

SOIL RESPONSE TO FIRE FREQUENCY IN THE NORTHERN COLUMBIA BASIN
SAGEBRUSH STEPPE

by

Leslie C. Nichols



A thesis

submitted in partial fulfillment

of the requirements for the degree of

Master of Science in Biology

Boise State University

December 2020

© 2020

Leslie C. Nichols

ALL RIGHTS RESERVED

BOISE STATE UNIVERSITY GRADUATE COLLEGE

DEFENSE COMMITTEE AND FINAL READING APPROVALS

of the thesis submitted by

Leslie C. Nichols

Thesis Title: Soil Response to Fire Frequency in the Northern Columbia Basin Sagebrush
Steppe

Date of Final Oral Examination: 14 May 2020

The following individuals read and discussed the thesis submitted by student Leslie C. Nichols, and they evaluated their presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

Marie-Anne de Graaff, Ph.D.	Chair, Supervisory Committee
Ian Robertson, Ph.D.	Member, Supervisory Committee
Douglas J. Shinneman, Ph.D.	Member, Supervisory Committee
Susan K. McIlroy, Ph.D.	Member, Supervisory Committee

The final reading approval of the thesis was granted by Marie-Anne de Graaff, Ph.D., Chair of the Supervisory Committee. The thesis was approved by the Graduate College.

ACKNOWLEDGMENTS

My sincerest gratitude goes to my advisor, Marie-Anne de Graaff, Ph.D., for her continued support and expertise, for her enduring patience and enthusiasm, and for her unwavering faith in me throughout this project, as well as my committee members Ian Robertson, Ph.D., Douglas Shinneman, Ph.D., and Susan McIlroy for their time, advice, and contributions.

This project would not have come to fruition without the many hours of invaluable, diligent assistance from my fellow lab members: Mariah Emele, Britt Eubanks, Ana Velasquez, McCall Barney, Tori Tripoli, James Duncan, Karina Guterrez, Kathleen Blackaller, Kinslee Sikes, Lola Klamm, Megan Kelly-slatten, Natalie Zeigler and Regan Denny.

I am indebted to my family for their perseverance in the field under the hot August sun and for their infinite confidence, encouragement, and patience throughout this journey.

ABSTRACT

Fire is one of the most significant disturbances in an ecosystem, as it is capable of altering the physical, chemical, and biological properties of soil, and the fire frequency in semi-arid ecosystems is increasing. These changes can potentially alter plant-soil feedbacks that may affect post-fire recovery of the native plant and soil communities and lead to an ecosystem state change. However, there is much uncertainty about the magnitude of change as soils are exposed to more fires, because soil recovery and changes in fire severity following a first fire mediate the impact of successive fires on soil properties. To improve understanding of fire frequency effects on the soil ecology of the northern Columbia Basin sagebrush steppe ecosystem, this study assessed the physical, chemical and biological properties of soil that are critical to plant communities (e.g. soil pH, C and N, respiration and extracellular enzyme activity) from four different fire frequencies (unburned, burned once, twice, and thrice). Our study yielded three main results: 1) fire reduced the soil C concentration relative to unburned soil, but only when soil was exposed to fire once, 2) soil pH and NO_3^- -N increased with fire frequency, whereas enzyme activity decreased, and 3) soil organic matter contents and microbial respiration were suppressed significantly in the once and thrice burned soils compared to the unburned and twice burned soils. Taken together, our findings suggest that a one-time fire in this region of the sagebrush steppe is capable of significantly changing soil properties that alter plant-soil feedbacks and hinder ecosystem resilience, thus contributing to ecosystem change particularly when fire frequency increases.

TABLE OF CONTENTS

ACKNOWLEDGMENTS	iv
ABSTRACT	v
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	x
INTRODUCTION	1
METHODS	6
Study Area and Fire History	6
Site Selection	7
Soil Sampling	7
Soil pH.....	8
Soil Organic Matter.....	8
Soil Fractions	9
Soil Nitrogen and Carbon.....	10
Mineral Nitrogen.....	10
Microbial Extracellular Enzyme Activity	11
Microbial Respiration.....	12
Analysis	13
RESULTS	14
Soil Physical and Chemical Properties	14

Soil Biological Properties.....	16
DISCUSSION	18
REFERENCES.....	35

LIST OF FIGURES

Figure 1.	Location of the study site within the channeled scablands of the northern Columbia Basin, Lincoln County, Washington (47.590383N 118.540236W). Map Data 2018 Google.....	24
Figure 2.	Aerial view of heterogenous landscape within the channeled scablands showing mounds (solid line) and flats (dotted line). Imagery date: 05/29/2014, Google Earth.....	24
Figure 3.	Outline of fire events that occurred in the study area. Fires occurred in 2003 (blue outline), 2007 (yellow outline), and 2012 (red outline). The overlap of fires provides the fire frequencies: 0x, unburned; 1x, once burned (2012); 2x, twice burned (2012, 2003); 3x, thrice burned (2012, 2007, 2003). Imagery date: 07/01/2017, Google Earth.	25
Figure 4.	Mound stratified sampling design. All cores were a minimum of 1 m from the mound perimeter and 1 m from adjacent soil cores. Soil cores were divided into two soil depths: 0-5 cm and 5-10 cm.	25
Figure 5.	Soil pH across fire frequencies for 0-5 cm soil depth. Values represent means with error bars as \pm SE. Lowercase letters indicate significant differences among fire frequencies from post hoc analysis ($p < 0.05$, $n = 10$). Means (0x, 5.7; 1x, 6.2; 2x, 6.3; 3x, 6.5).....	26
Figure 6.	SOM across fire frequencies for 0-5 cm soil depth. Values represent means with error bars as \pm SE. Lowercase letters indicate significant differences among fire frequencies from post hoc analysis using transformed data ($p < 0.05$, $n = 10$). Graphical representation based on non-transformed data.	27
Figure 7a-e.	Carbon concentration or C-richness, the carbon present within each fraction, for water stable and occluded soil fractions of the 0-5 cm soil depth across fire frequencies. Values represent means with error bars as \pm SE. Lowercase letters indicate significant differences among fire frequencies within each fraction from post hoc analysis using transformed data ($p < 0.05$, $n = 10$). Graphical representation based on non-transformed data.	28
Figure 8a-b.	Carbon content, the relative abundance of soil aggregate-derived carbon within bulk soil for water stable and occluded soil fractions of the 0-5 cm	

	soil depth across fire frequencies. Values represent means with error bars as \pm SE. Lowercase letters indicate significant differences among fire frequencies within each fraction from post hoc analysis using transformed data ($p < 0.05$, $n = 10$). Capital letters represent significant differences among fire frequencies of C-content of bulk soil, as represented by the sum of water stable fractions. Graphical representation based on non-transformed data.....	29
Figure 9a-d.	Available mineral nitrogen, both nitrate (NO ₃ -N) and ammonium (NH ₄ -N), across fire frequencies for both soil depths (0-5 and 5-10 cm). Values represent means with error bars as \pm SE. Lowercase letters indicate significant differences among fire frequencies from post hoc analysis using transformed data ($p < 0.05$, $n = 10$). Graphical representation based on non-transformed data.....	30
Figure 10a-d.	Extracellular hydrolytic enzyme activity across fire frequencies for the 0-5 cm soil depth. Values represent means with error bars as \pm SE. Lowercase letters indicate significant differences among fire frequencies from post hoc analysis ($p < 0.05$, $n = 10$). Tukey post hoc did not differentiate the significant differences within BG. Graphical representation based on non-transformed data.....	31
Figure 11a-c.	Rate of microbial CO ₂ evolution through one week, two months, and one year of incubation for 0-5 cm soil depth across fire frequencies ($n = 10$). Inset graphs represent cumulative soil respiration for the same time periods ($n = 10$). Values represent means with error bars as \pm SE. Lowercase letters indicate significant differences among fire frequencies from post hoc analysis using transformed data ($p < 0.05$, $n = 10$). Graphical representation based on non-transformed data.....	32
Figure 12a-c.	Rate of microbial CO ₂ evolution through one week, two months, and one year of incubation for 5-10 cm soil depth across fire frequencies ($n = 10$). Inset graphs represent cumulative soil respiration for the same time periods (0x, 2x, 3x $n = 10$; 1x $n = 9$). Values represent means with error bars as \pm SE. Lowercase letters indicate significant differences among fire frequencies from post hoc analysis using transformed data ($p < 0.05$, $n = 10$). Graphical representation based on non-transformed data.....	33
Figure 13.	Without sufficient recovery time between successive fires, altered plant-soil feedbacks and associated state changes will persist. Fire frequency promotes elevated soil pH and mineral N, which promotes establishment of invasive plant species. Invasive plant species increase the risk of subsequent fire which exacerbates the cycle. In addition, fire frequency contributes to further depletion of soil organic matter which leads to the suppression of microbial processes. These changes hinder soil ecosystem resilience contributing to progressive ecosystem state changes.	34

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BG	β -D-glucoopyranoside
BSU	Boise State University
C	Carbon
CBH	β -D-cellobioside
CO ₂	Carbon dioxide
GC	Graduate College
MANOVA	Multivariate analysis of variance
MUB	Methylumbelliferyl
N	Nitrogen
NAG	N-acetyl- β -D-glucosaminide
NCB	Northern Columbia Basin
NH ₄ ⁺ -N	Ammonium associated nitrogen
NO ₃ ⁻ -N	Nitrate associated nitrogen
PHOS	Phosphate
SOC	Soil organic carbon
SOM	Soil organic matter
TDC	Thesis and Dissertation Coordinator
0x	Unburned
1x	Once burned

2x Twice burned

3x Thrice burned

INTRODUCTION

Sagebrush steppe, a cold desert shrubland and one of the largest ecosystems in North America, spans nearly 500,000km² across eleven western US states and three provinces in southwestern Canada (Pyke et al., 2014). Although it occurs across environmental gradients with elevations ranging from 90 to 2750m above sea level, its climatic niche is characterized by hot-dry summers and cold-wet winters (Chambers et al., 2014a) with the majority of annual precipitation occurring as either rain or snow. Summer precipitation, usually the result of thunder or hailstorms, is intermittent. Sagebrush steppe is typically dominated by sagebrush species (*Artemisia* spp.) with wide-ranging big sagebrush (*Artemisia tridentata* Nutt.) the most common and with a substantial component of perennial bunchgrasses, including species such as bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve) and Sandberg bluegrass (*Poa secunda* J. Presl). Other common plants include shrubs, such as rabbitbrush (*Chrysothamnus* and *Ericameria* spp.) and bitterbrush (*Purshia tridentata* (Pursh) DC.), while forbs typically account for the greatest number of vascular plants in sagebrush steppe communities (Pyke et al., 2015b). Sage grouse (*Centrocercus urophasianus* (Bonaparte)) and more than 350 other vertebrate species rely on sagebrush steppe ecosystems for food, cover, and breeding sites (Chambers et al., 2017). As a result of declining sagebrush steppe habitat, many plant and animal species are at risk of local or regional extirpation (Thompson, 2007).

A unique landscape within the broader sagebrush steppe semi-arid ecosystem occurs in the “channeled scablands” of northern Columbia Basin (NCB). The channeled scablands are the result of multiple Pleistocene mega-flooding events from glacial Lake Missoula in present-day western Montana and northern Idaho (Baker, 2009). High energy water flows during flooding scoured the landscape, stripping away topsoil, picking apart bedrock, and carving an immense channel system, leaving behind a pitted basalt landscape with meager soil (Baker, 2009). Channeled scablands are characterized by relatively flat, rocky areas with poor soil development that primarily support sparsely vegetated scabland sagebrush (*A. rigida* (Nutt.) A. Gray) communities, and mounds that support big sagebrush communities. Mounds initially developed under Pleistocene periglacial frost activity and contain the majority of soil across the channeled scablands (Fryxell, 2012). Further mound development is suggested to have occurred through the gradual accumulation of aeolian (wind) sediment by patches of vegetation, thus mound soils are comprised of loess (fine, windblown sediments) overlying basalt bedrock (Fryxell, 2012; Shinneman and McIlroy, 2016). Typically, mounds are circular to elliptical and have a center height of generally less than 2 m (Fryxell, 2012). Vegetation, including big sagebrush and deep-rooted perennial bunchgrasses, is generally more abundant on mound soils than across the entire landscape. Nonnative annual grasses (e.g. cheat grass - *Bromus tectorum* L.) are also more abundant on mounds. Wetlands, characterized by pothole lakes and sedges (*Carex* spp.), are also interspersed across the channeled scablands. Elevation across the NCB study area ranges from ~170 to 850m above sea level with annual precipitation ranging from 20 to 49cm (Shinneman and McIlroy, 2016).

The NCB of Washington has less than 50% of its historic sagebrush steppe remaining. Much of what remains is degraded, fragmented, and/or isolated from other similar habitats (Dobler et al., 1996). Furthermore, fire frequency has increased. Whereas historical fire intervals in sagebrush steppe ecosystems ranged between 50 to more than 300 years, the contemporary fire interval is often far shorter, declining to less than 10 years in some portions of the sagebrush steppe (Pyke et al., 2015a; Whisenant, 1990). Fire is a significant disturbance in ecosystems, removing substantial aboveground biomass and directly altering physical, chemical, and biological soil properties (Chambers et al., 2014a; Miller et al., 2013; Neary et al., 2005). Annually, fires worldwide release approximately 2×10^{15} g of carbon (C) as carbon dioxide (CO₂) and convert another 50×10^{12} g of labile C into forms that are more resistant to microbial decomposition (Caon et al., 2014; Certini, 2005; Knicker, 2007). Nitrogen (N) can be lost during fire via volatilization (Zavala et al., 2014) or converted from organic N into mineral N (Certini, 2005; Hobbs and Schimel, 1984; Knicker, 2007; Neary et al., 2005), which is prone to leaching (Certini, 2005; Knicker, 2007) or volatilization during subsequent fires (Carreira et al., 1994; Fuentes-Ramirez et al., 2015; Jones et al., 2015). Fire affects soil physical properties by decreasing aggregate distributions and stability through the loss of soil organic matter (SOM) via combustion or by breaking aggregate bonds via dehydration (Albalasmeh et al., 2013; Girona-García et al., 2018). Finally, fire can change biological soil properties through heat-induced microbial mortality particularly in the top 5cm of soil (Certini, 2005; Fontúrbel et al., 2012; Hart et al., 2005; Neary et al., 2005, 1999). Together, these changes are likely to affect water retention and decomposition processes that regulate nutrient availability to plants, thus affecting plant

reestablishment (Knicker, 2007). Importantly, fire-induced changes in plant-soil feedbacks may persist in the long-term. Fire can thus be the trigger that moves sagebrush steppe ecosystems across thresholds into new alternative states that may not sustain the native community of organisms once supported by the previous systems (McIver et al., 2010; Miller et al., 2013).

In sagebrush steppe ecosystems specifically, changes in soil properties driven by frequency of fire can prevent re-establishment of slow-recovery plant species such as sagebrush (*Artemisia* spp.) (Baker, 2006; Lesica et al., 2007) and promote annual grass dominance, creating a new dynamic wherein soil processes and plant communities may be permanently altered (Allen et al., 2011; Chambers et al., 2014b). While we know that fire impacts the physical, chemical, and biological properties of soil, it is uncertain how an increase in fire frequency will compound these effects in the sagebrush steppe of the NCB. Based in part on the resistance and resilience model for sagebrush steppe (Chambers et al., 2014a), we would expect that if such effects are compounded under increases in fire frequency in this region, then ecosystem state changes are more likely to occur. While much post-fire recovery research in sagebrush steppe systems has been conducted in the Great Basin and scattered locations around the Rocky Mountains (Baker, 2006; Menakis et al., 2003; Mitchell et al., 2017; Rau et al., 2014, 2008, 2007), post-fire recovery research specific to the NCB, a region that contains unique climatic and edaphic conditions at the northwestern extent of the sagebrush steppe biome and is potentially critical to long-term conservation of sagebrush steppe ecosystems and associated wildlife, is still limited (Shinneman and McIlroy, 2016). Thus, it is uncertain how an increased occurrence of fire in the NCB affects the physical, chemical, and

biological soil properties that underpin successional trajectories and plant communities that will ultimately inhabit the NCB ecosystem. To answer our research question, how does frequency of fire affect the physical, chemical, and biological properties of soil that are critical to sagebrush steppe communities within the NCB, we investigated fire frequency effects upon soil pH, carbon and nitrogen content, and microbial activity across four fire frequencies (unburned, burned once, twice, and thrice) in the NCB.

METHODS

Study Area and Fire History

The study area is located in the NCB of Washington, about 100km west of Spokane (47.590383N 118.540236W) (Figure 1). The site encompasses approximately 2,500ha of sagebrush steppe habitat primarily managed by the U.S. Bureau of Land Management. The landscape is characterized by Anders-Bakeoven-Rock outcrop complex overlying basalt plateaus of the channeled scablands (NRCS, 2015). The study site was divided into “mounds” and “flats”, two distinct land types that are representative of the channeled scabland’s heterogenous landscape (Baker, 2009). Mounds, which are areas characterized by Anders silt loam, are circular to elliptical dome-like areas ranging from 2-25m in diameter with a center height of generally less than 2m (Figure 2). Flats, which are areas characterized by Bakeoven and Rock outcrop, surround the mounds and feature basalt cobbles and stones intermixed with very cobbly loam (Figure 2). Mounds have experienced soil development, while flats feature sparse areas of meager soil development. Consequently, soils were sampled from mounds only.

Fire events of interest in the study area occurred in 2003, 2007, and 2012 (Figure 3). The 2003 Hatten Road fire burned approximately 2,933ha. The 2007 Highline Road fire occurred entirely within the 2003 fire boundary, reburning 475ha. The 2012 Apache Pass fire burned approximately 10,000ha, reburning the 2007 fire area as well as approximately 2,000ha of the 2003 fire area. All burned sites therefore experienced fire in 2012, which provided a common, recent disturbance event for comparison.

Site Selection

A stratified-random sampling design was used to select sites based on fire frequency. Fire frequencies included in our analysis are described as unburned (0x), burned once (1x; 2012), burned twice (2x; 2003, 2012), and burned thrice (3x; 2003, 2007, 2012). Site selection involved choosing cells across a 100m grid in ArcGIS. To limit the potential for spatial autocorrelation while maximizing sampling area, selected mounds were separated by 200m or more. A complimentary assessment of MTBS (Monitoring Trends in Burn Severity; <https://www.mtbs.gov/>) fire severity maps and Google Earth (<https://www.google.com/earth/>) imagery was conducted to assess fire history and other past disturbances within each 100m cell. The following criteria were used for mound selection: 1) > 100m from fire perimeter and major roads, 2) >200m from known land treatments, and 3) mounds were not immediately adjacent to wetlands. All mounds selected were at least 10m² in area. Prior to sampling in 2016, the burned-unburned condition was verified for each site by assessing evidence of fire occurrence (e.g. charred sagebrush snags, charcoal in soil). While unburned areas in our study showed no indication of fire, these areas could have burned prior to the beginning of MTBS data collection in 1984 (<https://www.mtbs.gov/>).

Soil Sampling

In August 2016, 10 mounds from each fire frequency were sampled. Eight soil cores (5cm diameter, 10cm depth) across each mound were collected using a stratified sampling design (Figure 4). All core samples collected from a mound were at least 1m from the mound perimeter to minimize edge effects and at least 1m from adjacent core samples to capture soil variability across the mound. Before sampling, dead plant

material was removed from the soil surface and standing vegetation was clipped back. Soil cores were separated into two depth increments (0-5cm and 5-10cm) (Figure 4), kept on ice in the field and during transportation, then frozen (-20°C) until analysis. Within each soil depth (0-5cm and 5-10cm) the soil cores from each mound were composited in the lab.

Soil pH

Soil pH for each fire frequency was quantified using 15g of field moist soil from each of the composited soils from the 0-5cm soil depth. These subsamples were added to 30mL of DI water, agitated for one minute to create a soil slurry, then allowed to rest for 30 minutes. To reduce variability in pH measurements after the 30 minute rest period, we agitated the soil slurry again immediately prior to using an Oakton pH 6 Acorn Series meter with pH probe (Oakton Instruments, Vernon Hills, Illinois). The process was repeated three times for each soil sample, after which we used the mean value as our measure of soil pH.

Soil Organic Matter

SOM was quantified using 20g subsamples of field moist soil from each of the composited soils of the 0-5cm soil depth. A loss-on-ignition method was used to determine SOM (Hoogsteen et al., 2015; Wright et al., 2008). Briefly, subsamples were passed through a 2mm sieve to homogenize the soil. Any particles larger than 2mm, including rocks, roots, and litter were removed. Five grams of the homogenized soil were placed into a ceramic crucible and oven dried at 105°C for 24 hours. Crucibles were then placed into a Thermolyne FD1535M muffle furnace (Thermo Fisher Scientific,

Waltham, Massachusetts) for four hours at 550°C. Weights before and after burn were used to determine SOM.

Soil Fractions

Soils of the 0-5cm depth were fractionated using the wet sieving and aggregate isolation protocols as described by Six et al. (2000). An initial wet-sieving step was used to separate fractions into large and small water stable macroaggregates (>250µm), free microaggregates (53-250µm), and free silt and clay (<53µm). Compositied soil subsamples of 80g were placed in a 250µm sieve then submerged in 1cm of de-ionized water and slaked for five minutes prior to wet sieving. Soils were then wet-sieved, moving the sieve up and down with an amplitude of approximately 3cm for two minutes using a digital metronome, after which water stable macroaggregates (remaining on top of the 250µm sieve) were washed into small aluminum pans. Free microaggregates were collected using the same wet-sieving protocol with a 53µm sieve from the material that passed through the 250µm sieve. The free silt and clay fraction, which passed through the 53µm sieve, was then washed into large aluminum pans. All soil fractions were dried at 105°C in a forced air oven.

Ten gram subsamples collected from oven-dried macroaggregates were further separated using a microaggregate isolator into microaggregates (53-250µm) occluded within macroaggregates and silt and clay (<53µm) occluded within macroaggregates. Macroaggregate subsamples were placed in a beaker with approximately 50mL of de-ionized water for 20 minutes, after which soils were transferred to the microaggregate isolator mounted on an Eberbach E6000 reciprocal shaker (Belleville, Michigan). To break apart the macroaggregates, subsamples were shaken in the microaggregate isolator

at low speed (150 rpm) in a steady stream of de-ionized water on a 250 μ m screen with 50 stainless steel, 2mm diameter beads for five minutes. The material was emptied onto a 53 μ m sieve and wet-sieved using the same wet-sieving procedure as described above and processed in a similar fashion as the water stable microaggregate and silt and clay fractions. All fractions were dried at 105°C in a forced air oven.

Soil Nitrogen and Carbon

All composited soil subsamples (5g for bulk soil, 1g for all soil fractions) were dried at 105°C in a forced air oven for 24 hours, then ground in a SPEX Sample Prep Mixer/Mill (Metuchen, New Jersey). An acid test was conducted to check for the presence of inorganic C. Three random samples were chosen from bulk soil for each fire frequency. Several drops of 12M hydrochloric acid were added to 0.5g soil. Soils did not effervesce in the presence of acid; therefore, we did not test for inorganic carbon. All bulk soil samples at 0-5 and 5-10cm depths, as well as fractions at 0-5cm depth, were processed for total C and total N with a FlashEA 1112 NC analyzer (Thermo Fisher Scientific, Waltham, Massachusetts) using aspartic acid for calibration and internal standards. Every tenth sample was run in duplicate with replicate error <10%.

Mineral Nitrogen

In specimen cups (118mL), 20g of field moist soil sample were added then brought to 60% water holding capacity after which 100mL of 2M potassium chloride solution was added. Soil water holding capacity was determined by the difference in weight for soils at saturation and at oven dry (105°C). The samples were placed on an Eberbach E6000 reciprocal shaker for 45 minutes. After shaking, the slurry was poured through Whatman #1 filter paper, and the filtrate collected. Extraction filtrates were kept

at -20°C until ready for processing. Each extraction filtrate was processed via a manual vanadium (III) reduction and a Berthelot reaction in 96-well plates to assess concentrations of nitrate associated N (NO_3^- -N) and ammonium associated N (NH_4^+ -N), respectively (Doane and Horwath, 2003; Forster, 1995; Miranda et al., 2001; Poulin and Pelletier, 2007). The absorbance of each reaction solution was determined using a BioTek Synergy Mx plate reader and Gen5 v1.11.5 software (Winooski, Vermont) at 540nm (NO_3^- -N) and 650nm (NH_4^+ -N) wavelengths. Final available mineral N amounts were calculated and expressed as μg available N (NH_4^+ -N or NO_3^- -N) per gram of soil (μgNO_3^- -N g^{-1})

Microbial Extracellular Enzyme Activity

A hydrolytic enzyme assay using fluorescent methylumbelliferyl substrates was conducted to assess the rates of microbial enzyme-catalyzed reactions involved in the breakdown of plant materials. Enzyme analyses were conducted according to methods outlined in van Diepen et al. (2015) and Weintraub et al. (2013) with minor modifications. Activities of the hydrolytic enzymes cellobiohydrolase (cellulose degradation releasing cellobiose), β -glucosidase (cellobiose degradation releasing glucose), phosphatase (protein degradation releasing phosphorus compounds), and β -N-acetylhexosaminidase (chitin/glycoprotein degradation releasing nitrogen compounds) were assayed using the methylumbelliferyl (MUB) linked substrates β -D-cellobioside (CBH), β -D-glucopyranoside (BG), phosphate (PHOS), and N-acetyl- β -D-glucosaminide (NAG), respectively.

Briefly, 1g of field moist soil was homogenized for 30 seconds in 100mL of 50mM sodium acetate buffer at the pH determined from the pH analysis. A Waring Pro

SB10 immersion blender (East Windsor, NJ) was used to homogenize soil samples. The homogenized sample (200 μ L) was transferred to a 96-well assay plate followed by substrate addition (50 μ L). Assay plates were incubated for 1 to 6 hours depending on the enzyme substrate. After incubation, fluorescence was measured at an excitation wavelength of 360 nm and an emission wavelength of 450 nm on a BioTek Synergy Mx plate reader using Gen5 v1.11.5 software (Winooski, Vermont). All enzyme assays were conducted with eight replicate wells per sample and corrected for background fluorescence of substrate (negative control). Final enzyme activity was calculated and expressed as μ mol of substrate converted per hour per gram soil (μ mol h⁻¹ g⁻¹).

Microbial Respiration

To assess the long-term microbial activity and SOM decomposition across the four fire frequencies, a one year incubation of the 0-5 and 5-10 cm soil layers was conducted in the laboratory. We placed 20g homogenized soil (no rocks, roots >2mm, or litter) into a specimen cup (118 mL), soil was brought to 60% water holding capacity, then placed into in a mason jar. Jars were sealed with lids containing a rubber septum in the center to allow gas removal with a syringe, then stored in the dark at room temperature. Soil CO₂ evolution was measured on days 1, 3, 5, 7, and 14, and thereafter every 30 days for one year, using a LI-7000 CO₂/H₂O infrared gas analyzer (LICOR Corp., Lincoln, Nebraska). After each gas sampling measurement, jars were opened for 30 minutes to allow gas exchange to prevent anoxic conditions within the jar. Soil water content was checked and maintained at 60% water holding capacity for the duration of the incubation. After 30 minutes, jars were again sealed and left to incubate until the next measurement.

Analysis

All statistical analyses were conducted in SPSS Statistics 24.0 (IBM, Armonk, New York) using the Univariate General Linear Model with significance level $p < 0.05$. Two-way ANOVAs with fire frequency and soil depth as fixed factors were conducted for soil properties (nitrogen, soil respiration) that included both soil depths (0-5 and 5-10cm). When the interaction term was not significant, one-way ANOVAs were conducted for each soil depth. One-way ANOVAs with fire frequency as the fixed factor were conducted for soil properties (pH, SOM, enzymes, C-concentration, C-content) that included only one soil depth (0-5cm). The Tukey HSD multiple comparison test was used for post hoc analysis when the one-way ANOVA revealed significant differences between treatments. Homogeneity of variance and normality of data were checked by Levene and Shapiro-Wilks tests, respectively. Variances were not homogenous for all data sets; however, we proceeded with parametric analysis due to the robustness of ANOVAs to deviations from this assumption (L. Bond, personal communication). Data that did not meet the assumption of normality was transformed using log base 10. Because transformations did not consistently improve the data to meet the assumption, statistical analyses were conducted on data that best met the normality assumption (non-transformed: pH, enzymes; transformed: soil organic matter, C-concentration, C-content, mineral N, soil respiration).

RESULTS

Soil Physical and Chemical Properties

Fire frequency affected pH, and SOM at the 0-5cm depth. Soil pH increased as a function of fire frequency with significant differences between unburned (0x) and both the twice (2x) and thrice (3x) burned soils as revealed by post hoc analysis (Figure 5; $F_{(3,36)}=7.088$, $p<0.05$).

Fire frequency was associated with an overall decrease in soil organic matter with significant differences between unburned (0x) and both the once (1x) and thrice (3x) burned soils as revealed by post hoc analysis (Figure 6; $F_{(3,36)}=7.292$, $p<0.05$).

There were significant overall effects of fire frequency on C-concentration for water stable macroaggregates (Figure 8a; $F_{(3,36)}=3.744$, $p<0.05$) and microaggregates (Figure 7b; $F_{(3,36)}=3.399$, $p<0.05$), but not for silt and clay (Figure 7c; $F_{(3,36)}=1.113$, $p=0.357$). In both the macroaggregate and microaggregate fractions, once (1x) burned soils had significantly lower C-concentrations than unburned (0x) soils as revealed by post hoc analysis. In addition, fire frequency resulted in a significant decrease in C-concentration for occluded microaggregates (Figure 7d; $F_{(3,36)}=3.311$, $p<0.05$) and silt and clay (Figure 7e; $F_{(3,36)}=3.065$, $p<0.05$). In the microaggregate fraction, post hoc analysis showed the once (1x) burned soils had significantly lower C-concentrations than unburned (0x) soils. In the silt and clay fraction, thrice (3x) burned soils had significantly lower C-concentrations than unburned (0x) soils.

We calculated soil C associated with each one of the soil fractions (hereafter called 'carbon content') by multiplying the relative abundance of a fraction in a gram of soil with its C-concentration ($[\text{g wt fraction sample} / (\text{total g wt of soil sample} - \text{moisture g weight of soil sample})] * \text{C-concentration of fraction} = \text{g C/kg soil fraction}$). Fire frequency had overall significant effects on C-content for water stable microaggregates ($F_{(3,36)}=4.430$, $p<0.05$) and silt and clay ($F_{(3,36)}=5.518$, $p<0.05$), but not for macroaggregates (Figure 8a; $F_{(3,36)}=1.880$, $p=0.150$). As revealed by post hoc analysis, the once (1x) burned soil had significantly lower C-content than unburned (0x) soil in the microaggregate fraction, as well as significantly lower C-content than unburned (0x) and twice (2x) burned soils in the silt and clay fraction. No significant differences in C-content were noted within the occluded microaggregate ($F_{(3,36)}=0.905$, $p=0.448$) or silt and clay fractions (Figure 8b; $F_{(3,36)}=0.150$, $p=0.929$).

There were significant effects of fire frequency on total soil C-content (Figure 8a; $F_{(3,34)}=3.169$, $p<0.05$) when total soil C is calculated as the sum of C associated with the water stable soil fractions. Significant decreases, as revealed by post hoc analysis, occurred in the once (1x) burned soil compared to the unburned (0x) soil.

Fire frequency affected mineral N resulting in a decrease in $\text{NH}_4^+\text{-N}$ and an accompanying increase in $\text{NO}_3^-\text{-N}$. At both soil depths (0-5 and 5-10cm) there was no significant effect of fire frequency on $\text{NH}_4^+\text{-N}$ (Figure 9a, $F_{(3,36)}=2.810$, $p=0.053$ and Figure 9b, $F_{(3,36)}=0.563$, $p=0.643$, respectively). $\text{NO}_3^-\text{-N}$ was significantly affected by fire frequency at both the 0-5 and 5-10cm soil depths (Figure 9c, $F_{(3,36)}=3.399$, $p<0.05$ and Figure 9d, $F_{(3,36)}=7.250$, $p<0.05$, respectively). $\text{NO}_3^-\text{-N}$ in the twice (2x) burned soil was significantly higher than unburned (0x) soil as revealed by post hoc analysis of the 0-5cm

soil depth. Post hoc analysis of the 5-10cm soil depth showed significant NO_3^- -N increases in the once (1x) and twice (2x) burned soils compared to unburned (0x).

Soil Biological Properties

In terms of enzymatic activity, there was a significant overall decrease in NAG and BG activity as fire frequency increased (Figure 10b, $F_{(3,36)}=3.094$, $p<0.05$ and Figure 10a, $F_{(3,36)}=3.294$, $p<0.05$, respectively). Significant decreases in NAG activity, as revealed by post hoc analysis, occurred between unburned (0x) and thrice (3x) burned soils, whereas in the BG samples, none of the pairwise comparisons were statistically significant. Decreases across all enzymatic activity were noted in both the once (1x) and thrice (3x) burned soils when compared to unburned (0x) soils. Specifically, enzyme activity in once (1x) burned soils decreased 20.5-31.6% (BG, 31.6%; CBH, 25.3%; NAG, 20.5%, PHOS 22.4%). Similarly, enzyme activity in thrice (3x) burned soils decreased 36.7-42.8% (BG, 36.7%; CBH, 37.1%; NAG, 42.8%; n.b., PHOS samples were excluded because they showed little change).

Fire frequency affected the soil microbial respiration rate in both the 0-5 and 5-10cm soil depths during the first week of incubation. On day 5 at the 0-5cm soil depth, soil respiration rate significantly decreased (Figure 11a; $F_{(3,36)}=4.977$, $p<0.05$) in the once (1x) burned soil compared to the unburned (0x) and twice (2x) burned soils, as revealed by post hoc analysis. At the 5-10cm depth on day 5, the twice (2x) burned soil respiration rate was significantly higher than the once (1x) burned soil (Figure 12a; $F_{(3,36)}=3.393$; $p<0.05$). In the 5-10cm depth there were no significant differences in respiration rate after day 14 (Figure 12b). In contrast, fire frequency affected soil respiration rates at the 0-5cm depth at two weeks (day 14) and at one month (day 34) of incubation (Figure 11b,

$F_{(3,36)}=5.326$; $p<0.05$ and $F_{(3,36)}=4.885$; $p<0.05$, respectively). As revealed by post hoc analysis, the soil respiration rate at two weeks of incubation (day 14) of the once (1x) burned soil was significantly lower than the unburned (0x) and twice (2x) burned soils, and at one month of incubation (day 34) soil respiration rates of the once (1x) and thrice (3x) burned soils were significantly lower than the unburned (0x) soil.

Cumulative soil respiration in both the 0-5 and 5-10cm soil depths was affected by fire frequency. On day 7 at the 0-5cm soil depth and on day 14 at both the 0-5 and 5-10cm soil depths, cumulative soil respiration was significantly higher (Figure 11a, $F_{(3,36)}=3.736$, $p<0.05$; Figure 11b, $F_{(3,36)}=4.344$, $p<0.05$; Figure 12b, $F_{(3,36)}=2.913$, $p<0.05$, respectively) for the twice (2x) burned soil compared to the once (1x) burned soil as revealed by post hoc analysis. This trend, the twice (2x) burned soil exhibiting higher cumulative respiration than the once (1x) burned soil, continues through the entire incubation, although the trend is no longer significant after two weeks for the 5-10cm soil depth and after two months for the 0-5cm soil depth.

DISCUSSION

Our study yielded three main results: 1) fire significantly reduced the soil C concentration relative to unburned soil, but only when soil was exposed to fire once, 2) soil pH and NO_3^- -N increased with fire frequency, whereas enzyme activity decreased, and 3) SOM contents and microbial CO_2 respiration were suppressed significantly in the once burned soil compared to the control and twice burned soils. Taken together, our findings suggest that a one-time fire in this region of sagebrush steppe is capable of significantly changing physical, chemical, and biological soil properties that feedback to alter the plant community structure from a shrub-dominated to a grass-dominated system. Although additional fires exacerbate these changes in soil properties and processes and serve to maintain and perpetuate the ecosystem state change, given sufficient time between fires, soil properties and processes can partially recover. Soil ecosystems in this sagebrush steppe landscape with its unique climatic and edaphic conditions are thus unlikely to be resilient to multiple fire events, particularly when a reduction in time between recurrent fires exacerbates fire effects on plant-soil interactions.

Fire reduced the soil C-concentration, but only at the site that burned once. The depletion of soil C at the once burned site, but not at the other sites, suggests that the first fire was more severe than subsequent fires. Higher intensity fires tend to lead to more soil organic C (SOC) loss (Allen et al., 2011; Certini, 2005; Neary et al., 1999), because SOM combustion and heat-induced root mortality are greater at higher temperatures (Allen et al., 2011; Neary et al., 1999). The greater burn severity in the once-burned site relative to

other sites may be explained by differences in plant communities at the time of the fire. Plant community surveys indicated that average shrub cover across unburned mounds was 48.2% (Susan McIlroy, personal communication), whereas the plant community at the previously burned sites was dominated by annual grasses (i.e. >90% cover exotic grasses and 0% shrub cover) (Susan McIlroy, personal communication). From these data we may deduce that the sites that burned for the first time in 2012 were dominated by shrubs, whereas the sites that had burned prior to 2012 (i.e. in 2003 and 2007) were dominated by exotic grasses. Fires in shrub dominated ecosystems may reach temperatures up to 760°C (Alexis et al., 2010; Bailey and Anderson, 1980; Rundel, 1983) whereas grass dominated systems burn at lower temperatures of <300°C (Bailey and Anderson, 1980; Neary et al., 1999; Rundel, 1983), leading to considerably different outcomes for SOC dynamics. The significant reductions in total C, SOM, and C associated with macro- and microaggregates, along with a decline in microbial CO₂ respiration in the once burned sites, reveal that a first fire in this ecosystem is capable of degrading soil structure and decomposition processes. Given that both of these soil properties are key predictors of plant communities through their controls on soil water holding capacity and nutrient retention and availability (Hao and Chang, 2002; Reynolds et al., 2003; Whalen and Chang, 2002), we postulate that the first fire served as a spring board to significantly alter plant-soil feedbacks and initiated a state-change in this ecosystem.

Increasing fire frequency exacerbated increases in soil pH. Soil pH has been shown to increase with fire frequency across studies and among different ecosystem types (Certini, 2005; Certini et al., 2011; Ulery et al., 1993; Vega et al., 2013; Zavala et al.,

2014), which may be explained by accumulation of ash containing alkaline ions (Chen et al., 2010; Kennard and Gholz, 2001; Neary et al., 2005; Ulery et al., 1993). Fire-induced changes in soil pH are likely to persist (Fonseca et al., 2017; Kennard and Gholz, 2001; Muñoz-Rojas et al., 2016), particularly in semi-arid soils (Allen et al., 2011) with consequences for microbial activity and soil nutrient cycling because a higher pH promotes the process of nitrification (Kennard and Gholz, 2001; Kyveryga et al., 2004; Zavala et al., 2014). In our study increasing fire frequency tended to enhance NO_3^- -N concentrations and decreased NAG activity, which indicates that microbes reduced enzyme production owing to sufficient resource availability (Allison and Vitousek, 2005). Elevated soil NO_3^- -N concentrations following fire have been observed across sagebrush steppe and other arid ecosystems (DeLuca and Sala, 2006; Martí-Roura et al., 2013; Neary et al., 1999; Rau et al., 2007). Within these systems elevated NO_3^- -N is a key driver of the success of invasive plant species (Chambers et al., 2007; Ehrenfeld, 2003; Norton et al., 2007; Saetre and Stark, 2005; Stark and Norton, 2015) as increased N favors plant species adapted to high resource availability, a trait associated with most invasive plant species (Perry et al., 2010; Vitousek et al., 1997), including *B. tectorum* (Blank et al., 2017; Blank and Morgan, 2011; Johnson et al., 2011).

The deleterious effects of both fire severity and fire frequency on soil properties was most clearly represented by our data on SOM contents as well as soil microbial CO_2 respiration and enzymatic activity. High severity fires are considered typical in big sagebrush steppe ecosystems (Baker, 2011) and tend to lead to greater SOM combustion at the higher surface and soil temperatures reported for fires in shrub dominated sites compared to those reported for grass dominated sites (Allen et al., 2011; McCauley et al.,

2005; Neary et al., 1999). Given that SOM is viewed as a foundational soil property, underpinning soil quality and microbial activity (e.g. decomposition and nutrient cycling) (Hao and Chang, 2002; Reynolds et al., 2003; Whalen and Chang, 2002), fluctuations in these data through time are indicative of ecosystem recovery trajectories and how they are influenced by fire frequency. Our data showed that SOM contents were significantly lower in the once burned and thrice burned sites than in the unburned site. As a result of SOM losses, decomposition rates in these soils were suppressed, as evidenced by decreased BG activity and CO₂ evolution (Bárcenas-Moreno et al., 2011; Knelman et al., 2017).

Conversely, SOM contents in the twice burned site were not statistically different from the that of the unburned site and supported higher rates of decomposition (e.g. CO₂ evolution) than the once and thrice burned sites. These results suggest that soil properties in the twice burned soil may have been recovering towards pre-fire levels before the most recent burn in 2012. The twice burned site burned for the first time in 2003 and for the second time in 2012, thus there was a nine year recovery period between the two fires. During this time grasses would have re-established and released C into soil through above- and belowground litter inputs. Despite an increased cover of exotic annual grasses in our burned sites, these sites retained a significant presence of perennial native bunchgrasses, including *Poa secunda* J. Presl and the deep-rooted species *Leymus cinereus* (Scribn. & Merr.) Á. Löve (Shinneman et al., n.d.), that are likely to contribute significant quantities of C to the soil, thus partially restoring SOM contents and microbial activity, perhaps providing a pathway for succession back to native soil properties in the absence of further fire activity (Bárcenas-Moreno et al., 2011; Dangi et al., 2010; Francos

et al., 2018; Guénon et al., 2013). In contrast, our thrice burned site that burned in 2003, 2007 and 2012 was unable to recover from the disturbances. SOM contents at this site were progressively depleted leading to suppression of microbial activity and an inability of soil properties and processes to recover.

This study capitalized on a unique, natural experiment that enabled us to disentangle how a recent fire differentially impacted soil properties and processes that depended on whether sites had been previously unburned, burned once, twice, or thrice. Our results suggest that an increase in fire frequency between fires in this semi-arid sagebrush steppe ecosystem of the NCB is producing changes in foundational soil properties and processes that are likely to undermine ecosystem stability and promote ecosystem state change (Guénon et al., 2013), even if soil properties and process are able to partially recover with sufficient time between fires. The change in pH and concomitantly higher NO_3^- concentrations in the twice burned site indicate that despite recovery of SOM and microbial activity, the resilience of soil is compromised and resistance to plant invasions has been reduced, leading to a reduction in ecosystem stability. Furthermore, while soil C and microbial decomposition processes are able to recover with sufficient time between fires, indicators of enhanced resource availability (i.e. increased NO_3^- -N contents or reduced enzyme activity) prevail in soil across all fire frequencies, indicating that fire has the capacity to reduce ecosystem stability, even when other soil properties recover. Moreover, even with evidence of soil recovery in twice burned plots (via greater fire interval), exotic annual grasses dominate mounds after any fire frequency (Shinneman et al., n.d.), consequently increasing future fire probability and likely initiating longer-term changes in soil properties (Bailey and Anderson, 2014).

Thus, we conclude that whole-ecosystem recovery in this semi-arid sagebrush steppe ecosystem of the NCB remains dubious or uncertain at best (Figure13).



Figure 1. Location of the study site within the channeled scablands of the northern Columbia Basin, Lincoln County, Washington (47.590383N 118.540236W). Map Data 2018 Google.



Figure 2. Aerial view of heterogenous landscape within the channeled scablands showing mounds (solid line) and flats (dotted line). Imagery date: 05/29/2014, Google Earth.

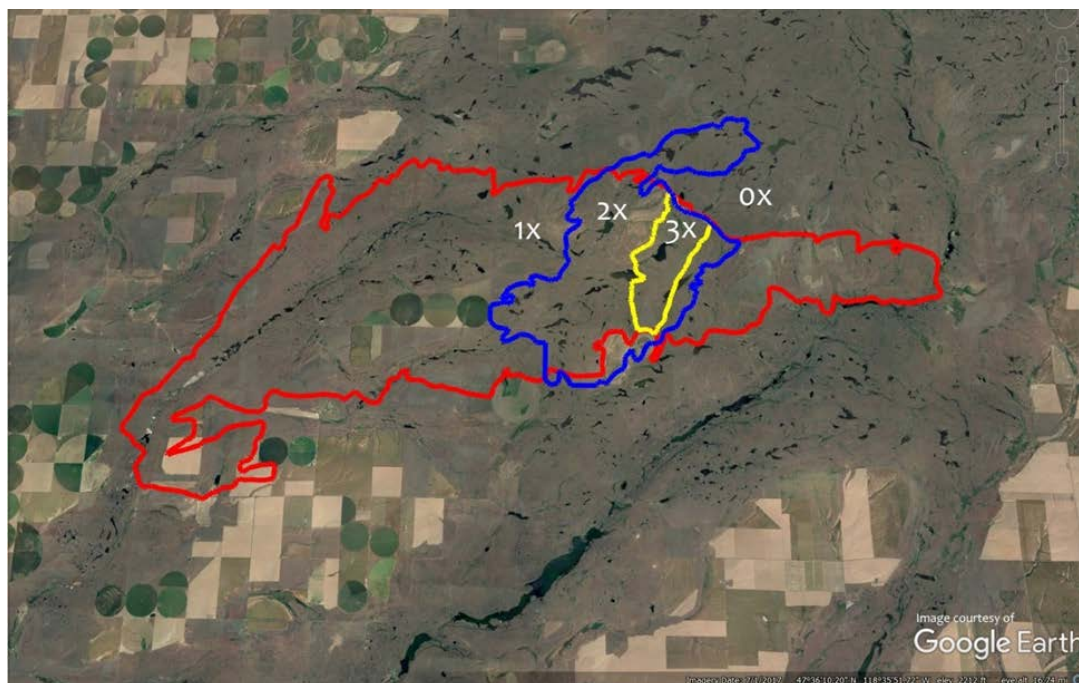


Figure 3. Outline of fire events that occurred in the study area. Fires occurred in 2003 (blue outline), 2007 (yellow outline), and 2012 (red outline). The overlap of fires provides the fire frequencies: 0x, unburned; 1x, once burned (2012); 2x, twice burned (2012, 2003); 3x, thrice burned (2012, 2007, 2003). Imagery date: 07/01/2017, Google Earth.

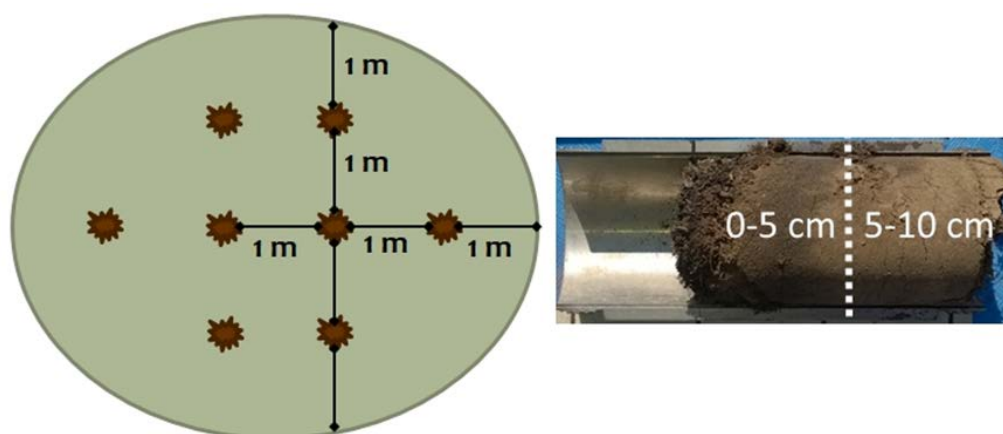


Figure 4. Mound stratified sampling design. All cores were a minimum of 1 m from the mound perimeter and 1 m from adjacent soil cores. Soil cores were divided into two soil depths: 0-5 cm and 5-10 cm.

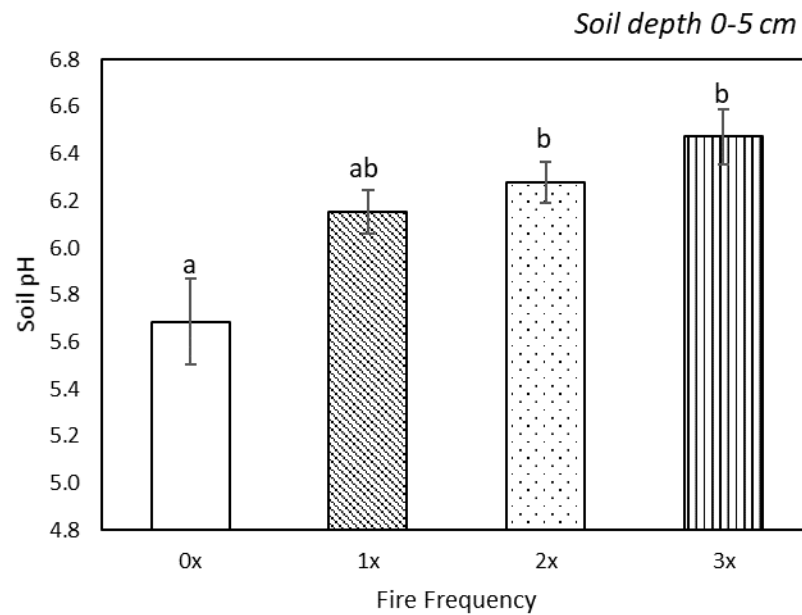


Figure 5. Soil pH across fire frequencies for 0-5 cm soil depth. Values represent means with error bars as \pm SE. Lowercase letters indicate significant differences among fire frequencies from post hoc analysis ($p < 0.05$, $n = 10$). Means (0x, 5.7; 1x, 6.2; 2x, 6.3; 3x, 6.5).

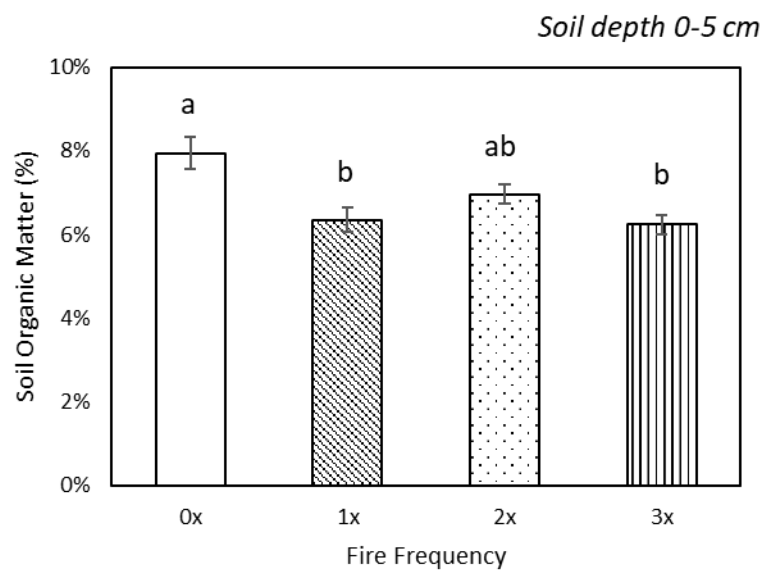


Figure 6. SOM across fire frequencies for 0-5 cm soil depth. Values represent means with error bars as \pm SE. Lowercase letters indicate significant differences among fire frequencies from post hoc analysis using transformed data ($p < 0.05$, $n = 10$). Graphical representation based on non-transformed data.

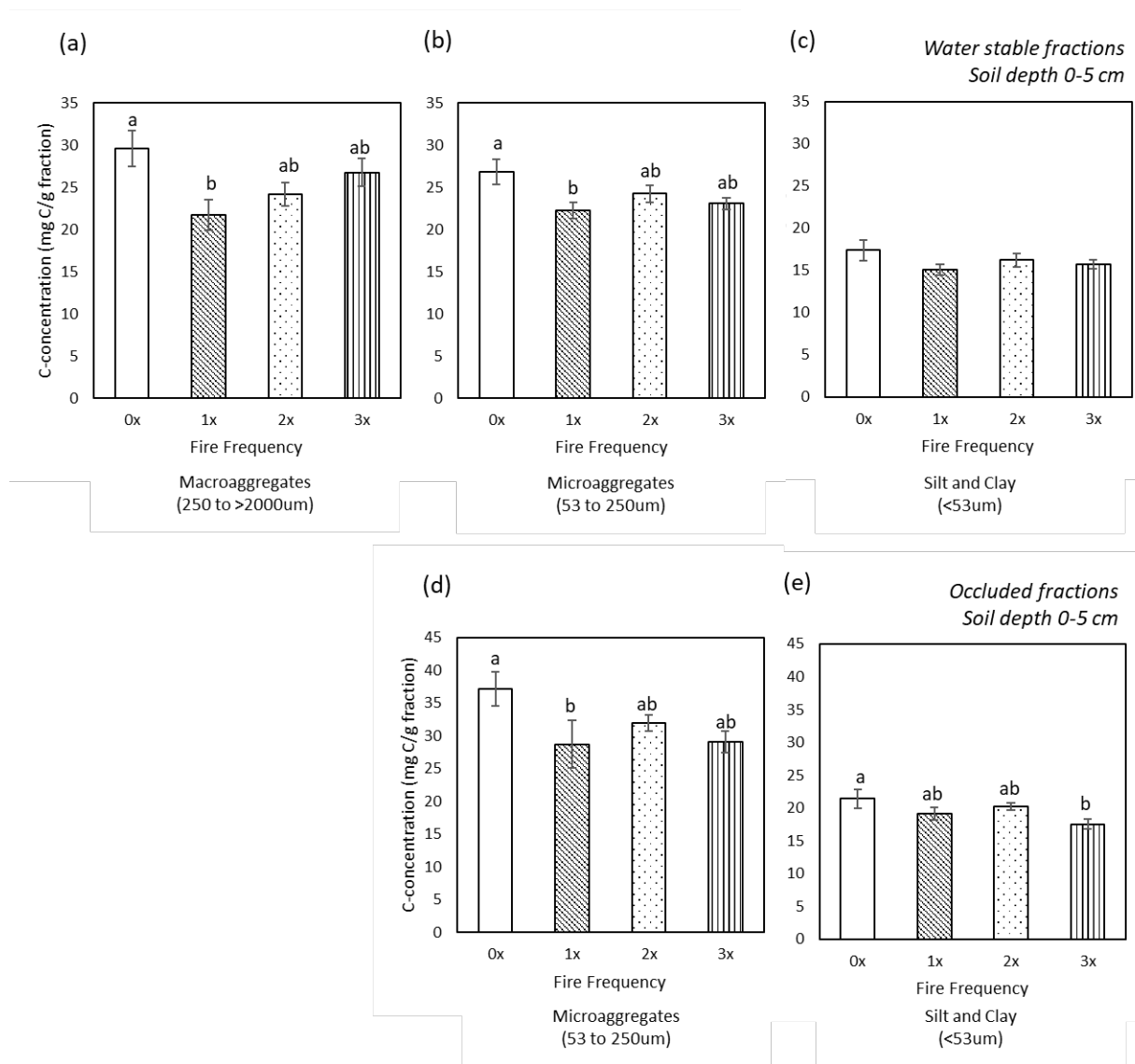


Figure 7a-e. Carbon concentration or C-richness, the carbon present within each fraction, for water stable and occluded soil fractions of the 0-5 cm soil depth across fire frequencies. Values represent means with error bars as \pm SE. Lowercase letters indicate significant differences among fire frequencies within each fraction from post hoc analysis using transformed data ($p < 0.05$, $n = 10$). Graphical representation based on non-transformed data.

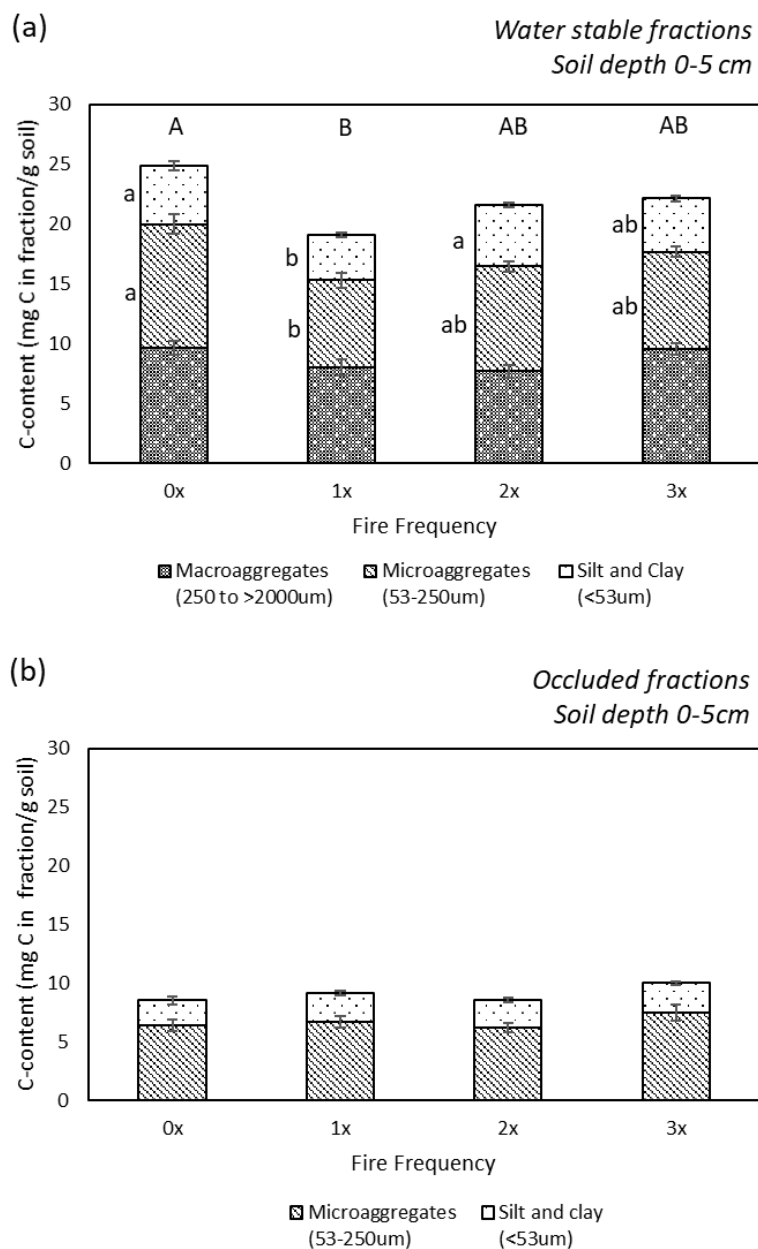


Figure 8a-b. Carbon content, the relative abundance of soil aggregate-derived carbon within bulk soil for water stable and occluded soil fractions of the 0-5 cm soil depth across fire frequencies. Values represent means with error bars as \pm SE. Lowercase letters indicate significant differences among fire frequencies within each fraction from post hoc analysis using transformed data ($p < 0.05$, $n = 10$). Capital letters represent significant differences among fire frequencies of C-content of bulk soil, as represented by the sum of water stable fractions. Graphical representation based on non-transformed data.

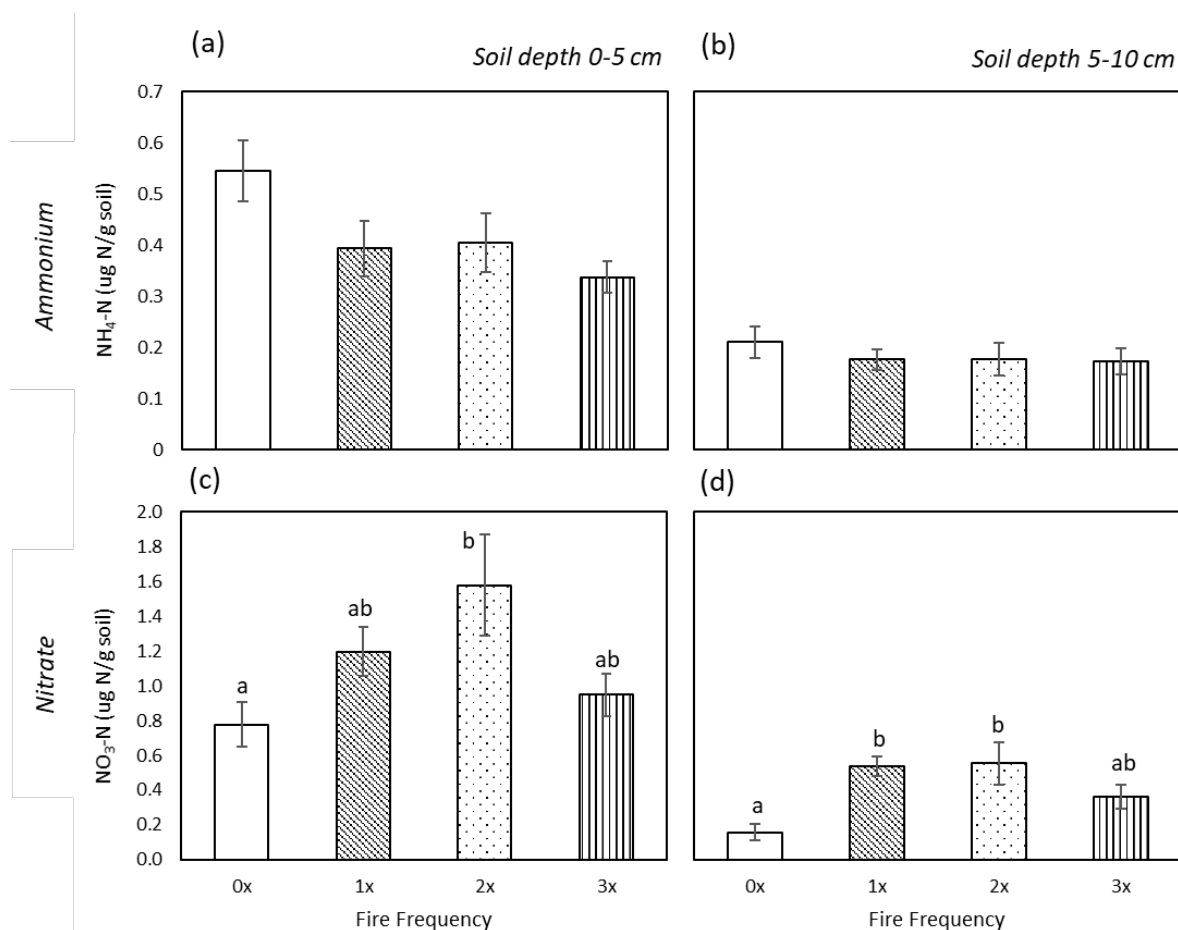


Figure 9a-d. Available mineral nitrogen, both nitrate (NO₃-N) and ammonium (NH₄-N), across fire frequencies for both soil depths (0-5 and 5-10 cm). Values represent means with error bars as \pm SE. Lowercase letters indicate significant differences among fire frequencies from post hoc analysis using transformed data ($p < 0.05$, $n = 10$). Graphical representation based on non-transformed data.

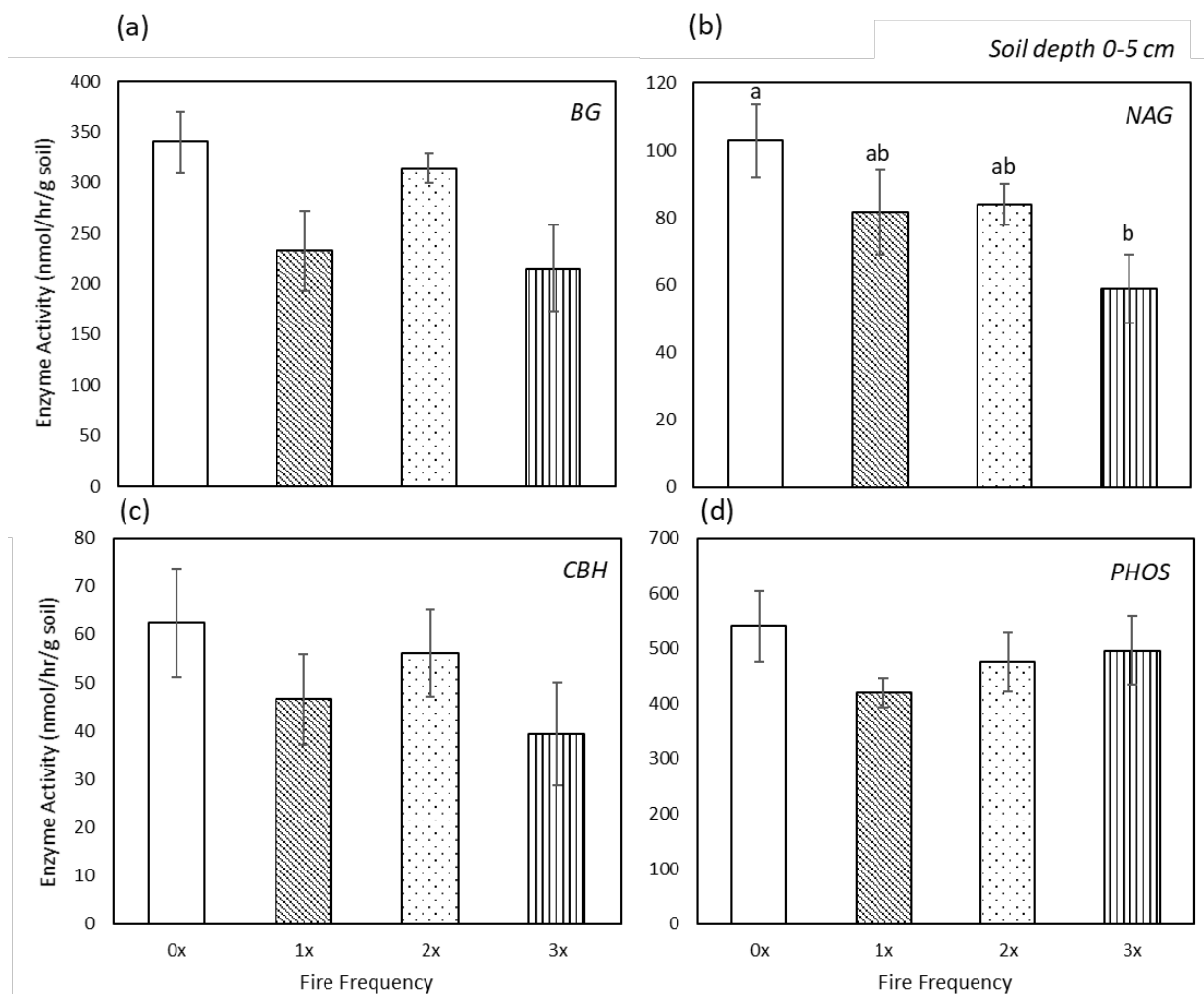


Figure 10a-d. Extracellular hydrolytic enzyme activity across fire frequencies for the 0-5 cm soil depth. Values represent means with error bars as \pm SE. Lowercase letters indicate significant differences among fire frequencies from post hoc analysis ($p < 0.05$, $n = 10$). Tukey post hoc did not differentiate the significant differences within BG. Graphical representation based on non-transformed data.

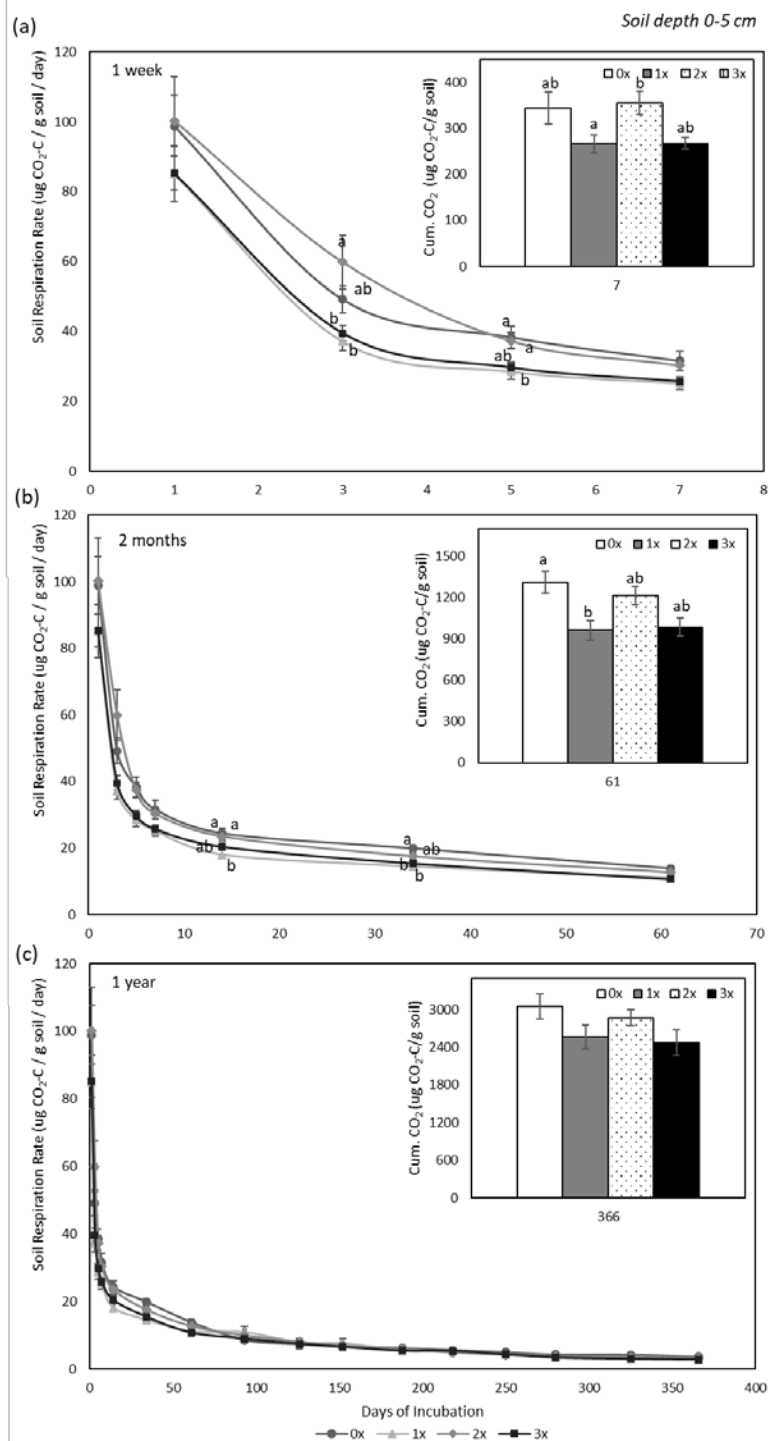


Figure 11a-c. Rate of microbial CO_2 evolution through one week, two months, and one year of incubation for 0-5 cm soil depth across fire frequencies ($n=10$). Inset graphs represent cumulative soil respiration for the same time periods ($n=10$). Values represent means with error bars as $\pm\text{SE}$. Lowercase letters indicate significant differences among fire frequencies from post hoc analysis using transformed data ($p < 0.05$, $n=10$). Graphical representation based on non-transformed data.

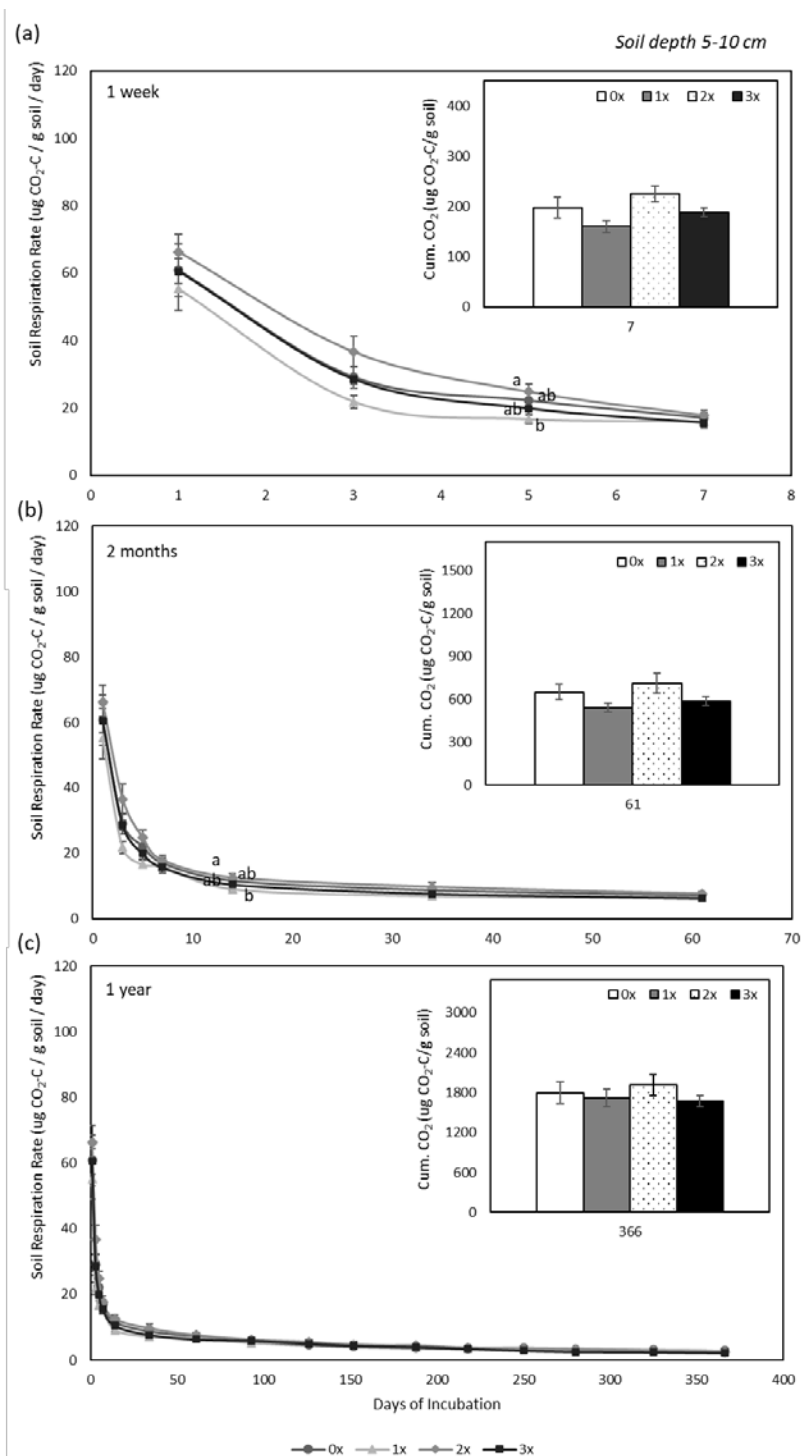


Figure 12a-c. Rate of microbial CO_2 evolution through one week, two months, and one year of incubation for 5-10 cm soil depth across fire frequencies ($n=10$). Inset graphs represent cumulative soil respiration for the same time periods (0x, 2x, 3x $n=10$; 1x $n=9$). Values represent means with error bars as $\pm\text{SE}$. Lowercase letters indicate significant differences among fire frequencies from post hoc analysis using transformed data ($p < 0.05$, $n=10$). Graphical representation based on non-transformed data.

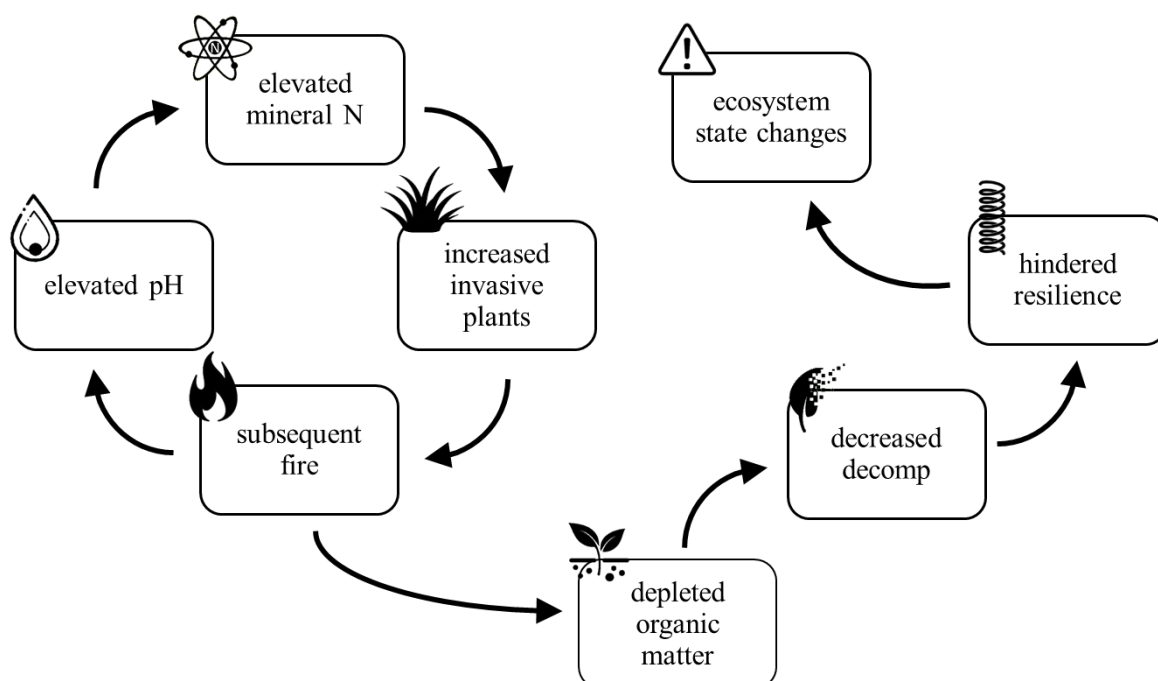


Figure 13. Without sufficient recovery time between successive fires, altered plant-soil feedbacks and associated state changes will persist. Fire frequency promotes elevated soil pH and mineral N, which promotes establishment of invasive plant species. Invasive plant species increase the risk of subsequent fire which exacerbates the cycle. In addition, fire frequency contributes to further depletion of soil organic matter which leads to the suppression of microbial processes. These changes hinder soil ecosystem resilience contributing to progressive ecosystem state changes.

REFERENCES

- Albalasmeh, A.A., Berli, M., Shafer, D.S., Ghezzehei, T.A., 2013. Degradation of moist soil aggregates by rapid temperature rise under low intensity fire. *Plant Soil* 362, 335–344. <https://doi.org/10.1007/s11104-012-1408-z>
- Alexis, M.A., Rumpel, C., Knicker, H., Leifeld, J., Rasse, D., Péchot, N., Bardoux, G., Mariotti, A., 2010. Thermal alteration of organic matter during a shrubland fire: A field study. *Org. Geochem.* 41, 690–697.
<https://doi.org/10.1016/j.orggeochem.2010.03.003>
- Allen, E.B., Steers, R.J., Dickens, S.J., 2011. Impacts of fire and invasive species on desert soil ecology. *Rangel. Ecol. Manag.* 64, 450–462.
<https://doi.org/10.2111/REM-D-09-00159.1>
- Allison, S.D., Vitousek, P.M., 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biol. Biochem.* 37, 937–994.
- Bailey, A.W., Anderson, M.L., 2014. Medium term evolution of soil aggregate stability, organic matter and calcium carbonate of a Mediterranean soil burned at two different fire intensities. *Geoderma* 6, 2680–2685.
<https://doi.org/10.1016/j.catena.2013.10.002>
- Bailey, A.W., Anderson, M.L., 1980. Fire Temperatures in Grass, Shrub and Aspen Forest Communities of Central Alberta. *J. Range Manag.* 33, 37–40.
<https://doi.org/10.2307/3898225>
- Baker, V.R., 2009. The Channeled Scabland: A Retrospective. *Annu. Rev. Earth Planet. Sci.* 37, 393–411. <https://doi.org/10.1146/annurev.earth.061008.134726>

- Baker, W.L., 2011. Pre–Euro-American and recent fire in sagebrush ecosystems, in: Knick, S.T., Connelly, J.W. (Eds.), *Greater Sage-Grouse: Ecology and Conservation of a Landscape Species and Its Habitats*. *Studies in Avian Biology* (Vol. 38). University of California Press, Berkeley, CA, pp. 185–205.
- Baker, W.L., 2006. Fire and Restoration of Sagebrush Ecosystems. *Wildl. Soc. Bull.* 34, 177–185. [https://doi.org/10.2193/0091-7648\(2006\)34\[177:FAROSE\]2.0.CO;2](https://doi.org/10.2193/0091-7648(2006)34[177:FAROSE]2.0.CO;2)
- Bárcenas-Moreno, G., García-Orenes, F., Mataix-Solera, J., Mataix-Beneyto, J., Bååth, E., 2011. Soil microbial recolonisation after a fire in a Mediterranean forest. *Biol. Fertil. Soils* 47, 261–272. <https://doi.org/10.1007/s00374-010-0532-2>
- Blank, R.R., ClementsTye, C., Harmon, M., 2017. Sagebrush Wildfire Effects on Surface Soil Nutrient Availability: A Temporal and Spatial Study. *Soil Sci. Soc. Am. J.* 81, 1203–1210. <https://doi.org/10.2136/sssaj2017.02.0039>
- Blank, R.R., Morgan, T., 2011. Evidence that Invasion by Cheatgrass Alters Soil Nitrogen Availability. *Nat. Resour. Environ. Issues* 17.
- Caon, L., Vallejo, V.R., Coen, R.J., Geissen, V., 2014. Effects of wildfire on soil nutrients in Mediterranean ecosystems. *Earth-Science Rev.* 139, 47–58. <https://doi.org/10.1016/j.earscirev.2014.09.001>
- Carreira, J.A., Niell, F.X., Lajtha, K., 1994. Soil Nitrogen Availability and Nitrification in Mediterranean Shrublands of Varying Fire History and Successional Stage. *Biogeochemistry* 26, 189–209.
- Certini, G., 2005. Effects of fire on properties of forest soils: A review. *Oecologia* 143, 1–10. <https://doi.org/10.1007/s00442-004-1788-8>
- Certini, G., Nocentini, C., Knicker, H., Arfaioli, P., Rumpel, C., 2011. Wildfire effects on soil organic matter quantity and quality in two fire-prone Mediterranean pine forests. *Geoderma* 167–168, 148–155. <https://doi.org/10.1016/j.geoderma.2011.09.005>

- Chambers, J.C., Beck, J.L., Bradford, J.B., Bybee, J., Campbell, S., Carlson, J., Christiansen, T.J., Clause, K.J., Collins, G., Crist, M.R., Dinkins, J.B., Doherty, K.E., Edwards, F., Espinosa, S., Griffin, K.A., Griffin, P., Haas, J.R., Hanser, S.E., Havlina, D.W., Henke, K.F., Hennig, J.D., Joyce, L.A., Kilkenny, F.F., Kulpa, S.M., Kurth, L.L., Maestas, J.D., Manning, M., Mayer, K.E., Meador, B.A., McCarthy, C., Pellant, M., Perea, M.A., Prentice, K.L., Pyke, D.A., Wiechman, L.A., Wuenschel, A., 2017. Science framework for conservation and restoration of the sagebrush biome: Linking the department of the interior's integrated rangeland fire management strategy to long-term strategic conservation actions Part 1: Science Basis and Applications. USDA For. Serv. - Gen. Tech. Rep. RMRS-GTR 1-217.
- Chambers, J.C., Bradley, B.A., Brown, C.S., D'Antonio, C., Germino, M.J., Grace, J.B., Hardegree, S.P., Miller, R.F., Pyke, D.A., 2014a. Resilience to stress and disturbance, and resistance to *Bromus tectorum* L. invasion in cold desert shrublands of western North America. *Ecosystems* 17, 360–375.
<https://doi.org/10.1007/s10021-013-9725-5>
- Chambers, J.C., Miller, R.F., Board, D.I., Pyke, D.A., Roundy, B.A., Grace, J.B., Schupp, E.W., Tausch, R.J., 2014b. Resilience and Resistance of Sagebrush Ecosystems: Implications for State and Transition Models and Management Treatments. *Rangel. Ecol. Manag.* 67, 440–454. <https://doi.org/10.2111/REM-D-13-00074.1>
- Chambers, J.C., Roundy, B.A., Blank, R.R., Meyer, S.E., Whittaker, A., 2007. What Makes Great Basin Sagebrush Ecosystems Invasible by *Bromus tectorum*? *Ecol. Monogr.* 77, 117–145.
- Chen, S., Peng, S., Chen, B., Chen, D., Cheng, J., 2010. Effects of fire disturbance on the soil physical and chemical properties and vegetation of *Pinus massoniana* forest in south subtropical area. *Acta Ecol. Sin.* 30, 184–189.
<https://doi.org/10.1016/j.chnaes.2010.04.010>
- Dangi, S.R., Stahl, P.D., Pendall, E., Cleary, M.B., Buyer, J.S., 2010. Recovery of soil microbial community structure after fire in a sagebrush-grassland ecosystem. *L. Degrad. Dev.* 21, 423–432. <https://doi.org/10.1002/ldr.975>

- DeLuca, T.H., Sala, A., 2006. Frequent fire alters nitrogen transformations in ponderosa pine stands of the Inland Northwest. *Ecology* 87, 2511–2522.
[https://doi.org/10.1890/0012-9658\(2006\)87\[2511:FFANTI\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2006)87[2511:FFANTI]2.0.CO;2)
- Doane, T.A., Horwath, W.R., 2003. Spectrophotometric determination of nitrate with a single reagent. *Anal. Lett.* 36, 2713–2722.
- Dobler, F.C., Eby, J., Perry, C., Richardson, S., Haegen, M. Vander, 1996. Status of Washington's Shrub-Steppe Ecosystem: Extent, ownership, and wildlife/vegetation relationships.
- Ehrenfeld, J., 2003. Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* 6, 503–523.
- Fonseca, F., de Figueiredo, T., Nogueira, C., Queirós, A., 2017. Effect of prescribed fire on soil properties and soil erosion in a Mediterranean mountain area. *Geoderma* 307, 172–180. <https://doi.org/10.1016/j.geoderma.2017.06.018>
- Fontúrbel, M.T., Barreiro, A., Vega, J.A., Martín, A., Jiménez, E., Carballas, T., Fernández, C., Díaz-Raviña, M., 2012. Effects of an experimental fire and post-fire stabilization treatments on soil microbial communities. *Geoderma* 191, 51–60.
<https://doi.org/10.1016/j.geoderma.2012.01.037>
- Forster, J.C., 1995. Soil nitrogen., in: Alef, K., Nannipieri, P. (Eds.), *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, San Diego, CA, USA, pp. 79–87.
- Francos, M., Úbeda, X., Pereira, P., Alcañiz, M., 2018. Long-term impact of wildfire on soils exposed to different fire severities. A case study in Cadiretes Massif (NE Iberian Peninsula). *Sci. Total Environ.* 615, 664–671.
<https://doi.org/10.1016/j.scitotenv.2017.09.311>
- Fryxell, R., 2012. The late Wisconsin age of mounds on the Columbia Plateau of eastern Washington., in: Horwath Burnham, J., Johnson, D. (Eds.), *Mima Mounds: The Case for Polygenesis and Bioturbation*. The Geological Society of America, Boulder (CO), pp. 161–172.

- Fuentes-Ramirez, A., Schafer, J.L., Mudrak, E.L., Schat, M., Parag, H.A., Holzapfel, C., Moloney, K.A., 2015. Spatio-temporal impacts of fire on soil nutrient availability in *Larrea tridentata* shrublands of the Mojave Desert, USA. *Geoderma* 259–260, 126–133. <https://doi.org/10.1016/j.geoderma.2015.05.016>
- Girona-García, A., Ortiz-Perpiñá, O., Badía-Villas, D., Martí-Dalmau, C., 2018. Effects of prescribed burning on soil organic C, aggregate stability and water repellency in a subalpine shrubland: Variations among sieve fractions and depths. *Catena* 166, 68–77. <https://doi.org/10.1016/j.catena.2018.03.018>
- Guénon, R., Vennetier, M., Dupuy, N., Roussos, S., Pailler, A., Gros, R., 2013. Trends in recovery of mediterranean soil chemical properties and microbial activities after infrequent and frequent wildfires. *L. Degrad. Dev.* 24, 115–128. <https://doi.org/10.1002/ldr.1109>
- Hao, X., Chang, C., 2002. Effect of 25 annual cattle manure applications on soluble and exchangeable cations in soil. *Soil Sci.* 167, 126–134. <https://doi.org/10.1097/00010694-200202000-00005>
- Hart, S.C., DeLuca, T.H., Newman, G.S., MacKenzie, M.D., Boyle, S.I., 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. *For. Ecol. Manage.* 220, 166–184. <https://doi.org/10.1016/j.foreco.2005.08.012>
- Hobbs, N.T., Schimel, D.S., 1984. Fire Effects on Nitrogen Mineralization and Fixation in Mountain Shrub and Grassland Communities. *J. Range Manag.* 37, 402–405. <https://doi.org/10.2307/3899624>
- Hoogsteen, M.J.J., Lantinga, E.A., Bakker, E.J., Groot, J.C.J., Tiftonell, P.A., 2015. Estimating soil organic carbon through loss on ignition: Effects of ignition conditions and structural water loss. *Eur. J. Soil Sci.* 66, 320–328. <https://doi.org/10.1111/ejss.12224>
- Johnson, B.G., Johnson, D.W., Chambers, J.C., Blank, R.R., 2011. Fire effects on the mobilization and uptake of nitrogen by cheatgrass (*Bromus tectorum* L.). *Plant Soil* 341, 437–445. <https://doi.org/10.1007/s11104-010-0656-z>

- Jones, R.O., Chambers, J.C., Board, D.I., Johnson, D.W., Blank, R.R., 2015. The role of resource limitation in restoration of sagebrush ecosystems dominated by cheatgrass (*Bromus tectorum*). *Ecosphere* 6, 1–21. <https://doi.org/10.1890/ES14-00285.1>
- Kennard, D.K., Gholz, H.L., 2001. Effects of high- and low-intensity fires on soil properties and plant growth in a Bolivian dry forest. *Plant Soil* 234, 119–129. <https://doi.org/10.1023/A:1010507414994>
- Knelman, J.E., Graham, E.B., Ferrenberg, S., Lecoivre, A., Labrado, A., Darcy, J.L., Nemergut, D.R., Schmidt, S.K., 2017. Rapid shifts in soil nutrients and decomposition enzyme activity in early succession following forest fire. *Forests* 8. <https://doi.org/10.3390/f8090347>
- Knicker, H., 2007. How does fire affect the nature and stability of soil organic nitrogen and carbon? A review. *Biogeochemistry* 85, 91–118. <https://doi.org/10.1007/s10533-007-9104-4>
- Kyveryga, P.M., Blackmer, A.M., Ellsworth, J.W., Isla, R., 2004. Soil pH effects on nitrification of fall-applied anhydrous ammonia. *Soil Sci. Soc. Am. J.* 68, 545–551.
- Lesica, P., Cooper, S. V, Kudray, G., 2007. Recovery of Big Sagebrush Following Fire in Southwest Montana. *Rangel. Ecol. Manag.* 60, 261–269.
- Martí-Roura, M., Casals, P., Romanyà, J., 2013. Long-term retention of post-fire soil mineral nitrogen pools in Mediterranean shrubland and grassland. *Plant Soil* 371, 521–531. <https://doi.org/10.1007/s11104-013-1714-0>
- McCauley, A., Jones, C., Jacobsen, J., 2005. Basic Soil Properties. *Soil Water* 1–12.
- McIver, J., Brunson, M., Bunting, S., Chambers, J., Devoe, N., Doescher, P., Grace, J., Johnson, D., Knick, S., Miller, R., 2010. The Sagebrush Steppe Treatment Evaluation Project (SageSTEP): A Test of State-and-Transition Theory (General Technical Report RMRS-GTR-237). Fort Collins, CO.
- Menakis, J.P., Osborne, D., Miller, M., 2003. Mapping the Cheatgrass-Caused Departure From Historical Natural Fire Regimes in the Great Basin, USA--USDA Forest Service Proceedings RMRS-P-29.

- Miller, R., Chambers, J.C., Pyke, D.A., Pierson, F.B., Williams, J.C., 2013. A review of fire effects on vegetation and soils in the Great Basin region: Response and site characteristics (General Technical Report RMRS-GTR-308). Fort Collins, CO.
- Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide - Biol. Chem.* 5, 62–71. <https://doi.org/10.1006/niox.2000.0319>
- Mitchell, R.M., Bakker, J.D., Vincent, J.B., Davies, G.M., 2017. Relative importance of abiotic, biotic, and disturbance drivers of plant community structure in the sagebrush steppe. *Ecol. Appl.* 27, 756–768. <https://doi.org/10.1002/eap.1479>
- Muñoz-Rojas, M., Erickson, T.E., Martini, D., Dixon, K.W., Merritt, D.J., 2016. Soil physicochemical and microbiological indicators of short, medium and long term post-fire recovery in semi-arid ecosystems. *Ecol. Indic.* 63, 14–22. <https://doi.org/10.1016/j.ecolind.2015.11.038>
- Neary, D.G., Klopatek, C.C., DeBano, L.F., Ffolliott, P.F., 1999. Fire effects on belowground sustainability: A review and synthesis. *For. Ecol. Manage.* 122, 51–71. [https://doi.org/10.1016/S0378-1127\(99\)00032-8](https://doi.org/10.1016/S0378-1127(99)00032-8)
- Neary, D.G., Ryan, K.C., DeBano, L.F. (Eds.), 2005. *Wildland fire in ecosystems: effects of fire on soils and water*, General Technical Report - Rocky Mountain Research Station, USDA Forest Service. Ogden, UT. <https://doi.org/10.2737/RMRS-GTR-42-V4>.
- Norton, J., Monaco, T., Norton, U., 2007. Mediterranean annual grasses in western North America: kids in a candy store. *Plant Soil* 298, 1–5.
- NRCS, 2015. Web Soil Survey [WWW Document]. Nat. Resour. Conserv. Serv. United States Dep. Agric. URL <https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm> (accessed 9.10.18).
- Perry, L.G., Blumenthal, D.M., Monaco, T.A., Paschke, M.W., Redente, E.F., 2010. Immobilizing nitrogen to control plant invasion. *Oecologia* 163, 13–24. <https://doi.org/10.1007/s00442-010-1580-x>

- Poulin, P., Pelletier, É., 2007. Determination of ammonium using a microplate-based fluorometric technique. *Talanta* 71, 1500–1506.
<https://doi.org/10.1016/j.talanta.2006.07.024>
- Pyke, D.A., Chambers, J.C., Pellant, M., Knick, S.T., Miller, R.F., Beck, J.L., Doescher, P.S., Schupp, E.W., Roundy, B.A., Brunson, M., McIver, J.D., 2015a. Restoration Handbook for Sagebrush Steppe Ecosystems with Emphasis on Greater Sage-Grouse Habitat — Part 1 . Concepts for Understanding and Applying Restoration Circular 1416.
- Pyke, D.A., Knick, S.T., Chambers, J.C., Pellant, M., Miller, R.F., Beck, J.L., Doescher, P.S., Schupp, E.W., Roundy, B.A., Brunson, M., McIver, J.D., 2015b. Restoration Handbook for Sagebrush Steppe Ecosystems with Emphasis on Greater Sage-Grouse Habitat - Part 2 Landscape Level Restoration Decisions.
- Pyke, D.A., Shaff, S.E., Lindgren, A.I., Schupp, E.W., Doescher, P.S., Chambers, J.C., Burnham, J.S., Huso, M.M., 2014. Region-Wide Ecological Responses of Arid Wyoming Big Sagebrush Communities to Fuel Treatments. *Rangel. Ecol. Manag.* 67, 455–467. <https://doi.org/10.2111/REM-D-13-00090.1>
- Rau, B.M., Blank, R.R., Chambers, J.C., Johnson, D.W., 2007. Prescribed fire in a Great Basin sagebrush ecosystem: Dynamics of soil extractable nitrogen and phosphorus. *J. Arid Environ.* 71, 362–375. <https://doi.org/10.1016/j.jaridenv.2007.05.006>
- Rau, B.M., Chambers, J.C., Blank, R.R., Johnson, D.W., 2008. Prescribed fire, soil, and plants: Burn effects and interactions in the central Great Basin. *Rangel. Ecol. Manag.* 61, 169–181. <https://doi.org/10.2111/07-037.1>
- Rau, B.M., Chambers, J.C., Pyke, D.A., Roundy, B.A., Schupp, E.W., Doescher, P., Caldwell, T.G., 2014. Soil Resources Influence Vegetation and Response to Fire and Fire-Surrogate Treatments in Sagebrush-Steppe Ecosystems. *Rangel. Ecol. Manag.* 67, 506–521. <https://doi.org/10.2111/REM-D-14-00027.1>
- Reynolds, H.L., Packer, A., Bever, J.D., Clay, K., 2003. Grassroots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology* 84, 2281–2291. <https://doi.org/10.1890/02-0298>

- Rundel, P., 1983. Impact of Fire on Nutrient Cycles in Mediterranean-Type Ecosystems with Reference to Chaparral, in: F.J., K., D.T., M., J.U.M., J. (Eds.), *Mediterranean-Type Ecosystems: The Role of Nutrients*. Springer-Verlag, Berlin, Heidelberg, p. 556. https://doi.org/10.1007/978-3-642-68935-2_11
- Saetre, P., Stark, J.M., 2005. Microbial dynamics and carbon and nitrogen cycling following re-wetting of soils beneath two semi-arid plant species. *Oecologia* 142, 247–260.
- Shinneman, D.J., McIlroy, S.K., 2016. Identifying key climate and environmental factors affecting rates of post-fire big sagebrush (*Artemisia tridentata*) recovery in the northern Columbia Basin, USA. *Int. J. Wildl. Fire* 25, 933–945. <https://doi.org/http://dx.doi.org/10.1071/WF16013>
- Shinneman, D.J., McIlroy, S.K., de Graaff, M.-A., n.d. Disentangling the effects of multiple fires on spatially interspersed sagebrush (*Artemisia* spp.) communities. *J. Veg. Sci.* (in-press).
- Six, J., Paustian, K., Elliott, E.T., Combrink, C., 2000. Soil Structure and Organic Matter: I. Distribution of Aggregate-Size Classes and Aggregate-Associated Carbon. *Soil Sci. Soc. Am. J.* 64, 681–689.
- Stark, J., Norton, J., 2015. The invasive annual cheatgrass increases nitrogen availability in 24-year-old replicated field plots. *Oecologia* 177, 799–809.
- Thompson, J., 2007. Sagebrush in western North America: habitats and species in jeopardy. *PNW Sci. Find.* 1–6.
- Ulery, A.L., Graham, R.C., Amrhein, C., 1993. Wood-ash composition and soil pH following intense burning. *Soil Sci.* 156, 358–364.
- van Diepen, L.T.A., Frey, S.D., Sthultz, C.M., Morrison, E.W., Minocha, R., Pringle, A., Peters, D.P.C., 2015. Changes in litter quality caused by simulated nitrogen deposition reinforce the N-induced suppression of litter decay. *Ecosphere* 6, 1–16. <https://doi.org/10.1890/ES15-00262.1>

- Vega, J.A., Fontúrbel, T., Merino, A., Fernández, C., Ferreiro, A., Jiménez, E., 2013. Testing the ability of visual indicators of soil burn severity to reflect changes in soil chemical and microbial properties in pine forests and shrubland. *Plant Soil* 369, 73–91. <https://doi.org/10.1007/s11104-012-1532-9>
- Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Human Alteration of the Global Nitrogen Cycle: Sources. *Source Ecol. Appl. Ecol. Appl. Ecol. Appl.* 7, 737–750. [https://doi.org/10.1890/1051-0761\(1997\)007\[0737:HAOTGN\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2)
- Weintraub, S.R., Wieder, W.R., Cleveland, C.C., Townsend, A.R., 2013. Organic matter inputs shift soil enzyme activity and allocation patterns in a wet tropical forest. *Biogeochemistry* 114, 313–326. <https://doi.org/10.1007/s10533-012-9812-2>
- Whalen, J.K., Chang, C., 2002. Macroaggregate Characteristics in Cultivated Soils after 25 Annual Manure Applications. *Soil Sci. Soc. Am. J.* 66, 1637–1647. <https://doi.org/10.2136/sssaj2002.1637>
- Whisenant, S.G., 1990. Changing fire frequencies on Idaho's Snake River plains: ecological and management implications. *Symp. Cheatgrass Invasion* 4–10. [https://doi.org/10.1016/0006-3207\(92\)90659-B](https://doi.org/10.1016/0006-3207(92)90659-B)
- Wright, A.L., Wang, Y., Reddy, K.R., 2008. Loss on Ignition Method to Assess Soil Organic Carbon in Calcareous Everglades Wetlands. *Commun. Soil Sci. Plant Anal.* 39, 3074–3083.
- Zavala, L.M., de Celis, R., Jordán, A., 2014. How Wildfires Affect Soil Properties. A Brief Review. *Cuad. Investig. Geográfica* 40, 311–331. <https://doi.org/10.18172/cig.2522>