénéa et al., 2006). The relative advantage of these different analytical techniques may vary according to sample type, but studies using PyGCMS to characterize soils from northern forests are scarce.

Water extractable organic matter (WEOM) is DOM extracted from soils under defined laboratory conditions (Guigue et al., 2014). Numerous studies have characterized WEOM using UV-vis and fluorescence spectroscopy techniques, including the assessment of WEOM variation with soil depth (Corvasce et al., 2006); and WEOM response to land disturbance following fire (Vergnoux et al., 2011) and changes in land management (Praise et al., 2020). Studies using PyGCMS to characterize DOC or WEOM are much more limited (Kaal et al., 2017; Monda et al., 2017), and an even lower number have focused on the soil environment. Currently there are gaps in our understanding of how environmental factors control WEOM quantity and quality under different land uses, and how WEOM composition varies between organic and mineral soil horizons. Further, double-shot PyGCMS has been underutilized as an analytical tool to characterize WEOM chemical composition. The following research aimed to fill some of these gaps by: (1) assessing different PyGCMS analytical techniques to determine which one is the most advantageous in producing a detailed WEOM chemical fingerprint from forest soil samples, (2) determining if there is an impact of harvesting and/or wildfire on the chemical character of WEOM within these forest soils, and (3) identifying how WEOM from forest floors may be related to WEOM from surficial mineral horizons under different tree canopy covers. In addition to investigating the chemical fingerprints produced using the different PyGCMS techniques with ordination and multivariate regression trees, this study took advantage of indicator species analyses to identify potential molecular markers of disturbance, vegetation, and soil horizon type.

3.2 Methods and Materials

3.2.1 Study Area and Sampling Strategy

This study was completed in the Montane Cordillera Ecozone of south-western Alberta, Canada, and included four watersheds located within the headwaters of the Oldman watershed, one of Alberta's main water supplies. The Star Creek and North York Creek watershed are situated in Crowsnest Pass near Coleman, Alberta (49.64° N, 114.50° W) (Figure 3-1), whereas Yarrow Creek and Cameron Creek are found near Waterton Lakes National Park (49.05° N, 113.91° W) (Figure 3-2). These watersheds span an elevation of 1200-2000 m on the eastern slopes of the southern Albertan Rocky Mountain range, and encompass lower and upper montane, sub-alpine, and alpine ecological zones (Natural Regions Committee, 2006). The climate is continental with precipitation ranging between 650 and 1900 mm/yr, with about half falling as snow. Average temperatures for

January range between -7 and -10°C, and the average June temperature is 11°C (Bladon et al., 2008; Coen & Holland, 1976). Vegetation at lower elevations is dominated by lodgepole pine (*Pinus Contorta*), with discrete stands of trembling aspen (*Populus Tremuloides*). The dominant vegetation at higher, subalpine elevations consists of mixed conifers, including lodgepole pine, subalpine fir (*Abies lasiocarpa*), with white spruce (*Picea glauca*) in the riparian areas. Soils in the area are weakly developed, as is typical of high elevation northern climates, and include Regosols, Eutric and Dystric Brunisols.

Ten sampling locations within the four watersheds were selected using ArcGIS to span two disturbances (harvesting, fire) as well as corresponding undisturbed (reference) sites (Table 3-1). For the disturbed sites, sampling was conducted three years following harvesting and one year following fire. At each sampling location, three cores of the forest floor were collected within a 3m radius with a hand auger and composited to yield one representative sample per site. The top 10 cm of the mineral soils were sampled using the same approach.

3.2.2 Laboratory Analyses

The forest floor and mineral soil samples were sieved to ensure a homogenous matrix by removing rocks and vegetation debris. The forest floor was sieved at 4 mm, and the mineral soil at 2 mm (Chantigny et al., 2007). Sieved samples were air-dried and finely ground using a Retsch MM200 ball grinder before they underwent a water extraction process adapted from Chantigny et al. (2008) to obtain the water extractable organic matter (WEOM). All samples were extracted and analyzed in duplicate. In brief, following addition of hexanedioic acid (dimethyl adipate-Aldrich 186252-100G) as a surrogate standard, ultra-pure water was added to the centrifuge tubes containing the samples. This was followed by sonification for 15 minutes and centrifugation at 4000 rpm for one hour. The extraction process was repeated three times until ~75ml of solution was collected resulting in a 1:15 soil:water ratio for the forest floor and a 1:7.5 ratio for the mineral soil samples. Solutions were filtered through a 0.45 µm filter (Kolka et al., 2008), frozen for 12 hours at -20°C, and then freeze-dried.

The WEOM freeze-dried samples underwent analysis by double-shot pyrolysis gas chromatography mass spectrometry (PyGCMS) using a Frontier - EGA/PY-3030D pyrolyzer. Separation was performed on an Agilent Technologies 7890B gas chromatograph (GC) equipped with a HP-5MS

UI column (60 m x 0.25 mm x 0.25 μm) and connected to a 5977A (Agilent Technologies) mass spectrometer. Each sample was analyzed with and without tetramethylammonium hydroxide, 25wt. % in methanol (TMAH). Approximately 1.5mg for forest floor and 2.5mg for mineral soil was loaded into a sample cup and 1.5μg of the instrument response standard, heptadecanoic acid, methyl ester (C17:0 internal standard) was added. For TMAH runs, 2μL of TMAH was added to the sample along with the instrument response standard. During the thermal desorption (TD) phase, the sample cup was introduced into the furnace at 100°C, where the temperature was raised to 320°C at a rate of 50°C min^{-1} and held at 320°C for 3 minutes, for a total time of 7.5 min. Once the thermal desorption was complete, the remaining sample fraction was ejected from the furnace into a cool zone to wait for the furnace to get to the pyrolysis temperature (600°C). The sample was then pyrolyzed at 600°C for 0.5 min, with the Py/GC interface and GC injector temperatures set at 300 °C (split ratio 25:1). Compounds were identified on the mass spectrometer in the electron impact (EI) mode at 70eV ionization energy, and acquisition scanned the mass range from *m/z* 28 to 650. Data were processed using Agilent Mass Hunter Qualitative Analysis B.07.00 software. Compounds were identified by searching the NIST14 and Wiley 17 reference libraries, as well as our custom-made library. Our library was built running external standards and calculating target ions using homologous series based on spectra found in the literature and open access libraries. A series of external standards were run to confirm retention time and their fragmentations were used to build our custom library. Standards included: fatty acid methyl esters (C6 to C30), phenolic monomers present in lignin or products of lignin degradation (p-hydroxybenzaldehyde, p-hydroxy acetophenone, vanillin, ethylvanillin, acetovanillone, syringaldehyde, acetosyringone, phenylacetic acid, vanillic acid, syringic acid, p-coumaric acid, ferulic acid), alkanes (C4-C40 -Sigma Aldrich), aromatics (BTEX mix, polyaromatic hydrocarbon mix -Sigma Aldrich), tannic acid, humic acid, catechol (Sigma Aldrich), carbohydrates (furan, D-glucose, D-galactose, D-glucuronic acid, D-galacturonic acid), and nitrogen containing compounds (4-aminobutyric acid, L-alanine). The GC C17:0 internal standard and the surrogate standard were used to calculate the amount of each identified chemical compound as μg g^{-1} of soil. Relative chemical concentrations (% total WEOM) were also calculated by dividing the concentration of each compound (μg g^{-1} of soil) by the sum of all detected compounds for that sample.

3.2.3 Data Analyses

All statistical analyses were completed using R Version 3.6.1 (R Core Team, 2018). The following six GCMS analytical techniques were considered during statistical analysis: 1. Thermal desorption phase with TMAH (TMAH-TDGCM), 2. Thermal desorption phase without TMAH (TDGCMS); 3. Pyrolysis phase with TMAH (TMAH-PyGCMS); 4. Pyrolysis phase without TMAH (PyGCMS); 5. Both thermal desorption and pyrolysis phases with TMAH (TMAH-double-shot PyGCMS), and 6. Both thermal desorption and pyrolysis phases without TMAH (double-shot PyGCMS). All chemical compounds identified by GC-MS were analyzed using a non-metric multidimensional scaling (NMDS) ordination. As laboratory duplicates were close in the ordination space (e.g., see Figure 3-11 for the double-shot PyGCMS data), they were averaged for all subsequent analyses. Statistical differences among the six analytical techniques for each of the main carbon functional groups (aliphatics, carbohydrates, nitrogen containing compounds, phenolics, and aromatics) were assessed using a PERMANOVA followed by a Tukey's Honest Significant Difference test.

Overall, three GCMS analytical approaches were compared (thermal desorption, pyrolysis and double-shot pyrolysis) with two chemical treatments (with and without TMAH), as well as three stand types (reference, harvest and fire), and two soil horizons (forest floor and mineral soil). Data underwent a Hellinger transformation to account for the chemical compounds that had low values or zeros. The NMDS ordinations were completed using chemical compound relative concentrations (% of total) following the Hellinger transformation using the MetaMDS function in the vegan package (Oksanen et al., 2022). The Sorensen (Bray-Curtis) distance was used in all NMDS analyses as determined by the MetaMDS function. Carbon functional groups were considered as potential correlation vectors. The main carbon functional groups (aliphatics, carbohydrates, nitrogen containing compounds, phenolics, and aromatics) were fitted to the ordinations as correlation vectors using the envfit function, which is also included in the R vegan package (Oksanen et al., 2022). Only vectors showing a significant correlation ($p<0.05$) are presented on the ordinations, with the length of the vector corresponding to the strength of the correlation.

A multivariate regression tree (MRT) analysis was performed to explore relationships between WEOM composition and potential explanatory variables (De'ath, 2002). Data were then transformed to % total (similar to the data used for the NMDS analysis) along with a Bray-Curtis distance measure. Chosen variables included the GCMS analytical techniques (TD and Py), the

TMAH treatments (present and not present), soil horizons (forest floor and mineral soil), disturbance type (reference, harvest and fire) and vegetation (lodgepole pine, white spruce, mixed conifers and trembling aspen). The MRTs were computed using the devtools package (Wickham et al., 2022), RTools software and the mvpart package (Therneau et al., 2009).

To investigate if specific chemical compounds (pyrolysates) were associated with distinct sample groups, several indicator species analyses (ISA) were explored using the indicspecies package (De Cáceres & Legendre, 2009) in R. The ISA is a form of cluster analysis (Dufrêne & Legendre, 1997) that provides a list of species (in our case chemical compounds) and their associated statistical values reflecting the strength of their associations with different representative groups. The representative groups for these analyses were the soil horizons (forest floor or top mineral soil), the dominant vegetation canopy types, fire and harvesting disturbance and presence/absence of TMAH. The calculated ISA index (stat value) includes two indicator components: A, known as the specificity or positive predictive value, which represents the probability that a given pyrolysate belongs to the targeted group; and B, known as the fidelity or sensitivity of the indicator, which represents the probability of finding the pyrolysate belonging to the targeted group (De Cáceres & Legendre, 2009).

3.3 Results

3.3.1 Analytical Techniques

A slightly greater number of pyrolysates was identified using TDGCMS than with PyGCMS, and as expected, the double-shot PyGCMS analysis, which combined results from TDGCMS and PyGCMS, yielded the most compounds. Overall, for PyGCMS, there was little difference in how many compounds were detected whether TMAH was added or not; a total of 451 compounds were compiled with TMAH and 444 without. However, for TDGCMS the addition of TMAH increased the total number of compounds identified from 360 (no TMAH) to 475 with TMAH. Similarly, the double-shot PyGCMS analysis yielded a greater number of compounds when TMAH was present (773) than without (674).

In addition to differences in the total number of compounds, clear differences were evident in the chemical fingerprints identified by the TDGCMS and PyGCMS techniques, and their associated TMAH treatments as evidenced in Figure 3-3. Figure 3-3 is a two-dimensional representation of the non-metric multidimensional scaling analysis of all pyrolysate concentrations (%), where a final stress of 11% was achieved after 20 iterations. The percentage of data variance explained by the two axes was 37% for the first axis (MDS1) and 12% for MDS2. There was a clear separation between the pyrolysis and thermal desorption results. Further, for PyGCMS, there was a tight clustering of all data points for both TMAH treatments, while the TMAH treatments clearly influenced the TDGCMS results. This clear separation between TDGCMS and PyGCMS results was further apparent from a MRT analysis completed on all compounds (Figure 3-4) where the first branching explaining over two thirds of the data variance was related to the GCMS technique used (PyGCMS or TDGCMS). A second branching was linked to the presence or absence of TMAH treatments for the TDGCMS, while no further split was detected for the PyGCMS results.

As seen in Figure 3-5, the relative amount of aliphatics was comparable across all GCMS techniques. However, there were clear differences between TDGCMS and PyGCMS for the carbohydrates and phenolics, where TDGCMS detected significantly more carbohydrates but less phenolics compared to PyGCMS. In addition, TDGCMS tended to detect relatively less N-containing compounds and less aromatics than PyGCMS, but the differences were not always statistically significant. For TDGCMS, the addition of TMAH significantly increased the relative concentrations of aromatics and N-containing compounds, and significantly decreased the relative concentration of carbohydrates.

An indicator species analysis was completed comparing the two TMAH treatments for TDGCMS (Table 3-5). There were 10 chemical compounds associated with TDGCMS and 29 when TMAH was present. Without TMAH, the majority of compounds identified as indicators were carbohydrates, but a wider range was identified as representative of the TMAH treatment, including aliphatics, aromatics and nitrogen containing compounds.

3.3.2 Environmental Factors

Forest floor and mineral soil samples showed distinct chemical fingerprints following TDGCMS regardless of whether TMAH was added or not (Figure 3-6 and Figure 3-7). Non-linear multi-dimensional scaling analysis of pyrolysate concentrations (%) determined using TDGCMS without TMAH reached a final stress of 10% after 20 iterations where the percentage of variance explained was 26% for MDS1 and 16% for MDS2 (Figure 3-6). The non-linear multi-dimensional scaling analysis of chemical compound concentrations (%) determined with TDGCMS with the TMAH treatment reached a final stress of 11% after 20 iterations; MDS1 explained 44% of the total data variance and MDS2 explained 13% (Figure 3-7). Vectors on both plots detailed significant relationships with the main carbon functional groups. Forest floor samples were associated with carbohydrates both with and without the TMAH treatment (Figure 3-6 and Figure 3-7). When TMAH was absent, forest floor samples were also associated with phenolics while aromatics and aliphatics tended to be more closely associated with the mineral soil samples (Figure 3-6). In addition, a MRT analysis was conducted on all TDGCMS data (Figure 3-8). As seen previously on Figure 3-4, the most important grouping factor was linked to the TMAH treatment, which explained 58% of the variance. With TMAH present, the first branching tree separated mineral soils from forest floors (Figure 3-8) but when TMAH was absent, the first branching separated fire samples from the reference and harvested sites. The two fire samples (C3) were also clearly separated from the rest on both NMDS analyses (Figure 3-6 and Figure 3-7). As the MRT analysis highlighted the separation between forest floors and mineral soils when samples were analyzed with TMAH-TDGCMS, an indicator species analysis (Table 3-2) was completed comparing the two soil horizons. There were 18 indicator chemical compounds associated with the forest floors and seven with the mineral horizons. The compounds associated with the forest floors were mostly aliphatic whereas the compounds associated with the mineral horizons were predominantly aliphatic and aromatic.

Figure 3-9 shows results of three-dimensional non-metric multidimensional scaling analysis of the pyrolysate concentrations (%) with both TMAH treatments present. Stress was 11%, and the two visible axes present explain most of the variability in the data with the breakdown in the axes being: MDS1 = 20% and MDS2 = 14%. Phenolics and carbohydrates were associated with the forest floor samples as seen previously for the TDGCMS ordination. However, the separation between forest floor and mineral soil samples was not as clear as shown in the TDGCMS ordinations (Figure 3-6 and Figure 3-7). On the other hand, different vegetation types, in particular samples from the lodgepole pine stands demonstrated a tight clustering. This observation was confirmed by results from the MRT analysis (Figure 3-10) where the main driver for the tree branching was the dominant canopy vegetation with a variance of 28%. An indicator species analysis (Table 3-3) was then completed on the dominant canopy vegetation for the PyGCMS data. With this analysis, two chemical compounds were associated with lodgepole pine, 13 with trembling aspen, one with white spruce, four with the mixed conifer and white spruce grouping and 21 with the mixed conifer, white spruce and trembling aspen group. The indicator compounds associated with trembling aspen were mostly aliphatic as were the compounds associated with the grouping of mixed conifer, white spruce and trembling aspen.

What was apparent across the different analytical techniques was the clear separation between the forest floor and mineral soil samples, including the fire-affected samples. However, as seen in the double-shot PyGCMS analysis (Figure 3-11), the fire-affected samples were separated but the fire-affected forest floor samples were more closely associated with the mineral soil samples, specifically the ones under aspen, than with the rest of the forest floor samples. The fire mineral soil samples were similar to the rest of the other mineral soil samples. An indicator species analysis (Table 3-4), completed on the double-shot PyGCMS data, identified 21 indicator chemical compounds associated with the fire-affected samples, primarily aromatic in nature.

3.4 Discussion

3.4.1 Analytical techniques

The first shot (thermal desorption), which allowed detection of the more thermolabile compounds present in the WEOM samples, generated a greater number of products than the subsequent pyrolysis. It also responded strongly to the addition of TMAH. Without TMAH, proportionally more carbohydrates were identified, including some key identified products such as furanones, which are typical products derived from cellulose (Quénéa et al., 2006). The addition of TMAH yielded a greater diversity of products and a greater number of aliphatic compounds, including several short-chain fatty acid methyl esters (C6-C9), N-containing products including pyrimidinediones, and substituted benzene compounds. This is similar to what Stewart (2012) reported when comparing PyGCMS and TMAH-PyGCMS analyses of tropical vegetation and soil, where she detected comparable lignin-derived aromatics when using TMAH.

The TMAH-TDGCMS method was the most sensitive in term of detecting differences between forest floors and mineral soil horizons. Specific compounds associated with forest floors included saturated and unsaturated fatty acids, some of which have been previously identified as components of soil microbial cell membranes, including: the ubiquitous methyl stearate (18:0); and 8-Octadecenoic acid, methyl ester (18:1ϖ9c) and 9-Octadecenoic acid, methyl ester (18:1 ϖ8c), both preferentially present in gram (-) bacteria. Docosanoic acid, methyl ester has been identified as a suberin monomer (Otto & Simpson, 2005). In addition, several abietic acids were detected for forest floors, which are the predominant constituents of higher plant resins, especially in conifers, such as pine and spruce. Diterpenoid acids such as abietic acid and dehydroabietic acid, in particular, are known to be the major constituents in conifer resin (Hjulström et al., 2006).

Statistical analysis confirmed that harvesting disturbance was not one of the main drivers for differences observed among WEOM chemical signatures. Instead, when PyGCMS was used as the analytical technique, differences linked to the dominant canopy vegetation were identified with a separation between lodgepole pine compared to the other tree stands (aspen, white spruce, mixed conifers). The specific compounds associated with the grouping of white spruce, mixed conifer, and trembling aspen included aliphatic compounds such as cycloalkenes and n-alkenes, carbohydrates in the form of an aldehyde and a saturated fatty acid as well as several N-containing products. Specific chemical compounds associated with mixed conifer and white spruce WEOM consisted of N-containing compounds which were either aromatic N heterocyclic or nitriles, and which commonly occur from secondary reactions of peptides during pyrolysis (Derenne & Quénéa, 2015). In addition, aspen WEOM generated a variety of compounds with the largest grouping being aliphatic in nature. The aliphatic compounds included n-alkanes, substituted alkenes, and n-alkanols. In particular, 1-Undecene could be a by-product of suberin (Mazzetto et al., 2019), while n-alkanes produced by plants tend to be odd-numbered carbon chains in the range of C_{23}-C_{33} (Girona-García et al., 2019; Quénéa et al., 2006). The n-alkanes associated with the aspen samples were dominated by shorter chain molecules C_{21}, C_{14} and C_{17}, which indicated that their origin was more likely microbial in nature.

Overall, results from the different GCMS methods were complementary in terms of the chemical information they provided. However, depending on the focus of the study, specific techniques may be chosen to highlight the presence of specific compound group or functional C-groups. While differences between forest floors and mineral horizons were best distinguished by their thermolabile fingerprints (i.e., through TMAH-TDGCMS), differences linked to vegetation were best distinguished when their pyrolysis products were included (i.e., through PyGCMS).

3.4.2 Environmental Factors

3.4.2.1 Fire

The effect of fire on soil is variable and depends on the type and intensity of the fire, the nature of the materials being burnt, soil moisture and many other soil properties such as thickness of the surficial litter layer, organic matter content of the mineral horizons, pH, and texture (González-Pérez et al., 2004). Depending on severity, a fire can result in volatilization of minor compounds, charring, or complete oxidation of the soil organic matter (Certini, 2005). Extensive research has focused on fire impacts on SOM composition using different methods including Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (NMR) and pyrolysis GCMS (Knicker, 2007) but only a few studies have evaluated WEOM. One such study by Vergnoux et al. (2011) used UV-vis and fluorescence spectroscopy to determine the impact of fire on WEOM fractions (hydrophilic, transphilic and hydrophobic) and humic substances (humic acids, fulvic acids and the non-humified fraction) in the top (0-5 cm) layer of Mediterranean soils. Both humic components and WEOM were affected by fire, with an observed increase in the aromaticity of the hydrophilic fraction along with a decrease in the less humified, lower molecular weighted aromatic compounds from the transphilic and hydrophobic fractions (Vergnoux et al., 2011). However, Vergnoux et al. (2011) did not investigate the impact on deeper soil layers primarily due to the lack of significant differences observed in total organic carbon (TOC) content between the burnt and the control sites. This finding aligns with our research results, where even though TOC analysis was not conducted, it was apparent that there was a similar composition between the fire-affected mineral soil samples and the reference mineral soil samples. Our findings reinforce the understanding that the impact of fire on SOM composition is more pronounced in surface layers, while deeper soil layers may remain relatively unaffected if they do not experience the same elevated temperature during fire.

As seen using double-shot PyGCMS, the fire impacted forest floor exhibited a chemical signature distinct from both the reference (conifer dominated) and harvested sites forest floors. The analysis further showed that several known black carbon makers, mostly aromatic in nature, were present in the fire affected WEOM samples. These black carbon markers included polycarboxylic benzoic acids, biphenyls, and a series of substituted PAHs, similar to what has been reported in the literature by Derenne and Quénéa (2015). Similarly, the research completed by Ide et al. (2020), which examined DOM composition in boreal forest soils with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR), indicated a greater concentration of condensed aromatic structures in DOM right after fire. In our study, the double-shot PyGCMS analyses also showed a similar chemical composition for the fire-impacted forest floor samples and the reference aspen mineral soil samples. This association was more apparent with the TMAH analysis. This similarity could be attributed to remnant charcoal present at the aspen site from previous slash burn harvesting or more likely the continuing legacy of a previous forest fire.

3.4.2.2 Forest Harvesting and Dominant Canopy Vegetation

Harvesting influence on soil organic matter composition has been studied in the past although previous research has focused mostly on forest floors. There are also no specific studies focusing on forest floor and mineral soil WEOM under harvesting disturbance using PyGCMS. For instance, the study completed by Hannam et al. (2005) examined the impact of harvesting on the composition of boreal forest floors from northwestern Alberta using cross- polarization, magic-angle spinning (CPMAS) ^{13}C nuclear magnetic resonance (NMR). This study showed that the composition of forest floors in harvested stands differed from that of uncut stands as they became more aromatic with increased decomposition and decreased fresh litter inputs following harvesting. Thiffault et al. (2008) also used CPMAS ^{13}C NMR to assess forest floor chemical composition and nutrient availability after wildlife and harvesting in Quebec boreal forest. They concluded that both wildfire and harvesting impacted the chemical composition of the forest floor, but that wildfire had a greater impact where there was a higher concentration of aromatic C attributable to charcoal and a lower concentration of labile C compared to the harvested sites. It is important to note that this research did not cover the impact of disturbance on organic matter composition in mineral soils. Overall, our study shows that there was little impact of harvesting on WEOM, and that the WEOM chemical signature from the harvested sites was comparable to the reference sites in both the forest floor and in the underlying mineral soil.

As opposed to the very limited impact of harvesting, the legacy of the dominant vegetative cover remained strong after clearcutting disturbance. Wickland et al. (2007) used vegetation leachates to demonstrate that DOC concentrations varied by vegetation species, while Yoshida et al. (2018) showed that DOM leaching under a coniferous cover was higher than that in a broadleaf forest. As indicated on all of the GCMS analyses within our study, the lodgepole pine reference and harvest sites clearly showed a similar WEOM chemical signature in both the forest floor and in the mineral soil that was different from the other samples. The PyGCMS analysis further showed that vegetation was one of the main drivers with regards to the WEOM chemical signature where the lodgepole pine samples were clustered away from the white spruce, mixed conifer and trembling aspen samples. These results are supported by the study by Ohno et al. (2014), who determined that forest composition impacted the chemical composition of subsoil WEOM, while Crecelius et al (2017) reported that differences in WEOM characteristics linked to different vegetation types could even be preserved following fire.

3.4.2.3 Soil Horizons

Several studies have investigated the vertical transport of DOC through soil profiles by characterizing WEOM composition from different soil horizons. For instance, Corvasce et al. (2006) attributed the change in WEOM signature with depth to the preferential migration of structurally simple molecules from the soil surficial horizons and to the preferential adsorption of aromatic moieties to the soil mineral matrix. Other processes controlling WEOM composition within a given soil mineral horizon may include biodegradation of DOM during leaching from surficial horizons, desorption from the mineral matrix, release from decomposing organic matter and microbial recycling at depth (Kalbitz et al., 2000). Leaching of fresh DOM from forest floors can be an important source for WEOM found in soil mineral layer due to simultaneous sorption and desorption of DOM, in other words the replacement of mineral-associated organic matter carbon by fresh DOM (Leinemann et al., 2018).

Our study results demonstrated a clear separation between the forest floor and mineral soil WEOM composition. Overall, the forest floor WEOM was relatively enriched in carbohydrates and phenolics, while the mineral soil WEOM showed more nitrogen containing compounds, aromatics and aliphatics. This increase in aromaticity with depth was previously reported by Ohno et al.(2014), who showed that under both deciduous and coniferous cover, WEOM aromaticity increased from the forest floor to the underlying E horizon (corresponding to the Ae horizon in the Canadian Soil Classification). Part of our results may be explained by the preferential adsorption of aromatics previously leached from the forest floors and subsequently released during the laboratory extraction used to obtain WEOM from the mineral soil samples. In addition, some of the differences in WEOM composition may be due to differences in SOM composition of the two soil layers. Higher carbohydrates and phenolics associated with the forest floor are most likely derived from decomposing aboveground litter, while the increase in nitrogen containing compounds in the mineral soils may be linked to an increase in microbial substrates.

3.5 Conclusion

There was not one GCMS technique that yielded a better overall WEOM chemical fingerprint compared to another. However, depending on the focus of a specific study, a specific technique may be chosen to highlight relevant differences. If the objective is to distinguish differences between forest floors and mineral soil horizons, the most sensitive method was TDGCMS with the addition of TMAH. Differences among dominant canopy vegetation types were best identified with PyGCMS, while fire chemical signature was most distinct with the use of double-shot PyGCMS.

The impact of harvesting on WEOM was negligible, as the WEOM chemical signature for both the forest floor and mineral soil under harvesting disturbance was comparable to the reference sites. This relates back to the legacy of the dominant canopy vegetation within the study sites, lodgepole pine, which was still clearly present following harvesting. Consequently, WEOM composition in the harvested lodgepole stands remained separate from the WEOM under different canopy composition, namely the aspen and mixed coniferous stands.

Fire disturbance had an impact on the forest floor WEOM chemical signature with less impact on the underlying mineral soil WEOM. Several black carbon biomarkers were identified in the fire impacted WEOM samples. These markers are good candidates for further research, as it would be interesting to investigate how long they may remain in these soils. Lastly, the forest floor WEOM signatures differed from those obtained from the underlying mineral horizons. The forest floor WEOM was relatively enriched with carbohydrates and phenolic compounds, while the mineral soil WEOM contained proportionally more of the nitrogen containing compounds as well as the aromatic and aliphatic compounds.

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Table 3-1: Key site characteristics including dominant vegetation, location, and disturbance.

Sample ID	Dominant Vegetation	Watershed	Easting* (m)	Northing* (m)	Elevation (m)	Disturbance
L1	Lodgepole Pine	Star Creek	675375	5497971	1596	Reference
L2	Lodgepole Pine	Star Creek	675282	5497386	1589	Reference
L3	Lodgepole Pine	Star Creek	675325	5497943	1600	Harvest
L4	Lodgepole Pine	Star Creek	675444	5497451	1562	Harvest
A	Trembling Aspen	Star Creek	675453	5497584	1559	Reference
C1	Mixed Conifer	Star Creek	675494	5497532	1551	Reference
S1	White Spruce	North York Creek	677775	5495295	1622	Reference
S2	White Spruce	North York Creek	677595	5495265	1624	Reference
C2	Mixed Conifer	Yarrow Creek	719162	5453459	1554	Reference
C3	Mixed Conifer	Cameron Creek	717305	5438303	1840	Fire

*: UTM Zone 11 North and East

Table 3-2: Chemical compounds preferentially associated with either forest floors or top mineral soil layers (0-10 cm) as identified using an indicator species analysis. Water extractable organic matter (WEOM) samples were analyzed using TMAH-TDGCMS. Only significant indicator values of $p<0.05$ and a stat value 0.7 or greater are shown. The values that are associated with 'A' are known as the specificity and the values associated with 'B' are known as the fidelity.

FOREST FLOOR

Chemical Compound	Chemical class/ origin	Stat	p-value	A	B
9-Hexadecenoic acid, methyl ester, (Z)-	Unsaturated fatty acid	0.86	<0.01	0.93	0.80
Docosanoic acid, methyl ester	Saturated fatty acid	0.84	<0.01	1.00	0.70
Nonanedioic acid, dimethyl ester	αω-alkanoic dioic acid	0.83	<0.01	0.77	0.90
9-Octadecenoic acid, methyl ester, (E)-	Unsaturated fatty acid	0.80	<0.01	0.91	0.70
Methyl stearate	Saturated fatty acid	0.78	0.01	1.00	0.60
Pentadecanoic acid, methyl ester	Saturated fatty acid	0.71	0.03	1.00	0.50
Hexacosanoic acid, methyl ester	Saturated fatty acid	0.71	0.03	1.00	0.50
8-Octadecenoic acid, methyl ester	Unsaturated fatty acid	0.71	0.03	1.00	0.50
Methyl dehydroabietate	Abietic acid	0.81	<0.01	0.73	0.90
7-Oxodehydroabietic acid, methyl ester	Abietic acid	0.75	0.02	0.80	0.70
15-Hydroxydehydroabietic acid, methyl ester	Abietic acid	0.74	0.02	0.79	0.70
Methyl 6-dehydrodehydroabietate	Abietic acid	0.74	0.02	0.78	0.70
Methyl 2-furoate	Furancarboxylic acid /Carbohydrate	0.86	<0.01	0.81	0.90
Furan, 4,5-diethyl-2,3-dihydro-2,3-dimethyl-	Substituted Furan/Carbohydrate	0.84	<0.01	1.00	0.70
Acetic acid, hydroxy-, methyl ester	Saturated hydroxy fatty acid/Carbohydrate	0.78	0.01	1.00	0.60
Ethanone, 1-(2-furanyl)-	Furan ketone/Carbohydrate	0.74	0.04	0.78	0.70
6H-Purin-6-one, 1,7-dihydro-1,7-dimethyl-	Purin ketone/N-containing	0.78	0.01	0.86	0.70
DL-Proline, 5-oxo-, methyl ester	Oxo-Proline/N- containing	0.71	0.04	1.00	0.50

MINERAL SOIL

Chemical Compound	Chemical class/ origin	Stat	p-value	A	B
Heptanoic acid, methyl ester	Saturated fatty acid	0.81	0.02	0.73	0.90
Nonanoic acid, methyl ester	Saturated fatty acid	0.78	0.02	0.76	0.80
Dibutyl phthalate	Polycarboxylic benzoic acid	0.88	<0.01	0.78	1.00
Benzaldehyde, 2,4-dimethyl-	Substituted Aromatic aldehyde	0.84	<0.01	1.00	0.70
1,2,3,4-Tetramethoxybenzene	Phenol	0.71	0.03	1.00	0.50
3-Furaldehyde	Furan-aldehyde/Carbohydrate	0.71	0.03	1.00	0.50
2-Pyrrolidinone, 1-methyl-	Substituted pyrrolidinone/N-containing	0.85	<0.01	0.90	0.80

Table 3-3: Pyrolysis products preferentially associated with dominant vegetation canopies as identified using an indicator species analysis. Water extractable organic matter (WEOM) samples were analyzed using PyGCMS. Only significant indicator values of $p<0.05$ and a stat value above 0.7 or greater are shown. The values that are associated with 'A' are known as the specificity and the values associated with 'B' are known as the fidelity.

LODGEPOLE PINE					
Chemical Compound	**Chemical Class/Origin**	**Stat**	**p-value**	**A**	**B**
Naphthalene, 1,6,7-trimethyl-	Substituted PAH	0.86	<0.001	0.84	0.88
TREMBLING ASPEN					
Heneicosane	n-Alkane	0.71	<0.01	1.00	0.50
Tetradecane	n-Alkane	0.71	<0.01	1.00	0.50
Heptadecane	n-Alkane	0.71	<0.01	1.00	0.50
1-Undecene	n-Alkene	0.71	<0.01	1.00	0.50
2-Pentene, 2-methyl-	Substituted alkene	0.71	<0.01	1.00	0.50
1-Dodecanol	n-Alkanol	0.71	<0.01	1.00	0.50
Ethanol, 2-(2-ethoxyethoxy)-	Aliphatic alcohol	0.71	<0.01	1.00	0.50
Benzaldehyde, 2,4-dimethyl-	Substituted aromatic aldehyde	0.71	<0.01	1.00	0.50
Dibutyl phthalate	Polycarboxylic benzoic acid	0.71	<0.01	1.00	0.50
Cyclopentanone, 2-methyl-	Substituted cyclic aliphatic ketone	0.81	<0.01	0.87	0.75
Cyclopentene	Cyclic aliphatic alkene	0.79	<0.01	0.62	1.00
2-Pyrrolidinone, 1-methyl-	Substituted pyrrolidinone/N-containing	0.71	<0.01	1.00	0.50
Semicarbazide	N-containing	0.71	<0.01	1.00	0.50
WHITE SPRUCE					
o-Xylene	Substituted benzene	0.84	<0.01	0.80	0.88
MIXED CONIFER + WHITE SPRUCE					
Butanedinitrile, 2,3-diamino-2,3-dimethyl-	Substituted aminonitrile/ N-containing	0.83	<0.01	0.91	0.75
1-Aza-2,4-cyclopentadiene	Pyrrole/N-containing	0.77	<0.01	0.85	0.70
Butanenitrile, 3-methyl-	Substituted nitrile/N-containing	0.74	<0.01	0.91	0.60
MIXED CONIFER + WHITE SPRUCE + TREMBLING ASPEN					

Chemical Compound	Chemical Class/Origin	Stat	p-value	A	B
Cyclopentadecane	Cyclic aliphatic alkane	0.80	0.01	0.89	0.71
2-Butene, (E)-	Alkene	0.92	<0.01	0.92	0.92
1-Hexene	n-Alkene	0.89	<0.01	0.99	0.79
1-Pentadecene	n-Alkene	0.86	<0.01	0.97	0.75
1-Octene	n-Alkene	0.83	<0.01	0.96	0.71
1-Tetradecene	n-Alkane	0.76	0.02	0.92	0.63
1-Pentene	n-Alkene	0.71	0.01	1.00	0.50
1-Nonene	n-Alkene	0.71	0.01	1.00	0.50
1,3-Cyclopentadiene	Cyclic aliphatic alkene	0.93	<0.01	0.86	1.00
1,3-Cyclohexadiene	Cyclic aliphatic alkene	0.89	<0.01	0.91	0.88
Acetaldehyde	Aldehyde	0.78	<0.01	0.92	0.67
Acetic acid, methyl ester	Saturated fatty acid	0.73	0.01	0.98	0.54
Propanenitrile	Nitrile/N-containing	0.96	<0.01	0.96	0.96
1H-Pyrazole, 4,5-dihydro-5-methyl-	Substituted pyrazole/N-containing	0.92	<0.01	0.92	0.92
Indol	Indole/N-containing	0.75	0.01	0.90	0.63
1H-Pyrrole, 2-ethyl-4-methyl	Substituted pyrrole/N-containing	0.73	0.01	0.97	0.54

Table 3-4: Chemical compounds preferentially associated with the fire disturbance as identified using an indicator species analysis. Water extractable organic matter (WEOM) samples were analyzed using double-shot PyGCMS. Only significant indicator values of $p<0.05$ and a stat value above 0.7 or greater are shown. The values that are associated with ‘A’ are known as the specificity and the values associated with ‘B’ are known as the fidelity.

Chemical Compound	**Chemical Class/Origin**	**Stat**	**p-Value**	**A**	**B**
1-Decene	n-Alkene	0.72	0.02	0.68	0.75
Benzaldehyde, 3-[4-(1,1-dimethylethyl)phenoxy]-	Biphenyl aldehyde	0.71	<0.01	1.00	0.50
Isobutyl methyl phthalate	Polycarboxylic benzoic acid	0.71	<0.01	1.00	0.50
3,4'-Diisopropylbiphenyl	Substituted biphenyl	0.71	<0.01	1.00	0.50
Acetophenone	Phenyl ketone	0.74	0.01	0.73	0.75
o-Xylene	Substituted benzene	0.71	0.02	0.68	0.75
Methyl dehydroabietate	Terpenoid-Abietic acid	0.75	0.01	0.74	0.75
Benzene, 1-ethyl-3-methyl-	Substituted benzene	0.72	0.02	0.69	0.75
1-Methyl-4-p-tolylnaphthalene	Substituted PAH	0.87	<0.01	1.00	0.75
Naphthalene, 1,4,5-trimethyl-	Substituted PAH	0.71	<0.01	1.00	0.50
Naphthalene, 1,8-dimethyl-	Substituted PAH	0.71	<0.01	1.00	0.50
Naphthalene, 3-(1,1-dimethylethyl)-1,2-dihydro-	Substituted PAH	0.71	<0.01	1.00	0.50
Anthracene, 9-(1-methylethyl)-	Substituted PAH	0.77	0.04	0.59	1.00
Anthracene, 2-(1,1-dimethylethyl)-1,2-dihydro-	Substituted PAH	0.71	<0.01	1.00	0.50
Naphthalene, 1,2-dihydro-3-methyl-	Substituted PAH	0.71	<0.01	1.00	0.50
Phenanthrene, 3,6-dimethyl-	Substituted PAH	0.71	<0.01	1.00	0.50
Phenanthrene	PAH	0.71	<0.01	1.00	0.50
Silane, 9-anthracenyltrimethyl-	Substituted PAH	0.71	<0.01	1.00	0.50
1,3,6,8-Tetramethylanthracene	Substituted PAH	0.71	<0.01	1.00	0.50

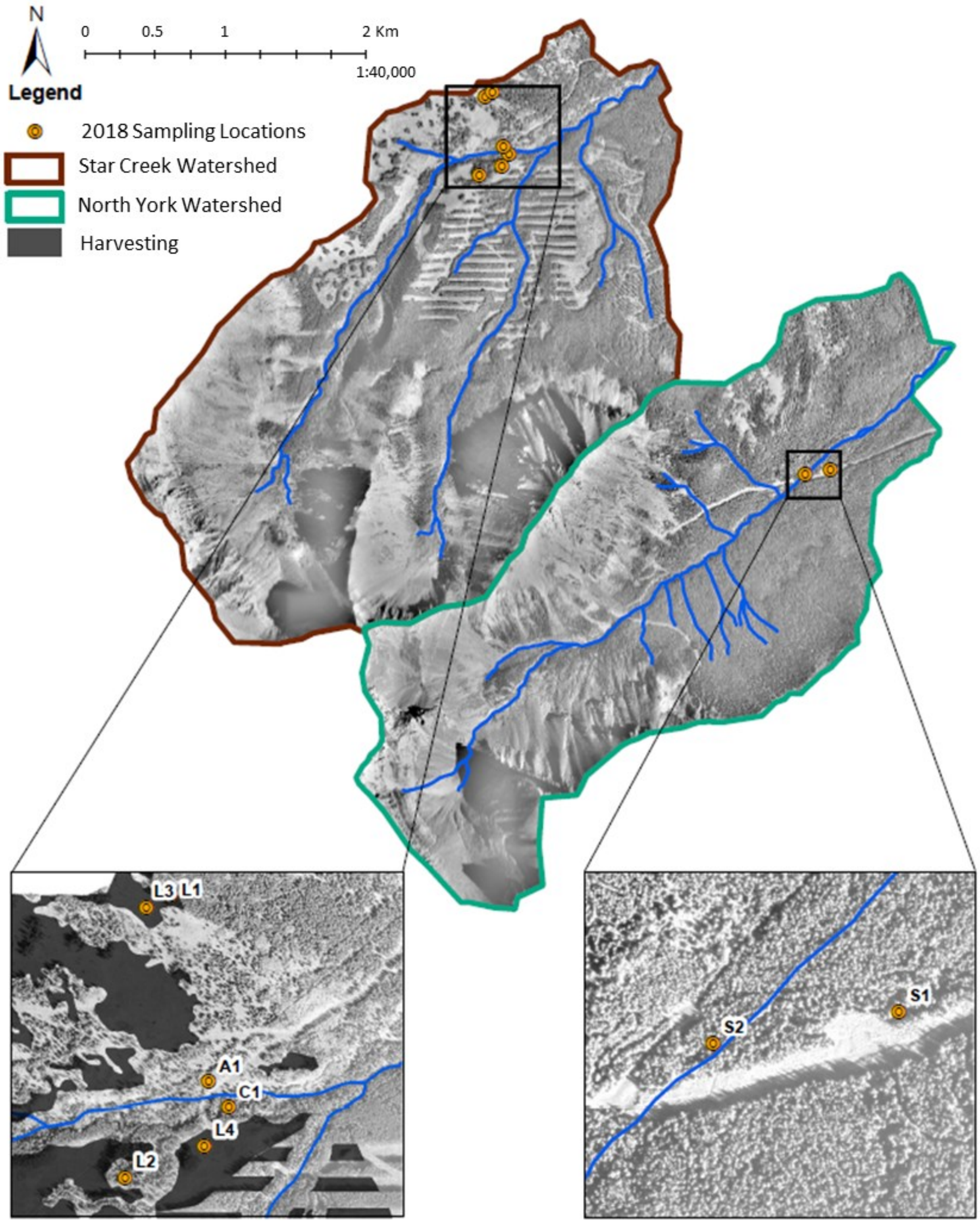

Figure 3-1: Map of the Crowsnest Pass study site. The Star Creek watershed has the brown outline and the left inset and the North York watershed has the green outline and the right inset. The sampling locations across both watersheds are in orange. The harvesting disturbance is in dark grey.

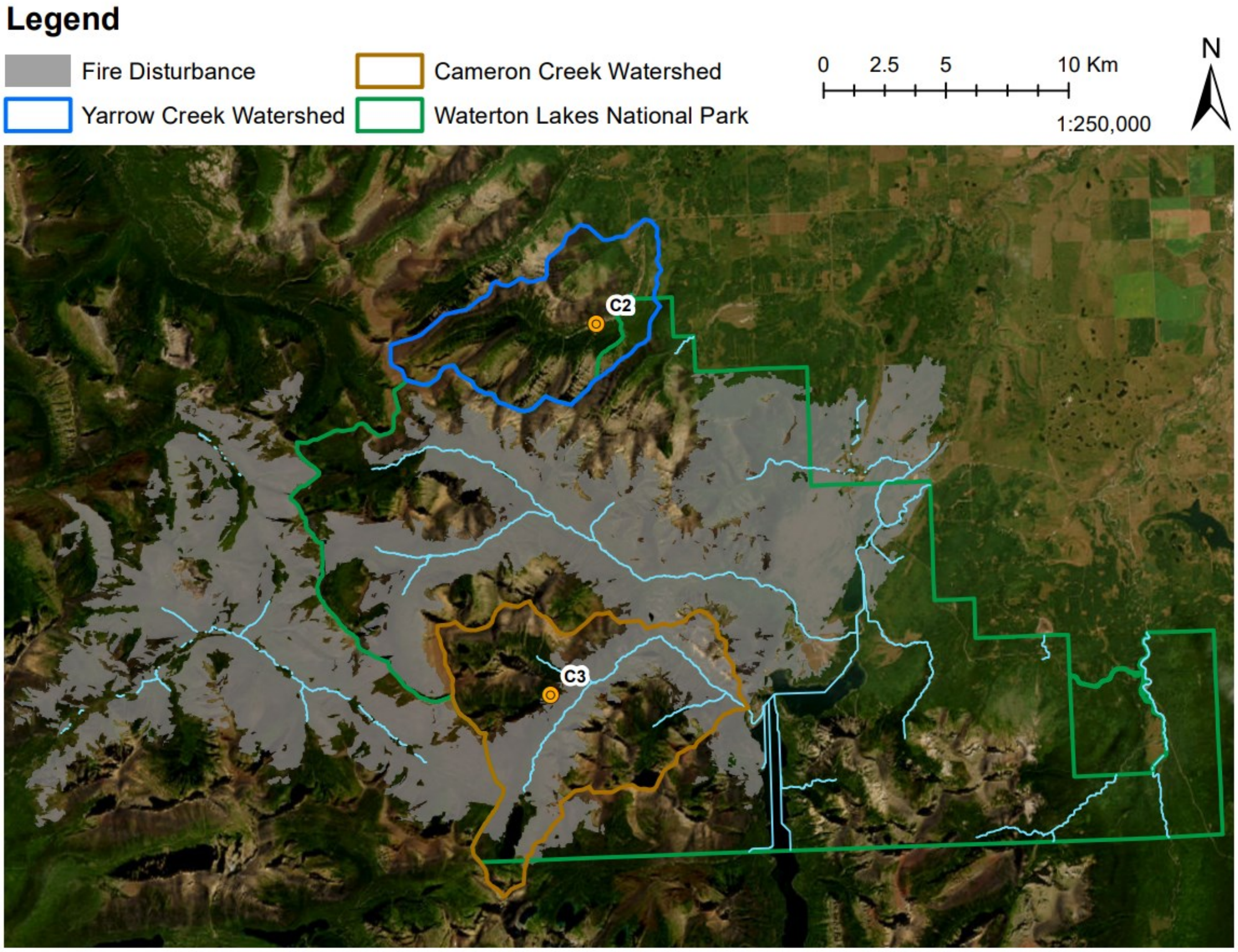

Figure 3-2: Map of the Waterton area study site. The Yarrow Creek watershed is outlined in blue, and the Cameron Creek watershed is outlined in brown. The sampling locations across both watersheds are in orange. The fire disturbance is in dark grey, and the Waterton Lake National Park is in green.

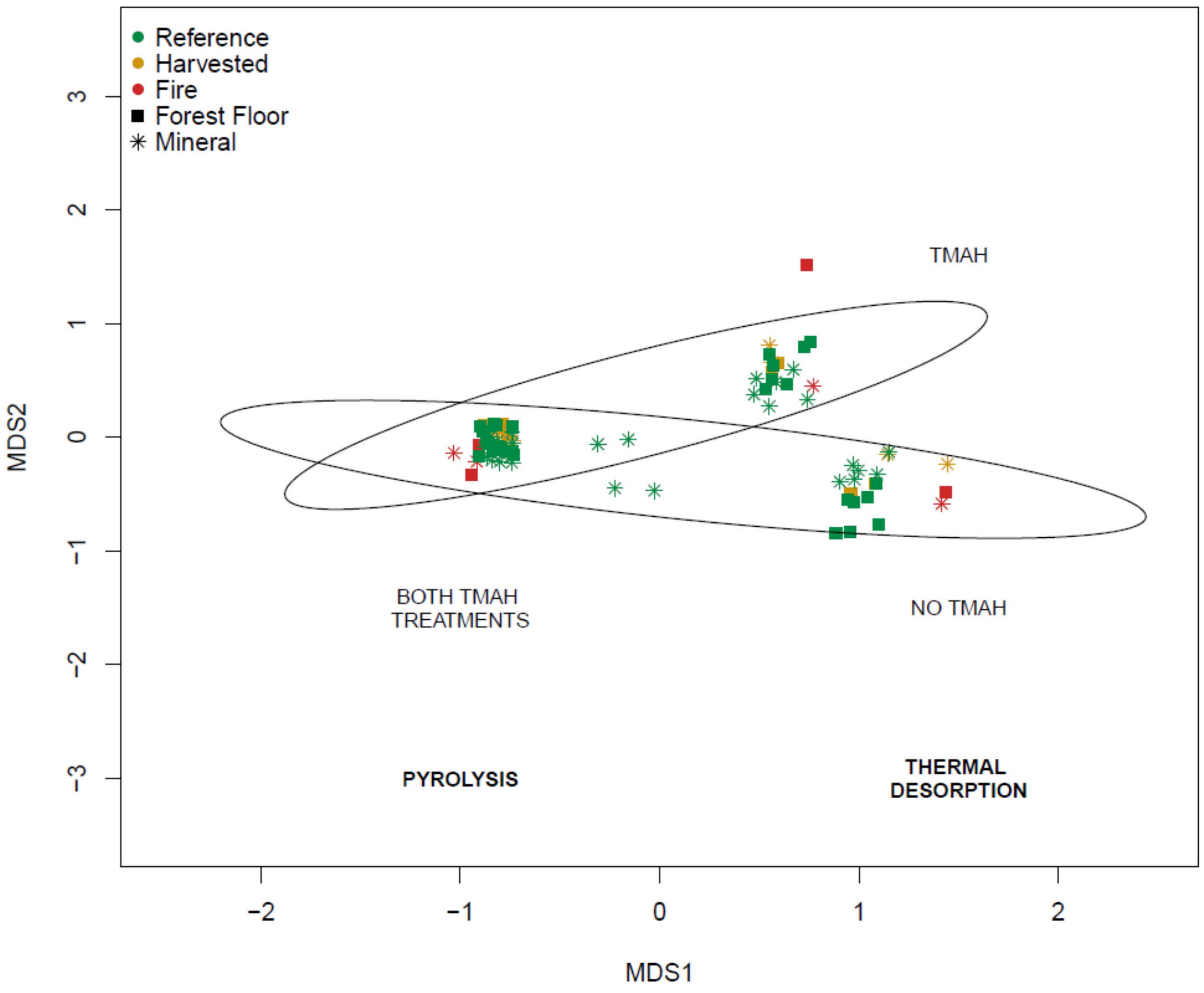

Figure 3-3: Two-dimensional non-linear multidimensional scaling (NMDS) ordination of chemical compound concentrations (% total) in water extractable organic matter (WEOM) analyzed using PyGCMS and TDGCMS. Ellipses show the 95%confidence intervals for the two TMAH treatments.

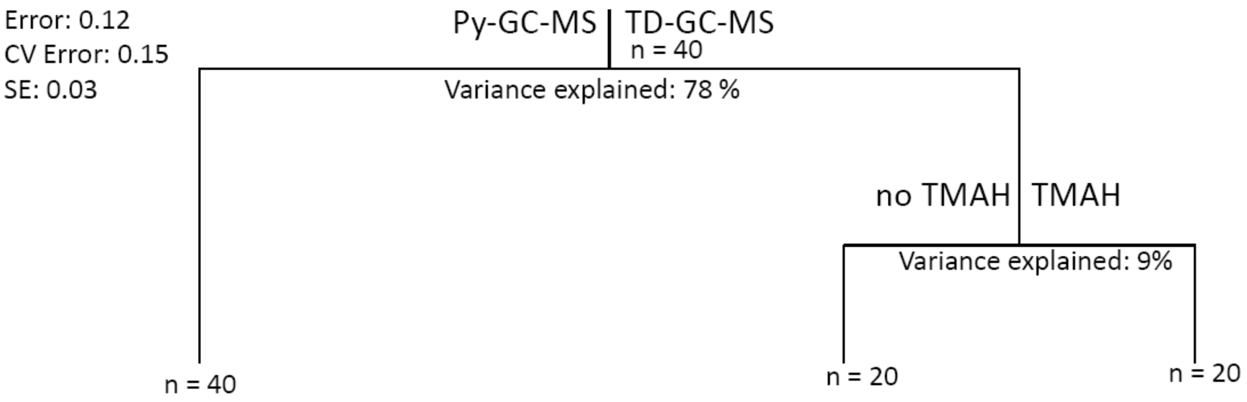

Figure 3-4: Multivariate regression tree (MRT) analysis of Hellinger transformed chemical compound concentrations (% total) in water extractable organic matter (WEOM) analyzed using PyGCMS and TDGCMS.

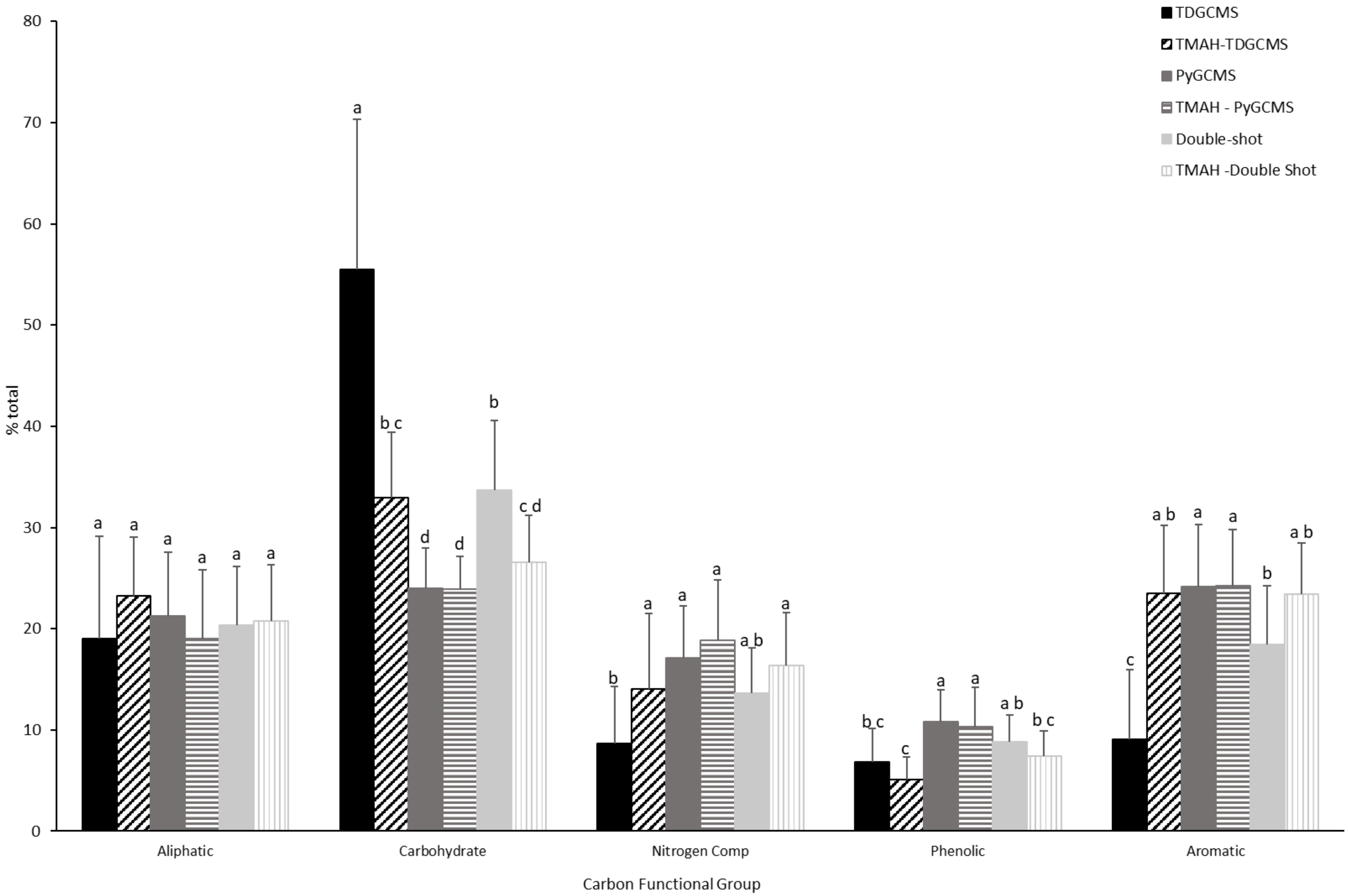

Figure 3-5: Relative concentrations (% total) of key carbon functional groups for all WEOM samples with error bars corresponding to standard deviations (n =20). Different letters indicate significant differences among analytical techniques for each carbon functional group.

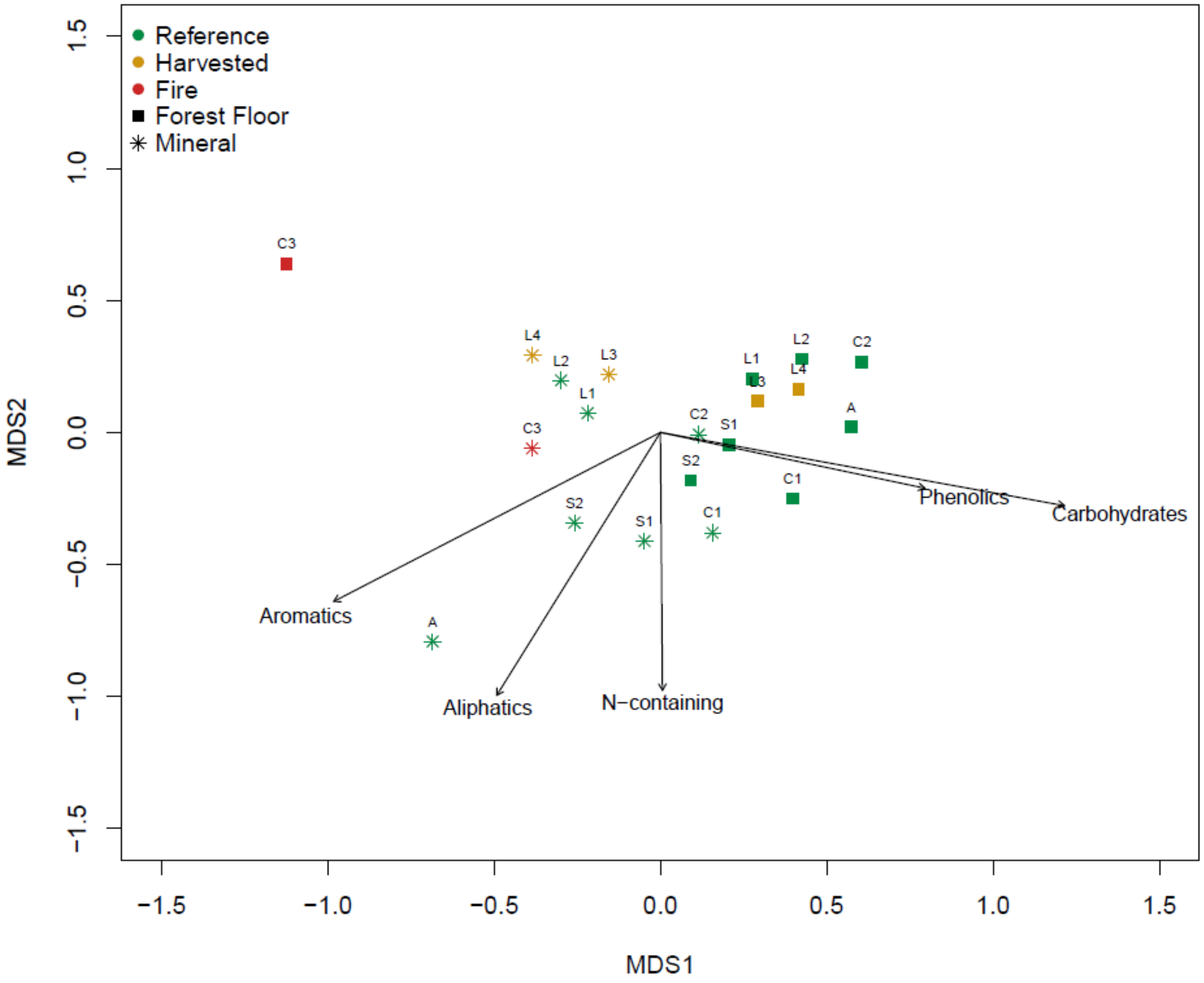

Figure 3-6: Two-dimensional non-linear multidimensional scaling (NMDS) ordination of chemical compound concentrations (% total) in water extractable organic matter (WEOM) analyzed using TDGCMS without TMAH. Symbol labels are associated with dominant vegetation; L lodgepole pine, S white spruce, A trembling aspen and C mixed conifer. Vectors ($p<0.05$) correspond to main carbon functional groups.

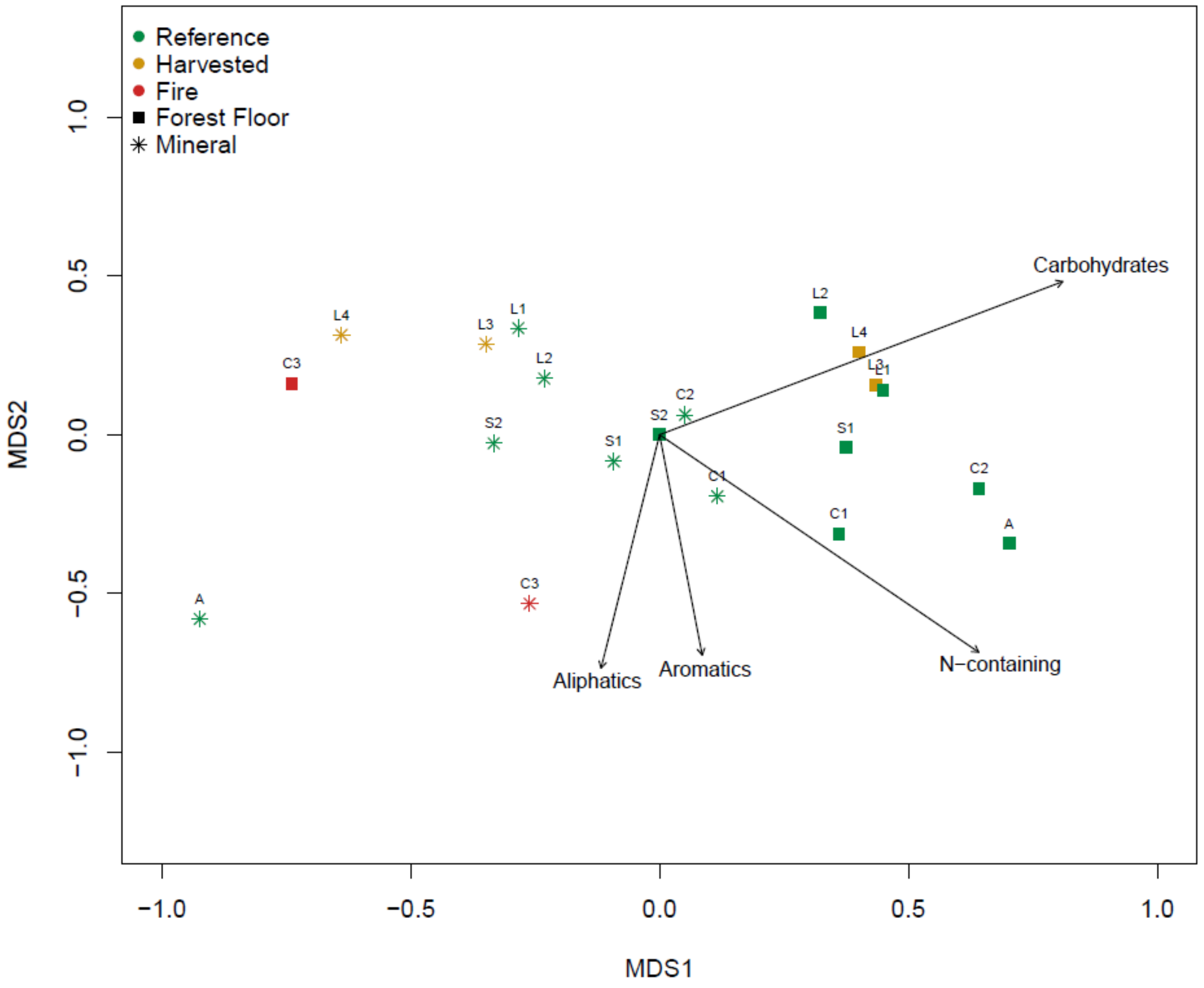

Figure 3-7: Two-dimensional non-linear multidimensional scaling (NMDS) ordination of chemical compound concentrations (% total) in water extractable organic matter (WEOM) analyzed using TDGCMS with TMAH. Symbol labels are associated with dominant vegetation; L lodgepole pine, S white spruce, A trembling aspen and C mixed conifer. Vectors ($p<0.05$) correspond to main carbon functional groups.

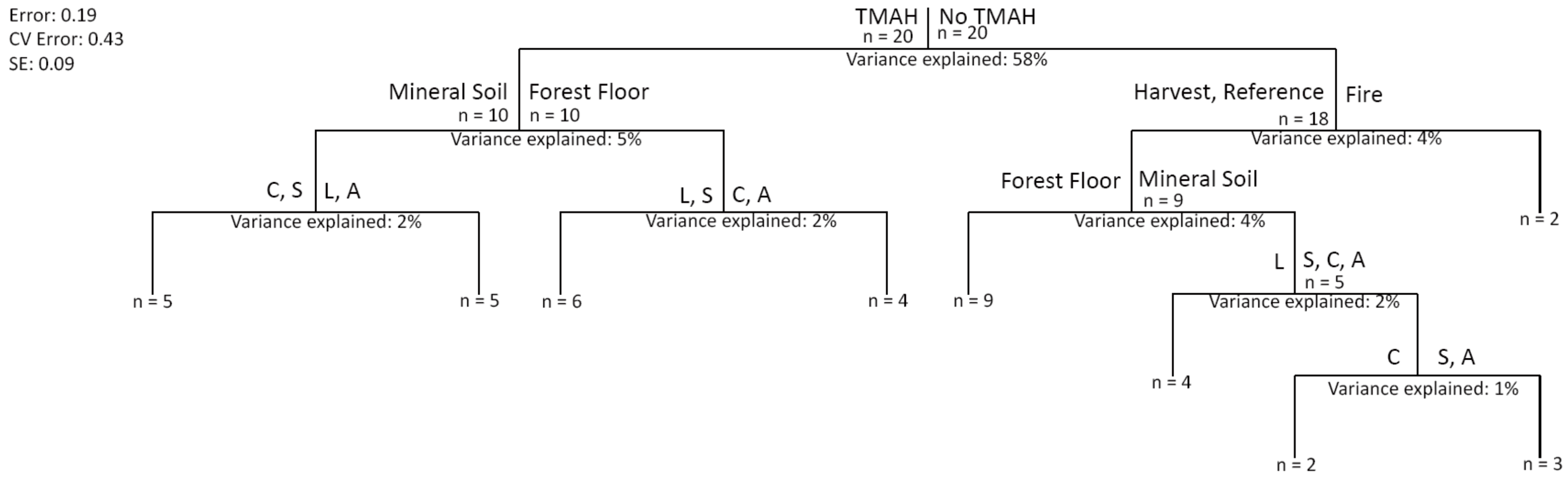

Figure 3-8: Multivariate regression tree (MRT) analysis of Hellinger transformed chemical compound concentrations (% total) in water extractable organic matter (WEOM) analyzed using TDGCMS.

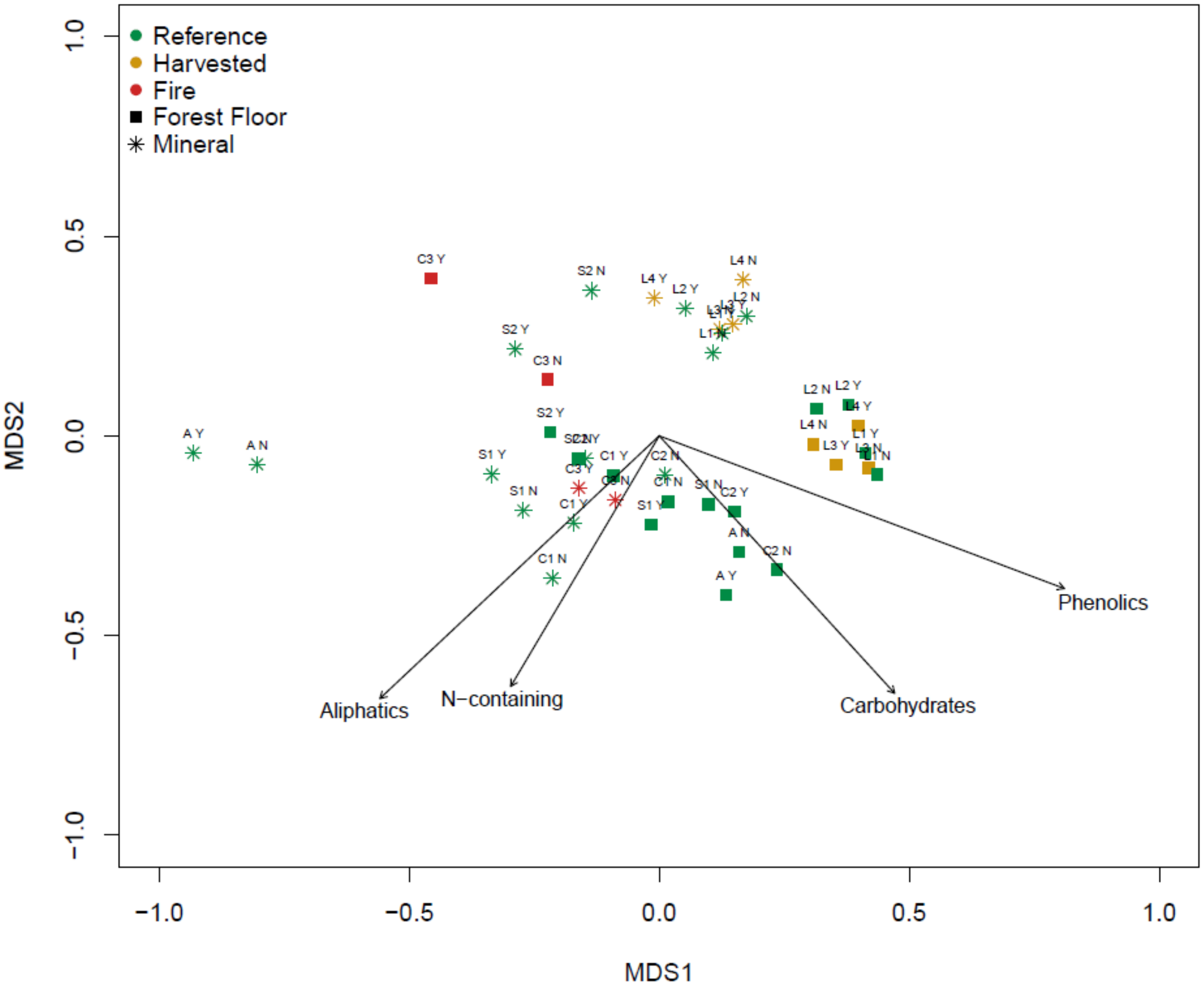

Figure 3-9: Three-dimensional non-linear multidimensional scaling (NMDS) ordination with Hellinger transformation of chemical compound concentrations (% total) in water extractable organic matter (WEOM) analyzed using PyGCMS with both TMAH treatments. Symbol labels are associated with dominant vegetation; L lodgepole pine, S white spruce, A trembling aspen and C mixed conifer and TMAH treatments; Y TMAH present and N TMAH not present. The vectors ($p<0.05$) are the main carbon functional groups.

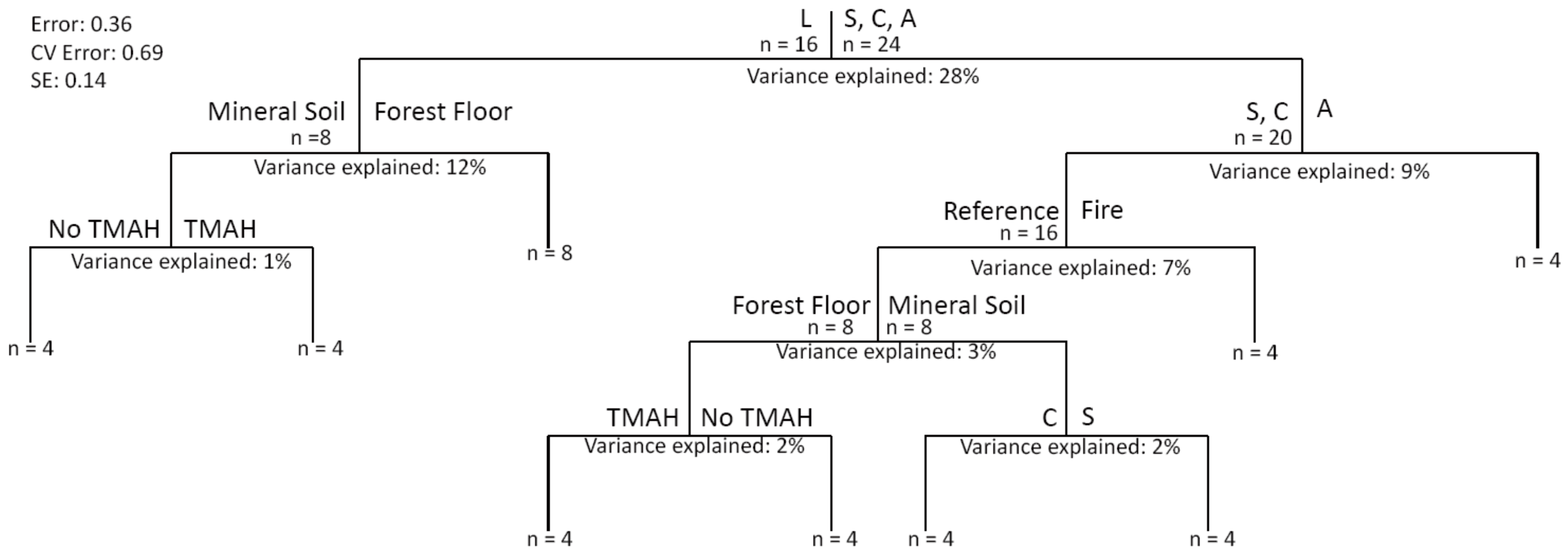

Figure 3-10: MRT analysis of Hellinger transformed chemical compound concentrations (% total) in water extractable organic matter (WEOM) analyzed using PyGCMS.

Table 3-5: Indicator species analysis comparing the two TMAH treatments for chemical compound concentrations (% total) in water extractable organic matter (WEOM) analyzed using TDGCMS. Only significant indicator values of $p < 0.05$ and a stat value above 0.7 are shown. The values that are associated with ‘A’ are known as the specificity and the values associated with ‘B’ are known as the fidelity.

NO TMAH

Chemical Compound	Chemical Class/Origin	Stat Value	p-Value	A	B
1,3-Butadiyne	Aliphatic alkyne	0.71	<0.001	1.00	0.50
Acetaldehyde	Aldehyde	0.95	<0.001	1.00	0.90
1,2-Cyclopentanedione	Cyclic aliphatic diketone/Carbohydrate	0.81	<0.001	0.87	0.75
2(3H)-Furanone, 5-methyl-	Substituted Furan Ketone/Carbohydrate	0.73	<0.001	0.88	0.60
Maltol	Substituted hydroxy pyran ketone/ Carbohydrate	0.73	0.003	0.88	0.60
Propanoic acid, 2-oxo-, methyl ester	Saturated oxo-fatty acid	0.73	<0.001	0.96	0.55
2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	Substituted hydroxy cyclic aliphatic ketone/Carbohydrate	0.71	0.002	0.91	0.55
4-Cyclopentene-1,3-dione	Cyclic aliphatic diketone/Carbohydrate	0.71	<0.001	1.00	0.50
2,4-Di-tert-butylphenol	Substituted Phenol	0.78	0.002	0.77	0.80

TMAH PRESENT

Chemical Compound	Chemical Class/Origin	Stat Value	p-Value	A	B
Hexadecanoic acid, methyl ester	Saturated fatty acid	0.97	<0.001	0.98	0.95
Hexanoic acid, methyl ester	Saturated fatty acid	0.95	<0.001	1.00	0.90
Dodecanoic acid, methyl ester	Saturated fatty acid	0.92	<0.001	1.00	0.85
Heptanoic acid, methyl ester	Saturated fatty acid	0.81	<0.001	1.00	0.65
Nonanedioic acid, dimethyl ester	αω-alkanoic dioic acid	0.78	<0.001	1.00	0.60
Nonanoic acid, methyl ester	Saturated fatty acid	0.71	<0.001	1.00	0.50
Benzoic acid, 3,4-dimethoxy-, methyl ester	Dimethoxy Benzoic acid	1.0	<0.001	1.00	1.00
Benzoic acid, 3,4-dimethyl-, methyl ester	Substituted Benzoic acid	0.99	<0.001	0.98	1.00

Chemical Compound	Chemical Class/Origin	Stat Value	p-Value	A	B
1,2,4-Trimethoxybenzene	Trimethoxybenzene	0.92	<0.001	1.00	0.85
Benzene, 1,2-dimethoxy-	Dimethoxybenzene	0.87	<0.001	1.00	0.75
Benzene, 1,4-dimethoxy-	Dimethoxybenzene	0.87	<0.001	1.00	0.75
Benzoic acid, 4-methoxy-, methyl ester	p-methoxybenzoic acids	0.85	<0.001	0.96	0.75
Methyl dehydroabietate	Terpenoic-abietic acid	0.71	0.001	0.91	0.55
Methylbenzoate	Benzoic acid	0.71	<0.001	1.00	0.50
Propanoic acid, 2-methoxy-, methyl ester	Saturated methoxy-fatty acid/Carbohydrate	1.00	<0.001	1.00	1.00
Acetic acid, methyl ester	Saturated fatty acid/Carbohydrate	1.00	<0.001	0.99	1.00
Acetic acid, methoxy-, methyl ester	Saturated methoxy fatty acid/Carbohydrate	0.84	<0.001	1.00	0.70
Methyl propionate	Saturated fatty acid/Carbohydrate	0.78	<0.001	1.00	0.60
Methyl 2-furoate	Furancarboxylic acid/Carbohydrate	0.78	<0.001	1.00	0.60
2-Methoxy-4,4,5-trimethyloxazoline	Substituted methoxy oxazole/N-containing	0.71	<0.001	1.00	0.50
2,4(1H,3H)-Pyrimidinedione, 1,3-dimethyl-	Substituted pyrimidine diketone/N-containing	0.95	<0.001	1.00	0.90
2-Pyrrolidone-5-carboxylic acid, N-methyl, methyl ester	Saturated fatty acid	0.92	<0.001	1.00	0.85
2,4(1H,3H)-Pyrimidinedione, 1,3,5-trimethyl-	Saturated fatty acid	0.81	<0.001	1.00	0.65
Phenol, 4,6-di(1,1-dimethylethyl)-2-methyl-	Saturated fatty acid	0.77	<0.001	0.98	0.60

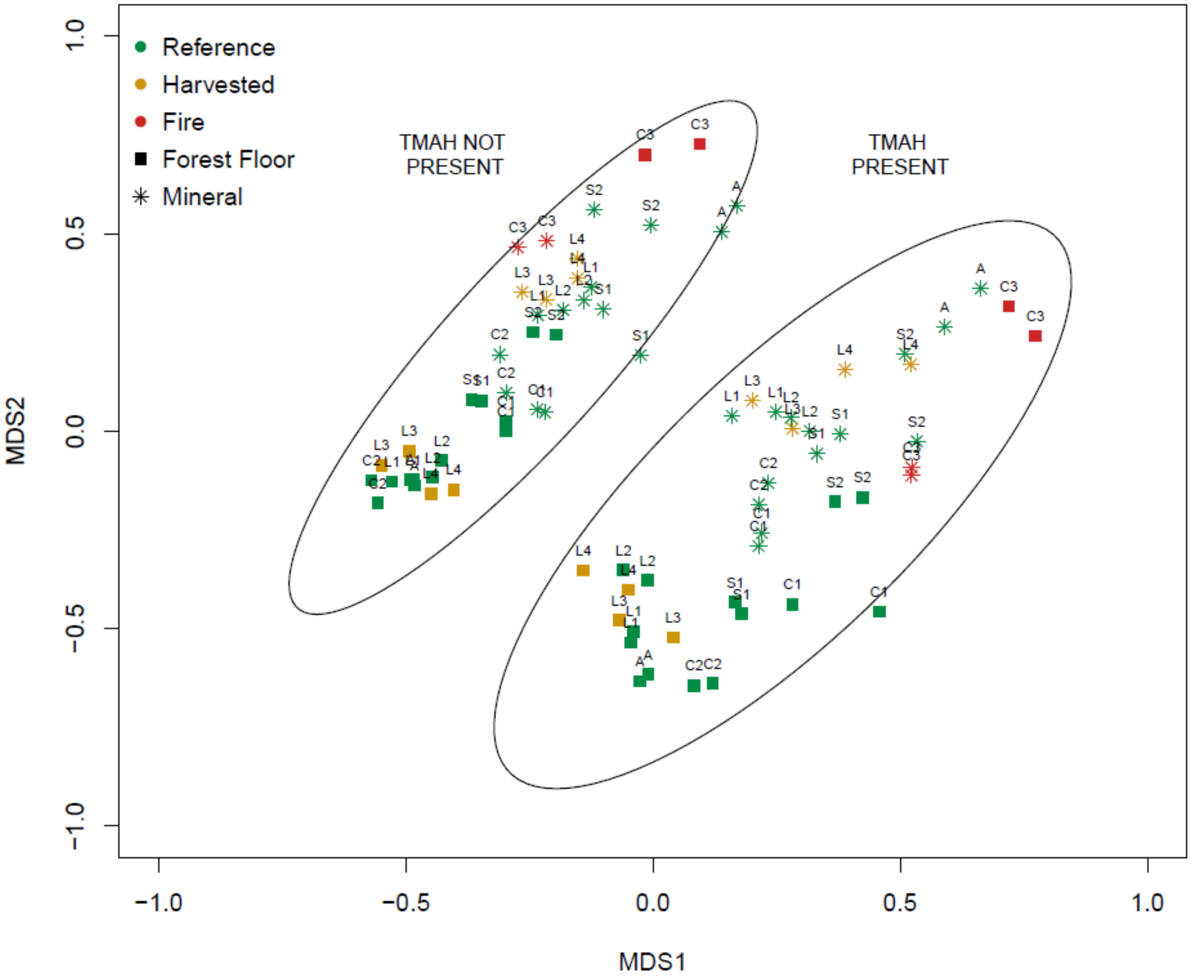

Figure 3-11: Three-dimensional non-linear multidimensional scaling (NMDS) ordination with Hellinger transformation of chemical compound concentrations (% total) as replicates in water extractable organic matter (WEOM) analyzed using double-shot PyGCMS. Symbol labels are associated with dominant vegetation; L lodgepole pine, S white spruce, A trembling aspen and C mixed conifer. The Ellipses are associated with the TMAH treatments.

Chapter 4: Synthesis

The soluble component of soil organic matter (SOM) plays a key role in ecological processes and has implications for water quality. Watershed disturbance from both fire and forestry practices are significant concerns for watershed managers because they can affect the quantity and quality of organic compounds – the aromatic component in particular – that can enter surrounding waterways. Potential effects on source water quality can also affect treatability of downstream drinking water supplies. Without a good understanding of the chemical composition and lability of these compounds, downstream water treatment options can be potentially expensive and challenging to implement (e.g., to prevent formation of regulated carbon compounds in drinking water). Dissolved organic matter (DOM) is a natural aqueous constituent of streams draining forested watersheds. However, little is known about how both wildfire and forest harvesting practices may affect DOM quantity and its chemical composition in northern regions of the Rocky Mountains. Water extractable organic matter (WEOM), derived from laboratory extraction, is a component of DOM and has been proposed as an early and sensitive indicator of changes in SOM dynamics.

In this thesis, forest floor and the underlying mineral soil horizon were sampled from four watersheds in the eastern slopes of the southern Canadian Rocky Mountains: a burnt and a reference watershed and a clear-cut harvested and a reference watershed. The dominant forest canopy vegetation over the sampling areas includes lodgepole pine (*Pinus Contorta*), white spruce (*Picea glauca*), sub alpine fir (*Abies lasiocarpa*) and trembling aspen (*Populus Tremuloides*). The chemical composition of the samples was characterized to assess if there was an impact by clear-cut harvesting and forest fire on WEOM as well as to determine if there was a link between the forest soils and the corresponding WEOM. Carbon chemical composition in WEOM was analyzed using three different methods: ^{13}C nuclear magnetic resonance (NMR), specific ultraviolet (UV) absorbance and gas chromatography mass spectrometry (GCMS).

In this chapter I summarize the findings associated with this study and discuss needs for further research.

4.1 Summary and assessment of Chapter 2

The objective of the research in Chapter 2 was to assess whether vegetation type, fire or harvesting altered forest floor chemical composition and how differences in forest floor chemical composition translated to their associated WEOM. This was completed by analyzing the forest floor OM and the associated WEOM using ^{13}C NMR spectroscopy in both direct polarization (DP) and cross polarization (CP) pulse sequence. This included evaluating the whole trace spectra as well as the seven chemical shift regions: alkyl, methoxyl, O-alkyl, di-O-alkyl, aromatic, phenolic, and carbonyl carbon. Part of the analysis also used specific UV absorbance analysis at a wavelength of 254nm ($SUVA_{254}$) which commonly uses as proxy for aromaticity indicators of aquatic systems. The $SUVA_{254}$ for the samples was compared to the % aromaticity estimated from the ^{13}C NMR aromatic shift region to determine if there was a correlation.

With the regression analysis there was a positive correlation in the DP analysis with the carbonyl region and with the CP analysis the positive correlations were with the methoxyl, di-O-alkyl and carbonyl regions. However, the average % of those regions were minimal, and together only showed a correlation between WEOM and the forest floor OM for less than 30% of the total signal intensity. Overall, the chemical composition in the forest floor OM was not similar to that of the associated WEOM. This was also visible with the PCA scores and loading plots. However, there was a positive relationship between the $SUVA_{254}$ results and the aromatic region of the ^{13}C NMR spectra. The WEOM was more oxidized with an enrichment in alkyl (short chain), methoxyl, O-alkyl C and carbonyl C, while the forest floor OM was enriched in aromatic and phenolic C. Harvesting had a negligible impact on carbon chemical composition, but forest fire had a noticeable impact with the presence of charcoal, carbonate and a higher aromaticity in both the forest floor OM and WEOM. With the different vegetation types there were similarities regarding the impact that they had on the forest floor OM and WEOM but there were differences also found between the trembling aspen and the rest of the conifer tree types.

It is important to note that by using the whole spectra any spectral differences that occurred that otherwise may have otherwise been masked were able to be identified clearly. The main factors governing the composition of the WEOM in this study link back to the solubility of OM and this is what was driving the differences between the composition of the forest floor OM and the associated WEOM.

4.2 Summary and assessment of Chapter 3

The objective of the research in Chapter 3 was to evaluate several gas chromatography mass spectrometry (GCMS) analytical techniques for their ability to provide a chemical fingerprint of the water extractable organic matter (WEOM) from soils under different disturbance regimes. The different GCMS analytical techniques included thermal desorption (TD) and pyrolysis (Py) along with the use of tetramethylammonium hydroxide (TMAH).

The soils that were analyzed were obtained from clear-cut harvesting and forest fire disturbances as well as reference forests in the eastern slopes of the southern Canadian Rocky Mountains. Soil samples were taken from the forest floor and mineral soil horizons and were sieved and ground. These soils then underwent an extraction process for the WEOM component before being analyzed with TDGCMS, PyGCMS and double shot GCMS all with and without TMAH.

The study determined that results from the GCMS methods were complementary in regard to the chemical information provided and that not one GCMS technique yielded better results than another. However, the best technique for distinguishing the differences between forest floors and mineral soil horizons was TDGCMS with TMAH. The best technique for distinguishing dominant canopy vegetation was PyGCMS as it showed the differences more clearly and with the impact of fire, a distinct chemical signature was visible with the use of double-shot PyGCMS.

Overall fire disturbance had an impact on WEOM chemical composition with several known black carbon biomarkers identified. These markers included polycarboxylic benzoic acid, biphenyls, and a series of substituted polycyclic aromatic hydrocarbons (PAHs). However, clear-cut harvesting disturbance did not have an impact on the WEOM chemical composition and this may be due to the legacy associated with the dominant canopy vegetation.

Overall, there were compositional differences between soil horizons where forest floor samples were dominated by carbohydrates and phenolics whereas the underlying mineral horizons were linked with higher concentrations of nitrogen containing compounds, aromatics and aliphatics.

4.3 Conclusion

In Chapter 2 the forest floor OM and associated WEOM was studied whereas in Chapter 3 the chemical composition of the WEOM for both the forest floor OM and underlying mineral soil was analyzed. Overall, disturbance type and dominant canopy vegetation can influence the WEOM composition. In this study, harvesting had a negligible impact on the WEOM however, forest fire had a clear impact with fire impacted sites exhibiting higher aromaticity. Regarding vegetation impact, with the GCMS analysis lodgepole pine samples were clustered away from the white spruce, mixed conifer and trembling aspen samples and with the ^{13}C NMR analysis there were differences between the aspen samples and the conifer samples.

The combination of using ^{13}C NMR, specific UV absorbance and GCMS over the course of the study resulted in a thorough assessment of the chemistry of the forest floor C and the associated WEOM. There are advantages and disadvantages with using all three techniques but for future research if the specific chemical fingerprint of WEOM is to be analyzed especially regarding biomarkers then GCMS is recommended however, if a more comprehensive understanding of the chemical structure and overall composition of OM and WEOM is to be determined then ^{13}C NMR is recommended.

4.4 Future Research

Results from this research have highlighted knowledge gaps regarding forest floor and its associated WEOM in response to forest fire, harvest, and different vegetation types. As stated in Chapter 2, further research into why there were greater differences in WEOM composition among sites than those observed among forest floors OM samples would help with a more in-depth understanding of WEOM chemical composition.

Regarding Chapter 3, further investigation into the black carbon makers, more specifically the PAHs was noted. These PAHs would be good markers to determine how long they remain in the soils samples along with if they are persistent in other soils impacted by wildfire with similar retention times.

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