

AN ABSTRACT OF THE THESIS OF

Lauren Matosziuk for the degree of Master of Science in Sustainable Forest Management presented on August 9, 2017.

Title: Effects of Season and Interval of Prescribed Burns on Pyrogenic Carbon Cycling in Ponderosa Pine Stands in Malheur National Forest.

Abstract approved:

Jeff A. Hatten

In ponderosa pine (*Pinus ponderosa*) forests of the western United States, prescribed fires are used to reduce fuel loads and restore historical fire regimes. The season in which prescribed burns are performed and the interval between burns can have complex consequences for the ecosystem, including soil carbon cycling through the production of pyrogenic carbon (PyC). PyC is a broad term that refers to a spectrum of thermally-altered organic matter. PyC plays a crucial role in soil carbon cycling, displaying turnover times that are orders of magnitude longer than unburned organic matter. This work investigated how the season of and interval between prescribed burns affects the formation and retention of pyrogenic carbon (PyC) in a ponderosa pine forest of eastern Oregon. In 1997 a season and interval of burn study was implemented in Malheur National Forest to examine the ecological effects of burning at 5 and 15-year intervals in either the spring or fall. In October 2015, both O-horizon and mineral soil (0-15 cm) samples were collected and analyzed for PyC concentration, content, and structure using the benzene polycarboxylic acid (BPCA) method. O-horizon depth, carbon and nitrogen concentration and content, pH, and bulk density were also measured. Compared to unburned controls, we estimate that fall burns increased the PyC concentration of the mineral soil by 8.4 g BPCA/kg C (95% CI: 4.2, 12.6 g BPCA/kg C). No change in PyC concentration of the O-horizon was detected for the plots burned in either the spring or fall compared to the unburned

controls; however, the chemical structure of the PyC in the O-horizon of the unburned control plots was significantly more condensed than that of the plots burned in either the spring or the fall.

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Effects of Season and Interval of Prescribed Burns on Pyrogenic Carbon Cycling in
Ponderosa Pine Stands in Malheur National Forest

by
Lauren Matosziuk

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented August 9, 2017
Commencement June 2018

Master of Science thesis of Lauren Matosziuk presented on August 9, 2017

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Lauren Matosziuk, Author

ACKNOWLEDGEMENTS

First and foremost I would like to thank my Major Professor, Jeff Hatten. Jeff took a huge gamble when he took me into his group – I was a chemist, not a forester, soil scientist, or an ecologist. Over the past two years, he taught me how to design and execute a field sampling study, interpret data in an ecological context, and think broadly. Any time I have had a question, he quickly offered sound advice. Most amazingly, I have always left meetings with him feeling like have a better understanding of what I'm supposed to be doing.

I would also like to thank my other committee members. Prof. John Bailey was my first contact in the College of Forestry at OSU, the person who was originally willing to try to teach a chemist how to work in the forest. He set me up with my first foray into fieldwork looking at fire histories, introduced me to Jeff, and has been a great source of advice over the past two years. Dr. Becky Kerns is a collaborator on the SIB project and has provided invaluable information about the history of the project as well as many years of vegetation and fuels data. Dr. Mark Johnson has been a fantastic resource for conversations about biochar, soil organic matter, and the use of chemical tools in ecological sciences. Prof. Brenda McComb gave several talks during CoF orientation and in my first classes at OSU. Her talks were fantastic, making me feel welcome in the College of Forestry and excited to start on my new career path.

I can't say enough good things about all of the members of the Forest Soils Lab. Yvan Alleau showed me how to use all of the equipment in the lab, helped me to figure out how to implement a new protocol for PyC quantification, fixed the lyophilizer at least a half a dozen times, instilled in me a new appreciation for organization and cleanliness, and did it all with a delightful sense of humor. I honestly would not have been able to finish this project without his help. Brett Morrisette not only helped to collect the soil samples for this project, but also showed me a hundred tips and tricks to be safer, more organized, and more efficient in the field. Adrian Gallo helped me collect the samples for this project and has been a fantastic resource for all of my questions about soils, geology, and forests. Dave Frey

has been the best labmate you could ever ask for. He has proofread almost everything I've written while at OSU, worked with me on countless class projects, talked for hours with me about the best ways to analyze data for my project, and listened all the times I was angry at either the HPLC or the lyophilizer. Franco Guerrero is a genius at science communication – his advice about science writing and figure making has greatly improved my own ability to talk about science. Mandy Allen-Kahl, Hayden England, and Ethan Donoghue have also been a great help - their help in keeping the lab running smoothly is much appreciated.

I would also like to thank our collaborators in the Forest Service who helped to implement the SIB project and keep it running. In addition to Becky, Doug Westlind and Michelle Day have been invaluable resources about the history of the project, helped me to determine an effective way to analyze the data, and provided me with all of the fuels data that is referenced in this work.

There are so many people in the College of Forestry that have been a great help to me on this project. Prof. Jeff Morell let me use the HPLC in his laboratory. Matt Konkler showed me the specifics of this machine and talked with me for hours about the best way to separate molecules, improve resolution, and the horrors of picking peaks by hand. Prof. Lisa Ganio and Ariel Muldoon have been a fantastic resource for data analysis and interpretation – their class in statistics and data analysis was the most useful course I have ever taken. Andrew Merschel taught me so much about developing fire histories and showed me how to core trees, date fire scars, and the importance of using multiple grades of sandpaper. Danielle Nelson has been a great resource for improving my statistical analysis skills, making my R-coding more efficient, and providing general enthusiasm for science.

Lastly, I would like to thank my friends and family, particularly Joanna Lee, Dr. Stephanie Walter, Jay Dicke, Laura Lilley, and my father, Ed Matosziuk. I was terrified to quit my job and go back to graduate school for something completely outside of my realm of expertise, but you guys supported me and encouraged me to pursue a career that would actually make me happy.

To all of you that have helped me along the way, thank you so much.

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1. Introduction

1.1 Prescribed fire and soils

Historically, wildfire has been an important component of the natural disturbance regime in much of the western United States, shaping the plant and animal communities of fire-adapted ecosystems such as the ponderosa pine (*Pinus ponderosa*) forests of the Pacific Northwest.¹⁻³ However, fire suppression policies adopted during the twentieth century have significantly altered the disturbance regime of these ecosystems, leading to a significant buildup of fuels and shifting forest vegetation communities toward less fire-tolerant species.^{1,4-8} This, combined with recent climactic trends has led to longer, drier fire seasons, that have left many ecosystems vulnerable to high-intensity, stand-replacing wildfires far outside the historical range of variability.⁹⁻¹³ Prescribed burns offer a way to reduce fuel loads and mimic the ecological benefits of the natural fire regime.^{4,14-18}

Prescribed fires are intentionally ignited under circumstances in which fire behavior can be more easily controlled.¹⁹ In the Pacific Northwest they are often performed in the spring, when fuel moisture levels are higher, or in the fall once temperatures have begun to decrease and moisture levels are expected to increase; however most wildfires historically burned in the summer.²⁰ There is some concern that burning outside of the natural fire season may fail to achieve management objectives, including the restoration of historical fire regimes.²⁰ Consequently, it is often necessary to perform multiple burns on the same site to achieve the desired reduction in fuels.^{21,22} As catastrophic wildfires become more widespread, and prescribed burning a more necessary tool for reducing fuel loads, it is imperative that we develop a better understanding of how specific characteristics of prescribed burns, specifically the season of burn and the interval between repeated burns, interact to affect various ecosystem properties, including soil organic matter (SOM).

1.1.1 Season and interval of burn

Fuel moisture can have profound, interconnected effects on fire behavior including fuel consumption, the temperature achieved during the fire, the rate of fire

spread, and fuel connectivity.²³⁻²⁵ Prescribed fires performed when fuel moisture content is high are characterized by less overall fuel consumption, lower temperatures, slower moving fires, and patchier, more heterogeneous burn patterns.^{20,23} Despite the fact that spring burns are performed when fuel moisture content is higher than in the fall,^{23,24} most studies thus far have indicated that the impact of prescribed burning season is rather minor.²⁰ However, many of these studies considered only one or two prescribed burns in a given area. Ponderosa pine forests in the Pacific Northwest east of the Cascade crest historically experienced low-to-moderate-severity fires every 5-35 years; however, most of these forests have not burned in nearly 100 years due to fire suppression policies.^{1,7,26,27} It is likely that sites will need to be burned multiple times to achieve and maintain desired fuel loads, as well as more closely emulate the historical fire return interval.^{21,22}

The effect of performing multiple, consecutive, burns outside the typical fire season has not been thoroughly studied.²⁸ When multiple burns are conducted on the same site, fire effects can accumulate with each successive burn. The interval between burns determines how long the ecosystem is allowed to recover between these disturbances. In this way, the burn interval can essentially determine the cumulative effect of multiple burns. Thus different combinations of season and interval of burn could potentially have important consequences for the ecosystem, including soil properties.

1.1.2 Fire effects on soils

Fire has multiple, interconnected effects on soil properties that depend on fire intensity and duration.^{29,30} Fire can cause changes in soil temperature and moisture regimes,³¹ erosion potential,³²⁻³⁴ nutrient content and cycling,^{29,35,36} soil pH,^{29,37-39} composition of the soil microbial community,^{30,40-42} and the turnover time of SOM.⁴²⁻⁴⁴ Specific factors that determine these effects include the amount and type of fuel consumed, the temperatures achieved during the fire, and the duration of time that elevated temperatures were maintained – all factors that can be affected by fuel moisture levels.³⁰

This study focuses on the way prescribed fires affect the quantity and chemical structure of SOM through either consumption or thermal alteration of

organic material. There is currently a great deal of interest in understanding the drivers that control the cycling of SOM.⁴⁵⁻⁴⁹ Soils represent a significant reservoir in the global carbon cycle, containing approximately 2300 petagrams (Pg) of carbon – nearly three times the amount stored in the atmosphere.⁵⁰ On an annual basis, microbial decomposition of SOM transfers approximately 60 Pg of carbon from soils back to the atmosphere.⁵⁰ Thus, even small shifts in the turnover of SOM can have significant consequences for atmospheric concentrations of carbon-based greenhouse gasses.

In general, fires have a larger impact on SOM when the fire temperature is higher, and when this temperature is maintained for a longer period of time. Hotter fires have the potential to combust more organic material, volatilize a greater proportion of nutrients, and thermally alter the SOM that remains.³⁰ The most immediate loss of SOM during fire comes from the consumption of the O-horizon (litter and duff layer).^{29,30,42} The O-horizon, which contains the highest concentration of SOM, acts as an insulating layer for the mineral soil. In high severity fires, this layer can be completely consumed resulting in a direct loss of SOM.^{29,31,42} Complete combustion of the O-horizon also exposes the mineral soil to direct rainfall, increasing the potential for loss of SOM through erosion.³²

The impact of fire on the carbon content of mineral soils is highly variable and can include competing effects.^{30,35} Substantial amounts of carbon can be lost to combustion, particularly in the O-horizon and even the upper centimeters of the mineral soil if soil temperatures exceed 200°C.³⁰ In contrast, fire-induced vegetation mortality can increase carbon inputs to the soil and cause fragmentation of biomass in the O-horizon that can facilitate the incorporation of organic material into the mineral soil.³⁹ Because of the large range of variables that can affect the impact of fire on soil carbon content (slope, aspect, geomorphology, fire intensity, etc.), it is difficult to draw conclusions from individual studies.³⁰ A recent meta-analysis determined that fire will often reduce the total carbon and nitrogen content of the O-horizon, while having little effect on carbon and nitrogen concentrations.³⁵ In contrast, in mineral soils, fire tends to reduce the carbon and nitrogen concentrations but leave the total content of carbon and nitrogen unchanged, suggesting that complex, counteracting

processes may be taking place.³⁵ This interesting result can only occur if the reductions in carbon and nitrogen concentration are accompanied by an increase in bulk density. Nave et al. suggest that this may occur as less dense SOM is lost during the fire, causing a collapse of soil structure and an increase in bulk density.³⁵

In addition to changing the quantity of SOM, fire can also alter the chemical structure of SOM. Nitrogen, phosphorus, and other plant nutrients found in organic compounds can be mineralized at elevated temperatures.^{30,31} Ash production and deposition during fires can increase the soil pH, which in turn can affect the protonation state of mineral surfaces and SOM as well as increase the cation exchange capacity of the soil.^{29,37,51} Lastly, the elevated temperatures achieved during fire can transform organic molecules into pyrogenic carbon or PyC – thermally altered organic matter that of great interest to the SOM cycling community.⁵²⁻⁵⁴

1.1.3 Formation of pyrogenic carbon (PyC)

The formation of pyrogenic carbon (PyC) during fires can have a profound effect on SOM cycling. PyC is a broad term that refers to a spectrum of thermally-altered organic matter formed either through biomass burning or fossil fuel combustion (Figure 1).^{53,55,56} This spectrum encompasses a heterogeneous mixture of fire-altered residues that differ in physical size, formation temperature, chemical composition, resistance to degradation, and mechanism of formation, making it somewhat challenging to define.^{53,57} Further complicating the issue, there are a host of other terms used to describe various subsets of the spectrum including pyrogenic carbon, char, soot, and biochar. Here, PyC will simply refer to the product of incomplete combustion of organic material or pyrolysis – the thermal alteration of organic material in a low-oxygen environment. Thus, PyC will refer to thermally altered material located anywhere along the PyC spectrum.

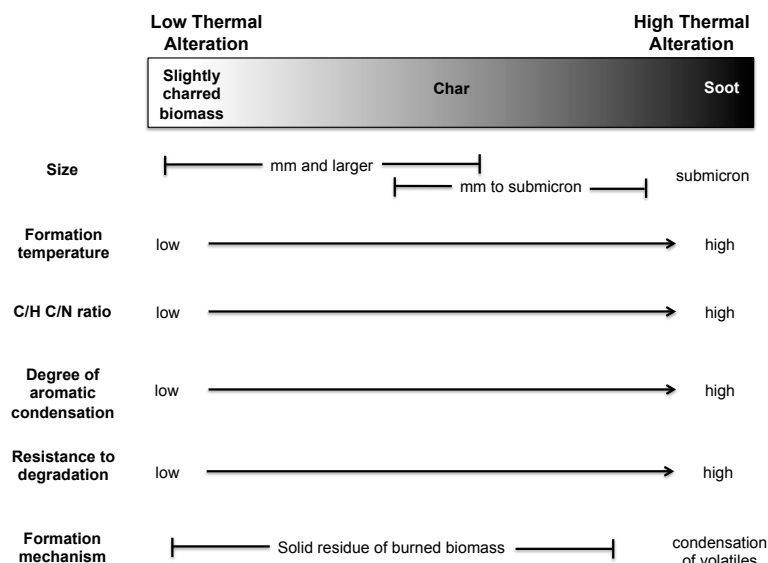


Figure 1: The PyC spectrum encompasses a wide range of heterogeneous materials. As the formation temperature increases, the chemical composition of the materials begins to change. Residues become more enriched in carbon and display higher levels of aromatic condensation (Figure 2 discusses aromatic condensation in more detail). These chemical changes are associated with higher resistance to decomposition. Figure adapted from Masiello, 2004.

Though PyC remains difficult to define, it is a crucial part of the global carbon cycle. It is ubiquitous in both terrestrial and marine environments. At least 6% of the carbon in marine sediments is estimated to be pyrogenic,⁵⁸ while the PyC content of terrestrial soils is estimated to range between 0 and 60%, with an average near 14%.⁵⁴ Moreover, PyC can display turnover times that are orders of magnitude longer than unburned organic matter, cycling on the order of millennia rather than decades or centuries.^{52,53,56} This originally led to the belief that PyC was a separate pool of soil carbon that was environmentally inert; however, recent developments in the field of SOM have led to a more nuanced view of the role of PyC in the carbon cycle.^{52,53} Evidence suggests that molecular structure and environmental factors work in combination to determine the turnover time of PyC.^{53,56,59,60}

The molecular structure of PyC is largely determined by the pyrolysis temperature. As organic matter is subjected to higher temperatures, water and carbon dioxide are driven off. The residual material becomes highly enriched in carbon, as evidenced by its low H/C and O/C ratios^{53,57}. Between 250°C and 350°C, the organic components of biomass are transformed into aromatic (or benzene) rings (Figure 2).⁶¹ As the temperature is further increased from 400-1000°C, these aromatic rings begin to fuse together into larger, more condensed aromatic structures that resemble

graphite.⁶¹ Studies have found that PyC that is composed of these larger, more condensed aromatic structures is more resistant to decomposition.^{53,56,59,62} This relationship between the molecular structure of PyC and its rate of decomposition suggests that a thorough understanding of the role that PyC plays in the carbon cycle will require accurate quantification of PyC produced and retained under different conditions, as well as information about the chemical structure of the material.

Estimates for stocks and fluxes of PyC in different environments can vary by orders of magnitude.^{54,57,63-65} While some of this range is due to natural variation, much results from uncertainty in the techniques used to quantify PyC. A wide variety of methods have been used for PyC quantification. These methods range from simple physical separation of charred material based on visual inspection,^{66,67} to intensive chemical treatment followed by analytical separation and spectroscopy.^{59,63,68-74} Certain techniques provide accurate quantification across the entire PyC spectrum, while others can only capture specific regions. Some techniques systematically overestimate certain regions of the spectrum while underestimating others.⁶³ Several of these methods can be applied to both terrestrial and aqueous samples, while others are limited to one or the other. While all methods provide estimates for the quantity of PyC in a sample, only a handful offer information about the chemical structure of the PyC that is present.^{63,73,75,76} In this study, the benzene polycarboxylic acid (BPCA) method is used to determine both the concentration and chemical structure of PyC present in soils. This method offers a number of advantages compared to other methods of PyC quantification. It is applicable to both terrestrial and aqueous samples, it provides information about PyC chemical structure as well as PyC concentration, and can quantify a wide range of the PyC spectrum.⁵⁷ A detailed discussion of the theory behind the method is included in Section 2.4.1.

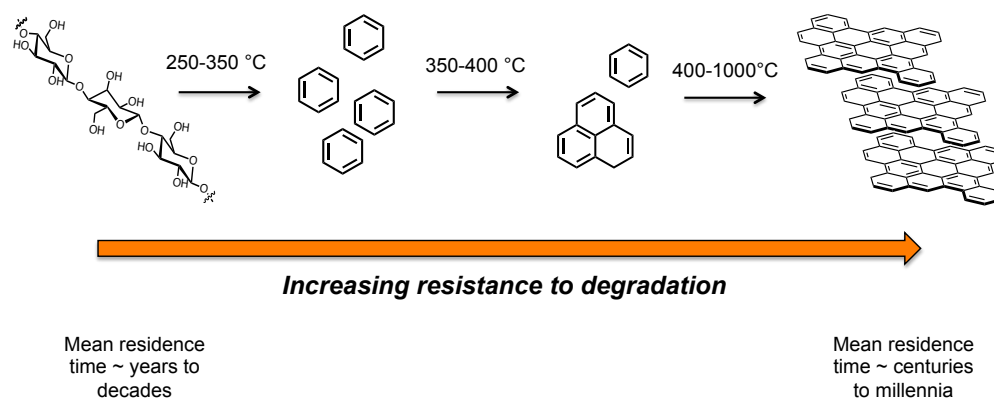


Figure 2: As biomass is exposed to elevated temperatures (250-350°C), molecular structures such as carbohydrates are transformed into individual benzene rings. As temperatures increase further (350-400°C), these benzene rings begin to fuse together into larger, more condensed aromatic structures. Above 400°C these aromatic structures condense further into graphite-like sheets of carbon. The more condensed aromatic structures associated with higher formation temperatures appear to provide protection from microbial decomposition.

1.2 Objectives

In 1997, a prescribed burn study was initiated in Malheur National Forest in Eastern Oregon (Figure 3) to investigate how different seasons or different intervals of prescribed burns affect a range of ecosystem properties including tree mortality,⁷⁷⁻⁷⁹ vegetation cover and richness,^{80,81} soil properties,^{31,51,82} and fuel levels.²² The purpose of this study is to examine how these various prescribed burn regimes affect the cycling of SOM, with a focus on the production and retention of PyC. SOM cycling is not likely to be a prominent factor when making management decisions regarding the timing and frequency of prescribed burns; however, because of the major role that SOM cycling plays in the global carbon cycle, and as the use of prescribed burns to reduce fuel loads increases, it will become important to understand how specific prescribed burn regimes affect soil carbon cycling.

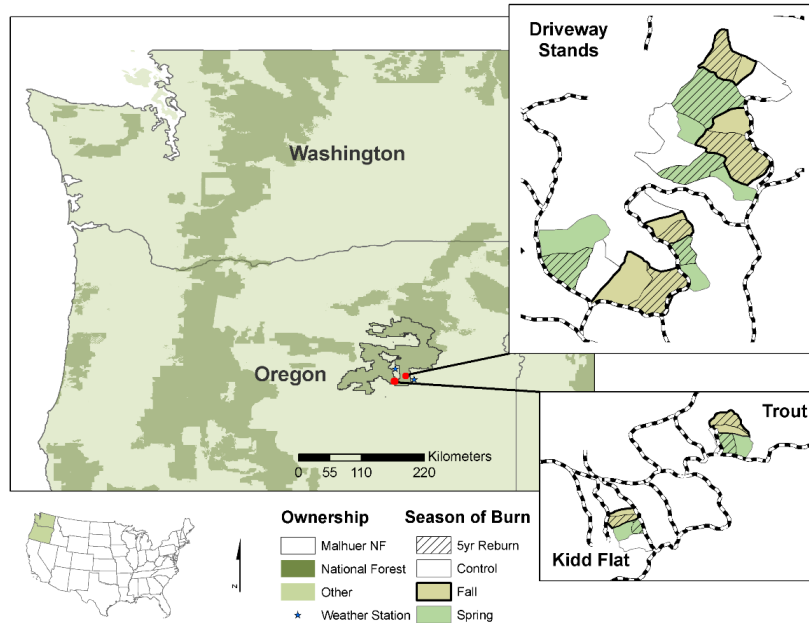


Figure 3: Map of study location in Malheur National Forest in eastern Oregon. Figure taken from Reference 80 (Kerns et al. 2011).

1.2.1 Research Questions

The soil-related response variables that were investigated in this study have been grouped into two broad categories: non-PyC soil properties and PyC-related variables. All response variables were measured separately for both the O-horizon and the top 15 cm of mineral soil. Non-PyC soil properties include O-horizon depth (cm), pH, bulk density, and carbon/nitrogen concentration (%) and content (Mg/ha and kg/ha for C and N, respectively). PyC-related variables include the PyC concentration (g BPCA/kg C), PyC content (g BPCA/m²) and aromatic condensation index (or ACI, discussed in further detail in section 2.4.1, g B6CA/g total BPCA).

Two specific research questions will be addressed in this study: 1) Do prescribed burns performed in either the fall or spring change the mean value of any of the response variables compared to the unburned control?; and 2) Does the season of burn or the interval between burns affect the mean value of any of the response variables that were investigated? The first question will investigate whether the reintroduction of fire to this ecosystem has begun to affect SOM cycling, while the second question investigates whether the specific characteristics of prescribed burns have differing effects on soil.

1.2.2 Scope of Inference

The findings of this investigation directly apply only to the study area due to the specific manner in which stands were selected. While several studies have shown that the PyC content of soils is strongly correlated to soil properties, historical fire regimes, and climatic variables,^{52,54,74} quantification of PyC following prescribed or wildland fire is not yet a widely used technique. Further investigations in other areas should be performed before extending inference even to ponderosa pine forests that undergo prescribed burns and have similar pedogenic properties, historical fire regimes, and climate. Additionally, this study used samples that were collected 2-3 years after the most recent burn; it is possible that the effects of erosion, bioturbation, and other ecological processes could produce different results if samples were collected at different times relative to the most recent burn.⁸³ For example, a proportion of PyC migrates deeper into the soil profile over time due to bioturbation, freeze-thaw cycles, etc. If samples had been collected almost immediately after the most recent burn, we would expect a greater proportion of the PyC to be located in the O-horizon than if samples were collected later.

2. Methods

2.1 Site characteristics

The study site is located within the Malheur National Forest in the Emigrant Creek Ranger District at the southern end of the Blue Mountains of eastern Oregon (43° 52' 41" N/ 118° 46' 19" W). All stands were located between 1585 and 1815 m elevation, with slopes ranging from 3-50%.^{31,81} Ponderosa pine is the dominant tree species; western juniper (*Juniperus occidentalis*) and mountain mahogany (*Cercocarpus ledifolius*) are present in drier areas with shallow soils. Typical stand age is approximately 80-100 years; all stands were thinned in 1994 or 1995. The understory is dominated by grasses and sedges.⁸¹ The dominant nitrogen fixers on the sites are tailcup lupine (*Lupinus caudatus*) and snowbrush (*Ceanothus velutinus*). Shrub cover is dominated by sage brush (*Artemesia tridentate*), Oregon grape (*Berberis repens*), and rabbitbrush (*Chrysothamnus* spp.).⁸⁰

Soils are well to poorly-drained gravelly loams and clay loams derived from basalt, andesite, tuffaceous interflow, altered tuffs, rhyolite, and breccia materials.⁵¹ Additionally, the soil has received inputs of ash from pre-historic eruptions of volcanoes in the Cascade Mountains to the west, including the eruption of Mount Mazama.⁸⁴ Mollisols are the dominant soil order found at the sites, though Inceptisols and Alfisols are also present.⁵¹ The average O-horizon depth ranges from 1-5 cm. Beneath this, the upper 30 cm of mineral soil typically contains 15 cm of A and AB horizons with an average bulk density of 1.17 g/cm³, followed by a Bw horizon with an average bulk density of 1.32 g/cm³, that also extends 15 cm.⁵¹ Surface horizons are typically coarser textured loams that grade into finer textured loams at depth.^{31,51}

The annual precipitation at the Rock spring SNOTEL station (44°0'N/118°50' W) located 25 km WNW of the study sites averages 460 mm. Eighty percent of precipitation falls as snow between November and April.⁵¹ Summers are dry and hot (17 °C mean air temperature in July–August), though diurnal temperatures can fluctuate widely.⁸¹ Winters are typically cold (−3°C mean air temperature in December–February), and significant snowfall accumulation can occur.⁸¹

2.2 Experimental design and sample collection

In 1997, a season of burn study was initiated in Malheur National Forest.⁷⁷ A diagram of the study is shown in Figure 4. Six replicate stands of mixed-age ponderosa pine (40-56 ha) were selectively chosen from a list of stands in the area scheduled for thinning and prescribed burning. The study was originally designed to examine how the season of prescribed burns affects the prevalence of black stain root disease (BSRD) in ponderosa pine; consequently, the six stands chosen for the study were selected for their proximity to BSRD affected areas.⁷⁷ Four of the stands are clustered next to one another, while the other two stands lie 18 km to the west.

Each stand was divided into three, contiguous plots (4.5-20.5 ha) that are similar in species composition, tree density, slope, and aspect.⁷⁷ Boundaries of the plots were established along topographical features such as roads or ridges to facilitate control of the burns. Within each stand, one of three season of burn treatments (fall burn, spring burn, or control) was randomly assigned to each plot. Following the first burns in the fall of 1997 and spring of 1998, six sampling locations (0.2 ha) were installed in each of the plots, with one exception in which the initial fall burn overachieved, reducing the size of the control plot. Sampling locations were specifically placed to represent average burn severity (subjectively determined by scorch height on trees, vegetation mortality, etc.) within the plot.

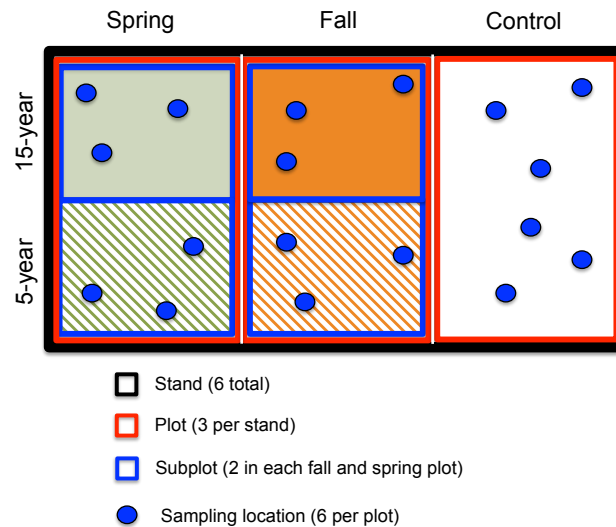


Figure 4: Schematic diagram of experimental design. The black box represents one of 6 stands. Each stand was divided into three plots (red box), each of which was assigned to one of three season of burn treatments: fall, spring, or control (unburned). The fall and spring plots were each bisected into two subplots (blue box) and assigned to one of two intervals of burn: 5 or 15 years. Thus within each stand

there is an unburned control plot (C), that was not subdivided as well as four subplots that were burned in one of four season-interval combinations: fall burning at 5-year intervals (F5), fall burning at 15-year intervals (F15), spring burning at 5-year intervals (S5), and spring burning at 15-year intervals (S15).

In 2002, the study was expanded to investigate the effects of both season and interval of burns.³¹ The fall and spring experimental plots within each stand were bisected into two experimental subplots such that 3 of the sampling locations fell within each subplot. Each subplot was then randomly assigned either a 5- or 15-year burn interval, to be burned in the season designated by the original plot assignment. Thus within each of the six replicate stands there is an unburned control plot that was not subdivided, as well as four subplots that were each burned in one of four season/interval combinations: in the fall at 15-year intervals (F15), in the fall at 5-year intervals (F5), in the spring at 15-year intervals (S15), or in the spring at 5-year intervals (S5). Each control plot contains 6 sampling locations (with the exception of one stand that contains only 5 sampling locations in the control plot), while each of the subplots contains 3 sampling locations for a total of 107 sampling locations.

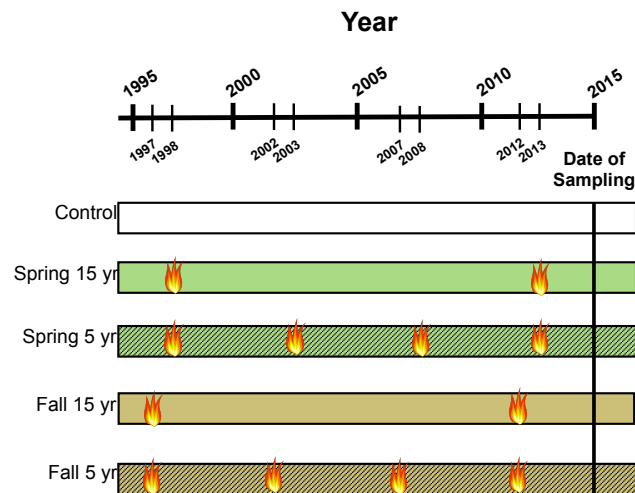


Figure 5: Timeline of prescribed burns. At the time of sampling, the subplots burned at 5-year intervals had been burned four times and the subplots burned at 15-year intervals had been burned twice.

Samples for this study were obtained in October 2015, at which point the fall 5-year and spring 5-year experimental subplots had been burned four times and the fall 15-year and spring 15-year experimental subplots had been burned twice (Figure 5). Two O-horizon samples and two 0-15 cm bulk density samples were collected at a

random point within each sampling location and composited by depth. Each of these composited samples was individually processed and analyzed for carbon and nitrogen content, pH, and PyC concentration/structure. O-horizons were collected using a 5x10 cm template. The O-horizon depth at each corner of the template was recorded and used in conjunction with the template area to obtain accurate estimates of O-horizon bulk density. Measurements of percent cover (bare ground, litter, coarse woody debris) were also recorded at each sample collection point.

2.3 Soil processing and characterization

Mineral soil samples were weighed and oven-dried at 50°C until a constant weight was achieved. Subsamples were dried overnight at 100°C to determine moisture corrections for bulk density calculations. The dried samples were sieved to 2 mm, and the individual coarse fractions weighed to determine coarse (>2 mm) content. The fine fractions (<2 mm) were ground to a fine powder using a roller grinder prior to subsequent analysis. O-horizon samples were oven-dried at 50°C and ground to a fine powder using an IKA grinder. Total carbon and nitrogen content of all mineral soil and O-horizon samples was determined using Thermo FlashEA 1112 series, NC Soil Analyzer. The pH of mineral soils was determined using a 2:1 (w/w) mixture of water/soil, while a 5:1 (w/w) mixture was used for O-horizons.⁸⁵

2.4 Pyrogenic carbon analysis – BPCA method

2.4.1 Conceptual explanation of BPCA method

The BPCA method, first introduced by Glaser et al., takes a molecular marker approach to PyC quantification.⁷⁵ Samples are digested in concentrated acid at elevated temperatures, oxidizing the carbon-carbon double bonds of the fused aromatic ring structures that form the molecular backbone of PyC. These aromatic structures are broken down into individual benzene rings substituted with carboxylic acids (i.e., benzene polycarboxylic acids or BPCAs, see Figure 6).^{75,86-88} Following several cleanup steps to remove interfering cations and hydrophobic compounds, these BPCAs can be separated and quantified using gas chromatography mass

spectrometry (GC-MS)^{75,86} or high performance liquid chromatography (HPLC, Figure 6).⁸⁷⁻⁸⁹ The total amount of BPCAs extracted from a sample can be used in conjunction with an empirically derived conversion factor to calculate the PyC content of the original sample.⁷⁵

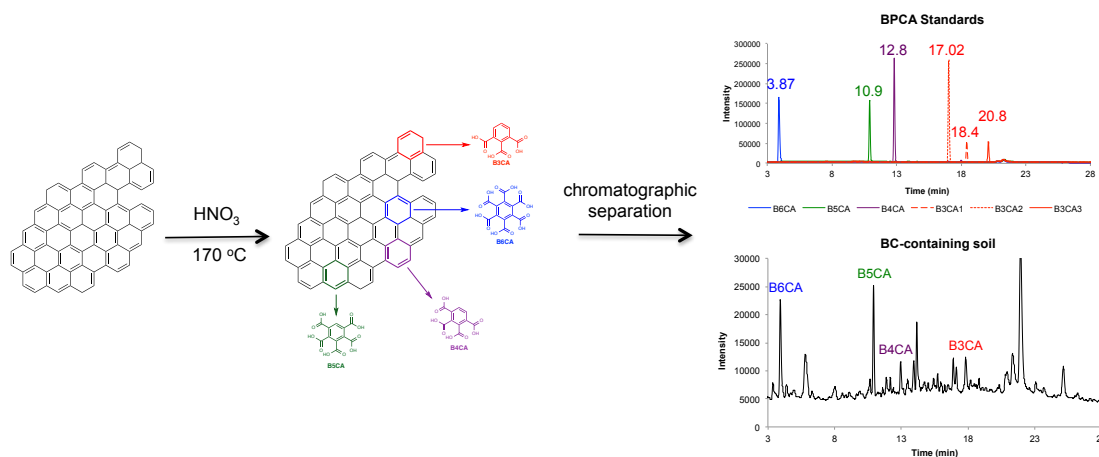


Figure 6: In the BPCA method, nitric acid is used to break down the large aromatic structures of PyC into individual, carboxylated benzene rings or BPCAs. When an individual benzene ring is cleaved from the larger aromatic sheet, its position in the original structure is reflected in the carboxylic acid substitution pattern of the resulting BPCA. These BPCAs can then be separated and quantified using GC-MS or HPLC. The top chromatogram is the HPLC trace of BPCA standards that are used to determine retention times and identify BPCA peaks from samples with unknown PyC content. The bottom chromatogram is the HPLC trace of a PyC-containing soil that has been treated with HNO_3 . Individual peaks from 3-, 4-, 5-, and 6-substituted BPCAs are clearly visible.

A primary advantage of the BPCA method is that in addition to quantifying the PyC content of a sample, it provides information about the molecular structure of PyC. When individual benzene rings are cleaved from a larger aromatic structure during the nitric acid digestion, each carbon-carbon double bond that is oxidized creates a carboxylic acid substitution on the resulting benzene ring.⁷⁵ Thus the carboxylic acid substitution pattern on individual BPCAs reflects the relative position the ring held in the original, extended aromatic structure (Figure 7). Rings near the edge of the aromatic structure can be cleaved by oxidizing only two or three carbon-carbon double bonds, resulting in BPCAs with only two or three carboxylic acid substitutions (B2CAs and B3CAs). In contrast, six carbon-carbon double bonds must be oxidized to cleave rings located near the interior of the aromatic structure, resulting in BPCAs with six carboxylic acid substitutions (B6CAs). Consequently, the B6CA:total BPCA ratio serves as an aromatic condensation index or ACI – a

quantitative measure of the molecular structure of PyC. Larger, more extended aromatic structures will have more interior benzene rings and thus a high B6CA:total BPCA ratio or high ACI.⁹⁰ Smaller, less extended aromatic systems will have a greater proportion of rings located on the edge of the structure and thus a lower ACI.

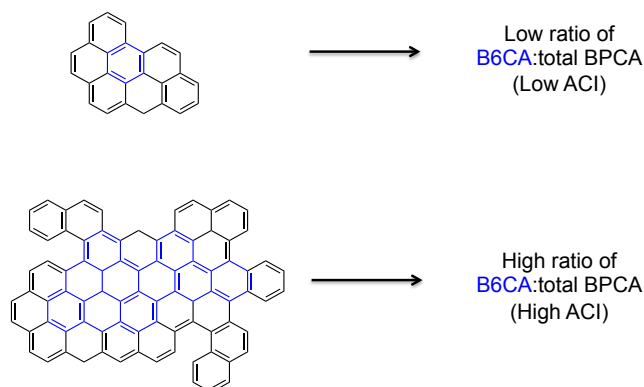


Figure 7: The BPCA method provides information about PyC molecular structure in addition to PyC content. Internal rings that could potentially produce B6CAs upon oxidation are shown in blue. Smaller, less extended aromatic structures (top) have a high proportion of rings on the edge of the structure and will result in a small ratio of B6CA:B3CA. Larger, more extended structures will have a larger proportion of interior rings, leading to a high ratio of B6CA:B3CA.

Though the method was initially developed for soil samples, modifications to sample preparation and chromatographic separation have extended it to aqueous samples with lower PyC content.^{87,89} Glaser's original method uses GC-MS to separate and quantify BPCAs following hydrochloric acid (HCl) digestion.⁷⁵ This process requires chemical derivitization of the isolated BPCAs with *N,O*-bis(trimethylsilyl)-trifluoroacetamide prior to chromatographic separation, a time-consuming step that often does not proceed to completion.^{87,89} Moreover, the use of HCl in the digestion step artificially introduces small quantities of BPCAs to samples from non-PyC sources, making quantification of low concentrations of BPCAs impossible.⁸⁶ In 2008, Dittmar modified the procedure to increase both sensitivity and sample throughput.⁸⁷ He also extended the method to aqueous samples, using solid phase extraction cartridges to isolate DOM from seawater. The extracted material was oxidized via microwave-assisted digestion in HNO₃ rather than HCl. The use of HNO₃ avoids the artificial production of BPCAs, decreasing the lower detection limit of the method. Moreover, because the digestion is performed in a microwave using pressurized vessels, it can be done at temperatures significantly above the boiling point of HNO₃. This improves the efficiency of the digestion and increases the overall

yield of BPCAs, reducing the volume of sample needed for accurate quantification. Following digestion, Dittmar's modification of the procedure uses HPLC rather than GC-MS for chromatographic separation and quantification of individual BPCAs. This eliminates the low-yielding, time-consuming derivitization step, improving both sensitivity and sample throughput.^{87,89}

2.4.2 Procedure

This procedure is based on the BPCA methods developed by Wiedemeier et al.^{88,91} and Dittmar,⁸⁷ with small modifications (Figure 8). Ground soil samples containing ~3.5 mg of carbon were digested in 5 mL HNO₃ at 170 °C for 8 hours using pressurized microwave vessels. Following digestion, samples were filtered through glass fiber filters. When analyzing mineral soils, the remaining solids were washed with 5 mL of NaOH (1 M). Samples were diluted to 50 mL with deionized water, flash frozen with liquid nitrogen, and freeze dried. The remaining residue was dissolved in 1 mL deionized water or 2 mL NaOH (1 M) for O-horizon and mineral soil samples, respectively. Samples were syringe filtered using 0.45 µm nylon filters. When performing mineral soil analysis, a 1 mL aliquot of sample was transferred to a clean vial and spiked with 600 µL of HCl (2 M) and analyzed on a Shimadzu LC-10AD analytical HPLC equipped with an SPD-M20A photodiode array capable of measuring wavelengths between 190-400 nm. An Agilent Poroshell 120 SB-C18 column was used with a mobile phase consisting of a binary gradient of H₃PO₄ (2% in water) and acetonitrile⁸⁸.

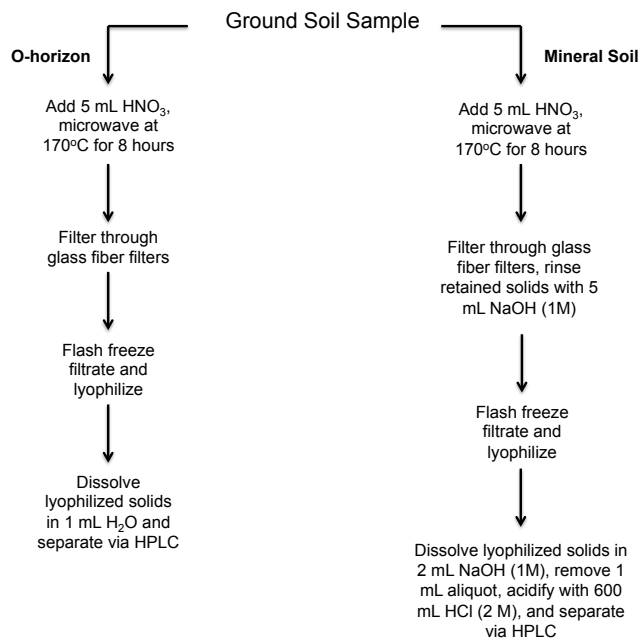


Figure 8: Flow chart of BPCA procedure for mineral soil and O-horizon samples.

External standards of pure BPCA solutions (Figure 9) were used to construct 6-point calibration curves to determine the concentrations of individual BPCAs. Two of the isomers of B4CA are not commercially available (mellophanic acid and prehnitic acid) and were not used in the quantification of PyC. Consequently, the B4CA content, and the PyC concentration in general, will be somewhat underestimated.

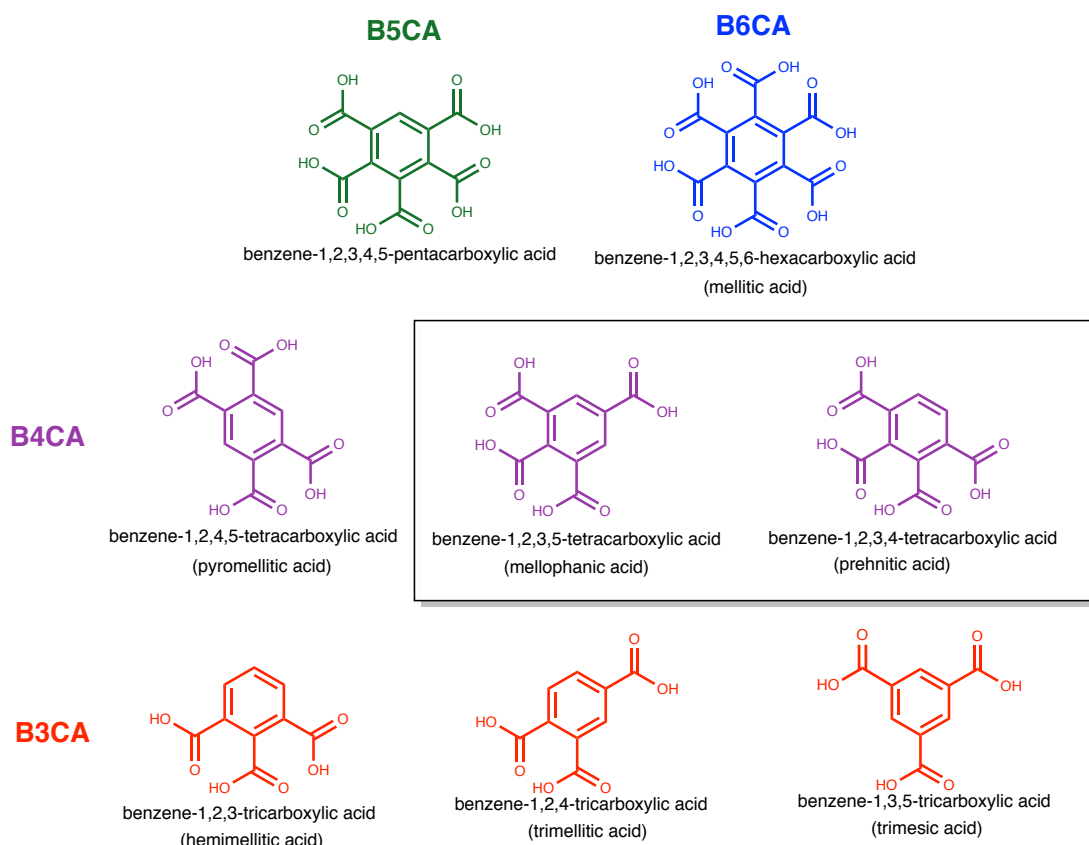


Figure 9: Individual BPCAs that were quantified by HPLC. The two isomers of B4CA enclosed in the black rectangle are not commercially available; consequently they were not used in BPCA quantification.

2.4.3 Method validation

There can be a wide range of lab-to-lab variability in the results from the BPCA method due to differences in specific pre-processing steps, equipment, and chromatographic settings. When the method is implemented in a new laboratory, it is recommended that a standard PyC-containing soil be analyzed to facilitate comparisons with the PyC literature. A PyC-containing Chernozem soil from the Hildesheim-Braunschweig region (Harsum, Germany) that was analyzed in a multi-laboratory ring trial comparing various methods for PyC quantification was used as a standard to assess the accuracy of the method implemented in this study.⁶³ This same soil has also been used as a standard in more recent studies that have adapted the BPCA method to quantification by HPLC rather than GC-MS.^{88,91}

In the original PyC ring trial performed by Hammes et al.,⁶³ the PyC concentration of the Chernozem as determined by the BPCA method ranged from ~10-25% of organic carbon in the sample. Because these values were obtained using a factor of 2.27 to convert from g BPCA/kg C to g PyC / kg C, this corresponds to approximately 45-110 g BPCA/kg C. (It has since been determined that the conversion between g BPCA and g of PyC is highly sample specific, and it is now recommended that PyC values obtained using the BPCA method be reported as g BPCA/kg C without any conversion factor.⁴³) Subsequent studies have found values ranging from 51-81 g BPCA/kg C⁹² to 90-110 g BPCA/kg C.⁸⁸ Using the method described above, we found values for this standard soil to range from 25-33 g BPCA/kg C ($n = 6$). While the absolute value is slightly below the range reported in the literature, the precision of the values is quite high.

There are a number of possible reasons for the reduction in the absolute value of PyC quantified in this study. First, previous methods that have quantified the BPCA content of the Chernozem soil have performed the nitric acid digestion step using a digestion block placed in an oven. In contrast, we performed the digest in pressurized microwave vessels. It is possible that microwave digestion is less efficient than the digestion block, leaving some sheets of PyC intact rather than breaking them into individual BPCAs. Conversely, it is also possible that the microwave digestion is *more* thorough than the digestion block, causing complete oxidation of some BPCAs. Dittmar found that the microwave digestion of PyC is extremely sensitive to temperature.⁸⁷ At a temperature of 180 °C extensive loss of BPCAs was observed due to excessive oxidation, while at a temperature of 160 °C the release of BPCAs into solution was kinetically hindered.⁸⁷ While a set temperature of 170 °C was used in this study, it is possible that fluctuations in temperature during the 8 hour digest resulted in some loss of BPCAs due to either over oxidation or incomplete digestion. Second, as mentioned above, two of the isomers of B4CA were not commercially available and thus could not be used as standards. Because quantification was performed on an HPLC that was not equipped with a mass spectrometer, we chose not to quantify these species. As a result, this method will consistently underestimate the total amount of BPCAs in a sample. Lastly, in an effort to improve the throughput

of the method and facilitate the analysis of a large number of samples, we did not clean the samples using a cation exchange column prior to HPLC separation. As a result some of the negatively charged BPCAs may have adsorbed to insoluble, positively charged surfaces in the mineral soil matrix and been lost during either of the filtration steps.

Because the numbers we obtained for the Chernozem soil were lower than expected, we wanted to confirm that the amount of BPCAs quantified by the method adequately and reproducibly detected differences in PyC concentration. To test this, samples of Chernozem soil (200 mg) were amended with increasing amounts of charred ponderosa pine wood and bark (0, 1, 2, or 3 mg) and analyzed for BPCA content as described above. Three replicates were performed for each concentration of char. The total amount of BPCAs detected in solution increased as a linear function of the amount of char that was added (Figure 10), confirming that the BPCA concentration detected in solution reflects the PyC concentration of the soil that was analyzed. Moreover, the BPCA concentration of char samples digested in the absence of mineral soil was 0.063 (± 0.018) mg BPCA/mg char. This is in excellent agreement with the slope of the line obtained from the linear regression in Figure 10, which suggests that for each additional mg of char added, an increase of 0.069 mg BPCA should be observed. Thus, while the absolute values for BPCA content of the samples in this study may be lower than if the same samples had been analyzed by a similar method in another laboratory, differences in BPCA content between these samples reflect differences in PyC concentration.

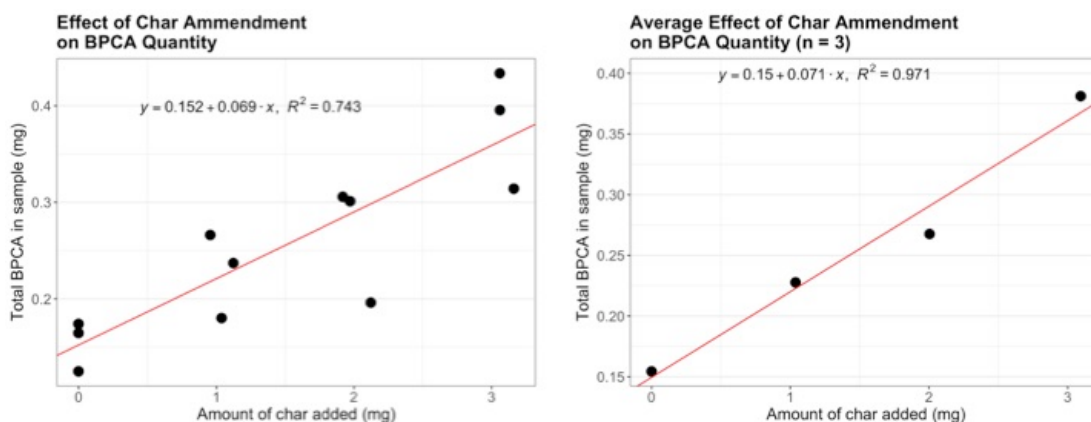


Figure 10: Chernozem soils were amended with increasing amounts of charred ponderosa pine. The amount of BPCAs detected in solution increased linearly with the amount of char that was added. Each

concentration of char was analyzed in triplicate. The figure on the left shows the individual points from these replicate samples; the figure on the right shows the average of the three points.

The samples from each stand were digested, filtered, and lyophilized as separate batches. To determine the precision of the method, measures of within-batch and batch-to-batch reproducibility were calculated. Each batch contained at least one sample duplicate to test the within-batch reproducibility of the method. A total of 16 duplicates were measured for 214 total samples for an average of 7.5% duplication. In addition, a sample of the Chernozem soil was analyzed with each batch of mineral soil samples to test for batch-to-batch reproducibility. In general both the within-batch and batch-to-batch reproducibility were quite high for both the measures of PyC content and PyC chemical structure (Table 1). The batch-to-batch reproducibility is reported as the standard deviation of the total amount of PyC (g BPCA/kg C) and the ACI (g B6CA/g total BPCA) of PyC measured in the standard Chernozem soil over 6 batches. The estimate of both of these metrics is also included. The within-batch reproducibility is reported as both the percent difference and absolute difference in the total amount of PyC and the ACI for between duplicate samples analyzed in the same batch. These values for reproducibility are on par with the most precise BPCA methods that have been reported in the literature.⁸⁸ The precision of the ACI is higher than that of the PyC concentration, as it is a ratio of two compounds that are likely affected by similar sources of error.⁹³

	Batch-to-Batch		Within Batch	
	Estimate	Standard Dev.	Percent Difference	Absolute Difference
PyC (g BPCA/kg C)	29.2	3.8	10.74%	3.09
ACI (g B6CA/g total BPCA)	0.33	0.037	2.38%	0.01

Table 1: Batch-to-batch and within-batch reproducibilities of the PyC concentration and ACI measured by the BPCA method used in this study.

2.5 Statistical Analysis

Multiple response variables were collected and analyzed in this study, including the O-horizon depth and the carbon/nitrogen content, C:N ratio, and pH of both the O-horizon and 0-15 cm samples. The primary focus of this study, however,

is the quantity and chemical structure of PyC found in the O-horizon and 0-15 cm samples, which will be referred to as PyC concentration (g BPCA/kg C) and ACI (g B6CA/g total BPCA), respectively. This study aims to answer multiple questions about the effect of different prescribed burning regimes on soil properties. Because the structure of the experimental design involves a 2x2 factorial study with an augmented control, two separate statistical analysis strategies were performed to answer the questions of interest.

To determine whether burning in the spring or fall substantially affected response variables compared to the unburned control (Question 1), the study was treated according to the original season of burn experimental design. The division of plots assigned to a given season of burn into subplots assigned to a given interval between burns was ignored. In this analysis, the measurements from each of the six sampling locations within a given plot in a given stand were combined to provide an average value of the response for that specific plot/stand combination, for a total of 18 data points. The average values of the response were then fit to a linear mixed effects model where ‘stand’ was used as a blocking factor:

$$Y_t = \alpha_0 + \alpha_1 I.fall_t + \alpha_2 I.spring_t + (b_r)_t + \epsilon_t \quad (\text{Eq. 2})$$

where:

Y_t is the observed response for the t^{th} observation in one of r stands burned in one of the i seasons; $i = \text{fall, spring}$; $r = 1-6$, $t = 1-18$

α_0 is the mean response of the unburned control plots

α_1 is the incremental effect of burning in the fall

α_2 is the incremental effect of burning in the spring

$I.fall$ is 1 when season of burn treatment is ‘fall’ and 0 otherwise

$I.spring$ is 1 when the season of burn treatment is ‘spring’ and 0 otherwise

b_r is the random effect of the r^{th} stand on mean response, $b_r \sim N(0, \sigma_b^2)$ and b_r and $b_{r'}$ are independent

ϵ_t is the random error associated with the t^{th} observation, $\epsilon_t \sim N(0, \sigma^2)$ and ϵ_t and $\epsilon_{t'}$ are independent. Additionally, it is assumed that ϵ_t and b_r are independent.

Values of each response variable were fit to this model or a similar model that included covariates (discussed below) using R version 3.2.2. Comparisons were performed to determine whether burns performed in the spring or fall change the mean value of any of the response variables compared to unburned controls. Tukey corrections for family-wise error rates associated with pairwise comparisons of 3 groups (spring, fall, and control) were applied for all statistical tests on a given

response variable using the LSmeans package in R.

To determine whether different seasons of burn or different intervals between burns affect the mean values of the response variables (Question 2), the study was treated as a 2x2 factorial experiment where each factor (season of burn and interval between burns) contains two levels (fall and spring or 5 and 15 years). The data from the control plots was not considered. In this analysis, the measurements from each of the three sampling locations within a given subplot in a given stand were combined to provide an average value of the response for that specific subplot/stand combination, for a total of 24 data points. The average values of the response were fit to a linear mixed effects model where ‘stand’ was used as a blocking factor.

$$Y_t = \alpha_0 + \alpha_1 I.15yr_t + \alpha_2 I.spring_t + \alpha_3 [(I.15yr)_t \times (I.spring)_t] + (b_r)_t + (c_s)_t + \epsilon_t \quad (\text{Eq. 1})$$

where:

Y_t is the observed response of the t^{th} subplot, in one of the s plots, in one of the r stands burned in one of the i seasons at j intervals; i = Fall or Spring, j = 15 year or 5 year; r = 1-6, s = 1-12, t = 1-24

α_0 is the mean response in subplots burned in the fall at 5 year intervals

α_1 is the incremental effect of burning at 15-year intervals on the mean response

α_2 is the incremental effect of burning in the spring on the mean response

α_3 is the further incremental effect of burning at 15-year intervals in the spring on mean response

$I.15yr$ is 1 when the interval between burns is 15-years and 0 otherwise

$I.spring$ is 1 when the burns are performed in the spring and 0 otherwise

b_r is the random effect of the r^{th} stand on the mean response, $b_r \sim N(0, \sigma_b^2)$ and b_r and $b_{r'}$ are independent

c_s is the random effect of the s^{th} plot on the mean response, $c_s \sim N(0, \sigma_c^2)$, c_s and $c_{s'}$ are independent

ϵ_t is the random effect associated with the t^{th} observation, $\epsilon_t \sim N(0, \sigma^2)$ and ϵ_t and $\epsilon_{t'}$ are independent. Additionally, it is assumed that ϵ_t and b_r are independent.

Again, values of each response variable were fit to this model or a similar model that included covariates (discussed in the section 2.4.2) using R version 3.2.2. For each response variable, an overall F-test was used to determine if there was an interaction between the factors of season and interval. If no interaction was detected, the main effects of season and interval were calculated using the LSmeans package in R. Multivariate t (MVT) corrections for family-wise error rate associated with two comparisons (spring vs fall and 5-year vs 15-year intervals) were applied to all statistical tests for each response variable.

The models used in this study assume that model residuals are normally distributed and that the variance is homogeneous between groups and across the response variable. Histograms and q-q plots of the residuals were used to evaluate the assumption of normality. While small deviations from normality were observed for some response variables, this is likely due to the small sample size. It is reasonable to assume that the residuals were drawn from a normal, or at least symmetric, distribution. Plots of the residuals as a function of the different grouping variables (season of burn, interval of burn, and stand) and as a function of the fitted values were used to evaluate the assumption of homogeneity of variance. In cases where it appeared as though the variance between groups was inhomogeneous, model assumptions were relaxed to allow for different estimates of variance for different groups.

3. Results

3.1 Factors influencing PyC concentration and structure

When fitting the PyC response variables (PyC concentration and ACI of the O-horizon and 0-15 cm samples) to statistical models, a number of covariates were considered. Westlind et al. reported measurements of both woody fuels (1, 10, 100, and 1000 hour fuels) and litter and duff at each sampling location before and after the most recent prescribed fires.²² Because fuels are the organic matter that is converted to PyC, we hypothesized that there would be a relationship between the amount of PyC in the soil and fuel consumption. However, neither the PyC concentration/content nor the ACI of either the O-horizon or mineral soil appear to be correlated to fuel consumption in the most recent set of burns (Table 2). Differences in vegetation patterns may also account for some of the variability in response. Kerns et al. have reported measurements of vegetation data at the study sites; however, these measurements were taken at only five of the six stands that were sampled. Consequently, vegetation measurements were not used as covariates.

Table 2: Pearson correlation coefficients between fuel consumption in the most recent prescribed burns and PyC response variables.

	O-horizon			0-15 cm		
	PyC concentration (g BPCA/kg C)	PyC content (g BPCA/m ²)	ACI (g B6CA/g total BPCA)	PyC content (g BPCA/kg C)	PyC content (g BPCA/m ²)	ACI (g B6CA/g total BPCA)
1 hr fuels	0.07	0.05	-0.13	0.03	-0.03	-0.31
10 hr fuels	-0.05	0.05	0.17	0.11	0.01	-0.09
100 hr fuels	0	-0.02	-0.06	-0.18	-0.31	0.02
1000 hr fuels	-0.06	-0.07	-0.03	-0.09	-0.05	0.03
total woody fuels	-0.06	-0.06	-0.01	-0.09	-0.09	0.02
litter and duff	0.07	0.03	-0.06	-0.27	-0.29	0.27

Correlations between the PyC-related response variables and several of the non-PyC response variables measured in this study (O-horizon depth, litter cover, bare ground cover, woody debris cover, pH, and carbon concentration) study were also examined (Table 3). When moderate correlations between some pairs of these variables were found ($R > 0.30$), models were constructed that included the non-PyC variable as a covariate. Only one non-PyC variable was considered as a covariate in any given model. AIC was used to compare these to models that included no covariates. In every case, the model without covariates resulted in a lower or comparable (<2 units) AIC value.

Table 3: Pearson correlation coefficients between the PyC-related response variables and the other response variables measured in this study.

	O-horizon			0-15 cm		
	PyC concentration (g BPCA/kg C)	PyC content (g BPCA/m ²)	ACI (g B6CA/g total BPCA)	PyC concentration (g BPCA/kg C)	PyC content (g BPCA/m ²)	ACI (g B6CA/g total BPCA)
O-horizon depth	-0.09	0.3	0.33	-0.12	-0.11	0.2
Litter	-0.04	0.12	0.25	-0.23	-0.1	0.31
Bare ground	0.01	-0.17	-0.27	0.19	0.1	-0.32
CWD	0.00	-0.01	-0.02	0.24	0.1	-0.13
pH (0-15 cm)	0.16	0.03	-0.20	0.45	0.19	-0.36
pH (O-horizon)	0.34	0.32	-0.11	0.3	0.24	-0.12
%C (0-15 cm)	-0.02	0.09	0.27	0.13	0.59	0.52
%C (O-horizon)	-0.27	-0.25	0.27	-0.10	-0.02	0.12

Lastly, because the PyC in the O-horizon is a primary source of PyC in the mineral soil, we examined the correlation between the PyC response variables measured in the O-horizon and the mineral soils. The correlation coefficient between the PyC concentration of the O-horizon and the 0-15 cm soils was 0.37, while the correlation coefficient between the ACI of the two was 0.34. Models that included PyC concentration, content, and ACI of the O-horizon as covariates for PyC concentration, content, and ACI of the 0-15 cm soils were constructed. These models were compared to models for PyC concentration, content, and ACI of the mineral soil that included no covariates using AIC. The model for PyC concentration of the mineral soil that included the PyC concentration of the O-horizon had a lower AIC than the model that included no covariates. The models for PyC content and ACI of the mineral soil that included the PyC content and ACI of the O-horizon, respectively, had approximately the same AIC as the models that included no covariates; however, the inclusion of these covariates significantly improved the normality of the model residuals. Consequently, the PyC concentration, content, and ACI of the O-horizon were used as covariates in models for PyC concentration, content, and ACI of the mineral soil, respectively. No covariates were used when fitting the PyC concentration and ACI of the O-horizon or any of the other non-PyC response variables.

3.2 Research questions

Fire can affect a wide range of soil-related properties. Here we investigated how 18 years of prescribed burn treatments have affected the O-horizon depth, pH,

bulk density, and carbon/nitrogen concentration and content of both the O-horizon and the 0-15 cm soils (Table 4).

Table 4: O-horizon and mineral soil properties. Values are reported as the average \pm the standard deviation. For the control plots, this represents the average over 6 sampling locations within each of 6 stands. For each of the subplots burned at different seasons/intervals, this represents the average over 3 sampling locations within each of 6 stands.

		Control	F15	F5	S15	S5
O-horizon	Depth (cm)	3.59 \pm 0.63	2.06 \pm 0.69	1.85 \pm 0.52	2.78 \pm 1.00	2.08 \pm 0.54
	%C	37.49 \pm 2.14	37.67 \pm 5.32	37.75 \pm 4.94	37.51 \pm 8.35	36.26 \pm 2.17
	C (Mg/ha)	32.59 \pm 9.04	16.85 \pm 7.18	14.28 \pm 10.98	21.53 \pm 5.72	16.08 \pm 6.02
	%N	1.12 \pm 0.14	1.02 \pm 0.24	0.92 \pm 0.14	1.09 \pm 0.25	1.09 \pm 0.20
	N (kg/ha)	992 \pm 368	473 \pm 279	356 \pm 238	652 \pm 209	500 \pm 187
	C:N	39.27 \pm 3.45	47.32 \pm 12.49	50.17 \pm 10.31	41.87 \pm 6.24	40.81 \pm 7.22
	pH	4.95 \pm 0.29	4.97 \pm 0.13	5.06 \pm 0.36	5.02 \pm 0.25	5.05 \pm 0.25
0-15 cm	BD (g/cm ³)	0.29 \pm 0.10	0.21 \pm 0.05	0.18 \pm 0.09	0.31 \pm 0.14	0.24 \pm 0.13
	%C	5.39 \pm 1.40	5.26 \pm 2.15	4.80 \pm 2.00	4.56 \pm 0.99	3.65 \pm 1.35
	C (Mg/ha)	45.07 \pm 4.53	48.41 \pm 14.86	46.62 \pm 20.53	46.18 \pm 4.75	35.16 \pm 8.65
	%N	0.29 \pm 0.07	0.29 \pm 0.08	0.28 \pm 0.11	0.25 \pm 0.07	0.20 \pm 0.07
	N (kg/ha)	2471 \pm 454	2650 \pm 414	2726 \pm 1205	2553 \pm 457	2031 \pm 681
	C:N	21.59 \pm 1.91	21.17 \pm 4.19	20.18 \pm 3.01	21.56 \pm 2.77	21.27 \pm 4.81
	pH	6.07 \pm 0.12	6.24 \pm 0.19	6.48 \pm 0.14	6.33 \pm 0.16	6.21 \pm 0.08
	BD (g/cm ³)	0.60 \pm 0.11	0.66 \pm 0.10	0.67 \pm 0.09	0.69 \pm 0.07	0.68 \pm 0.13

The primary focus of this study is to determine how various prescribed burn regimes affect the quantity and structure of PyC produced in ponderosa pine stands of Malheur National Forest in eastern Oregon, in order to better understand the effects of prescribed fire on SOM cycling in this region. Both the quantity and structure of PyC were measured using the BPCA method. The PyC concentration is expressed as g BPCA/kg C, and PyC content is expressed as g BPCA/m². Measurements of PyC structure is expressed in terms of the aromatic condensation index (or ACI), measured as g B6CA/g total BPCA, where a higher ACI is indicative of PyC with a more condensed aromatic structure. Average values for these PyC response variables are reported in Table 5. The remainder of the results section will be discussed in the context of the two research questions posed in Section 1.2.1.

Table 5: O-horizon and mineral soil PyC concentration, content, and ACI. Values are reported as the average \pm the standard deviation. For the control plots, this represents the average over 6 sampling locations within each of 6 stands. For each of the subplots burned at different seasons/intervals, this represents the average over 3 sampling locations within each of 6 stands.

		C	F15	F5	S15	S5
O-horizon	PyC concentration (g BPCA/kg C)	6.27 \pm 2.25	7.82 \pm 3.90	9.79 \pm 2.13	11.31 \pm 7.91	14.45 \pm 10.15
	PyC content (g BPCA/m ²)	62 \pm 56	34 \pm 29	52 \pm 78	86 \pm 83	82 \pm 93
	ACI (g B6CA /g total BPCA)	0.41 \pm 0.07	0.39 \pm 0.07	0.33 \pm 0.06	0.35 \pm 0.07	0.31 \pm 0.08
0-15 cm	PyC concentration (g BPCA/kg C)	31.06 \pm 5.46	39.18 \pm 8.37	43.51 \pm 7.70	38.73 \pm 9.46	36.03 \pm 9.33
	PyC content (g BPCA/m ²)	137 \pm 43	200 \pm 114	206 \pm 132	184 \pm 101	126 \pm 60
	ACI (g B6CA /g total BPCA)	0.31 \pm 0.04	0.29 \pm 0.02	0.28 \pm 0.03	0.30 \pm 0.01	0.26 \pm 0.03

3.2.1 Does burning in the fall or spring change the mean value of any of the response variables compared to the control?

To address this question the study was treated according to the original ‘season of burn’ experimental design, ignoring the division of the fall and spring plots into separate subplots.

3.2.1.2 O-horizon and mineral soil properties

The estimated differences between the mean soil properties of samples from the unburned control plots and those of the plots burned in either the fall or spring are given in Table 6. The mean O-horizon depth of the unburned control plots is estimated to be 1.6 cm deeper than that of plots burned in the fall ($p = 0.001$) and 1.16 cm deeper than that of plots burned in the spring ($p = 0.01$). Burning in either the spring or fall appears to have an effect on both the carbon and nitrogen stocks of the O-horizon (Figure 9). The mean carbon content of the unburned controls is estimated to be 16.2 Mg/ha greater than that of the plots burned in the fall and 13.2 Mg/ha greater than that of the plots burned in the spring ($p = 0.003$ and 0.01, respectively.) Similarly the mean nitrogen content of the unburned controls is estimated to be 561 kg/ha greater than that of plots burned in the fall and 394 kg/ha greater than that of

plots burned in the spring ($p = 0.002$ and 0.019 , respectively). In addition, the mean N concentration of the O-horizon for plots burned in the fall is estimated to be 0.15% lower than that of the unburned control plots ($p = 0.044$); as a result, the C:N ratio of the O-horizon in plots burned in the fall is estimated to be 9.27 ($p = 0.029$) units higher than the unburned control plots (Figure 11).

Table 6: Comparisons between unburned control plots and plots burned in either the spring or fall. Estimated differences are reported as the least squares mean of the estimated value \pm the 95% CI. Estimates of differences were calculated as (control – fall) and (control – spring). Differences that were statistically detectable at the $\alpha = 0.05$ level are shown in bold.

		Contrasts			
		Control - Fall		Control - Spring	
		Difference	p-value	Difference	p-value
O-horizon	Depth (cm)	1.63 \pm 0.85	0.001	1.16 \pm 0.85	0.01
	%C	0.06 \pm 5.3	1	0.6 \pm 5.3	0.948
	C (Mg/ha)	16.21 \pm 9.71	0.003	13.16 \pm 9.71	0.01
	%N	0.15 \pm 0.15	0.044	0.03 \pm 0.15	0.827
	N (kg/ha)	561 \pm 324	0.002	394 \pm 324	0.019
	C:N	-9.27 \pm 8.25	0.029	-2.07 \pm 8.25	0.775
	pH	-0.07 \pm 0.31	0.813	-0.1 \pm 0.33	0.714
	BD (g/cm ³)	0.07 \pm 0.14	0.351	0.02 \pm 0.14	0.936
0-15 cm	%C	0.46 \pm 0.95	0.408	1.28 \pm 0.95	0.011
	C (Mg/ha)	-2.33 \pm 16.4	0.92	4.4 \pm 16.4	0.749
	%N	0.02 \pm 0.05	0.648	0.06 \pm 0.05	0.015
	N (kg/ha)	-208 \pm 967	0.829	179 \pm 340	0.358
	C:N	0.96 \pm 2.21	0.484	0.17 \pm 2.21	0.975
	pH	-0.3 \pm 0.2	0.005	-0.2 \pm 0.2	0.05
	BD (g/cm ³)	-0.07 \pm 0.1	0.226	-0.08 \pm 0.1	0.107

Both the mean C and mean N concentrations of the mineral soils are estimated to be slightly lower in the plots burned in the spring than in the unburned control plots (Figure 11). The differences are estimated to be 1.28% and 0.06%, respectively ($p = 0.011$ and 0.015). Lastly, the pH of the mineral soil in unburned control plots is estimated to be 0.3 units lower than that of plots burned in the fall ($p = 0.005$) and 0.2 units lower than that of plots burned in the spring ($p = 0.05$).

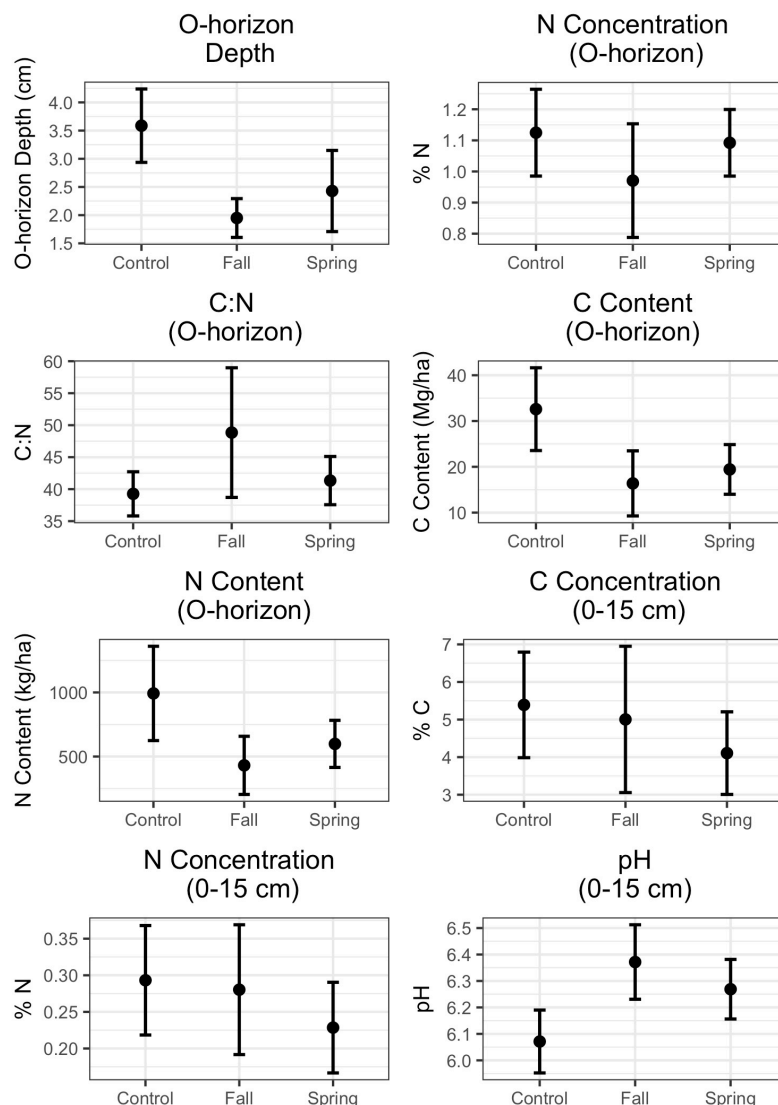


Figure 11: O-horizon and mineral soil properties where statistically detectable differences were found between the unburned control plots and the plots burned in the spring and/or fall. Circles represent the average values, error bars indicate the standard deviation.

3.2.1.3 O-horizon and mineral soil PyC

Estimated differences between samples from the unburned control plots and those from plots burned in either the fall or spring are shown in Table 7. The mean ACI of the PyC in the O-horizon of the unburned control plots is estimated to be 0.05 units higher than that of plots burned in the fall and 0.08 units higher than that of plots burned in the spring (p -values = 0.03 and 0.002, respectively, Figure 12). This suggests that on average, the PyC in the O-horizon of the unburned control plots has a more condensed aromatic structure than the PyC in the O-horizon of the plots that have undergone prescribed burns in either the spring or fall for the past 18 years.

Table 7: Comparisons of PyC concentration, content, and ACI between unburned control plots and plots burned in either the spring or fall. Estimated differences are reported as the least squares mean of the estimated value \pm the 95% CI. Estimates of differences were calculated as (control – fall) and (control – spring). Differences that were statistically detectable at the $\alpha = 0.05$ level are shown in bold.

		Contrasts			
		Control - Fall		Control - Spring	
		Difference	p-value	Difference	p-value
O-horizon	PyC concentration (g BPCA/kg C)	-2.58 \pm 3.67	0.182	-6.61 \pm 9.42	0.182
	PyC content (g BPCA/m ²)	18.91 \pm 45.3	0.511	-24.15 \pm 68.75	0.615
	ACI (g B6CA /g total BPCA)	0.05 \pm 0.04	0.03	0.08 \pm 0.04	0.002
0-15 cm	PyC concentration (g BPCA/kg C)	-8.42 \pm 4.19	0.001	-1.14 \pm 4.95	0.801
	PyC content (g BPCA/m ²)	-73.17 \pm 93.57	0.128	-6.24 \pm 95.11	0.982
	ACI (g B6CA /g total BPCA)	0.02 \pm 0.04	0.308	0.02 \pm 0.04	0.276

Additionally, the mean PyC concentration of the 0-15 cm soil is estimated to be 8.42 g BPCA/kg C higher in the plots burned in the fall than in the unburned control plots ($p = 0.001$). No difference was detected between the PyC concentration of the mineral soil in the unburned control plots and the plots burned in the spring. Additionally, burning in either the spring or fall does not appear to change the total PyC content of either the mineral soil or O-horizon compared to the unburned controls.

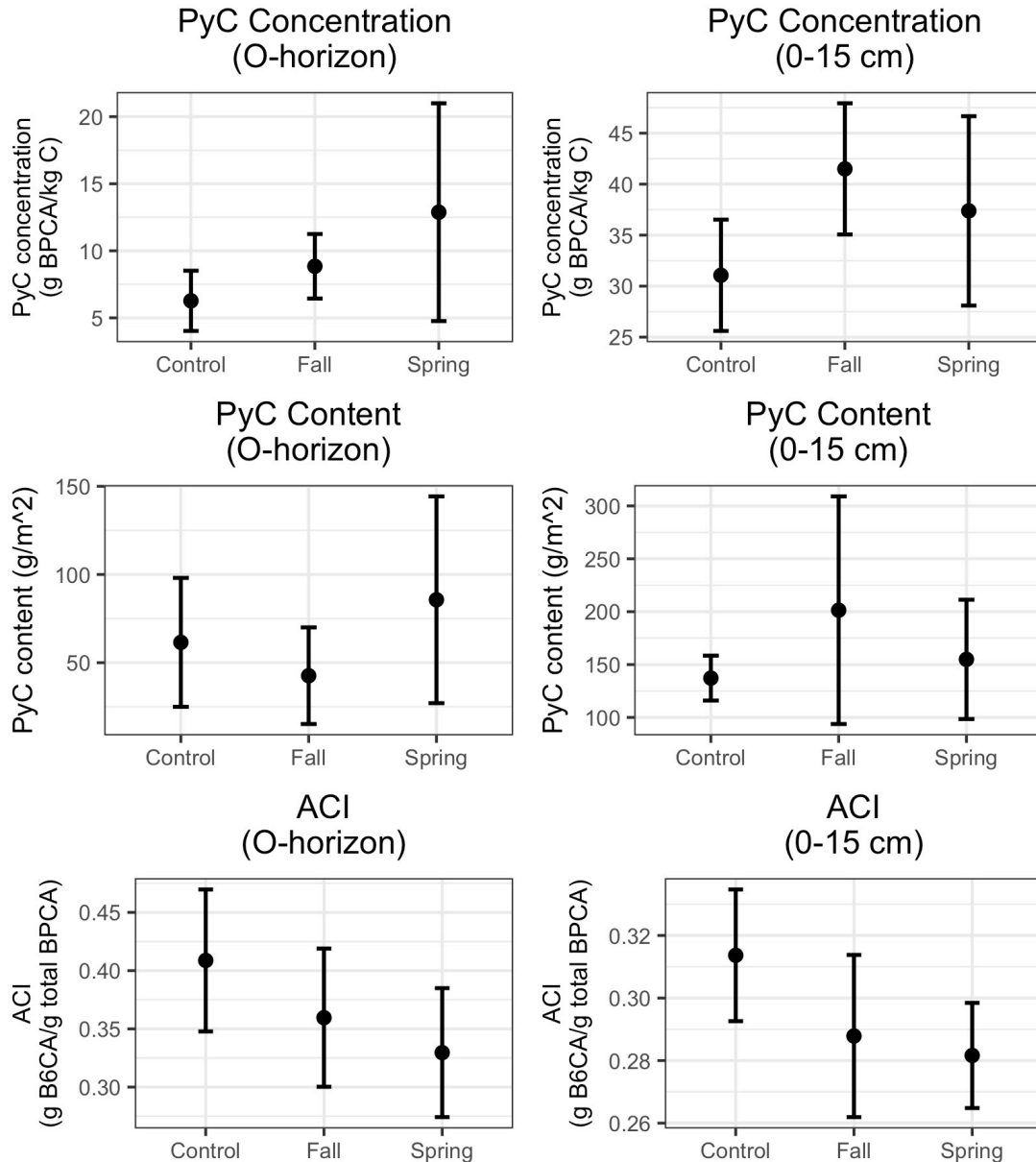


Figure 12: PyC-related response variables by seasons. Circles indicate raw averages, error bars indicate standard deviations.

3.2.2 Does the season of burn or the interval between burns affect the mean value of any of the response variables that were measured?

As discussed in section 2.4.1, to address this question the study was treated as a 2x2 factorial experiment where each factor (season of burn and interval between burns) contains two levels (fall and spring or 5 and 15 years).

3.2.2.1 O-horizon and mineral soil properties

Using an overall F-test, no interactive effect between season and interval of burn was detected for any of the non-PyC response variables, with one exception. Consequently, the main effects of ‘season’ and ‘interval’ were primarily used to answer this question. For the pH of the 0-15 cm soils, where an interactive effect was detected ($F = 15.5$, $p = 0.003$), the effect of interval was determined separately for each season and the effect of season was determined separately for each interval.

The estimated contrasts between spring and fall and 5 and 15-year intervals are reported in Table 8. No main effects of either season of burn or the interval between burns were detected for any of the response variables that were analyzed.

Table 8: Main effect comparisons (fall vs spring and 5-year vs 15-year intervals) of O-horizon and mineral soil properties analyzed in this study. No differences in season or interval were statistically detectable for any of the response variables. Estimates of differences are reported as the least squares mean value of the estimate \pm the 95% confidence interval. Differences were calculated as (fall – spring) and (15 yr – 5 yr).

		Contrasts			
		Fall - Spring		15 yr - 5 yr	
		Difference	p-value	Difference	p-value
O-horizon	Depth (cm)	-0.47 \pm 0.91	0.291	0.46 \pm 0.66	0.184
	%C	0.55 \pm 6.07	0.952	0.31 \pm 4.97	0.983
	C (Mg/ha)	-2.73 \pm 7.77	0.524	3.5 \pm 6.56	0.341
	%N	-0.12 \pm 0.24	0.305	0.05 \pm 0.2	0.793
	N (kg/ha)	-167 \pm 183	0.069	139 \pm 155	0.077
	C:N	7.2 \pm 11.01	0.177	-1.1 \pm 8.32	0.928
	pH	-0.01 \pm 0.29	0.997	-0.08 \pm 0.25	0.678
	BD (g/cm ³)	-0.05 \pm 0.2	0.667	0.03 \pm 0.06	0.434
0-15 cm	%C	0.82 \pm 1.35	0.212	0.58 \pm 0.83	0.182
	C (Mg/ha)	6.85 \pm 19.96	0.538	6.41 \pm 6.46	0.052
	%N	0.05 \pm 0.07	0.145	0.02 \pm 0.05	0.402
	N (kg/ha)	396 \pm 1008	0.458	223 \pm 562	0.533
	C:N	-0.79 \pm 2.95	0.672	0.59 \pm 2.49	0.787
	pH	Not analyzed as main effect differences			
	BD (g/cm ³)	-0.02 \pm 0.12	0.872	0 \pm 0.1	0.997

Because of the interaction between season and interval of burn on the pH of the 0-15 cm samples, the effects of these factors were determined separately. The

mean 0-15 cm pH of the F5 subplots is estimated to be 0.22 higher than that of the F15 subplots ($p=0.043$). No statistical difference in pH was detected between any of the other pairs of subplots at the $\alpha = 0.05$ level.

3.2.2.2 O-horizon and mineral soil PyC

An overall F-test confirmed that there were no interactive effects between ‘season’ and ‘interval’ for any of the PyC-related response variables that were analyzed. Thus, only the main effects of ‘season’ and ‘interval’ were analyzed to address this question.

The estimated contrasts between spring/fall and 5/15-year intervals are reported in Table 9. No main effects of either season of burn or the interval between burns were statistically detectable at the $\alpha = 0.05$ level; however, the p -value associated with the main effect of season on the PyC concentration of the 0-15 cm samples is below 0.1 ($p = 0.071$). Moreover, the estimate of the effect size is fairly large. The mean PyC concentration in the 0-15 cm soil is estimated to be 6.46 (± 7.16) g BPCA/kg C higher in samples collected at plots burned in the fall compared to those burned in the spring.

Table 9: Main effect comparisons (fall vs spring and 5-year vs 15-year intervals) of O-horizon and mineral soil PyC properties analyzed in this study. Estimates of differences are reported as the least squares mean value of the estimate \pm the 95% confidence interval. Differences were calculated as (fall – spring) and (15 yr – 5 yr).

		Main Effects Contrasts			
		Fall - Spring		15 yr - 5 yr	
		Difference	p-value	Difference	p-value
O-horizon	PyC concentration (g BPCA/kg C)	-4.07 \pm 8.74	0.354	-2.56 \pm 6.86	0.57
	PyC content (g BPCA/m ²)	-41.74 \pm 71	0.226	-6.77 \pm 45.51	0.91
	ACI (g B6CA /g total BPCA)	0.03 \pm 0.06	0.323	0.04 \pm 0.05	0.112
0-15 cm	PyC concentration (g BPCA/kg C)	6.46 \pm 7.16	0.071	0.76 \pm 5.92	0.93
	PyC content (g BPCA/m ²)	38.1 \pm 103.77	0.498	22.49 \pm 33.26	0.193
	ACI (g B6CA /g total BPCA)	0 \pm 0.02	0.93	0.02 \pm 0.02	0.082

4. Discussion

4.1 Factors influencing PyC concentration and structure

The results of the covariate selection process suggest that within the context of this study, there is not a simple relationship between fuel consumption and the PyC concentration, content, or chemical structure found in the O-horizon or mineral soil, a somewhat surprising result. We assumed that the amount of fuel consumed would reflect the proportion of organic matter exposed to elevated temperatures, which in turn would be correlated to concentration of PyC in the O-horizon and further down the soil profile.

While several of the O-horizon and mineral soil properties measured in this study showed some weak to moderate correlation with either PyC concentration or chemical structure (notably, the carbon concentration and pH of the 0-15 cm samples), these variables did not help to explain the observed variation in the PyC-related response variables. This absence of a strong correlation between PyC concentration and other soil variables is in agreement with the findings of Jauss et al., who found the distribution of PyC in eight sites along a vegetation gradient in Oregon to be highly variable and not strongly correlated to total soil carbon or other soil properties.⁷⁴

Slightly different methods were used for O-horizons and mineral soil samples during the BPCA extraction process. Because of this difference in procedure, and the possibility of mineral matrix effects on the BPCA extraction process, no direct comparisons were made between the PyC concentration, content, and ACI of the O-horizon and mineral soil. However, correlations between these properties at the two different depths can provide some insight into the movement of PyC through the soil profile. During a fire, thermally altered organic matter (i.e., PyC) is deposited on the soil surface. Over time this material can migrate downward through the soil profile through bioturbation, leaching, or other ecological processes. Consequently, PyC in the O-horizon can serve as a primary source of PyC in the mineral soil. If this is the case, we would expect to see a strong, positive correlation between the quantity

(concentration and total PyC content) and quality (ACI) of PyC in the O-horizon and the mineral soil.

There is a weak-to-moderate positive correlation between the PyC concentration of the O-horizon and the mineral soil ($R = 0.37$, Figure 13.A), and a very weak positive correlation between the total PyC content of the two depths ($R = 0.14$, Figure 13.B). This suggests that there is some mass movement of PyC from the O-horizon to the mineral soil, but other factors or sources of PyC in the mineral soil may be affecting the connection between the two depths. It is possible that surface PyC in the O-horizon can be consumed in subsequent fires, while PyC in the mineral soil is protected. If this is the case, and if a significant portion of the PyC in the O-horizon migrates deeper into the soil profile on a shorter time scale than the fire frequency, it could alter the expected relationship between the amount of PyC in the O-horizon and the mineral soil. Compared to the expected value, the amount of PyC in the O-horizon would be reduced by both the migration of PyC deeper into the profile and the losses due to combustion. The relationship between the concentration of PyC (g BPCA/kg C) of the O-horizon and the mineral soil is further complicated by the fact that the two depths receive carbon inputs from different sources.

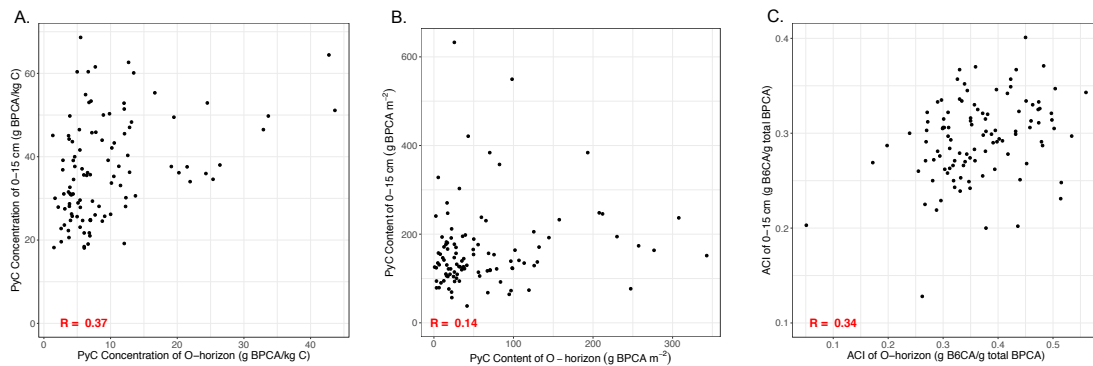


Figure 13: Correlation between the PyC concentration (A), content (B), and ACI (C) of the mineral soil and the O-horizon. Pearson correlation coefficients are shown in red.

There is also a weak-to-moderate correlation between the ACI of the O-horizon and the mineral soil ($R = 0.34$, Figure 13.C). If PyC does not undergo significant processing and decomposition, or if this processing happens at the same rate regardless of the initial chemical structure of the PyC, we would expect the structural features of the PyC to be preserved as it moves downward through the soil profile. This would result in a strong correlation between the ACI of the O-horizon

and the mineral soil. The weakness of the correlation observed here suggests that under the conditions of this study, PyC undergoes significant processing during the transition through the soil profile and that this processing may happen at different rates depending on the ACI of the initial material as well as other factors including physical structure, particle size, and exposure to photooxidation.

4.2 Does burning in the fall or spring change the mean value of any of the response variables compared to the control?

4.2.1 O-horizon and mineral soil properties

As expected, the mean O-horizon depth in plots burned in either spring or fall is lower than that of the unburned control plots. This is in good agreement with the work of Westlind et al., who found that all prescribed burn treatments used in this study reduced the depth of the forest floor compared to unburned controls, though no differences were observed between the different prescribed burn regimes.²²

The influence of fire on C and N concentrations of soils is complex, involving many competing drivers that may exert opposing effects.³⁵ The reduction in carbon and nitrogen content of the O-horizon is largely driven by a reduction in the O-horizon depth. The small decrease in mean N concentration in the O-horizons of plots burned in the fall compared to the unburned controls may be due to volatilization of organics during fire or the consumption of the more decomposed layers of the O-horizon (Oe and Oa) with lower C:N ratios.³⁵

It is similarly difficult to ascribe a specific reason for the decrease in mean C and N concentration of the 0-15 cm soil in the plots burned in the spring compared to the unburned controls. However, Hatten et al.⁵¹ did not observe this difference, suggesting that repeated burning has compounded the drivers responsible for the reduction in organic matter concentration.

The carbon and nitrogen results found here are in excellent agreement with the results found by Nave et al.³⁵ In a meta-analysis of 468 soil carbon observations from 57 publications, they found that fire tends to reduce carbon and nitrogen stocks in the O-horizon, but leave the carbon and nitrogen concentrations largely unchanged. In

contrast, fire tends to decrease the carbon and nitrogen concentration of the mineral soil, while having little impact on carbon stocks.

The increase in mean pH of the 0-15 cm soils that was observed in plots burned in the spring and fall compared to the unburned controls is likely due to the deposition of carbonate-containing ash during the fires.³⁹ While Hatten et al. did observe an elevated mineral soil pH in the subplots burned in the fall at 5-year intervals, this trend appears to have become more pronounced with repeated burning.⁵¹ Moreover, the observed increase in mean pH occurs within a range that could be beneficial for plant growth (from 6.07 in the control plots to 6.37 and 6.27 in the fall spring plots, respectively), possibly improving both phosphorus availability and base saturation.^{29,38} In fact, Hatten et al. observed an increase in available phosphorus in the F5 subplots compared to the unburned control even in samples collected in 2004, seven years after initiation of the study.³¹

4.2.2 O-horizon and mineral soil PyC

The ACI of the PyC in the O-horizon is significantly higher in the unburned control plots than in the plots burned in fall or spring, suggesting the PyC in the unburned plots is more highly condensed. This is likely due to differences in the source of PyC in burned and unburned plots. For plots that have undergone intermittent prescribed burns for the last twenty years, the primary source of PyC in the O-horizon is solid, charred biomass. In contrast, in the control plots, which have not been burned in approximately a century, the primary source of O-horizon PyC is soot deposition from nearby fires (multiple wildfires have occurred in the region in recent decades in addition to the prescribed burns performed in the study) and fossil fuel combustion. Soot, which is formed by gas phase condensation of volatiles during combustion, is the most highly condensed form of PyC.^{53,57} This would explain why the PyC found in the O-horizons of control plots is more highly condensed.

In contrast to the O-horizon results, in the 0-15 cm soils, no difference in mean ACI was detected between the unburned control plots and those burned in either the spring or fall. This suggests that much of the PyC present in the 0-15 cm soils of the unburned control plots is legacy PyC produced during naturally occurring

fires that burned prior to the introduction of fire-exclusion policies in the early twentieth century.

A recent fire history study in Malheur National Forest concluded that the mean fire return interval for the region was between 10 and 30 years.⁹⁴ The sites sampled in this study are dry Ponderosa pine stands in the southern region of Malheur National Forest, where the fire return interval lies closer to 10 years. Many centuries of such frequent fire likely built up significant stocks of PyC in the mineral soils of the area. Thus it is somewhat surprising that the fall burn plots already display an increase in mean PyC concentration of the 0-15 cm soils compared to unburned controls after only 18 years of prescribed burns. Despite the increase in PyC concentration, no differences in the overall PyC content of either the O-horizon or mineral soil were detected between the unburned control plots and either the spring or fall burn plots. This lack of a detectable difference could potentially arise from increased variability in the estimates of PyC content that incorporate variability in bulk density and PyC concentration.

Compared to the unburned controls, no increase in PyC concentration of the O-horizon was detected for the plots burned in either the spring or fall. This may be because accumulation of PyC over the course of multiple prescribed burns is required to produce statistically detectable increases in PyC. However, the samples analyzed here were collected 2-3 years after the most recent burns. Thus it is also possible that much of the PyC that was originally present in the O-horizon immediately following the 2012-2013 burns has begun to move deeper into the soil profile, dampening the treatment effects on O-horizon PyC concentrations.⁸³

The mean PyC concentration of the 0-15 cm soils in the spring burn plots is not detectably different from that of the unburned control plots. This may be due to patchiness of spring burns. During such burns, fuel moisture levels are higher. This can reduce fuel connectivity and results in a patchier, less continuous burn.^{20,23,24,95} The PyC concentrations of the O-horizon in the spring burn plots seem to reflect this. While the mean value for the O-horizon PyC concentrations is highest in the spring burn plots (12.88 g BPCA/kg C compared to 6.27 and 8.85 g BPCA/kg C for the fall burn and control plots, respectively), the standard deviation in the spring burn plots is

nearly four times higher than in either the fall burn or control plots (8.12 g BPCA/kg C, compared to 2.4 and 2.25 g BPCA/kg C for the fall burn and control plots, respectively). These results are consistent with a discontinuous burn pattern that produces concentrated pockets of fire-altered material interspersed with unaffected areas. If the lower standard deviation of the O-horizon PyC concentration in the fall burn plots does reflect more homogeneous, continuous fires, it seems logical that increases in the PyC concentration of the 0-15 cm soils would be statistically detectable in the fall burn plots before the spring. O-horizon PyC is a source for PyC in the mineral soils. In a more continuous burn pattern, all areas would receive similar amounts of PyC input in each repeated burn, leading to a continuous accumulation of PyC in the 0-15 cm soils across the landscape. In patchier, more heterogeneous burn patterns, repeated fires may burn different areas in different years depending on a complex interaction of fuel loads, fuel moisture, and weather patterns, resulting in a lower accumulation of PyC at a given location over the same number of repeated burns.

4.3 Does the season of burn or the interval between burns affect the mean value of any of the response variables that were measured?

4.3.1 O-horizon and mineral soil properties

Neither the season of burn nor the interval between burns had an effect on any of the O-horizon or mineral soil response variables that were investigated, with the exception of the mineral soil pH. The mean 0-15 cm pH of the F5 subplots was estimated to be higher than that of the F15 subplots ($p = 0.043$). This result is not unexpected. The carbonates present in ash are largely responsible for the increased pH that is often observed after a fire. It is reasonable that this effect is the most pronounced for the prescribed fires that burn more frequently (at 5-year intervals) and at hotter temperatures (fall burns), as this would likely result in the greatest ash production.

In samples collected from the same study location in 2004, Hatten et al. detected an effect of both season and interval of burn on the O-horizon depth;

however, at the time of sampling, the plots burned at 5-year intervals had been burned twice with 1-2 years of recovery and the plots burned at 15-year intervals had been burned once with 6-7 years of recovery.⁵¹ In contrast, here, the subplots burned at 5-year intervals had been burned 4 times while the subplots burned at 15-year intervals had been burned twice. Moreover, the most recent burns had included both 5-year and 15-year burns and were performed 2-3 years prior to sampling. The additional year of recovery time combined with equivalent recovery times for the different intervals likely dampened the effects of both season and interval on the O-horizon depth. In fact, the findings here are consistent with those of Westlind et al.,²² who found no differences in forest floor depth for different seasons or intervals of burn at the same study site following the most recent burns.

Hatten et al.⁵¹ also observed an effect of season on the carbon concentration of the O-horizon, with fall burns reducing the concentration by approximately 4%. No such effect was detected in this study. Again, this difference may be due to the time of sampling relative to the most recent burns – the litterfall that occurred during the additional recovery time may dampen the differences between prescribed burns performed in different seasons. Alternatively, it is possible that repeated burning has reduced the effect of season on the carbon concentration of the O-horizon.

4.3.2 O-horizon and mineral soil PyC

While no statistical effects of either season or interval of burn were detected at the $\alpha = 0.05$ level for any of the PyC-related response variables (PyC concentration or ACI of either the O-horizon or 0-15 cm soil) the p -value associated with the main effect of season on the PyC concentration of the 0-15 cm soil was below 0.1 ($p = 0.071$) and the estimate of the effect size was considerable. To explore whether this suggestive result reflected a true difference, we compared the PyC concentration of the plots burned in the spring and fall using the season of burn analysis described in Equation 2. While this analysis was primarily used to compare the fall and spring burns to the unburned controls, it also lends itself to comparisons between the spring and fall. Using this season of burn model (with Tukey's corrections for pairwise comparisons in families of 3), a statistically significant effect was detected. It was estimated that the mean PyC concentration of 0-15 cm soils is 7.27 (± 4.4) g BPCA/kg

C higher for plots burned in the fall than plots burned in the spring ($p = 0.003$). Considering the results of both models, it seems likely that the fall burns result in higher PyC concentration in the 0-15 cm soils than spring burns; however, the results are inconclusive.

The lack a difference in ACI between the spring and fall burns is an interesting negative result. It is well accepted that the degree of aromatic condensation of char derived from wood or bark increases as a function of the charring temperature.^{56,90} This phenomenon has been readily detected in muffle furnace experiments using the BPCA method for PyC characterization – as the charring temperature increases, the proportion of B6CA (i.e., the ACI) also increases.^{76,90,92} This has led researchers to propose that the ACI of PyC collected during wildfires could serve as a sort of thermometer, reflecting the temperatures that were achieved during the burn.⁹² However, this does not appear to be the case in this study. In general, prescribed burns performed in the fall are known to burn hotter than those burned in the spring,²⁰ and measurements of tree mortality within the study area suggest that was true in this specific case,^{77,79} yet no effect of season of burn on the ACI of either the O-horizon or the 0-15 cm soils was detected.

There are a number of potential reasons for this result. One possibility is that the charring conditions present in a muffle furnace differ dramatically from those that occur in a prescribed fire - the relationship between aromatic condensation and charring temperature may not be as strong when char is formed in an open-air fire. The type of fuel consumed in these fires may also be responsible. On a per mass basis, the majority of fuel consumed by the prescribed burns in this study was litter and duff, not woody debris.²² The correlation between charring temperature and aromatic condensation is weak to non-existent for chars derived from leaf and needle biomass.^{90,92} It is possible that the ACI may serve as a better temperature indicator for higher intensity wildfires that are more likely to produce wood-derived char.

The lack of difference in the mineral soil and O-horizon PyC concentration between 5 and 15-year burn intervals is also interesting. There is some debate within the PyC community as to whether consumption of PyC in subsequent fires represents a significant mechanism of PyC loss from the environment.^{25,67,96} The lack of an

effect of 'interval' on the PyC concentration of the O-horizon and mineral soil suggests that within the context of this study, PyC consumption in subsequent fire was not a major mechanism of loss. It is possible that in this ecosystem, even a 5-year recovery period between burns is enough time for much of the PyC to migrate from the O-horizon into the mineral soil where it would likely be protected from combustion in subsequent fire. Alternatively, it is possible that the relatively low temperatures achieved during prescribed burns are not sufficient for PyC consumption, and this mechanism of loss is more likely in higher intensity wildfires.

5. Conclusions

Our results suggest that the application of prescribed fire to the study sites has had a number of effects on both the quantity and quality of SOM. The total stocks of carbon and nitrogen in the O-horizon of plots burned in both spring and fall are lower than that of the unburned control plots, largely due to a decrease in O-horizon depth; however, the carbon and nitrogen concentrations have remained largely the same. In contrast, the total stocks of carbon and nitrogen in the mineral soil seem unaffected by either spring or fall burns, though compared to the unburned controls, the concentration of both carbon and nitrogen is lower for plots burned in the spring. The prescribed burns have also increased the pH of the mineral soil, potentially improving the base saturation of the soil and availability of base cations.

The prescribed burns have also affected the PyC properties of the soil. The fall burns have increased the PyC concentration in the mineral soil compared to the unburned controls. The slow turnover of PyC may lead to an increase in the PyC stock as well as the overall carbon stock of the mineral soil in these plots. The PyC concentration of the O-horizon was extremely variable in the plots burned in the spring, characterized by low to moderate concentrations at most areas and punctuated by occasional areas of high concentration. This is consistent with current models of the effect of fuel moisture on burning – while moist fuel may result in greater amounts of PyC²⁵ it also reduces fuel continuity, resulting in patchier, more heterogeneous burns.^{23,24}

While we detected a number of differences between the SOM characteristics of the unburned control plots and those burned in either the spring or fall, neither the season of burn nor the interval between burns appears to have had a strong effect on SOM quantity or quality. While we did detect a difference in the PyC concentration of the mineral soil between the plots burned in the spring and the fall using the season of burn model, these results were inconclusive. Future work should further investigate this result.

In addition, future investigations should seek to develop a greater understanding of SOM dynamics within the context of this study. The work reported here is a snapshot of the SOM quantity and quality at a specific point in time relative

to the most recent burns. A more thorough understanding of how SOM dynamics are affected by prescribed burns could be achieved from measurements of soil properties and PyC collected at specific time points: immediately before burning, immediately after burning, and several years following burning.

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