FIRE EFFECTS ON SOIL PROPERTIES: AMENDING POST-FIRE SOILS WITH NATIVE MICROBIAL COMMUNITIES AND BIOCHAR TO IMPROVE SAGEBRUSH PERFORMANCE

by

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DEDICATION

Rooted in my deepest passion to conserve and enhance habitat so that wildlife, as well as human health and enjoyment, may prosper, this work is rightfully dedicated to the vastly beautiful wild places that I cherish and live to protect. Additionally, I dedicate this work to my supportive loved ones, who have encouraged me to pursue these unstoppable passions.

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ABSTRACT

Within the sagebrush steppe, fire has been shown to affect biogeochemical properties and the microbial community composition in soils. However, there is uncertainty about the magnitude and direction of these effects, since they vary by sites that differ in abiotic and biotic conditions. Moreover, differences in post-fire management strategies are likely to mediate the effect of fire on soil properties, thus further compounding this uncertainty. Any changes in soil biogeochemical properties following fire can prevent successful restoration of *Artemisia tridentata* sp. *wyomingensis* (sagebrush), leading to variable outcomes of restoration success in the sagebrush steppe. Previous research has shown that addition of native soil microbes and biochar can improve ecosystem restoration efforts, but the effects of these soil amendments on post-fire soil properties and sagebrush performance across sites are uncertain. With this study, I investigated how fire impacts soil properties (i.e., soil organic matter (SOM), soil structure, carbon (C) and nitrogen (N) concentrations, soil pH, net mineral N, microbial richness and composition) at a variety of sites that differ in time since exposure to fire, post-fire plant communities, and post-fire site management. I then implemented a greenhouse study to evaluate how amending soils with native microbial community inocula and biochar impact soil properties of the post-burn sites and sagebrush germination and growth. Taken together, these findings capture the influences of multiple fires and separate management strategies on soil properties, and how certain soil amendments may redirect soil recovery to aid in sagebrush restoration.

In my first chapter, I asked two questions: (1) how does fire affect soil biochemical properties across sites that differ in fire history, post-fire plant communities, and post-fire site management, and (2) how does fire affect soil microbial richness and community composition across sites that differ in post-fire plant communities, and post-fire site management. To assess these questions, soils were collected from three south of Boise, Idaho within the Orchard Combat Training Center (OCTC) that contrasted in fire history, plant community, and post-fire management. The northern part of the Union Fire (180 acres; hereafter: UFN2011) burned in 2011, and was treated with a mix of imazapic and glyphosate during the spring of 2019, after which sagebrush was handplanted 8 months later that yea. The southern part of the Union Fire (160 acres; hereafter: UFS2011) burned in 2011 and seeded with native grass species and planted with sagebrush. The Christmas Fire (hereafter: CF2018) burned in 2018, and was subjected to the same seeding and handplanting treatments as UFS2011. At each site, I selected five locations within the perimeter of the burn, and five locations outside the burn, representing the unburned control plots. In these unburned control plots, the five locations were stratified by sagebrush canopy and interspace microsites separately. At each one of the five locations, I collected four soil cores (10cm depth, 2.5cm diameter). I evaluated differences in soil pH, soil organic matter (SOM), soil carbon (C) and nitrogen (N) contents, soil structure, N cycling, and soil microbial communities between burned and unburned sites. Fire reduced SOM and soil C contents, and these losses were greater in burned areas that received an herbicide treatment. This suggests that suppression of plant growth using herbicides may limit the recovery of soil properties that are foundational to sagebrush steppe ecosystem functioning. Furthermore, I found a loss of

arbuscular mycorrhizal fungi (AMF) richness with fire and significant changes in soil microbial community structure when herbicide had been used. Finally, increased soil mineral N concentrations across all burned sites indicate that fire may significantly reduce ecosystem stability and increase the risk of invasion. These changes in soil properties are likely to lead to a persistent ecosystem state-changes in the sagebrush steppe, and future studies should evaluate which management approaches could be used to restore both soils and plant communities.

In my second chapter, I investigated two management approaches that may be used to restore the soils and plant communities impacted by fire. I asked (1) How does a live native soil microbial inoculum impact sagebrush performance and soil properties, (2) how do biochar additions impact sagebrush performance and soil properties, and (3) how does prior management (e.g., herbicide) mediate the impact of soil microbial inoculation and biochar amendment on sagebrush performance? A full factorial greenhouse experiment was conducted for three months with soils collected from the three post-burn sites described in Chapter 1. In the greenhouse experiment I incorporated the following treatments: (1) sterile native inoculum [-Inoculum] and no biochar [-Biochar], (2) live native inoculum [+Inoculum] and no biochar [-Biochar], (3) sterile native inoculum [-Inoculum] and biochar [+Biochar], and (4) live native inoculum [+Inoculum] and biochar [+Biochar]. Inocula was derived from sagebrush canopies at unburned sites and either added as live native inocula or autoclaved to sterilize the microbial community. Biochar was crushed into <1 cm pieces and distributed onto the surface of pots that contained that treatment. Ten sagebrush seeds where planted, watered daily until cotyledons showed, and continually monitored throughout the growing period. Germination, soil moisture

content and pH, above- and below-ground measurements, total mineral N, fungal root colonization proportional abundances, and microbial richness and composition were assessed. I found that inoculations did not significantly benefit sagebrush performance, most likely due to the ratio of inocula administered. In contrast, biochar consistently enhanced soil moisture, pH, sagebrush germination and other performance variables while its effects on total mineral N and fungal root colonization varied by site location. Lastly, presence of herbicide in post-burn soils significantly altered soil bacterial and fungal community composition, and its effects persisted enough to inhibit sagebrush performance. Together, my data show that addition of biochar has a greater positive impact on sagebrush germination and performance than addition of soil microbial inocula, and that herbicide addition has persistent negative impacts on sagebrush performance.

My study captured the varying levels at which fire impacts ecosystem structure and function, and how different soil amendments affected sagebrush performance at these post-fire soils. My findings support the notion that soil properties will remain degraded without appropriate management strategies supporting restoration, and herbicide may actually suppress successful restoration, residing longer in the soil than previously documented. When growing sagebrush in post-burn soils within the greenhouse, biochar enabled soil recovery, and this benefited sagebrush performance. However, herbicide impacts persisted and decreased sagebrush biomass even when soil amendments were incorporated. Fire can have profound, yet vastly different, influences on soil properties, and soil amendments may be able to augment soil recovery. Future studies should investigate various soil amendments and their impacts on sagebrush performance in the

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AMF	arbuscular mycorrhizal fungi
BSA	bovine serum albumin
C	Carbon
CNPY	canopy (within the dripline of mature sagebrush)
$C_7H_5NaO_3$	sodium salicylate
CF2018	Christmas Fire of 2018
FDR	false discovery rate
HCl	hydrochloric acid
HRP	horseradish peroxide
IANG	Idaho Army National Guard
INSP	interspace (areas in between mature sagebrush)
ITS	internal transcribed spacer
KCl	potassium chloride
N	Nitrogen
NaOH	sodium hydroxide
OCTC	Orchard Combat Training Center
PBS	phosphate buffer solution
PCR	polymerase chain reaction
RDP	Ribosomal Database Project
SOM	soil organic matter

SV	sequence variant
UFN2011	Union Fire North of 2011
UFS2011	Union Fire South of 2011
USA	United States of America
VCl_3	vanadium chloride solution
WHC	water holding capacity

CHAPTER ONE: FIRE EFFECTS ON SOIL PROPERTIES IN THE SAGEBRUSH STEPPE ECOSYSTEM

1. Introduction

Sagebrush-steppe ecosystems of the intermountain west in North America have been dramatically affected by increased fires, influencing resilience of native species (Knapp, 1996; Xian et al., 2012; Bradford et al., 2014). Invasive, non-native species, such as cheatgrass (*Bromus tectorum* L.) and Russian thistle (*Kali tragus* (L.) Scop.), introduced centuries ago have heightened these negative landscape-altering fires, perpetuating a positive feedback loop that ultimately excludes reestablishment of native species, specifically Wyoming big sagebrush (*Artemisia tridentata* sp. *wyomingensis* Nutt., hereafter: sagebrush) (Knapp, 1996; Anderson and Inouye, 2001). Historic fire regimes now experience more severe and frequent fires, occurring every 10 years or less occasionally (Brooks et al. 2015), inhibiting sagebrush from recovering and reestablishing on the landscape (Johnson et al., 2011; Chambers et al., 2014). Although restoration initiatives have been implemented within burned, invaded areas, the degree of success has been highly variable and causes that underpin these impediments are largely unknown (Pyke et al., 2013; Chambers et al., 2019). Improving the understanding of how fire and subsequent changes in plant communities impact soil biochemical properties in the sagebrush steppe may aid in the restoration of these ecosystems, because above- and below-ground ecosystem components are tightly linked (Kardol and Wardle, 2010). While previous studies have assessed impacts of fire and changes in plant communities

on soil biochemical properties (Allen et al., 2011; Dickens and Allen, 2014; Jones et al., 2015a; Caspi et al., 2019), many studies focus on a limited number of soil properties, and have measured those at individual sites (Boxell and Drohan, 2009; Allen et al., 2011; Nichols et al., 2021). However, fire impacts a multitude of soil properties that together regulate feedbacks to plants, and these impacts on soil properties vary across sites which differ in abiotic and biotic site characteristics at the time of the fire.

Fire followed by non-native plant species invasion can drastically impact soil biogeochemical properties in semi-arid regions, and promote further invasion (Chambers et al., 2007; Blank et al., 2013; Nichols et al., 2021). Fire tends to reduce soil organic matter (SOM) contents (Norton et al., 2004a; Jones et al., 2015b; Nichols et al., 2021), which is a foundational soil property, because it controls soil carbon (C) and soil nitrogen (N) concentrations (Blank et al. 2017). SOM also promotes soil aggregate formation, structure, and moisture and nutrient retention (Mataix-Solera et al., 2011). In addition, fire-induced changes in the chemical composition of SOM, including greater presence of pyrogenic and aromatic compounds (Knicker, 2007; Caon et al., 2014), can increase soil pH, suggesting increased liming (Certini, 2005; Vega et al., 2012). While sagebrush can tolerate a wide range of pH fluctuations, a change in pH can impact biological functioning and soil nutrient dynamics (Fierer et al., 2012), thus impacting sagebrush indirectly. Fire also increases mineral N availability, which promotes the establishment of invasive annuals at enhanced rates after repeated fires (Chambers et al., 2007; Chambers et al., 2014; Jones et al., 2015a,b). For example, cheatgrass is able to profit quickly from the brief influx of N, but sagebrush, a species adapted to low-resource environments, cannot compete with this trait (Ehrenfeld, 2003; Saetre and Stark, 2005; Chambers et al.,

2007; Hooker et al., 2008; MacKown et al., 2009; Stark and Norton, 2015). In addition, as an early successional species, the litter quality of cheatgrass contains higher quantities of N compared to most native species (Evans et al., 2001), thus catalyzing the positive feedback loop that underlies the ecosystem state change (Gasch et al., 2015). Compounding all these factors can inhibit native plants from establishing.

Fire also impacts soil microbial community composition in semi-arid regions (Hamman et al., 2007; Capogna et al., 2009; Collins et al., 2016), and these changes can promote further invasion (Gehring et al., 2016; Rodriguez-Caballero et al., 2020). Changes in microbial community composition, such as a shift from fungal to bacterial dominated microbial communities (Klein et al., 1995; Fultz et al., 2016) have been observed across a range of fire severities (Hamman et al., 2007) and can be a direct result of fire through heat-induced microbial mortality, particularly in the top 5 cm of soil (Certini, 2005; Fontúrbel et al., 2012; Hart et al., 2005; Neary et al., 2005). However, some studies have indicated that soils are capable of recovering just years after fire has occurred (Dangi et al., 2010; Mũnoz-Rojas et al., 2013; Pérez-Valera et al., 2019). Longer term fire outcomes are often caused by indirect effects on the microbial community structure due to conversions in plant community compositions (Norton et al., 2004a) and associated soil property alterations (i.e., pH, soil moisture) (Prendergast-Miller et al., 2017). Particularly in the sagebrush steppe, there are spatially distinct microsites fertile with SOM that occur beneath sagebrush canopies, which provide more substrates to microbial organisms relative to barren interspaces between sagebrush canopies (Housman et al., 2007). Severe fires can remove these canopy microsites and alter microbial composition permanently (Bolton Jr. et al., 1993). This typically happens

due to changes in vegetation assemblies featuring more invasive annuals (Batten et al., 2006; Weber, 2015). Thus, stratification of soil microbial structure and composition can also be significantly reduced within invaded areas, and if established long-term, legacy effects can present major challenges to native shrub reestablishment (Pickett et al., 2019).

The arbuscular mycorrhizal fungal (hereafter: AMF) composition can also be impacted by fire and invasion, with possible significant consequences for native species like sagebrush, which rely heavily on the symbiotic relationship for photosynthesis, nutrient acquisition, and water uptake (Safir et al., 1972; Menge et al., 1978; Lambert et al., 1979; Clark and Zeto, 2000; Carter et al., 2014). AMF (i.e., Glomeromycetes) is an obligate root symbiont for sagebrush that facilitates resource exchange through extensions of hyphae, vesicles, and arbuscules throughout plant roots (Smith and Read, 2008). Studies have observed that even moderate fires can eliminate fungal propagules from soil surfaces and temporarily reduce fungal colonization abilities (Pattinson et al., 1999). This can inhibit sagebrush reestablishment and promote the establishment of plants that do not exhibit mycorrhizal dependency, such as cheatgrass (Allen and Allen, 1984). In addition, even at low temperatures, fire disturbance can change the AMF community composition immediately post-fire relative to unburned areas in shrub-dominated ecosystems (Lozano et al., 2016). While direct fire effects on AMF community composition may be transient, invasion-induced displacement of AMF that are important for sagebrush survival, such as species within the Claroideoglomeraceae and Glomeraceae families, may have long-term consequences for sagebrush survival (Carter et al., 2014; Dierks et al., 2019; Reinhart and Rinella, 2021). Given the profound importance of AMF for native plant resilience and for restoration outcomes (Hovland et

al., 2019), quantification of changes in AMF community composition across distinct sites and management practices is essential to understanding native sagebrush communities and implementing successful restoration methods.

Restoration practices have varied and continue to vary in strategy and intensity within post-fire sagebrush communities (Keeley, 2006; Davies et al., 2011; Ott et al., 2017; Copeland et al., 2018). Herbicide is a common approach to controlling invasive species, targeting seedbanks and hindering establishment (Applestein et al., 2018; Davies et al., 2020). Studies have shown that administering an imazapic herbicide can significantly reduce invasive species seedbank densities, one of the major solutions for long-term management of cheatgrass suppression in the sagebrush steppe (Reinwald, 2013; Sebastian et al., 2017). Another benefit of herbicide treatment is the potential for sagebrush growth and survival to increase, as shown in a recent study by Grant-Hoffman and Plank (2021). However, herbicide treatments may also suppress restoration efforts and ultimately sagebrush reestablishment because of direct negative residual effects on native plants, or because herbicide-induced vegetation suppression decreases soil organic matter and resource availability to plants (Rau et al., 2014). Thus, in addition to evaluating effects of fire and changes in plant communities, it is important to assess the effect of post-fire herbicide applications on soil properties.

The aim of my study was to quantify how fire and plant community changes impact (1) soil biochemical properties (i.e., SOM contents, bulk and aggregate C and N concentrations, soil pH, and N mineralization), (2) microbial community structure (i.e., bacterial, fungal, and AMF community richness and composition), and (3) how post-management (e.g., herbicide treatment) affected these properties. The sagebrush steppe

sites selected within this study have experienced a multitude of fires in recent years, and were subjected to different post-fire restoration treatments, including differences in herbicide use. These different approaches, along with site-level characteristics, have led to highly variable restoration outcomes in terms of post-fire plant community composition. I hypothesized that burned areas would have altered soil structure due to decreased SOM content and soil C and N concentrations, increased soil pH and N mineralization, and a significantly different microbial community composition relative to unburned areas. Additionally, I hypothesized that herbicide would have significant effects on all soil properties due to decreased SOM content available. Pre- and post-fire SOM, soil structure (i.e., relative abundance of aggregates), bulk soil C and N, soil pH, N mineralization, and soil microbial communities were analyzed across three sites that varied in fire history, plant community composition, and site management (i.e., herbicide). By exploring multiple, contrasting fires, my study provides insight into the variability in soil property responses to fire and post-fire site management.

2. Methods

2.1 Study Site

The study site included areas impacted by fire and their adjacent unburned counterparts within the Orchard Combat Training Center (OCTC), approximately 32 km south of Boise, Idaho, USA. This region is part of the semiarid Great Basin Desert, it is located at approximately 823 m in elevation, and has a mean annual temperature of 11.0°C, a mean annual rainfall precipitation of approximately 29.79 cm, and a mean annual snowfall of 50.04 cm (Western Regional Climate Center, https://wrcc.dri.edu/). The geographic region is also part of the Snake River Plain, an arc-shaped depression

across southern Idaho shaped by balsaltic volcanism from erupting rhyolite lava fissures, and then filled with sedimentation from the Pliocene Lake Idaho (Shevaris et al., 2002). Volcanic rocks are scatted about the soils complex consisting of two prevalent soil types from the Chilcott series known as Chilcott-Catchell-Chardoton and Chilcott-Chardoton, which are silt loams very similar to one another. These soils are characterized by eroded volcanic ash over loamy alluvium and are well-drained with slow permeability (Ada County Soil Survey, https://www.nrcs.usda.gov/Internet/FSE_MANUSCRIPTS/idaho/AdaID1980/AdaID_1980.pdf).

Utilized mostly for pastureland at lower elevations, this area has experienced historic grazing, and is now primarily used for military combat training by the Idaho Army National Guard (IANG). Natural vegetation that typically supports this landscape includes big sagebrush (*Artemisia tridentata*, Nutt.) communities with other associating species, such as rubber rabbitbrush (*Ericameria nauseosa*, (Pall. ex Pursh) G. L. Nesom and G. I. Baird), and common grasses that occur here include bluebunch wheatgrass (*Pseudoregneria spicata*, (Pursh) Á.Löve) and Sandberg bluegrass (*Poa secunda*, J. Presl). With the increase in fire frequency, invasive species, such as cheatgrass and Russian thistle, have established considerable patches interspersed throughout the native vegetation. Many different entities manage parcels of OCTC area besides IANG, including the U.S. Bureau of Land Management and Idaho Department of Lands with varying management objectives, providing a diversity of strategies for operating on the landscape.

Three fires that varied in fire history, post-fire management treatments, and post-fire plant community composition were selected, and paired with an adjacent area of unburned sagebrush steppe (Map 1.1). The Christmas Fire (43° 19' 00.8" N, 116° 12' 08.6" W, hereafter: CF2018) was approximately 24 acres and burned in July of 2018. This site was broadcast-seeded post-fire with a seed mix containing native sagebrush and grasses that had little success establishing. Additional hand-plantings of 1-year old sagebrush seedling plugs were incorporated into the restoration effort. Total survival of sagebrush at this site was ~85% (IANG, personal communication, September 6, 2019), yet field observations in early November of 2019 confirmed obvious decreases in recruitment. The Union Fire (43° 31' 04.5" N, 116° 06' 03.4" W) burned approximately 340 acres in 2011, and has been fenced off into two separate sites, based on landownership and differences in post-fire management. Based on these differences in post-fire management, this burn was treated as two separate fires and referred to as: Union Fire North (hereafter: UFN2011) and Union Fire South (hereafter: UFS2011). Eight years following fire, the 180 acres of UFN2011 was contracted out by IANG to be broadcast treated via tractor with a mixture of 12.36 oz./hectare of imazapic (~0.08 lbs Panoramic 2SL a.i./acre) and 14.83 oz./hectare (~0.14 lbs Glyphosate 4 Plus a.i./acre) during the spring of 2019; however, only 50% of the total area was covered successfully (Zoe Duran, personal communication, June 15, 2021). Drill seeding and hand-plantings of 1-year old sagebrush seeding plugs took place in early November of 2019, after sufficient time had passed for the herbicide half-life. Three years post-fire, the 160 acres of UFS2011 was subjected to drill and broadcast seeding coupled with planting of live plants as described previously, but no herbicide was applied at this site. Sagebrush

recruitment following these interventions was ~12% as of 2019 and decreasing based on field observations.

2.2 Site Selection and Sampling

QGis (OSGeo, 2020) was used to explore spatially explicit fire shapefiles provided by IANG for sample plot selection, where a 100 x 100 m grid was overlayed on each fire shapefile. To represent post-fire conditions, five sample cells (i.e., plots) were randomly selected from each fire that were at least 100 m from fire boundary and 200 m apart from other plots. Because herbicide impacts were to be captured and only 50% of the total area at UFN2011 was administered herbicide, additional criteria for sample plot selection included selecting cells within sprayed zones that were mapped out by IANG. Since CF2018 was a small fire (i.e., 24 acres), replicate sample plots were positioned 60 m apart instead of 200 m to account for adequate independence among the replicates. Each fire site was paired with an unburned, adjacent area to represent pre-fire conditions; UFS2011 and UFN2011 shared the paired unburned site because that unburned adjacent area was closest for both. Five sampling plots were selected in the unburned areas based on the previously mentioned selection criteria for burned areas for each fire. Although these areas could have experienced prior burning, the mature sagebrush shrub community suggested that fire had not occurred for many years, which was supported through field observations.

Soil samples were collected to a depth of 0-10 cm using a hammer core (diameter ~2.5 cm, depth 10 cm) from each of the five replicated sampling plots dispersed throughout each burned and adjacent unburned pair for each site in early November 2019. Each replicate sampling plot within the burned and unburned locations consisted of five

composited subsamples arranged in cardinal directions from a central subsample point representing the sample plot. Subsamples were located within a 5 m radius surrounding the recorded sample point (i.e., central subsample) to ensure that spatial heterogeneity in soil properties was captured. Particularly for the unburned areas, two distinct microsites were stratified to account for differences in vegetation cover: (1) canopy (CNPY), and (2) interspace (INSP; Figure 1.1). Canopy samples were located by selecting five mature sagebrush plants located within a 5 m radius from the original recorded sample point and extracting soil samples from directly beneath their canopy crown. Interspace samples were located by sampling soil adjacent to each selected mature sagebrush plant that was >1 m from the canopy. Samples were stored on ice for transfer and kept at -20°C until further analysis.

2.3 SOM Content

SOM (0-5 cm depth) was quantified using loss-on-ignition method (Wright et al., 2008; Hoogsteen et al., 2015). Briefly, subsamples were sieved (2 mm) to homogenize soil. Rocks and roots greater than 2 mm were removed. A subsample of soil (10 g) was then placed into a ceramic crucible and oven dried at 105°C for 24 hours. Crucibles were then placed into a muffle furnace for four hours at 550°C. Percent SOM was calculated using the following equation:

$$\%SOM = (OD - FD) / (OD - CRU)$$

where, OD means oven dry (g), FD means furnace dry (g), and CRU means weight of crucible (g).

2.4 Bulk C and N Concentrations

Oven dried subsamples (40 µg) of bulk soil and of fractioned soils from all field-collected samples were weighed into titanium capsules for hydrochloric acid (HCl) fumigation for 24 hours to remove presence of inorganic C. Subsamples were redried at 60°C in an oven and then analyzed for total C and N concentration using a Carlo –Erba 1500 NA Elemental Analyzer (Milan, Italy), utilizing internal standard soils and aspartic acid for calibration. For these concentration measurements, unburned interspace microsites were excluded from these calculations.

2.5 Soil Structure

Soil aggregates are heterogeneous assemblages of organic and mineral particles operationally distinguished by size as macroaggregates (>250 µm), microaggregates (53-250 µm), and silt and clay (< 53 µm). Fractionating soil into aggregates allows for quantifying of soil structure, C allocation, and SOM distribution, which respectfully drives C storage, soil water availability, and nutrient retention. I fractionated the top 10 cm of soil for burned and unburned canopy microsite soils to determine C in macroaggregates, microaggregates within macroaggregates (i.e., water stable microaggregates), silt and clay size fractions. Aggregate-size separations were conducted on 100 g subsamples of soil by a wet-sieving method adapted from Elliott (1986). Three sieves (2 mm, 250 µm, and 53 µm) were used to obtain large and small macroaggregates, microaggregates within macroaggregates, and silt and clay-size isolated fractions, respectively. Before wet-sieving, soil samples were submerged in room temperature water on top of the 2 mm sieve for 5 min. During sieving, water-stable aggregates (i.e., resistant to slaking) were separated by manually moving the sieve up and down 3 cm

with 50 repetitions during a period of 2 min. Rocks and litter that remained on the sieve were transferred to individual tin containers for oven drying. Using the 250 µm sieve, large and small macroaggregates were isolated for in a tin container for oven drying, and the remaining subsample retained within the tub (i.e., water-stable aggregates, silt and clay-size factions) were transferred to the microaggregate isolator 53 µm sieve. Minimal breakup of microaggregates isolated from the macroaggregates was ensured by continuous water flow flushing the 250 µm sieve and by reducing scraping on the bottom of the tub. Microaggregates (>53 µm) were mechanically isolated using the microaggregate isolation-method described by Six et al., (2001), and material retained on the sieve was transferred to a tin container for oven drying. Remaining fraction that passed through the 53µm sieve were considered silt and clay particles and immediately were transferred to a separate tin for oven drying as well. Soil samples were oven dried at 160°C for 24 – 48 hours, and homogenized. Dry weights of fractions were recorded before being ball milled with the X Sample Prep Mixer/Mill (Metuchen, New Jersey) for further analyses.

2.6 Soil pH

Soil pH was quantified on three subsamples (15 g soil, field moist) from each of the composited soils. Subsamples were suspended in 30 mL deionized water, agitated for one minute to create a soil slurry, then allowed to rest for 30 minutes. To avoid variability in pH measurements after the 30-minute rest period, I agitated the soil slurry again immediately prior to reading with a pH meter (Oakton WD-35613-52 pH, Illinois, U.S.A.) and averaged reading three values.

2.7 Mineral N

N mineralization quantities was assessed by extracting soil samples with potassium chloride (KCl) before and after 7-day incubations. For both assays, subsamples of the soil (10 g fresh weight) were rewetted with water to attain 60% water holding capacity (WHC). Day 0 samples were extracted immediately after rewetting. Day 7 samples were incubated at 60% WHC, covered by plastic wrap in specimen cups in a dark undisturbed area for seven days. For mineral N extraction, samples were suspended in 50 mL of 2M KCl, and placed on a reciprocal shaker for 45 minutes. After shaking the samples, the slurry was poured through filter paper (Whatman No. 1, Germany) and the extract was collected in a vial. Extracts were kept at -20°C until ready for processing. Each extract was processed via a manual vanadium chloride solution (VCl_3) reduction for nitrate analysis, and a sodium salicylate ($C_7H_5NaO_3$) and bleach/sodium hydroxide (NaOH) solution for ammonium analysis (Miranda et al., 2001; Doane and Howarth, 2003; Poulin and Pelletier, 2007). Standards and samples were pipetted 0.6 mL and 0.8 mL into individual cuvettes to assess concentrations of nitrate-associated N (NO_3^--N) and ammonium-associated N (NH_4^+-N), respectively. For NO_3^--N, 0.6 mL of VCl_3 solution was administered, and for NH_4^+-N, 0.2 mL of each reagent was added. The absorbance of each reaction solution was determined with a spectrophotometer at 540 nm (NO_3^--N) and 650 nm (NH_4^+-N) wavelengths. Final available mineral N amounts were calculated and expressed as mg available N (NO_3^-N or NH_4^+-N) per gram of soil ($mgNO_3^-$-N g^-1 or $mgNH_4^-$-N g^-1). Total mineralized N was calculated using total Day 0 values, and net mineral N was calculated by subtracting total mg N g^-1 after incubation from mg N g^-1 before incubation.

2.8 Soil Community Analyses

Total community DNA was recovered from homogenized soil samples using the DNAeasy PowerSoil® Pro Kit (Qiagen Inc., Maryland, U.S.A.) with elution buffer. Sequence data was processed for bacterial and fungal material obtained from soil samples. Fungi was delineated into general fungi, which is defined as all the soil fungi present using an ITS primer set, and only AMF, which was defined as a subset of mycorrhizal fungi within the Glomeraceae families using an FLR primer set. For bacteria 16S rRNA, the V3-V4 hypervariable region was amplified using the universal bacterial primer set 341f (5'-CCTACGGGNGGCWGCAG-3') and 785r (5'-GACTACHVGGTATCTAATCC-3') (Klindworth et al., 2013). For general fungi using ITS, the internal transcribed spacer (ITS2) region was amplified using 5.8S-FUN (5'-AACTTTYRRCAAYGGATCWCT-3') and ITS4-FUN (5'-AGCCTCCGCTTATTGATATGCTTAART-3') primer set (Taylor et al., 2016). For only AMF FLR sequencing, the D2 region of the LSU rDNA was amplified using the FLR3 (5'-TTGAAAGGGAAACGATTGAAGT-3') and FLR4 (5'-TACGTCAACATCCTAACGAA-3') primer set (Lekberg et al., 2013). Primers included an overhang adapter to enable downstream addition of dual indexes for sample identification.

To amplify the 16S and ITS regions, the Q5 high-fidelity master mix (New England Biolabs M0492L) was used with the following thermocycler conditions: denaturation at 98°C for 1 minute followed by 30 cycles of denaturation at 98°C for 10 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension of 2 minutes. All polymerase chain reaction (PCR) products were cleaned

with magnetic beads (Rohland and Reich, 2012). Gel electrophoresis verified successful amplification and cleanup. In a second PCR, each sample PCR product was indexed with a unique pair of 8-nucleotide barcodes attached to the forward and reverse strands (Hamady et al., 2008). Reagents and thermocycler conditions were identical to the initial amplification except that the annealing temperature was decreased to 55°C and 8 amplification cycles were performed for each sample.

Library preparation methods for the FLR region were the same as those described above except for two modifications. First, in the initial PCR, samples were amplified for 35 cycles instead of 30 cycles. Second, because this primer set created primer dimers that were not removed by bead cleanup, gel extraction was instead performed on each first PCR product. Bands of the accepted size (~425-475 bp) were excised from the gel and purified using the GeneJET Gel Extraction Kit (Thermo K0692). The resulting gel extracts were used as a template for the final PCR.

After a second bead cleaning, the dual-indexed 16S rRNA, ITS, and FLR amplicons were quantified fluorometrically (Qubit DS DNA HS assay, Qiagen). For each primer set, an equal mass of DNA from each sample was pooled into a library. The three resulting libraries were each bidirectionally sequenced on an Illumina Mi-Seq platform at the University of Texas at Arlington using 2 × 300 chemistry.

2.9 Sequence Data Processing

The *cutadapt* package (Martin, 2011) was utilized to remove gene priming regions and adapters from the ends of the sequences. The resulting reads were processed using the *DADA2* package in R (Callahan et al., 2016). Reads were truncated at the first instance of a quality score less than or equal to 2 and reads that contained unassigned

bases were discarded. Next, low-quality reads were filtered out and filtering parameters were selected on a run-by-run basis. For the all MiSeq Illumina runs, forward reads with a maximum expected error rate (maxEE) greater than 3 and reverse reads with a maxEE greater than 8 were discarded.

To infer and correct for remaining sequencing errors, the *DADA2* algorithm was used to learn the run-specific sequencing error rates for forward and reverse reads and to clean the reads accordingly. Forward and reverse reads were merged, allowing up to three mismatches in overlapping regions. The *removeBimeraDenovo* function was used to detect and remove chimeras. For bacterial data, taxonomy was assigned using the ribosomal database project (RDP) classifier with the SILVA bacterial 16S database (ver. 138) as the training set (Wang et al., 2007; Quast et al., 2013). For ITS, the UNITE v8.2 database (Abarenkov et al., 2020) was used as the training set. For FLR, representative sequences were searched against the NCBI database using BLAST with a minimum e-value of 1e-50. For all runs, sequences that were respectively identified as non-bacterial (e.g., chloroplast & mitchondria), non-fungal (e.g., plant or animal), or non-arbuscular mycorrhizal were discarded. Finally, I used the R *decontam* package to identify and remove contaminants based on their presence in blanks.

2.10 Statistical Analyses

All statistical analyses were conducted in R. To assess SOM, bulk and aggregate soil C and N, pH, and N mineralization, three-way ANOVAs were conducted with site (i.e., CF2018, UFN2011, and UFS2011), burn treatment (i.e., burned and unburned), and microsite (i.e., unburned canopy, unburned interspace, burned) as fixed factors for soil properties, and interaction terms were checked for significance. When no interactions

were present, two-way ANOVAs were conducted on sites with burn treatment and microsite as fixed factors. Microsite names were abbreviated in figures such that unburned canopy is CNPY, unburned interspace is INSP, and burned is BURN. Tukey HDS multiple comparison tests were conducted for post-hoc analysis when site differences were evident. Levene's test for homogeneity of variance and Shapiro-Wilks test for normality of data were checked, and when variances were not homogenous, and data was not normal, data was log transformed in order to accommodate for normality.

Bacterial and fungal richness and composition were tested to assess differences among sites, between fire and herbicide treatments, and among microsites. Prior to data exploration, all datasets had rarefaction curves generated to identify appropriate rarefying depth and determine dominant bacteria and fungi. To adequately normalize the data, bacterial, general fungi, and only AMF data were rarefied to a depth of 6,662 base pairs, 13,619 base pairs, and 1,227 base pairs, respectively. Richness was evaluated by using chi-square linear models against fire, sites, herbicide, and microsites. Dissimilarity between bacterial and fungal species among sites, fire and herbicide treatments, and microsites were assessed using a non-parametric multivariate PERMANOVA test assuming equal dispersion within groups from the adonis test in the R package *vegan*. Finally, differential abundance of individual sequence variants (SVs) between sample groups was tested using the R package *DESeq2*.

3. Results

3.1 SOM

Fire reduced SOM content across all sites when compared to unburned CNPY microsites ($F_{1,35} = 21.58$, $p < 0.05$, Figure 1.2), but percent SOM in burned sites was

equal to percent SOM in unburned INSP microsites across all sites. Percent SOM averaged 4.2% for burned microsites, 3.7% for unburned INSP and 5.5% for unburned CNPY.

3.2 Bulk soil C and N

Bulk soil C-concentrations were affected by fire, but not across all sites ($F_{2,35}$ = 23.79, $p < 0.05$, Figure 1.3a). Reductions in bulk soil C-concentrations occurred in the UFN2011 site only, and only when the burned soil C-concentration was compared to the soil C-concentration in unburned CNPY microsites. In addition, bulk soil C was consistently higher in unburned CNPY than in unburned INSP microsites across all sites. There were few differences in bulk soil C-concentrations among sites, but within burned areas, CF2018 had a higher bulk soil C-concentration (13.0 mg C/g soil) than UFN2011 (6.8 mg C/g soil; $F_{2,35}$ =4.52, $p = 0.02$).

Similar to bulk soil C-concentrations, bulk soil N-concentrations were reduced by fire in the UFN2011 site only, and only when burned soil N-concentration was compared to bulk soil N-concentrations of unburned CNPY microsites ($F_{1,35} = 26.697$, $p < 0.05$, Figure 1.3b). Overall, higher concentrations of N were detected in unburned CNPY microsites than in unburned INSP microsites. Bulk soil N-concentrations in CF2018 or UFS2011 sites were similar for burned and unburned sites.

3.3 Soil aggregates

Soil fractionations were conducted only with soils collected from burned sites and their unburned CNPY microsite counterparts. Fire had no impact on the relative abundance of macroaggregates across sites (Figure 1.4a), but the relative abundance of microaggregates and silt and clay differed between burned and unburned areas in some

sites. Fire significantly decreased the relative abundances of microaggregates, but only within the UFN2011 site ($F_{2,23} = 3.87$, $p = 0.04$; Figure 1.4b). While fire did not impact the relative abundance of silt and clay in CF2018 and UFS2011 sites, the relative abundance of silt and clay in burned areas was higher than in unburned areas in the UFN2011 site ($F_{2,23} = 5.15$, $p = 0.01$; Figure 1.4c).

There were some effects of site on the relative abundance of soil fractions. Specifically, the relative abundance of macroaggregates differed by site, and was greater in CF2018 compared to UFN2011 for burned areas ($F_{2,23} = 4.91$, $p = 0.02$, Figure 1.4a). In addition, the relative abundances of microaggregates was greater in UFS2011 compared to UFN2011 ($F_{2,23} = 3.87$, $p = 0.04$, Figure 1.4b), and *vice versa* the relative abundances of silt and clay particles were smaller in UFS2011 compared to UFN2011 ($F_{2,23} = 5.15$, $p = 0.01$, Figure 1.4c), but only in burned sites.

The C-concentration of microaggregates (i.e., mg C / g fraction) was significantly reduced by fire at all sites ($F_{1,23} = 6.38$, $p = 0.02$, Figure 1.5a). The C-concentration in silt and clay fractions was only significantly reduced by fire within the UFN2011 site compared to unburned areas ($F_{1,23} = 4.77$, $p = 0.04$, Figure 1.5b).). In addition, soil N-concentrations of microaggregates were lower for UFN2011 than CF2018 ($F_{1,23} = 4.35$, $p = 0.03$; Figure 1.5c), but N-concentrations for silt and clay showed no significant differences between burned and unburned areas (Figure 1.5d).

The soil C-content of both microaggregates and silt and clay (i.e., the relative abundance of fraction of soil by weight multiplied by its C-concentration) was negatively impacted by fire within the UFN2011 fire ($F_{1,23} = 6.92$, $p = 0.03$; Figure 1.6).

3.4 Soil pH

Soil pH was not significantly impacted by fire when compared to unburned areas across all sites (Figure 1.7). There were differences among sites ($F_{2,35} = 5.47$, $p < 0.05$), where CF2018 had higher overall pH than other sites.

3.5 Soil mineral N

Total field NO_3^-- and NH_4^+-associated N was consistently greater in burned areas than unburned areas across all sites ($F_{1,35} = 23.15$, $p < 0.05$; Figure 1.8). After incubation, N mineralization in burned areas decreased by 114.88%, 97.83%, and 95.05% while mineral N in unburned areas decreased by only 53.00%, 36.61% and 36.61% in CF2018, UFN2011, and UFS2011, respectively.

3.6 Bacterial and Fungal Community Composition

A total of 4,684 SVs with 149 families encompassing 271 genera of bacteria were identified across all samples. The most common bacterial genus detected across all samples was *Streptomyces* sp. followed by a member of the order Thermomicrobiales, a member of the Bacilliaceae family, *Pseudarthrobacter* sp., and *Afipia* sp. There were no significant differences in bacterial richness among treatments or microsites ($p > 0.05$; Figure 1.9a). Bacterial species composition significantly differed between the UFS2011 and UFN2011 microsites (R^2 =0.16, $p < 0.05$; Figure 1.10a), but not at other sites or among treatments. There were four differentially abundant sequence variants among sites, and more specifically, at CF2018, 22 taxa differed between burned and unburned treatments (all $p < 0.001$ after FDR correction), and 16 taxa differed between UFS2011-burned, and UFN2011-burned, and unburned treatments (all $p < 0.001$ after FDR correction).

There were 3,925 SVs with 217 families and 389 genera of fungi identified across all samples. The most common fungal genus detected was *Penicillium* sp. followed by a member of the Dothideomycetes, *Penicillium decumbens, Naganishia vaughanmartiniae,* a Chaetomiaceae ASV*,* and *Coniochaeta* sp. Across all sites, fungal species richness was significantly decreased in burned treatments compared to unburned treatments ($F_{2,79} = 6.8$, $p < 0.05$; Figure 1.9b) and in burn microsites versus canopy and interspace microsites ($F_{2,79} = 7.8$, $p < 0.05$). Fungal species composition was significantly different between treatments ($R^2 = 0.12$, $p < 0.05$), microsites ($R^2 = 0.07$, $p < 0.05$), and sites ($R^2 = 0.04$, $p < 0.05$; Figure 1.10b). A total of 31 SVs differed between sites. At CF2018, 65 taxa differed between burned and unburned treatments, while at UFS2011-burned and UFN2011-burned, 80 taxa differed from the unburned treatment.

3.7 AMF Community Composition

When evaluating phylum Glomeromycota (i.e., AMF), a total of 277 SVs were identified across all samples, the most common genus being *Claroideoglomus* sp., followed by *Rhizophagus* sp., and *Septoglomus* sp. Across all sites, phylum Glomeromycota species richness was significantly decreased in the herbicide-treated area ($F_{2,79} = 4.13$, $p = 0.03$; Figure 1.9c). Additionally, composition only differed between CF2018 microsites ($R^2 = 0.30$, $p = 0.02$, Figure 1.10c), but not between burned and unburned treatments ($p = 0.25$). There were differential abundances of AMF microbiota between UFS2011-burned and UFN2011-burned compared to the unburned area, with a total of 13 taxa differing. Additionally, four AMF microbiota differed between UFS2011-burned and the herbicide site UFN2011-burned. Fifteen sequence variants differed in relative abundance between treatments at CF2018.

4. Discussion

This study yielded four major findings. First, fire significantly decreased SOM in burned sites relative to unburned sites, but only when compared to canopy microsites in unburned areas. Second, the combination of fire and herbicide application reduced bulk C and N and aggregate C-concentrations relative to canopy microsites. Third, N mineralization was increased post-fire across all sites. Fourth, fire decreased soil general fungal richness and altered its composition, but the addition of the herbicide treatment increased bacterial richness and changed AMF composition. These findings support the original hypothesis that fire and herbicide treatments alter soil characteristics.

Fire reduced SOM contents across all sites in burned areas relative to unburned canopy microsites. However, no reduction was observed relative to unburned interspace microsites, indicating that SOM in burned sites reflected SOM in unburned interspace microsites. Thus, fire created a more uniform landscape lacking the "islands of fertility" created by sagebrush canopies which are characteristic of the structure of this ecosystem (Minnick and Alward, 2015). My results also show that fire-induced loss of SOM is both an instantaneous and persistent process. Namely, I observed SOM loss in soils collected one-year post-fire (i.e., in CF2018 soils), and in soils collected 8 years post-fire (i.e., in UFN2011 and UFS2011 soils). Since SOM is a foundational property for soil quality, fire induced changes in SOM are likely to induce significant and persistent changes in ecosystem functioning (Neary et al., 1999; Norton et al., 2004b; Fanin et al., 2020). This can lead toto long-term nutrient depletion (Burke et al., 1989; Norton et al., 2004b), reduced soil structure, aeration, water-holding capacity, and cause fluctuations in pH (Hamblin and Davies, 1977; Acker, 1992). All of these changes in soil structure and

functioning are likely to influence native plant species ability to recolonize a burned site (Wilder et al., 2019).

The reduction in SOM content was not accompanied by a loss of soil C at the CF2018 and UFS2011 sites. Other studies have also found that fire-induced SOM loss does not necessarily affect soil C-concentrations, and this can be explained by low C combustion at the time of the fire (Neary et al., 1999; Allen et al., 2011; Flanagan et al., 2020), or by repayment of soil C following colonization by early successional plants (Brockway and Lewis, 1997; Bardgett and Wardle, 2003; Cleary et al., 2010). At the CF2018 site, the lack of a reduction in soil C might be explained by a relatively low fire severity as suggested by acreage (i.e., 24 acres total) and ample sagebrush skeletons remaining on the landscape following the fire, thus leading to low combustion of soil C and undetectable changes in the soil C pool. In addition, at the UFS2011 site, growth of early successional species may have repaid some of the C loss. In contrast, fire significantly reduced soil C in microaggregates and in silt and clay fractions at the UFN2011-site, likely owing to an herbicide-induced reduction in SOM from aboveground plant biomass and a reduction in soil C input through roots. These data suggest that fire impacts on soil C may be minimal, but that management practices, such as herbicide addition, can exacerbate the impact of fire on soil C.

Net soil nitrate- (NO_3^--N) and ammonium-associated (NH_4^+-N) N concentrations were significantly greater across all burned relative to unburned sites. Higher concentrations of NO_3^- following fire have been observed in sagebrush steppe ecosystems before (Neary et al., 1999; DeLuca and Sala, 2006; Rau et al., 2007; Martí-Roura et al., 2013) and they may increase the risk of exotic plant species invasions (Ehrenfeld, 2003;

Saetre and Stark, 2005; Chambers et al., 2007; Norton et al., 2007; Stark and Norton, 2015). Increased N availability favors plant species adapted to high resource availability, a trait associated with most invasive plant species (Vitousek et al., 1997; Norton et al., 2007; Leonard et al., 2008; Perry et al., 2010; Blank and Morgan, 2011; Johnson et al., 2011; Blank et al., 2017). These data indicate that fire across these sites is likely to significantly reduce ecosystem stability and increase the risk of invasion, and this risk may increase over time, regardless of site management following fire.

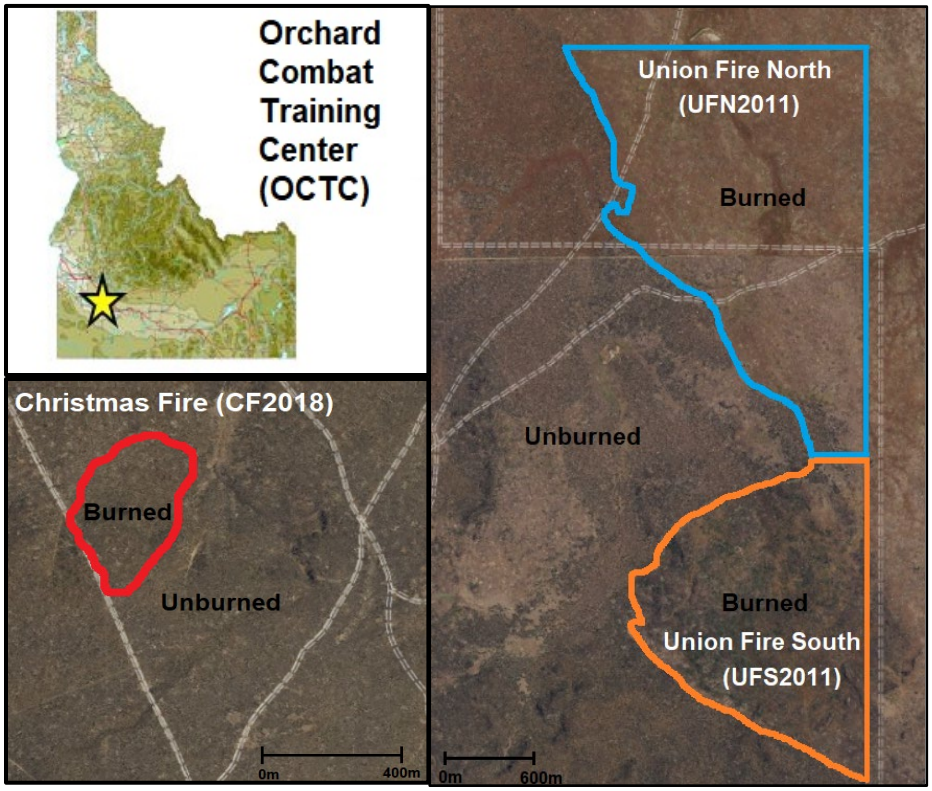
Fire significantly decreased fungal richness and composition across all study sites. Depending on heat intensity and resident time, fire can kill soil fungi within the top 5 cm, thus reducing richness and influencing composition (Smith et al., 2016; Bruns et al., 2020). Given that fungal richness in the UFS2011 and CF2018 sites were similarly impacted, my study indicates that fire can alter the fungal complex for years post-fire. This long-term effect of fire on the fungal community may be caused by persistent soil N increases and plant community shifts that induce acclimatization of the fungal community to favor the novel above-ground ecosystem (Weber et al., 2015; Dove and Hart, 2017). Indeed, the plant community composition at my UFS2011 and UFN2011 sites were completely altered relative to the unburned site, where the unburned site was dominated by sagebrush and the UFS2011 burned site was dominated by cheatgrass (*B. tectorum*, L.), burr buttercup (*Ceratocephala testiculata*, Crantz (Roth)) and Russian thistle (*K. tragus,* (L.) Scop.), while the UFN2011 burned site contained traces of these invasive species (personal observation). Future research should aim to understand how changes in soil fungal compositions may feedback to plant communities and ultimately to ecosystem resiliency.

Herbicide application increased bacterial richness relative to sites that burned and did not receive herbicide treatments. In contrast, post-fire herbicide treatments decreased AMF richness compared to both unburned and burned sites. Changes in the microbial community composition in response to herbicide applications have been shown in other studies (Wardle and Parkinson, 1990; Ratcliff et al., 2006; Puglisi, 2012), owing to direct toxicological impacts, or reductions in substrate availability to the soil microbial community (Thiour-Mauprivez et al., 2019). Many studies have shown that herbicide does not induce lasting effects on the microbial community because these substances are metabolized by bacteria that can even be utilized as a nutrient growth enhancement temporarily (Singh et al., 2020), but our study demonstrated that a number of bacterial and AMF SVs significantly changed in richness and maintained this change in the presence of herbicide during a time period that exceeded the half-life. A probable reason for this result may be that herbicide promoted certain strains of bacteria, such as the phylum Chloroflexi, which depending on the species, can metabolize halogenated organics sometimes found in herbicides (Tas et al., 2010). Although glyphosate and imazapic are not known to contain these toxic chlorinated ethenes or polychlorinated biphenyls, bacteria can thrive on new fuel sources and continue to proliferate even after degradation, or perhaps that degradation was slow in this water-limited ecosystem (Austin, 2011; Xiao et al., 2017). However, herbicide toxicity, even when applied at low concentrations, can inhibit spore production to various species within Glomeraecae, possibly explaining the reduction in AMF richness (Dodd and Jeffries, 1989). Herbicide-induced changes in microbial community structure has been shown to affect important ecosystem functions, such as N cycling and nutrient and water provision to plants via

fungi (DeCrappeo et al., 2017; Ockleford et al., 2017). Thus, the changes in microbial community structure observed in this study may signal that herbicide application in the sagebrush steppe ecosystem has consequences for plant-soil feedbacks that may ultimately impact the ability of native species to reestablish or persist (Baker et al., 2009).

Combining the effects of fire history, consequent vegetation changes, and post-fire management remediation provides greater interpretation of the significant impacts to soil properties to the scope of restoration. Short- and long-term fire effects are evident across all three environmentally different study sites, and in some instances, herbicide intensifies these impacts. Although certain fire factors (i.e., fire intensity and severity) and other abiotic factors (i.e., amount of precipitation) could have further explained my data, there is strong support fire presence or absence in an ecosystem can influence soil properties, and herbicide can dramatically impact ecosystem resilience. The differences in fires compared to their unburned counterparts provides further insight into the variability of recovery for independent ecosystems, as well as the implications associated with modern management strategies.

5. Maps and Figures



Map 1.1 Map of fire site and adjacent unburned locations within the OCTC located south of Boise, Idaho. Top left: State of Idaho indicating approximate sites within southwestern Idaho. Bottom left: Inset map of the 2018 Christmas Fire (CF2018) boundaries. Right: Inset map of the 2011 Union Fire, where 2011 Union Fire North (UFN2011) is outlined in blue, and 2011 Union Fire South (UFS2011) is outlined in orange

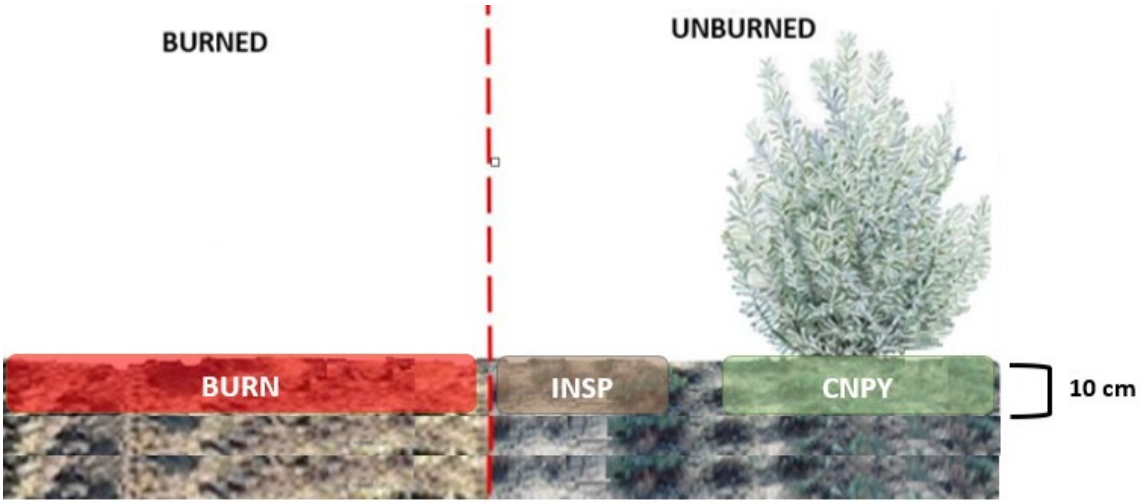


Figure 1.1 Field sampling procedure for soil collection of burned and unburned sites at the three site locations in the OCTC south of Boise, Idaho. Microsite-level characteristics of burned areas were sampled anywhere within the burn (BURN). Microsite-level characteristics of unburned areas were collected by sampling underneath mature sagebrush plants in unburned areas (CNPY) and between mature sagebrush plants in the interspace in unburned areas (INSP).

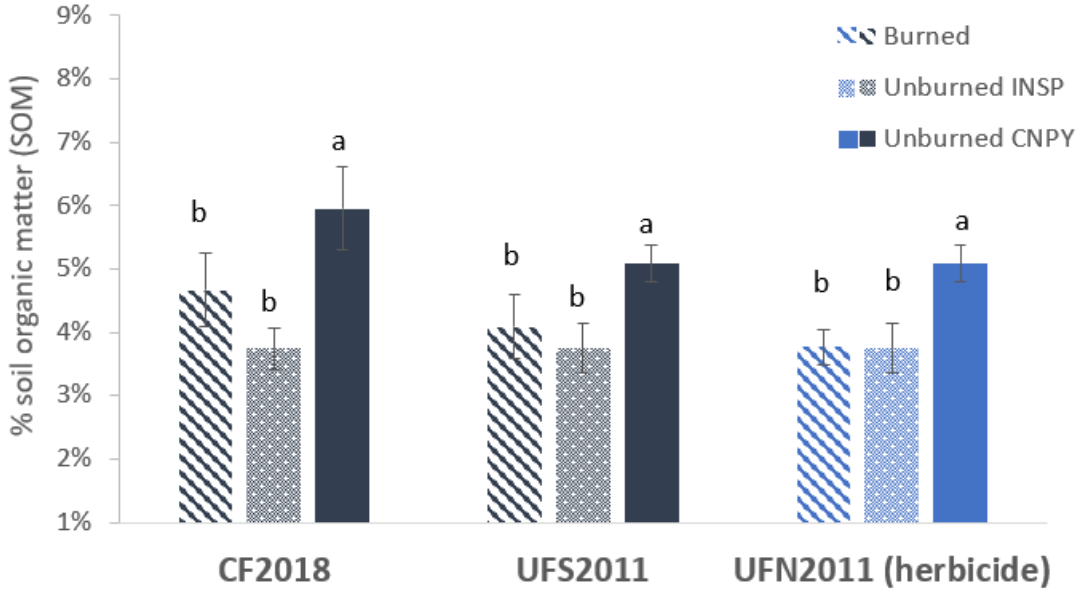


Figure 1.2 Percent soil organic matter (SOM) in burned [BURN] and unburned sites. Burned sites are subdivided into interspace (INSP, between mature sagebrush) and canopy (CNPY, underneath mature sagebrush) microsites. Lowercase letters represent differences between burned and unburned INSP and CNPY microsites within a site ($p < 0.05$). Values represent means with error bars as ± SE ($n = 5$).

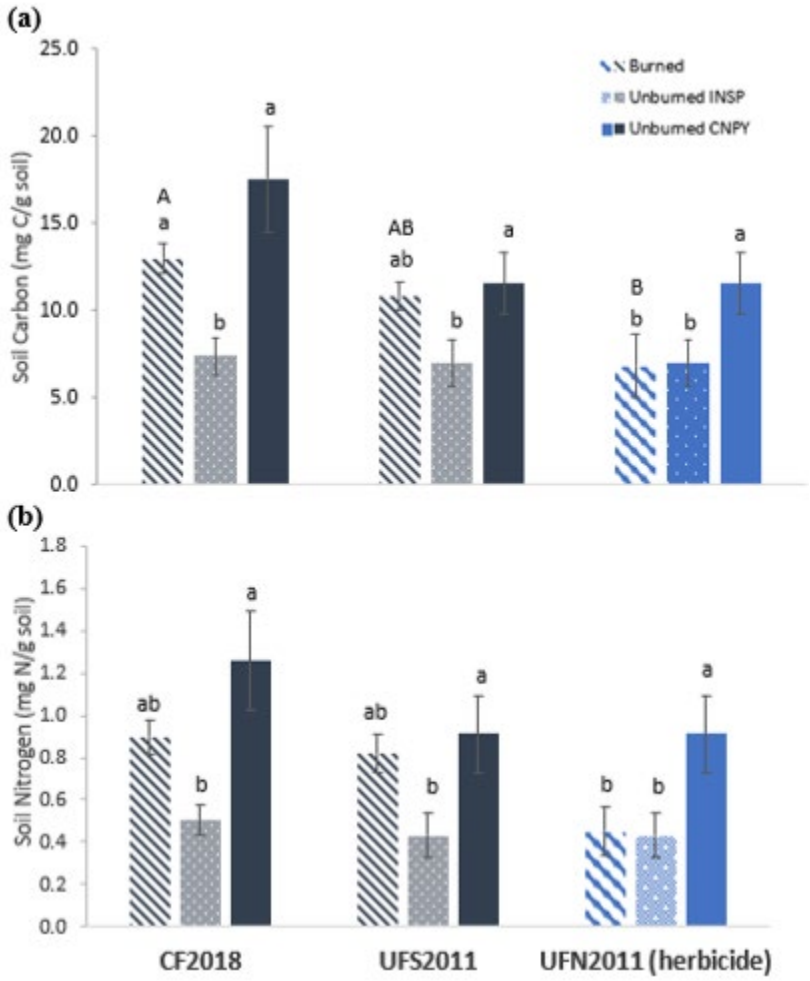


Figure 1.3 Bulk carbon- (*a*) and bulk nitrogen-concentrations (*b*) in burned [BURN] and unburned sites. Burned sites are subdivided into interspace (INSP, between mature sagebrush) and canopy (CNPY, underneath mature sagebrush) microsites. Lowercase letters represent differences between burned and unburned INSP and CNPY microsites within a site ($p < 0.05$). Uppercase letters represent differences among sites within burned and unburned treatments ($p < 0.05$). Values represent means with error bars as ± SE ($n = 5$).

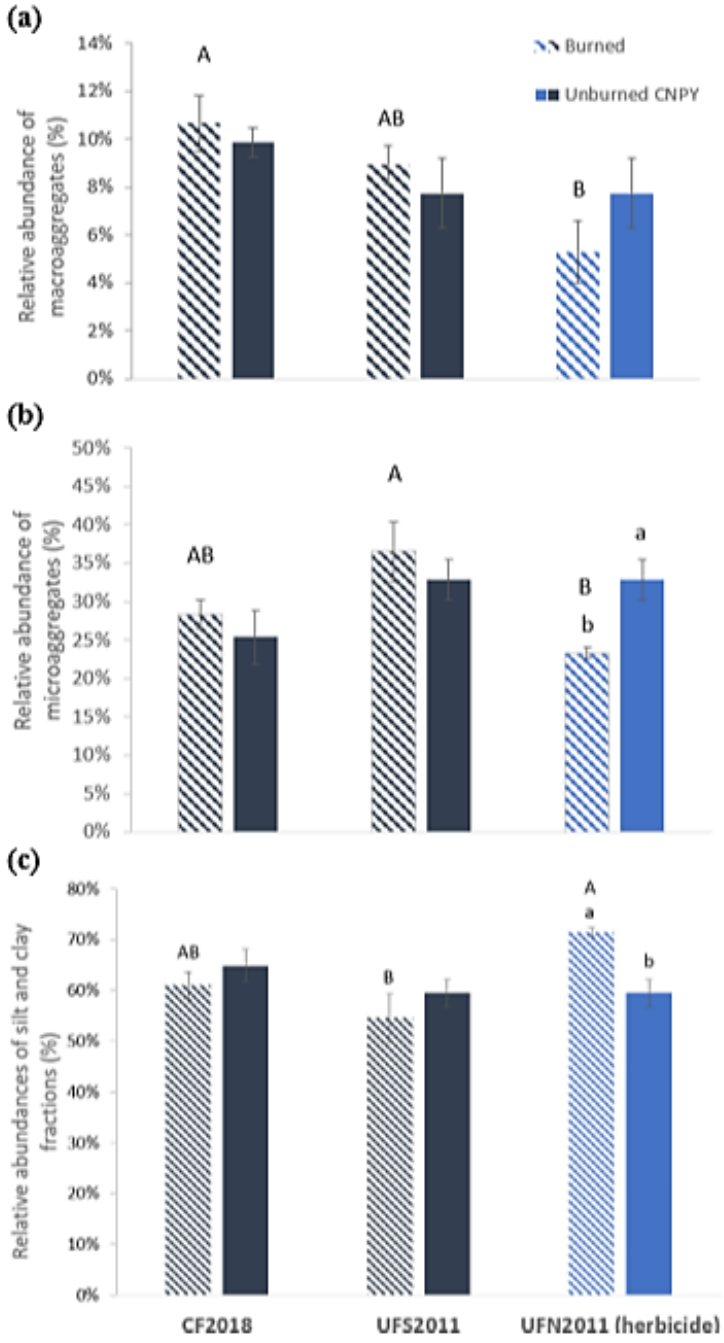


Figure 1.4 Relative abundance of macroaggregates (*a)*, microaggregates (*b)*, and silt and clay fractions (*c*) within burned and unburned sites. Lowercase letters represent differences between burned and unburned canopy (CNPY) treatments within a site ($p < 0.05$). Uppercase letters represent differences among sites within burned and unburned treatments ($p < 0.05$). Values represent means with error bars as ± SE (n = 5).

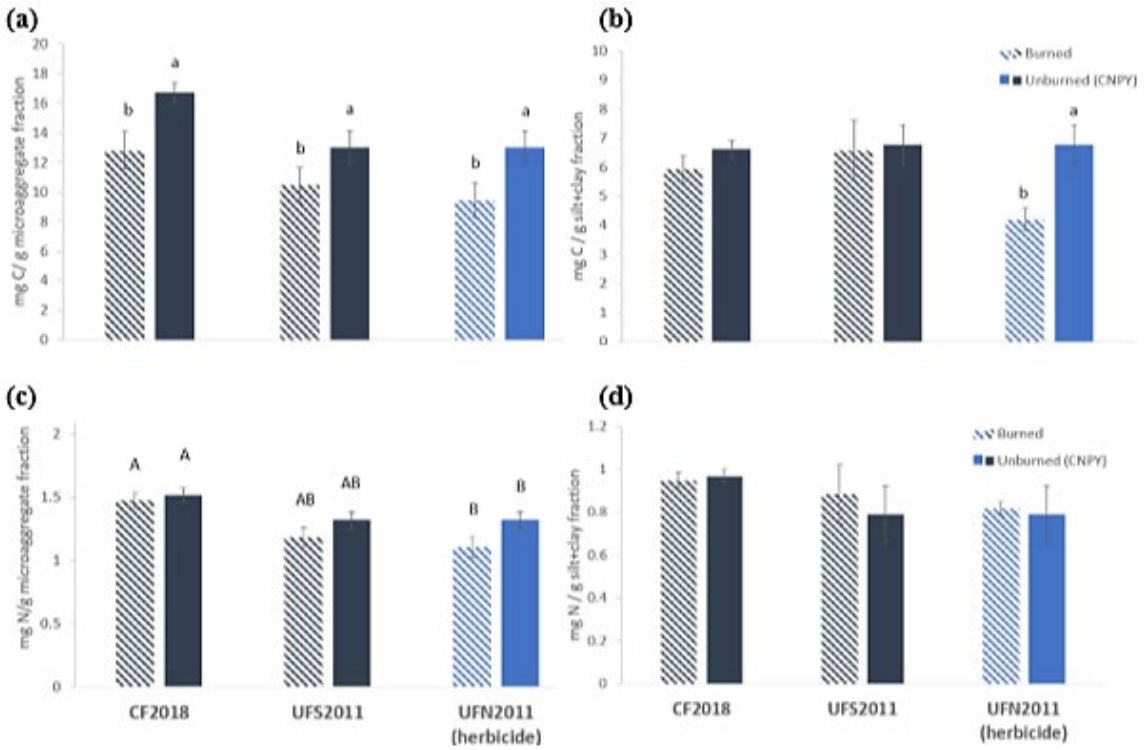


Figure 1.5 C- and N-concentration of microaggregates (*a, c)*, silt and clay fractions (*b, d)*, respectively, within burned and unburned sites. Lowercase letters represent differences between burned and unburned canopy (CNPY) microsites within a site ($p < 0.05$). Uppercase letters represent differences among sites within burned and unburned treatments ($p < 0.05$). Values represent means with error bars as ± SE ($n = 5$).

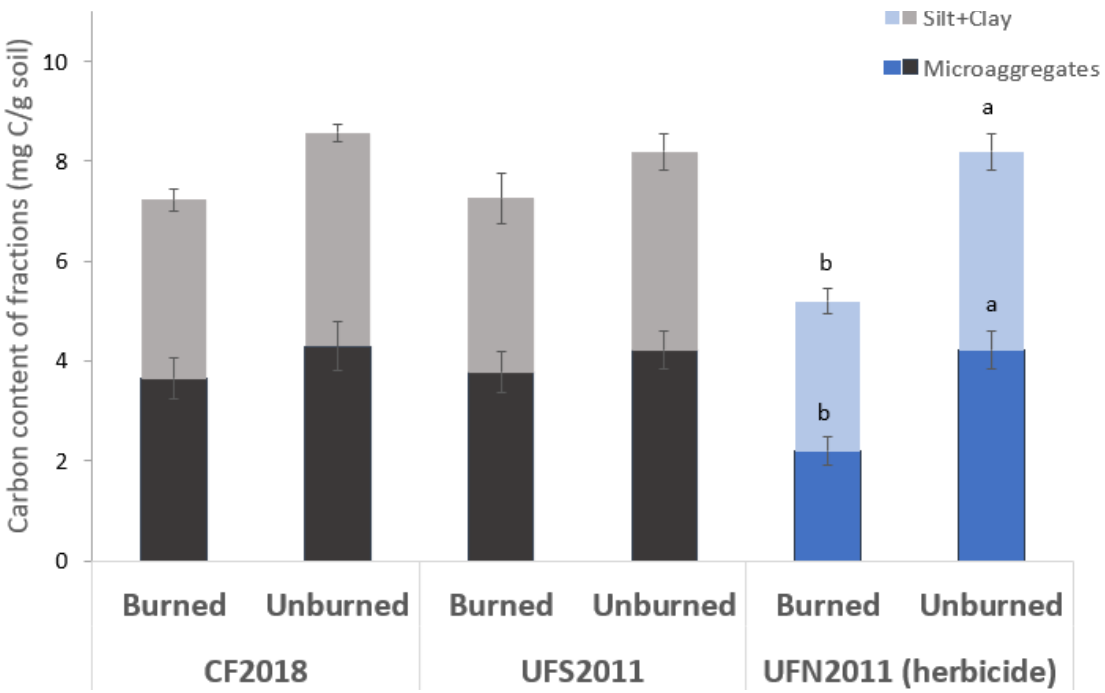


Figure 1.6 C-content in microaggregate and silt and clay fractions (i.e., relative abundance of fraction in soil by weight multiplied by its carbon concentration) within burned and unburned sites. Lowercase letters represent differences between burned and unburned canopy (CNPY) treatments ($p < 0.05$). Values represent means with error bars as ± SE ($n = 5$).

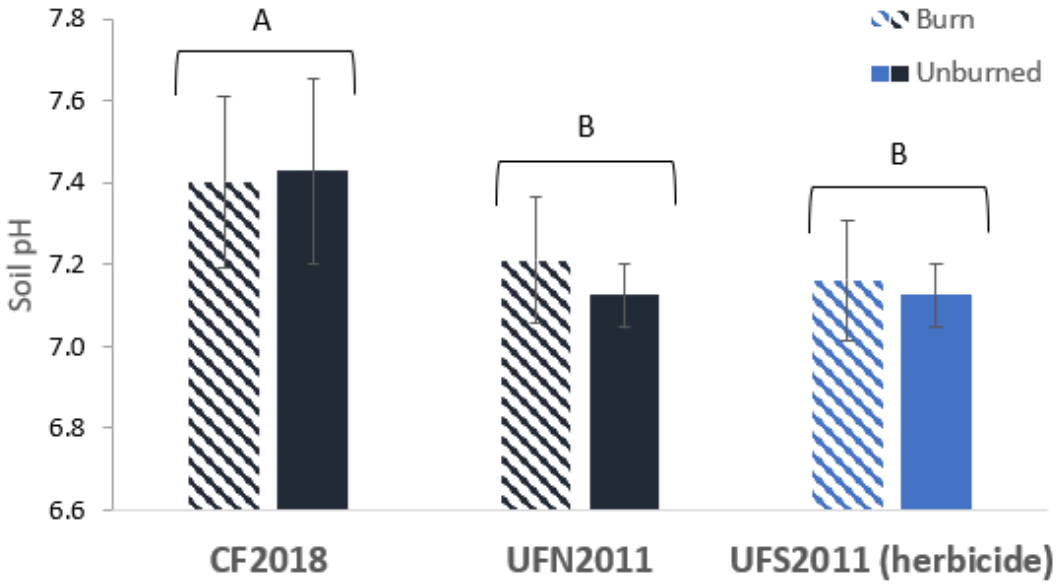


Figure 1.7 Soil pH among sites within the OCTC south of Boise, Idaho. Uppercase letters represent differences among sites ($p < 0.05$). Values represent means with error bars as ± SE ($n = 5$).

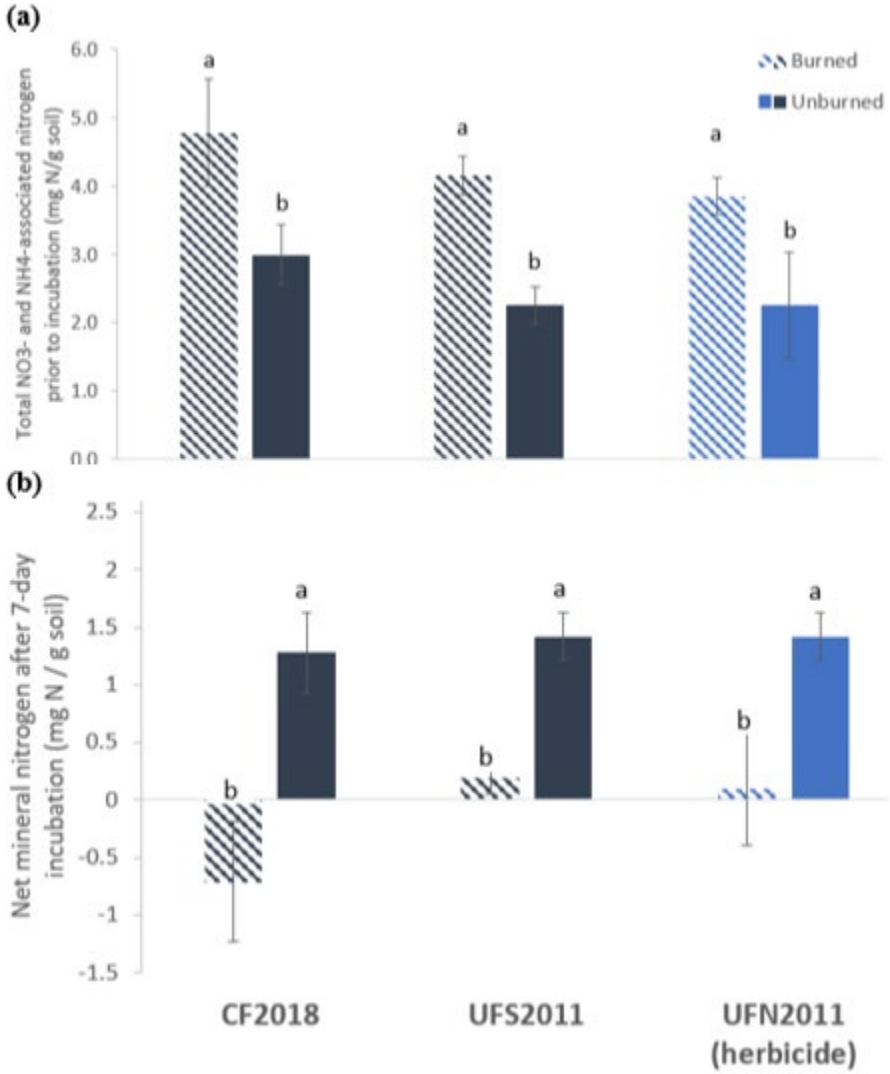


Figure 1.8 Total soil nitrate- (NO_3^--N) and ammonium-associated (NH_4^+-N) nitrogen (*a)* and net soil nitrogen mineralization (*b)* within burned and unburned sites. Lowercase letters represent differences between burned and unburned canopy (CNPY) treatments. within a site ($p < 0.05$). Values represent means with error bars as ± SE (n = 5).

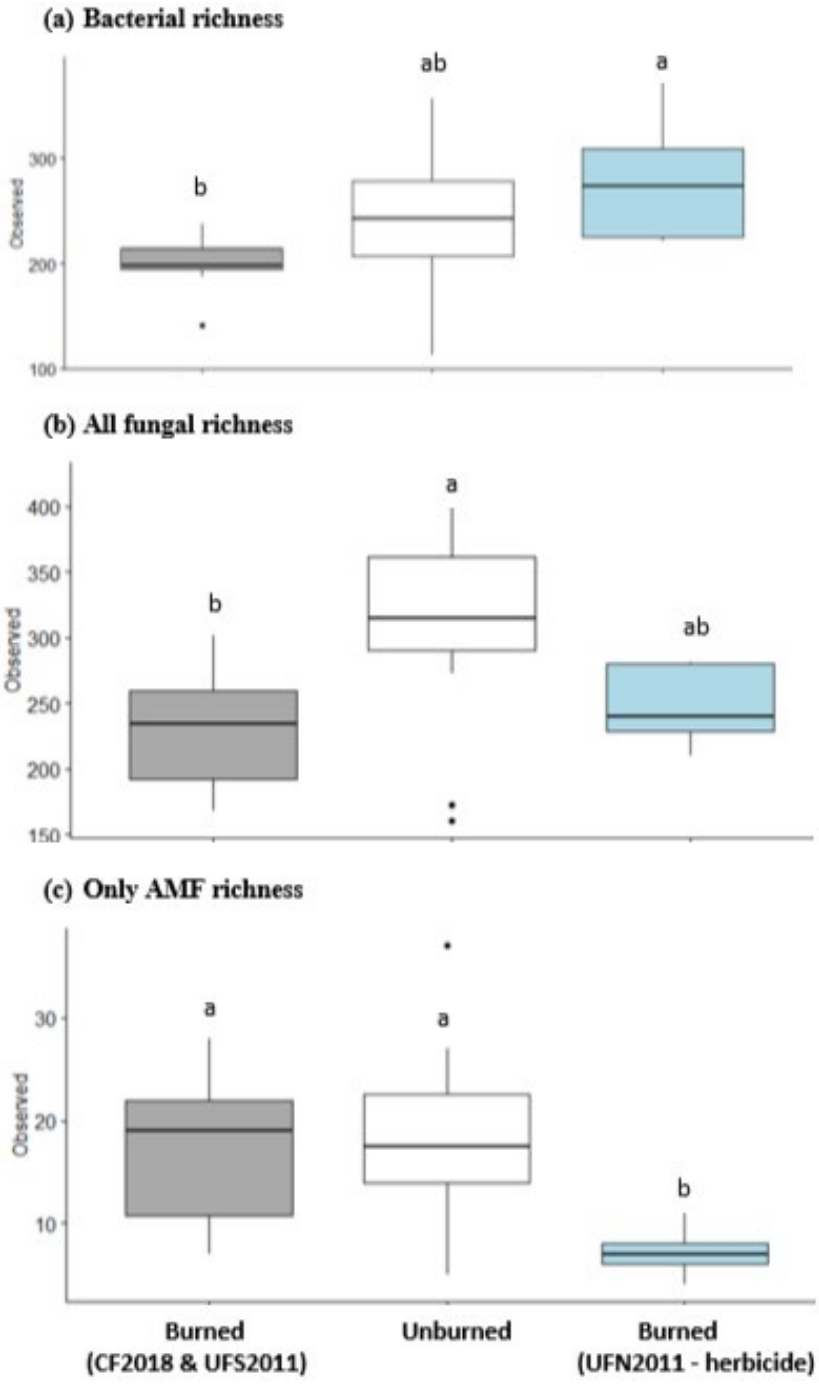
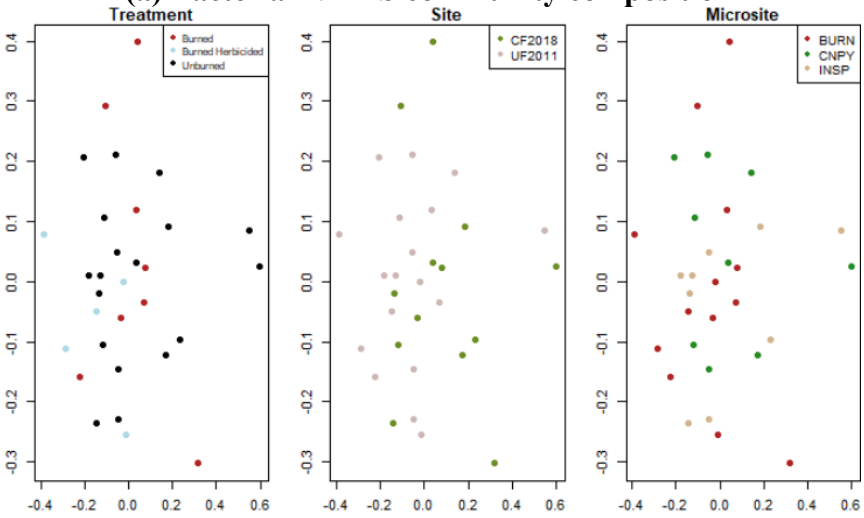
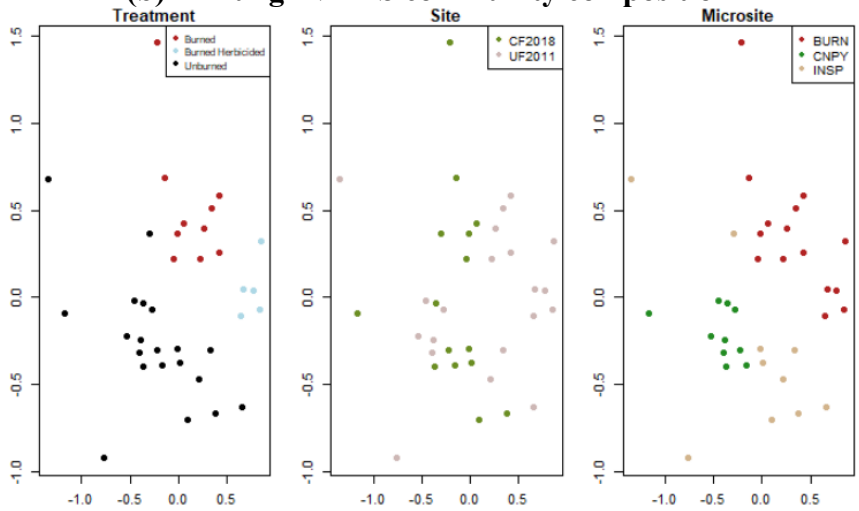


Figure 1.9 SV richness boxplot of bacteria (*a*), all fungi (*b*), and only AMF (*c*) differences by treatment across all sites within the OCTC south of Boise, ID. Note: Burned – herbicide refers to UFN2011 samples only (n=5) compared to Burned (n=10) and Unburned (n=20).

(a) Bacterial NMDS community composition



(b) All fungi NMDS community composition



(c) Only AMF NMDS community composition

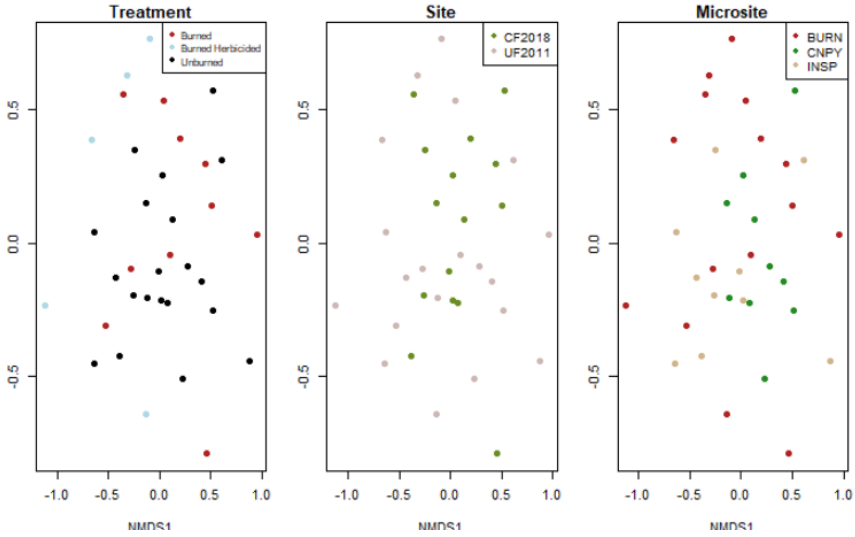


Figure 1.10 NMDS ordination results of bacteria (*a*), all fungi (*b*), and only AMF (*c*) organized by treatment (*left*), microsite (*middle*), and microsite (*right*). On the treatment legend, Burned refers to sites CF2018 and UFS2011 and Burned Herbicide refers to UFN2011, and Unburned refers to all unburned paired sites. For bacteria and AMF, only microsites differed in composition. For all fungi, treatment, site, and microsites differed in composition.

CHAPTER TWO: AMENDING POST-FIRE SOILS WITH NATIVE MICROBIAL COMMUNITIES AND BIOCHAR TO IMPROVE SAGEBRUSH PERFORMANCE FOR RESTORATION PURPOSES

1. Introduction

Native vegetation of the sagebrush-steppe ecosystem is threatened by increased fire and subsequent invasive species, like cheatgrass (*Bromus tectorum,* L.) (Knapp, 1996; Anderson and Inouye, 2001). Effective sagebrush (*Artemisia tridentata* sp. *wyomingensis*, Nutt.) restoration is required to maintain the services that the ecosystem provides, but reseeding efforts have been met with variable and often negligible success (Johnson et al., 2011; Pyke et al., 2013; Chambers et al., 2014; Chambers et al., 2019). Progressive changes in soil microbial and biochemical properties induced by fire, management (e.g., herbicide treatments), and invasive species establishment following fire may be limiting the ability for sagebrush to return to the landscape fully. Enhancing the native soil community and altering biochemical properties in degraded soils could be a possible mechanism for increased sagebrush reestablishment and retention.

Native soils of the sagebrush steppe encompass an array of microbial diversity with specific microbial properties that promote long-term preservation of native vegetation. They are involved in soil biochemical processes, like decomposition of organic matter, that are essential for vegetation growth and survival, especially in litter-limited semi-arid environments (Burke et al., 1989; Norton et al., 2004a; Ingham, 2009). For instance, the diverse bacteria actinomycetes, including the genus Streptomyces, have

a dominant presence in the sagebrush steppe (Jiménez-Esquilín and Roane, 2005), and are producers of chemically important secondary metabolites (Basil et al., 2004) that interact within the rhizosphere. The mutualistic actinomycete relationship has been known to enhance plant growth (Singh and Dubey, 2018; Selim et al., 2021) and protect against pathogenic fungi (Atalan et al., 2000; Basil et al., 2004) by secreting antibiotics and other metabolites. As indicated in laboratory settings by Grasso et al. (1996), bacterial community shifts can be significantly altered immediately post-fire, although recovery is typically short-term if native vegetation is able to reclaim the landscape. Likewise, shifts in predominating fungal genera have been evident when invasive species become increasingly established over time (Weber et al., 2015). For instance, in a study conducted by Gehring et al. (2016), invasive spread of cheatgrass led to increases in abundance of dark septate endophytes (DSE), classified as melanized, septate Ascomycetes that tended to colonize in disturbed areas, and gradually displace beneficial AMF. DSE has been noted to occur in sagebrush ecosystems and colonize their roots, but the effects of their dominance are relatively unknown (Carter et al., 2014). Fire and the subsequent changes in colonization by invasive species can potentially inflict harmful long-term effects to the microbial community during the process of succession by altering compositional levels during transitional stages (Klein et al., 1995). These compositional shifts in microbial communities within the rhizosphere – narrow region between and within plant roots – in which they can form important functional relationships with plant roots could have major impacts on restoration because certain functional aspects may no longer be present (Hinsinger & Marschner, 2006; Pierret et al., 2007; Jones & Hinsinger, 2008; Hinsinger et al., 2009).

Approximately 74% of terrestrial plants, including sagebrush, form beneficial relationships with the underground mycorrhizal complex (Smith and Read, 2008). Arbuscular mycorrhizal fungi (hereafter: AMF) exist in the Glomeromycota clade and in general, exhibit varying levels of mutualistic, or in some cases parasitic, dependence (Chomicki et al., 2020). Sagebrush associate primarily with Claroideoglomeraceae and Glomeraceae families within that clade (Carter et al., 2014). Since sagebrush is highly associated with specific AMF that promote tolerance to stress, fire- and invasion-induced changes in the soil microbial community may reduce the capacity of the ecosystem to withstand invasion by non-native species (Neary et al., 1999; Wolfe and Klironomos, 2005; Reinhart and Callaway, 2006; Norton et al., 2007; Busby, 2011; Dierks et al., 2019). Invasive species have been noted to induce negative growth responses with AMF colonization (Rowe et al., 2007), corresponding to compositional shifts and decreases in viable AMF propagules (Hawkes et al., 2006). There is evidence that inoculation with native AMF into greenhouse-grown seedlings can increase survival of sagebrush by 24% in drought-prone timespans (Davidson et al., 2016). Other studies showed that in grassland settings, incorporating various soil amending properties, such as native AMF inoculation, can benefit seedlings biomass growth (Hammer et al., 2015; Gebhardt et al., 2017). While evident that AMF communities can contribute to sagebrush performance, other factors may impede successful reestablishment attempts that should be considered.

Many studies have shown that increased rates of N cycling following fire and invasion catalyze the positive feedback loop that promotes the reestablishment and performance of invasive species, like cheatgrass (*Bromus tectorum* L.; Ehrenfeld, 2003; Saetre and Stark, 2005; Chambers et al., 2007; Hooker et al., 2008; Stark and Norton,

2015). Thus, for restoration of native sagebrush communities to be successful, nutrient availability should be reduced (Blumenthal et al., 2003; Brunson et al., 2010), which may be achieved by applying woody biochar. Biochar is a solid bio-carbon product developed by immediate thermal decomposition of plant organic matter at temperatures of up to 500°C, which can be utilized as a soil amendment on ground surfaces (Lehmann, 2007; Beesley et al., 2011). Applying woody biochar to highly degraded soils in semiarid ecosystems may help reduce N availability to plants owing to its high C:N ratio (Laird et al., 2010; Van Zwieten et al., 2010; Streubel et al., 2011; Kameyama et al., 2012; Anderson et al., 2013; Ippolito et al., 2015). Biochar also impacts soil pH, which can affect soil microbial composition and enzyme activity (Lehmann and Joseph, 2009), and has the capability to promote AMF colonization in roots (Hashem et al., 2018; Sales et al., 2020). Additionally, biochar can improve soil aggregation (Anderson et al., 2013), with positive impacts on hydraulic conductivity (Glaser et al., 2002; Basso et al., 2013; Ouyang et al., 2013). Increases in soil moisture in semiarid ecosystems can greatly improve performance for native sagebrush, which grows in a water-limited environment. Thus, applying biochar, pyrolyzed from woody material to highly degraded soils may moderate nutrient availability and increase resistance to non-native invasion, while improving physical soil characteristics that promote the reestablishment and performance of sagebrush.

This study sought to answer three questions: 1) How does a live native soil microbial inoculum impact sagebrush performance and soil properties, 2) how do biochar additions impact sagebrush performance and soil properties, and 3) how does prior management (e.g., herbicide) mediate the impact of soil microbial inoculation and

biochar amendment on sagebrush performance? A full factorial greenhouse experiment was conducted with soils collected from three post-burn sites within the Orchard Combat Training Center (OCTC) south of Boise, Idaho. Native soil community inoculums were collected from unburned sites adjacent from burned locations underneath canopies of sagebrush within the rhizosphere. Fast-pyrolyzed biochar was obtained from the Rocky Mountain Research Station located in Moscow, Idaho and further crushed to be applied to designated biochar pots within the greenhouse experiment. I hypothesized that treatments containing both inoculum and biochar would have the greatest sagebrush performance levels (germination, above-ground biomass, root length, proportional abundance of mycorrhizae in roots) and that inoculation and biochar addition enhances soil properties (soil moisture, pH, total N, microbial richness and composition). Additionally, I hypothesized that post-burn soils with an inoculum would have a higher abundance of AMF composition and colonization, while biochar plots would attain greater soil moisture, and harbor more diverse microbial communities. Lastly, I hypothesized that herbicide addition would reduce the positive effects of inoculation and biochar addition on soil biogeochemical properties and sagebrush performance. By investigating these questions relating to the importance of plant-soil feedbacks on restoration practices, future management decisions can be made for the greater benefit of the species that utilize this critically important, but oftentimes undervalued, ecosystem.

2. Methods

2.1 Experimental Design

To evaluate how a native soil inoculum and biochar additions would affect sagebrush performance, I created a full factorial greenhouse experiment by growing

sagebrush seedlings in post-burn soils from fires in the OCTC (i.e., CF2018, UFN2011, UFS2011, described in the previous chapter), which received either combinations of a live (+Inoculum) or sterilized (-Inoculum) native soil inoculum, or biochar (+Biochar) or no biochar (-Biochar; Figure 2.1). Soil up to 15 cm in depth (diameter ~2.5 cm, 15 cm depth) was collected from original burned sampling sites in late June 2020. Adjacent to each of the burn sites, unburned soil was collected from directly below sagebrush canopies to a depth of 15 cm using a 7/8" soil corer (AMS Samplers, Idaho) to represent the natural microbial sagebrush community within the rhizosphere. This soil was separated into +Inoculum and -Inoculum treatments (Figure 2.2). Following collection, soils were stored in the fridge at 4°C for a week prior to planting. Treatments representing -Inoculum were autoclaved for one hour at 15 psi at 121°C to pasteurize vegetative microbes and germinating spores before being stored in the fridge at 4°C. Biochar was prepared by using a mortar and pestle to grind up the biochar into pieces smaller than 0-1 cm in diameter, 0 indicating complete ash.

Pots were prepared by covering drainage holes with sterilized rock-filled nylon socks placed at the bottom of the pots, and then combining 110 g post-burn soil and 40 g sterile sand (Mosser Lee ML 111 White Sand Soil Cover) in cone-tainers (8u876 & Sons Inc., Ray Leach Cone-tainers, SC10RA, volume = ~150 mL). Soil +/-Inoculum and +/-Biochar were then added: (1) 5 g sterilized native inoculum from sagebrush rhizosphere without biochar, (2) 5 g of live native inoculum from sagebrush rhizosphere without biochar, (3) 5 g sterilized native inoculum from sagebrush rhizosphere with biochar, and (4) 5 g of live native inoculum from sagebrush rhizosphere with biochar (Figure 2.1). Pots requiring biochar were administered ~1.5 g of crushed biochar manually and mixed

using a cleansed stir rod to a depth of no more than 2.5 cm. Then, each pot was administered 5 mL of water to soak the soil for 5 minutes and inoculum was infused by scooping out 5 cm of the center out and depositing the appropriate +/-Inoculum treatment (Figure 2.3). Blunt-tipped tweezers were used to individually hand-pick 10 sagebrush seeds obtained from Granite Seed Company (Lehi, Utah) and carefully planted within the diameter of the inoculated area, no more than 0.25 cm deep. All these treatments were duplicated, totaling 120 pots appropriately labeled pots with identification tags, and placed into respective replicate trays for monitoring and caring.

To address if present microbial communities in post-burn soils modulated the impact of a live native soil community inoculum on germination and performance, additional soil was collected from CF2018 only and segregated for sterile potting soil treatments. Due to the timing of the CF2018 burn (i.e., most recent), this site was selected assuming that the soil microbial community had not been given ample time to stabilize the microbial community, and thus, the regulating impacts of post-burn bacteria and fungi could be investigated. Additionally, this experiment would yield any differences occurring between the post-burn microbial community and the live native soil microbial community (+Inoculum) because the inoculum would be isolated into sterilized post-burn potting soil (+/-Sterile). Furthermore, actual microbes of the inoculum could be assessed as well. Soil was autoclaved for 2 hours at 15 psi at 121°C due to the large quantity to be utilized as base potting soil. The following treatments were included: (1) 5 g of sterile native inoculum deposited into post-burn soil, (2) 5 g of live native soil inoculum deposited into post-burn soil, (3) 5 g sterile native inoculum deposited into sterilized

post-burn soil, and (4) 5 g of live native soil inoculum deposited into sterilized post-burn soil. A total of 40 pots were created for this experiment.

2.2 Monitoring for Germination, Survival, and Mortality

Pots were housed in the Boise State research greenhouse under ambient conditions with no artificial lighting for three months. For the first month, watering occurred daily, pipetting ~1.25 mL to each pot for the first month. Many seedlings germinated within the first week, and cotyledons began forming. Survival and condition of seedlings were tracked every other day for the first month until most had established (not all pots grew sagebrush). After a month, seedlings that persisted out of the cotyledon stage and expanded their leaf area upward were granted 2 mL of water every other day. For the remaining month, seedlings with more leaf area received 5 mL of water every other day. Replicate trays were rearranged every other day for the entirety of the experiment to prevent any bias from general greenhouse atmospheric concentrations. Over a month after beginning the experiment, a single beetle invaded one replicate tray and consumed six seedlings before expiring in one of the pots, which reduced the total count of alive plants. Seedlings were not thinned, and all surviving stems were counted. By the end of three months, 112 pots contained seedlings out of the 160 total, accounting for 70% total occupation.

Sagebrush seedlings were initially monitored for germination and survival every other day during the first month. For the remaining two months, progress and survival were monitored on a weekly basis through presence/absence abundance counts. Analyses could only be completed for germination rate, as the other measurements were final percentages based on occupancy within the pots. Germination rate was determined by

dividing the total number of seed originally planted by the number of shoots that emerged over the first month.

2.3 Soil Moisture Content and pH

To assess the effects of biochar treatments on moisture properties, 2 g of the first top centimeter from each sample pot was scraped off and oven dried the samples at 105°C for 24 hours. I quantified gravimetric soil moisture by calculating the differential weights between the samples that were not dried and those that were oven-dried. Soil pH was calculated by agitating slurry mixtures containing 15 g of soil and 30 mL of deionized water from all samples, measuring pH via pH reader (Oakton WD-35613-52 pH, Illinois, USA) and averaging three reads.

2.4 Above- and Below-ground Biomass and Root Morphology

Seedlings were snipped at the root collar and shoots were dried at 65°C for 24 hours, then weighed to determine biomass. Roots were carefully picked from soil contents and rinsed thoroughly to remove chunks of litter or aggregates that formed. Entire root morphology was captured using a WinRHIZO root scanner (Regent Instruments Inc., Canada). I recorded root morphology indices pertaining to root length (cm) and average diameter of roots (mm) to measure whether specific treatments impacted these properties. I then subsampled, utilizing half of the wet root for drying to determine biomass and the other half for AMF colonization staining. The biomass subsample was placed into the oven at 65°C for 24 hours and retrieved to be weighed for biomass growth. Both subsamples were weighed and configured to determine total dried root biomass.

2.5 Total Mineral N

Total mineral N was derived using the same methods as described in Chapter 1, but I adapted a new method to achieve 60% water holding capacity (WHC). WHC was calculated for independently for +Biochar and -Biochar treatments using 20 random samples (15 g) for each treatment (i.e., +Biochar = 20, -Biochar = 20). Samples were placed into a filter-lined funnel that was suspended above a specimen cup filled with water that contacted the filter paper. Water imbibed into the soil until the surface glistened from saturation, and then water was removed to allow the sample to drain for 15 minutes. Samples were then weighed and averaged based on +Biochar and -Biochar treatment to obtain corrected WHC values to administer to mineral N samples. No incubation ensued because only total mineral N was assessed to reflect sagebrush performance observations. After KCl filtration, samples were stored in the -20°C freezer until further analysis. This time, each filtrate was processed in 96-wellplates to assess concentrations of nitrate-associated N (NO_3^--N) and ammonium-associated N (NH_4^+-N) using manual VCl_3 reduction and sodium salicylate ($C_7H_5NaO_3$) and bleach/sodium hydroxide (NaOH) solutions, respectively. Absorbance of each reaction solution was calculated using a BioTek Synergy Mx plate reader using Gen5 v1.11.5 software (Winooski, Vermont) at 540 nm for NO_3^--N and 650 nm for NH_4^+-N. Final available mineral N was calculated by combining both values and being expressed as mg N g-1.

2.6 AMF and DSE Colonization

Roots were stained for fungal colonization presence using wheat germ agglutinin. Washed roots were cut into 1-2 cm segments and autoclaved in 5% potassium hydroxide (KOH) for 3 minutes. After cooling for 10 minutes, roots were rinsed three times and

soaked in deionized water for three minutes each time before soaking in phosphate buffer solution (PBS) for 20 minutes. Roots were rinsed and incubated in a lectin solution containing 50 mL of PBS + 1% bovine serum albumin (BSA) + 0.5µg/mL horseradish peroxidase (HRP) wheat germ agglutinin for 16-20 hours. After incubation, roots were rinsed in PBS three times for 5-minute washes each. Roots were then deposited into clean petri dishes and saturated with a staining solution containing 5 mL PBS + 3 drops of reagents 1, 2, 3, and 4 (Vector VIP peroxidase-HRP substrate Kit, Vector Laboratories, Inc., Burlingame, CA) for two minutes until roots began to change a purple tinge. Roots were washed immediately to inhibit further color penetration, which could potentially dye roots and decrease detectability of fungal presence. Once washed and soaked in deionized water for 5 minutes, roots were cleared for mounting in a 50% glycerol + 50% deionized water solution. Roots were carefully placed horizontally onto microscope slides top left to bottom right and gaps between roots were reduced between segments to prevent loss of intercepts when counting fungal presence. An identification tag and coverslip was applied to each completed slide and placed into a microscope slide-holding container until further analysis.

Once all roots were stained and prepared for counting fungal structures, slides were viewed using the intercept method described by McGonigle et al. (1990). Aligned parallel to the long axis, roots were viewed at 40X magnification using a stereoscope (Leica Microsystems, Wetzlar, Germany) every 5 mm. At each 5 mm intersect, AMF (e.g., spores, hyphae, vesicles, arbuscules) and DSE structures (e.g., hyphae and sclerotia) were counted with a presence/absence identification (Figure 2.4). I was advised to record DSE because my post-burn soils were once stocked with invasive species and most likely

were going to remain a strong presence in the soil. Because a technician also counted fungal structures, counts were averaged before running statistics. Unknown structures were photographed and compiled into a lab folder for further investigation, but most structures remained unidentified and were not analyzed.

2.7 Soil Community and Sequence Data Processing

Total community DNA was recovered from homogenized soil samples using the DNAeasy PowerSoil® Pro Kit (Qiagen Inc., Maryland, USA) with elution buffer. Sequence data was processed for bacterial 16S rRNA, general fungi ITS, and only AMFFLR material obtained from soil samples, as described in Chapter 1. Bacteria, general fungi, and only AMF rarefaction depths were 7,145 base pairs, 11,237 base pairs, and 1,184 base pairs, respectively. While processing our samples, I also performed DNA extraction on four empty tubes. These blank extractions were processed simultaneously with the samples, including all downstream lab work and sequencing, allowing me to identify and remove contaminants from the data. Data sequencing was processed according to the descriptions provided in Chapter 1.

2.8 Statistical Analyses

Similarly to Chapter 1, all statistical analyses were conducted in R. To evaluate sagebrush performance (i.e., pH, shoot biomass, root biomass, root length, root diameter, total N availability, microbial community), three-way ANOVAs were conducted with site (i.e., CF2018, UFN2011, and UFS2011), inoculation (i.e., +Inoculum and -Inoculum), and biochar (i.e., +Biochar and -Biochar) as fixed factors for soil properties, and interaction terms were checked for significance. An independent samples t-test was used for assessing soil moisture since only +/- Biochar were evaluated. Tukey HDS multiple

comparison tests were conducted for post-hoc analysis when site differences were evident. When the interaction terms were not significant, two-way ANOVAs were conducted to evaluate treatment impacts of inoculum and/or biochar based on each site. Assumptions for homogeneity and normality were checked by using Levene's test and Shapiro-Wilks test, respectively. Additionally, Spearman's non-parametric correlations were plotted to compare fungal AMF and DSE root colonization relationships across the entirety of the dataset, and were log transformed since measures were not normal prior to analyses. Bacterial and fungal richness were generated through boxplots and bacterial and fungal compositions were visualized with ordination plots across sites and treatments (i.e., +/-Inoculum, +/-Biochar. Barplots of AMF sequence variants (SVs) were also generated to show differences between treatments. Dissimilarity between bacterial and fungal species among sites and treatments were assessed using PERMANOVA assuming equal dispersion within groups from the adonis test in the R package *vegan*. Finally, differential abundance of individual SVs between sample groups was tested using the R package *DESeq2*.

3. Results

3.1 Seedling Occupancy and Germination

At termination of the 3-month greenhouse experiment, 112 of the total 160 pots had sagebrush occupants, equating to about 70% total occupancy. Overall, +Biochar treatments significantly increased germination rates for the first month of growth among all sites ($F_{1,108} = 15.51$, $p < 0.05$; Figure 2.5). +Inoculum treatments had no effect on germination rate at any of the sites. However, when post-burn soils were sterilized prior

to inoculum addition, base soil sterility (i.e., +Sterile) significantly increased germination rate ($F_{1,36} = 5.55$, $p = 0.02$, Table 2.1).

3.2 Soil Moisture Content and pH

+Biochar had significantly increased soil moisture by 31% across all sites ($F_{1,70} = 26.055$, $p < 0.05$, Figure 2.6a). Site and inoculum treatments had no impact on soil moisture. Soil pH increased significantly across all sites that contained +Biochar treatments ($t_{1, 30} = 4.73$, $p < 0.05$, Figure 2.6b).

3.3 Above-ground and Root Biomass

All biomass data presented here are representative of all sagebrush seedlings which emerged in the pots. Thus, in pots with a higher germination rate, biomass is measured across more seedlings, than in pots with a lower germination rate. +Inoculum did not impact aboveground biomass, but +Biochar increased sagebrush seedling aboveground biomass at the CF2018 and UFN2018 sites, but not in UFS2011 ($F_{1,70} = 10.28$, $p < 0.05$; Figure 2.7a). Above-ground biomass increased by 37.53% in +Biochar amendments compared to -Biochar amendments collectively across all sites. Root length was significantly greater in +Biochar treatments for the UFN2011 site only ($F_{1,70} = 6.98$, $p = 0.01$; Figure 2.7b), while root diameter was significantly decreased by +Biochar treatment at this site ($F_{1,70} = 3.22$, $p = 0.04$; Figure 2.7c). There were differences among sites, where UFN2011 had significantly less above-ground biomass compared to UFS2011, but only for +Biochar treatments ($F_{2,70} = 13.34$, $p < 0.05$; Figure 2.7a). In addition, root length was significantly shorter in UFN2011 compared to CF2018 and UFS2011 for both +Biochar and -Biochar treatments ($F_{2,70} = 9.69$, $p < 0.05$; Figure 2.7b).

Base sterility (i.e., +Sterile) significantly increased shoot biomass at CF2018 ($F_{1,27}$ = 4.39, p = 0.05, Table 2.1).

3.4 Total Mineral N

A significant three-way interaction effect occurred for total mineral N (i.e., NO_3^--N + NH_4^+-N expressed in µg N / g soil) ($F_{6,70}$ = 3.47, p = 0.03; Figure 2.8). Total mineral N was increased when +Biochar treatments were administered at site CF2018 ($F_{1,24}$ = 14.45, p < 0.05), but the opposite effect was shown in site UFS2011 ($F_{1,22}$ = 13.23, p < 0.05). Additionally, +Inoculum treatments within the UFS2011 site increased mineral N-concentrations by 16.63% relative to the –Inoculum treatments ($F_{1,22}$ = 5.72, p < 0.05). Treatments did not affect mineral N in post-burn soils of UFN2011.

3.5 AMF and DSE Colonization

DSE hyphal structures and sclerotia (i.e., persistent resting fungal structures associated with DSE) presence were more prevalent than AMF structures across all treatments, occurring in 96 % root samples compared to 37% root samples, respectively. Proportional abundances of AMF increased at the UFN2011 and CF2018 sites when +Biochar treatments were administered, but was negatively affected by +Biochar at site UFS2011 ($F_{2,70}$ = 4.24, p = 0.02; 2.9a). Likewise, proportional abundances of DSE abundance generally increased with biochar addition at the UFN2011 and CF2018 sites, but was negatively affected by +Biochar at site UFS2011 ($F_{2,70}$ = 5.174, p < 0.05; 2.9b). In addition, AMF proportional abundances positively correlated with DSE proportional abundances across all samples (R^2 = 0.167, p < 0.05, Figure 2.10). Treatments lacking base sterility (i.e., -Sterile) had greater absolute proportions of AMF abundance compared to other treatments ($F_{1,27}$ = 7.89, p < 0.05; Table 2.1), but +Inoculum did not

contribute significantly to AMF abundance. Base sterility and inoculum had no impact on colonization abundances of DSE.

3.6 Bacteria and Fungi Community Composition

A total of 9,191 SVs with 176 families encompassing 330 genera of bacteria were identified across all samples. More specifically, there were 138 more SVs in +Inoculum than -Inoculum, and 420 more SVs in +Biochar than -Biochar. The most common bacterial genus detected was an unnamed genus in the order Thermomicrobiales, followed by *Tumebacillus*, a Kallotenuales genus, *Streptomyces* sp., and a Gemmatimonadaceae genus. Bacterial strain composition was significantly different among all sites ($R^2 = 0.21$, $p < 0.05$), and were noted between +/-Inoculum treatments ($R^2 = 0.03$, $p < 0.05$) and between +/-Biochar ($R^2 = 0.03$, $p < 0.05$; Figure 2.11a). Additionally, herbicide also significantly impacted composition of bacterial species compared to all other burned pots ($R^2 = 0.12$, $p < 0.05$; Figure 2.11a). No significant differences in SV richness were found for any of the variables tested.

There were 2,937 fungal SVs with 18 families across 319 genera identified across all samples. More specifically, there were 474 more SVs in +Inoculum than -Inoculum, and 636 more SVs in +Biochar than -Biochar. The most common fungal genus detected was *Penicillium* sp., followed by Chaetomiaceae genus, an Onygenales genus, *Chaetomium* sp., and *Aspergillus* sp. Fungal richness was significantly increased with +Inoculum treatments ($F_{1,79} = 21.1$, $p < 0.05$, Figure 2.12). Composition of fungi was significantly affected by +Inoculum treatments ($R^2 = 0.05$, $p < 0.5$), +Biochar treatments ($R^2 = 0.02$, $p < 0.05$), and herbicide applications ($R^2 = 0.23$, $p < 0.05$) across all burned soils relative to -Inoculum, -Biochar treatments, and no herbicide, respectively (Figure

2.11b). Among +Inoculum and +Biochar treatments, CF2018, UFN2011, and UFS2011 had 10, 11, and 9 differing SVs, respectively.

3.7 AMF Community Composition

When evaluating phylum Glomeromycota (i.e., AMF), a total of 184 SVs were identified across all samples, with 2 more SVs in +Inoculum than -Inoculum and 4 more SVs in +Biochar than -Biochar. Phylum Glomeromycota composition was significantly different among all sites ($R^2 = 0.052$, $p < 0.05$), between +/-Inoculation ($R^2 = 0.023$, $p = 0.04$), and between +/-Biochar treatments ($R^2 = 0.022$, $p = 0.03$; Figure 2.11c). Additionally, herbicide significantly influenced AMF community composition relative to other sites ($R^2 = 0.052$, $p < 0.0.5$; Figure 2.11c). +Biochar within CF2018 soils were significantly different than -Biochar treatments ($R^2 = 0.081$, $p = 0.04$). SV richness was also not affected by any of the treatments applied.

When analyzing base sterility and inoculation effects, there were differences detected within inoculation treatment. Bacterial species richness was not impacted by +Inoculum treatment, but compositional differences did exist based on +Inoculum or -Inoculum ($R^2 = 0.27$, $p < 0.05$). However, fungal richness ($F_{1,26} = 14.97$, $p < 0.05$) and composition ($R^2 = 0.07$, $p < 0.05$) was impacted by inoculation. For AMF-specific Glomeromycetes, richness, was significantly impacted by +Inoculum treatments ($F_{1,26} = 3.43$, $p < 0.05$), but not composition ($p = 0.14$). To understand which genera of AMF were introduced to sagebrush seedlings, +Sterile+Inoculum (i.e., inoculation contribution only) and -Sterile-Inoculum (i.e., burned field conditions without inoculation) were analyzed independently (Table 2.2). +Sterile+Inoculum only contained one SV,

Claroideoglomus sp., while -Sterile-Inoculum contained four additional identified SVs of Glomeromycetes: *Rhizophagus* sp., *Septoglomus* sp., *Diversispora* sp., and *Otospora* sp.

4. Discussion

My study yielded three major conclusions. First, biochar addition consistently enhanced soil moisture, pH and germination, while the effects on soil N availability and fungal colonization varied by site and location. Second, adding a soil microbial inoculum derived from an intact native sagebrush ecosystem did not consistently impact soil biogeochemical properties or sagebrush performance, but it did increase fungal richness and affect microbial community composition. Third, herbicide presence in post-burn soil altered bacterial, fungal, and AMF community composition, and inhibited total sagebrush biomass. Additionally, I found that the vast majority of root colonizing fungi were DSE and not AMF, and that DSE and AMF increased proportionally to each other in some samples. These findings indicate that adding biochar to sagebrush seedlings may enhance establishment and resiliency in post-burn settings to a greater degree than adding native soil microbial inocula, and herbicide addition to control invasive species could counteract these benefits to performance.

Amending degraded soil with a native soil inoculum treatment did not enhance sagebrush germination or performance, but biochar consistently improved soil moisture retention, while also increasing pH, sagebrush germination, and performance. The biochar used within my greenhouse study was derived from mixed coniferous species consisting of beetle damaged Douglas-fir (*Psuedotsuga menzeisii*, Mirbel) and lodgepole pine (*Pinus contorta*, Douglas) created through the fast pyrolysis method. Biochar is naturally porous to enable improved water adsorption (Weber and Quicker, 2018) and

alkalinity of soils (Yuan et al., 2011; Anderson et al., 2013). The biochar I used had a pH of 8.9, and other studies confirm that woody biochar with a high pH increases soil pH and moisture (Lehmann, 2007; Duku et al., 2011; Karim et al., 2020). These biogeochemical changes likely contributed to greater above-ground biomass and changes in below-ground root morphology in our sagebrush seedlings, a phenomenon that has been observed previously (Albuquerque et al., 2014). Although biochar is primarily used in agricultural settings for increased crop yields and biomass, these results are indicative that sagebrush restoration projects could benefit from biochar applications.

The effect of biochar and inocula on N mineralization varied by site. In pots with CF2018 post-burn soil, biochar significantly increased total N mineralization, but inoculation had no effect. Conversely, biochar significantly decreased N mineralization and inoculum addition increased N mineralization in UFS2011. Neither biochar nor inoculation impacted N mineralization in UFN2011 for either treatment. Typically, when biochar with a high C:N ratio is added to soil (i.e., this biochar had a C:N ratio of 87), the biochar will promote microbial immobilization relative to N mineralization (Lehmann et al., 2003). As such, it is difficult to explain why biochar addition increased N mineralization in the CF2018 post-burn soils. A similar effect was observed in a grassland restoration study by van de Voorde et al. (2014) where biochar-induced increases in N mineralization were attributed to higher plant biomass accumulation. The differential impact of live soil inocula on N mineralization is likely due to differential interaction of native soil microbial communities with biogeochemical properties in the post-burn soils and the occurring plant communities (DeCrappeo et al., 2017). Greater mineral N availability in UFS2011 post-burn soils following inoculum addition may be

caused by greater substrate availability for ammonia oxidizers and nitrifiers owing to the relatively large abundance of invasive species at this site compared to CF2018 or UFN2011 sites. My study demonstrates that the impacts of biochar and inocula on soil N cycling processes vary considerably among different post-fire soils, but the mechanisms that underpin these variable responses remain speculative (Mia et al., 2014). I suggest that more studies evaluate the impacts of various biochar feedstock properties on post-fire soils and warrant further evaluation of inocula impacts on soil N cycling, since this variable is an important predictor of invasion potential of ecosystems.

The application of inocula significantly impacted bacterial and fungal community composition, as well as decreased fungal richness across all post-burn soils. Adding an inoculum to soil can shift microbial communities, because targeted microbes are typically introduced to intentionally benefit plant performance (Trabelsi and Mhamdi, 2013; Dove et al., 2021). To this end, I collected my inocula directly from the rhizosphere of mature sagebrush, which harbors a rich and diverse microbial community (DeCrappeo et al., 2010). Inoculation of soil with this inoculum increased the relative abundance of bacterial taxa by 7.31%, and fungal taxa by 19.41%; however, this did not increase the performance of sagebrush seedlings. The lack of a plant growth response to changes in the microbial community composition may have been caused by the lack of a concomitant increase in AMF, or DSE proportional abundances with addition of the live native soil inoculum. Indeed, when I added the inoculum to sterile soils, just one AMF SV (i.e., a Claroideoglomus genus) was present, suggesting low AMF propagation following inoculation. Future research should investigate whether dosage impacts AMF spore propagation and proliferation of sagebrush seedlings for restoration.

The application of biochar had a significant effect on fungal and bacterial composition across all post-burn soils. Biochar amendment increased the relative abundances of bacterial taxa by 6.69%, and fungal taxa by 25.20%. For bacteria, compositional shifts were found particularly in order classifications (i.e., Solirubrobacterales to Bacillales), and these changes can be explained by biochar-induced changes in soil chemistry and soil moisture (Cheng et al., 2018; Sheng and Zhu, 2018; Palansooriya et al., 2019). In addition, biochar increased the number of Glomeromycetes (i.e., AMF) by four SVs. Since AMF is an obligate root symbiont for sagebrush which aids in the transfer of nutrients and water, and increases disease resistance, this can enhance plant performance (Stahl et al., 1988; Frost et al., 2010; Begum et al., 2019). Increasing the number of Glomeromycetes is especially important in my post-burn soils, which were dominated by Ascomycetes (i.e., DSE). DSE typically colonize plants that do not exhibit mycorrhizal dependency (Mandyam and Jumpponeon, 2005) such as cheatgrass, which dominated my post-fire plant communities, and this may suppress sagebrush performance and other restoration efforts (Owen et al., 2013; Weber, 2015; Weber et al., 2015). Our results support a study by Jin (2010) who demonstrated that biochar addition can cause a shift from a fungal community dominated by Basidiomycota and Ascomycota (i.e., DSE), to a community dominated by Zygomycota and Glomeromycota. The effects of this change in microbial community are still uncertain in terms of sagebrush performance. Given that biochar addition increased sagebrush germination and performance in my study, my findings suggest that biochar-induced shifts in the soil microbial community composition have the potential to benefit sagebrush establishment.

Biochar consistently altered the fungal community composition, but fungal communities were clearly differentiated by sites. Site-specific effects on fungal community composition is most likely due to differences in plant communities, and in physical (i.e., structure and soil moisture), and chemical (i.e., pH) properties of soil among sites (Belnap et al., 2005; Kilvin and Hawkes, 2010; Lehmann et al., 2011; van der Putten et al., 2013). For example, in the previous chapter, I showed significant differences in the relative abundances of aggregates among sites. These differences likely contributed to variation in the effect of biochar addition on fungal root colonization among sites (Yin et al., 2021). Biochar addition reduced proportional fungal colonization of DSE, and AMF, in UFS2011 post-burn soils, but did not affect other post-burn soils from other sites. Earlier studies have shown that biochar can increase AMF colonization (Saito, 1990; Saito and Marumoto, 2002), but more recent studies indicate that properties of biochar may negatively interact with microbial communities due to its chemical formulation and its interaction with particular soils (Brtnicky et al., 2021). Warnock et al. (2010) observed either declines or no changes in AMF colonization across five chemically different biochar and 10 different application rates on different soil types due to decreased phosphorus availability. Thus, biochar has the potential to stimulate fungal persistence that could be more conducive to sagebrush establishment, but more robust research into the chemical properties and application rates of biochar and its interaction with the microbial community are needed to promote beneficial relationships.

Herbicide significantly decreased sagebrush above- and below-ground performance and changed microbial composition. Glyphosate, the most commonly used herbicide (i.e., RoundUp®), claims to have a half-life ranging from 3 – 249 days (USDA

1997) and imazapic (i.e., Plateau®) has claimed varying residual times (Tu et al., 2001; Durkin and Follensbee, 2004), one study reportedly observing herbicide persistence for up to 2,972 days (Jarvis et al., 2006). The UFN2011 post-fire soil was collected over 500 days after application in March 2019, perhaps persisting in the soil due to the decreased sagebrush performance. Planting native species before herbicide degradation could inhibit the formation of a large taproot and canopy, both of which are important for sagebrush establishment and resiliency (Sturges and Trlica, 1978). Hitchmough and de la Fleur (2006) found that prairie plant cover declined significantly from year to year due to phytotoxicity of paraquat and diquat (Weedol®) herbicide treatments administered in 1999. Similarly, at my site, I observed a reduction in plant cover in the field relative to the site that received no herbicide additions, and these observations are corroborated by lower overall biomass of sagebrush in soils containing herbicide in the greenhouse study. My findings enhance the knowledge surrounding the dangers of herbicide as an effective means for restoration.

Overall, my results suggest that biochar treatments enhance sagebrush performance in greenhouse settings, and soil-derived inoculums are not a definitive means for incorporating beneficial mycorrhizae. Furthermore, herbicide effects remain persistent within soils beyond the expected degradation time, influencing both plant growth and microbial community composition. To further understand the applications of these two treatments, it is recommended that this study is commissioned to the field to broaden the realistic bearings toward restoration practices.

5. Tables and Figures

Table 2.1 Sagebrush performance measurements (i.e., germination rate, shoot biomass, root length, AMF and DSE proportional abundances) for all combinations of treatments. Values represent means with error bars as ± SE.

Site	Treatment	Germination rate (%)	Shoot biomass (mg DW)	Root length (cm)	Root diameter (mm)	AMF Prop Abundance	DSE Prop Abundance
CF2018	+Inoculum	13.67 ± 2.67	.78 ± 0.79	33.18 ± 5.81	0.39 ± 0.01	.03 ± 0.01	0.03 ± 0.02
	-Inoculum	13.83 ± 2.65	.82 ± 0.55	25.63 ± 5.42	0.39 ± 0.01	.03 ± 0.02	0.17 ± 0.04
	+Sterile	17.90 ± 4.50 a	6.28 ± 0.68 a	31.18 ± 6.31	0.40 ± 0.01	0.00 ± 0.00 b	0.15 ± 0.03
	-Sterile	10.00 ± 2.25 b	5.23 ± 0.75 b	27.43 ± 5.37	0.39 ± 0.01	0.06 ± 0.02 a	0.11 ± 0.03
ANOVA							
Site							
Inoculum							
Sterile	$p < 0.05$						
Site x Inoculum x Sterile							

Table 2.2 Identified SVs of Glomerales present within the inoculum (i.e., +Sterile+Inoculum) and burned conditions within the field (i.e., -Sterile-Inoculum) treatments with total number of reads, percent reads, number of samples, and percent of samples (n = 7).

Site	Treatment	Genus observed	Number of reads	Percent reads	Number of samples	Percent samples
CF2018	+Sterile+Inoculum	*Claroideoglomus sp.*	53,063	29.39	4	57.14
	-Sterile-Inoculum	*Claroideoglomus sp.*	9,370	22.60	5	71.43
		Diversispora sp.	128	0.31	2	28.57
		Otospora sp.	286	0.69	5	71.43
		Rhizophagus sp.	6,215	14.99	2	28.57
		Septoglomus sp.	2,199	5.30	2	28.57
		Unknown	7,476	18.03	3	42.85

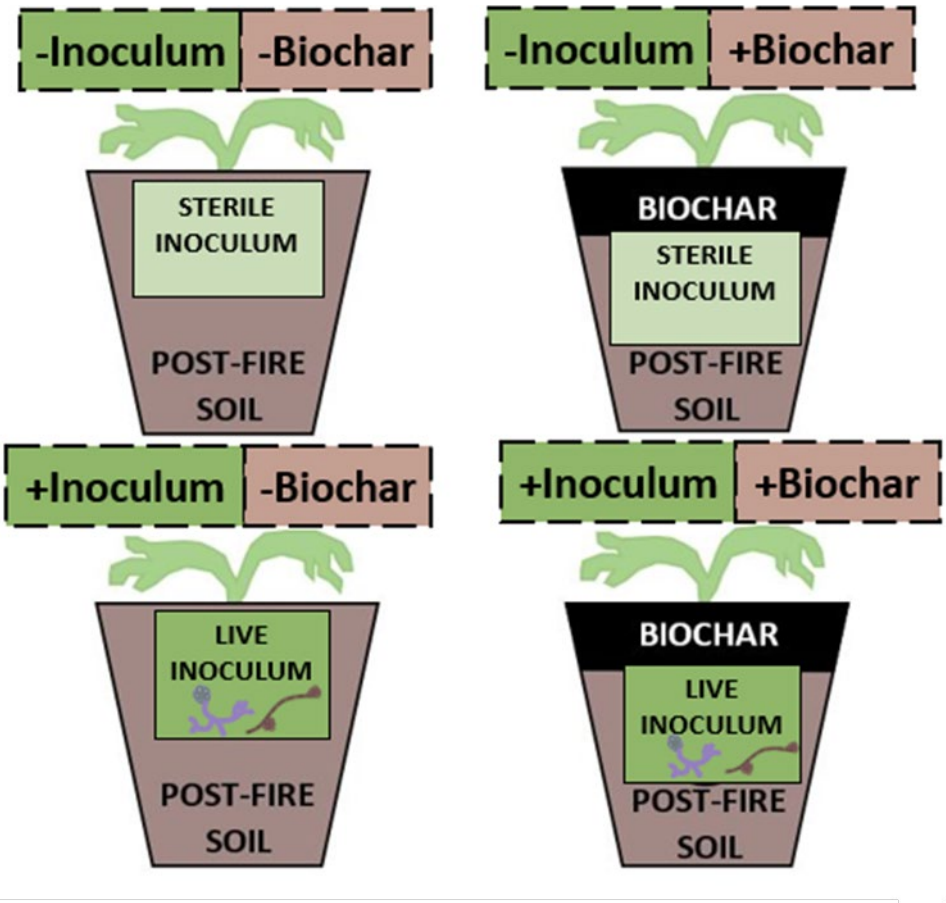


Figure 2.1 Full factorial greenhouse design consisting of native soil microbial community treatments (+Inoculum) and biochar treatments (+Biochar) implemented at the Boise State University research greenhouses in Boise, Idaho. Controls were added by sterilizing native soil microbial communities (-Inoculum) and by not including biochar (-Biochar).

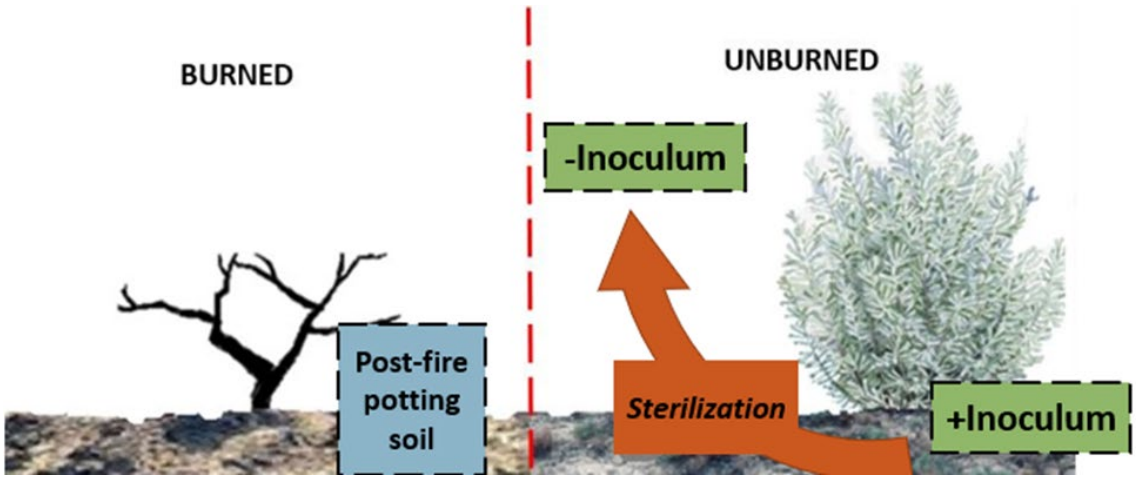


Figure 2.2 Inoculum collection for greenhouse experiment from each fire (i.e., CF2018, UFN2011, and UFS2011) located in the OCTC south of Boise, Idaho. Post-fire potting soil was collected from burned areas. Native inoculum was collected underneath mature sagebrush plants in unburned areas to represent a native soil microbial community treatment (+Inoculum) and was sterilized via autoclaving to represent control treatments (-Inoculum).

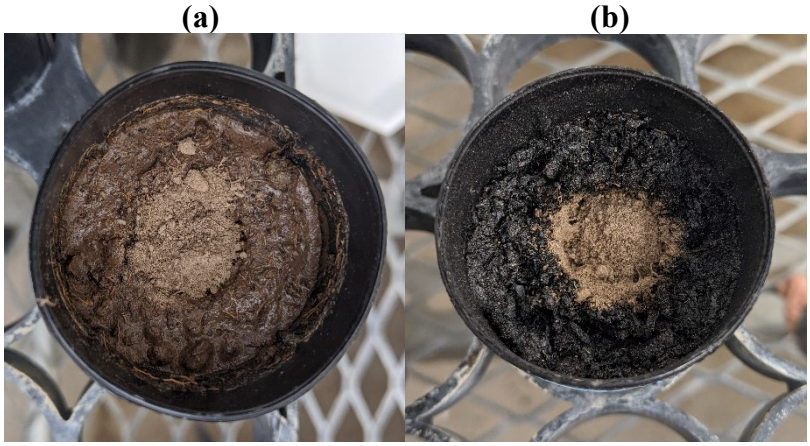


Figure 2.3 Image showcasing inoculation procedure where 5 g of +Inoculum are added to either +Inoculum-Biochar or -Sterile/+Sterile+Inoculum (*a*) or +Inoculum+Biochar (*b*) pots within the Boise State greenhouse at Boise State University.

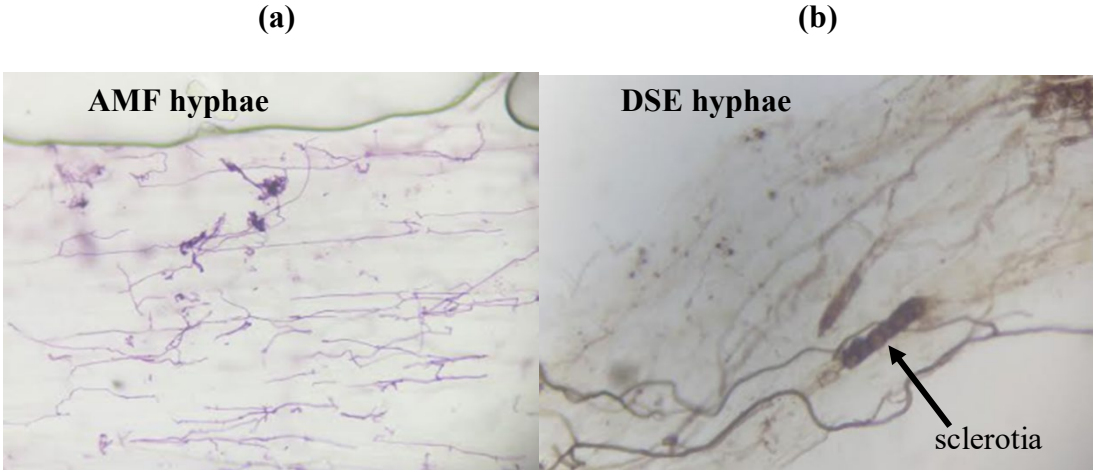


Figure 2.4 Stained AMF (*a*) and DSE (*b*) structures counted for proportional abundance measures on sagebrush roots (x40). Fungal colonization was recorded via magnified intersections (McGonigle 1990) to estimate presence and absence counts of fungal structures.

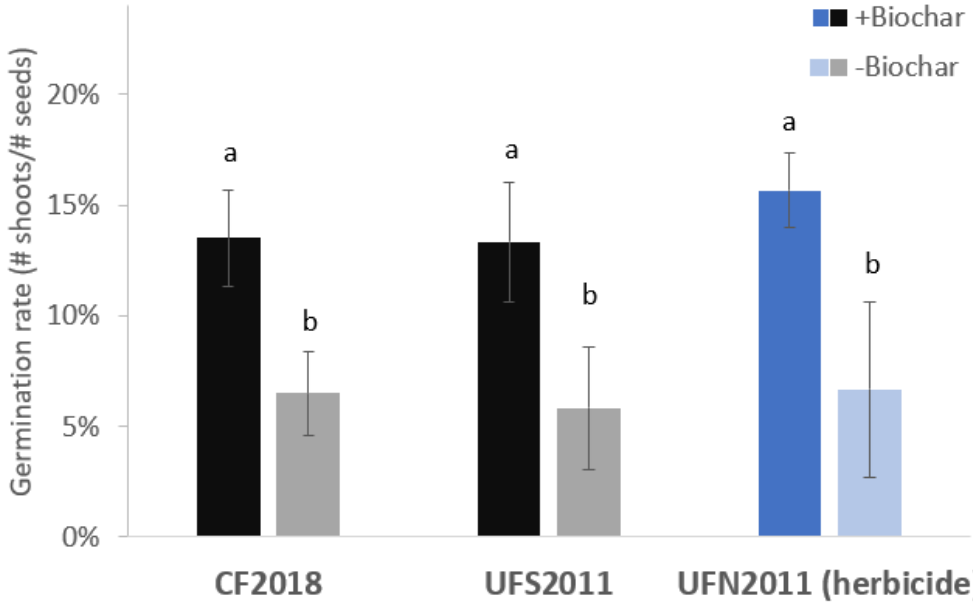
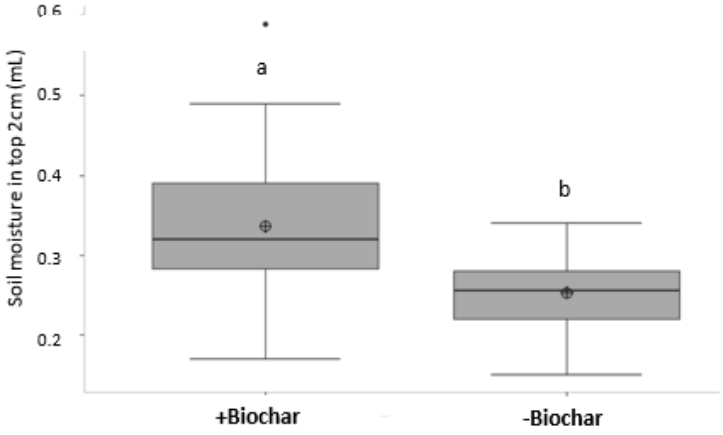


Figure 2.5 Germination rate of sagebrush seedlings (i.e., number of shoots present divided number of seeds planted) exposed to biochar treatments during the first month of growth in the greenhouse experiment. Significant differences existed between +Biochar treatments and -Biochar treatments ($p < 0.05$) and are indicated by lowercase letters. Values represent means with error bars as ± SE (n=20).

(a)



(b)

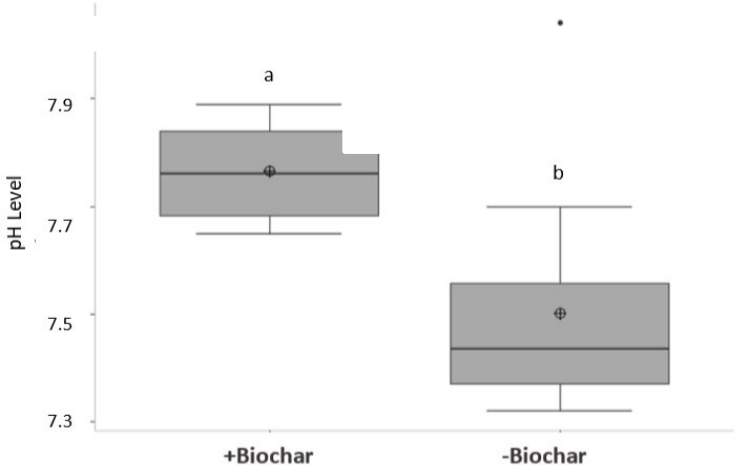


Figure 2.6 Soil moisture (*a*) and pH (*b*) in +Biochar and -Biochar treatments during the 3-month greenhouse study. Lowercase letters represent significant differences between treatments ($p < 0.05$).

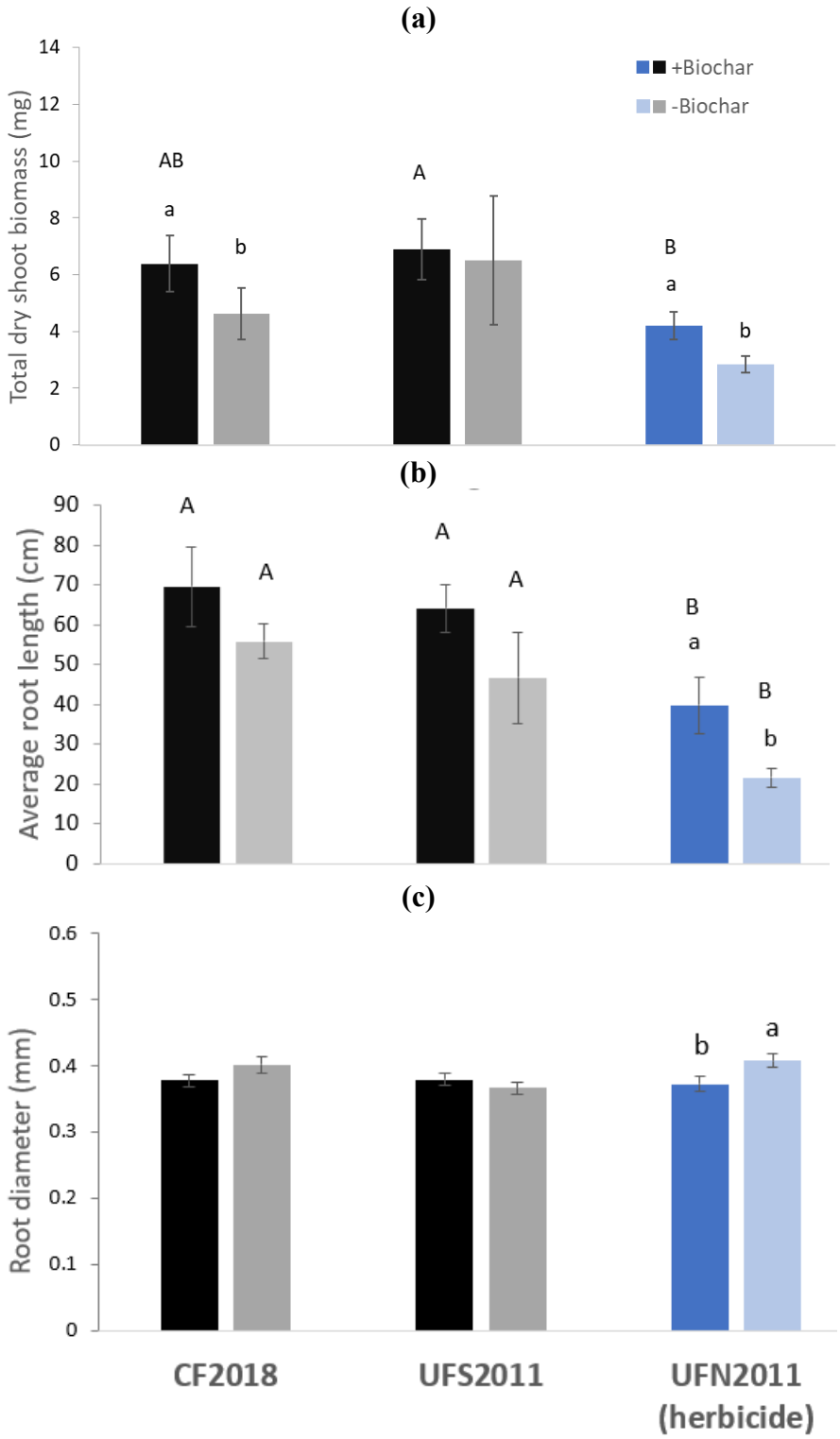


Figure 2.7 Shoot biomass (DW) of sagebrush seedlings (*a*), root length (*b*), and root diameter (*c*) during the 3-month greenhouse experiment. Significant differences between +Biochar treatments and -Biochar treatments ($p < 0.05$) are indicated by lowercase letters. Uppercase letters represent significant differences across sites ($p < 0.05$). Values represent means with error bars as ± SE (n=20).

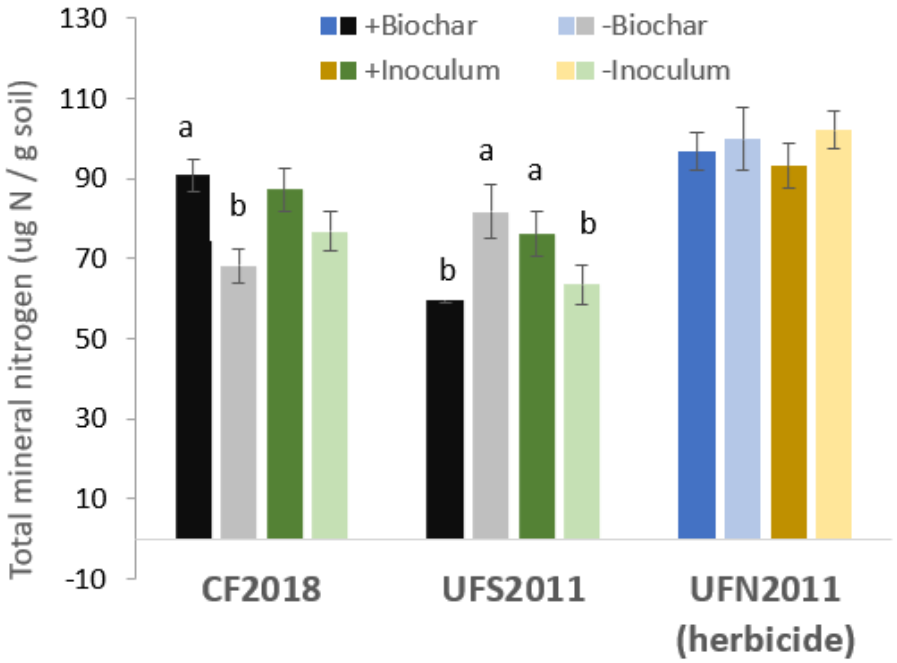


Figure 2.8. Total mineral N (NO_3^- + NH_4^+) at termination of the 3-month greenhouse experiment. Significant differences between biochar and inoculum treatments are indicated by lowercase letters. Values represent means with error bars as ± SE.

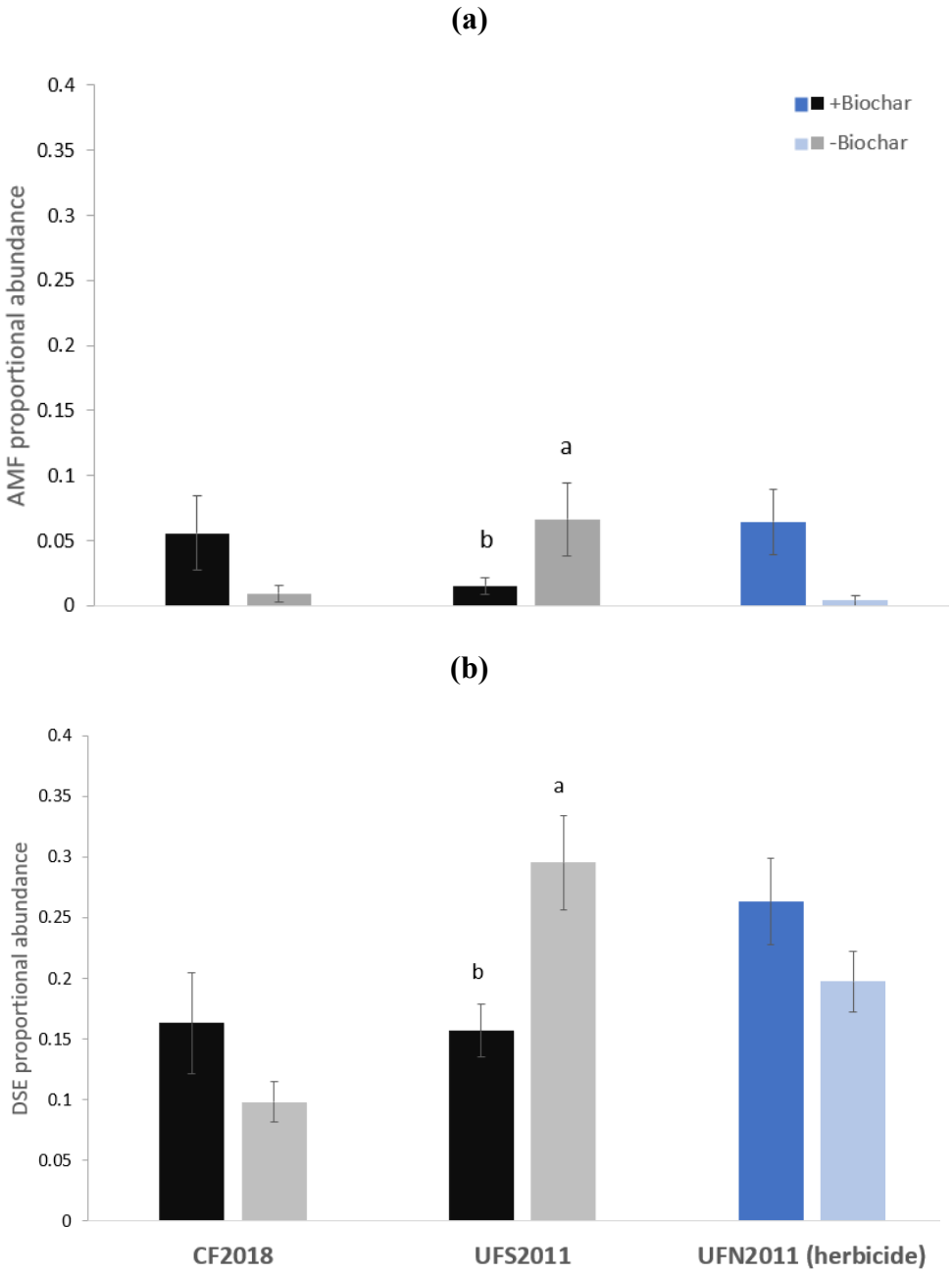


Figure 2.9 Proportional abundances of AMF (*a*) and DSE (*b*) colonization on sagebrush seedling roots at termination of the 3-month greenhouse experiment. Inocula treatments were included in this graph, but biochar had the main effect. Significant differences between biochar treatments ($p < 0.05$) are indicated by lowercase letters. Values represent means with error bars as ± SE.

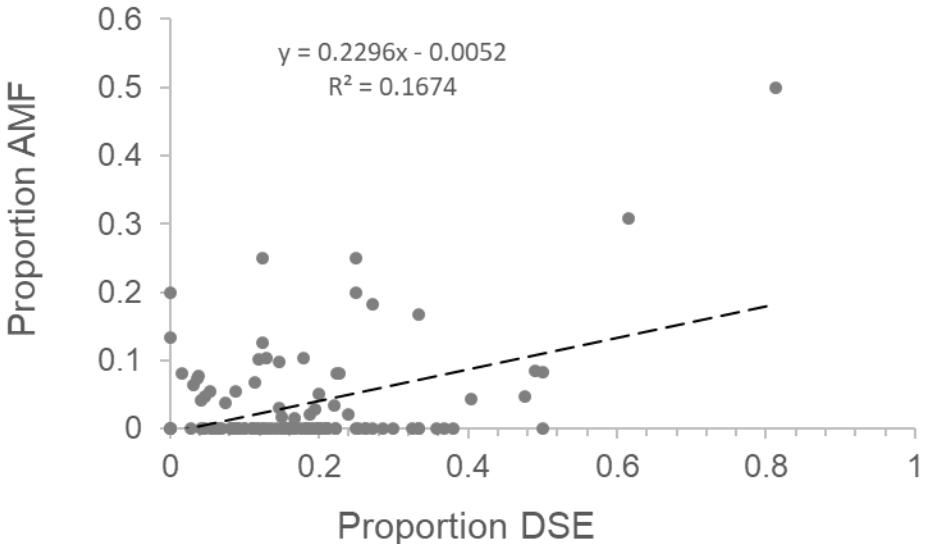
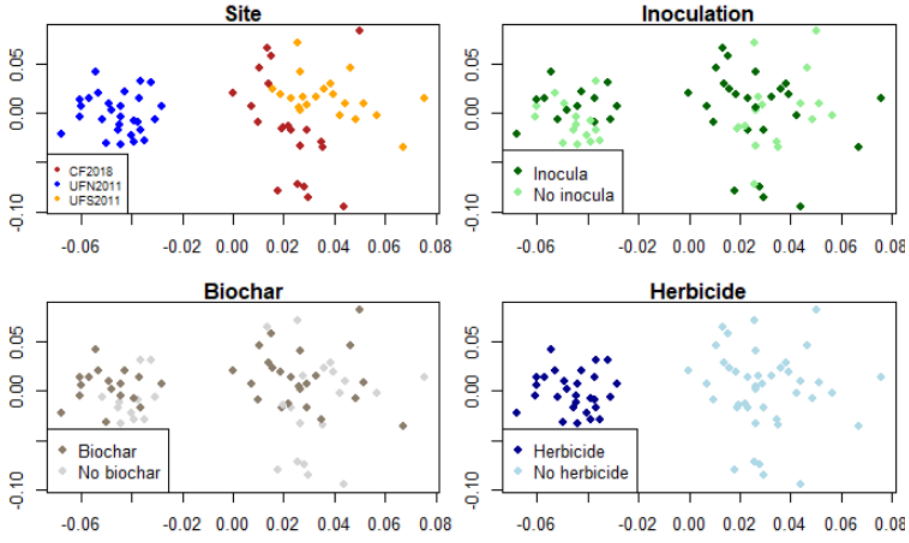
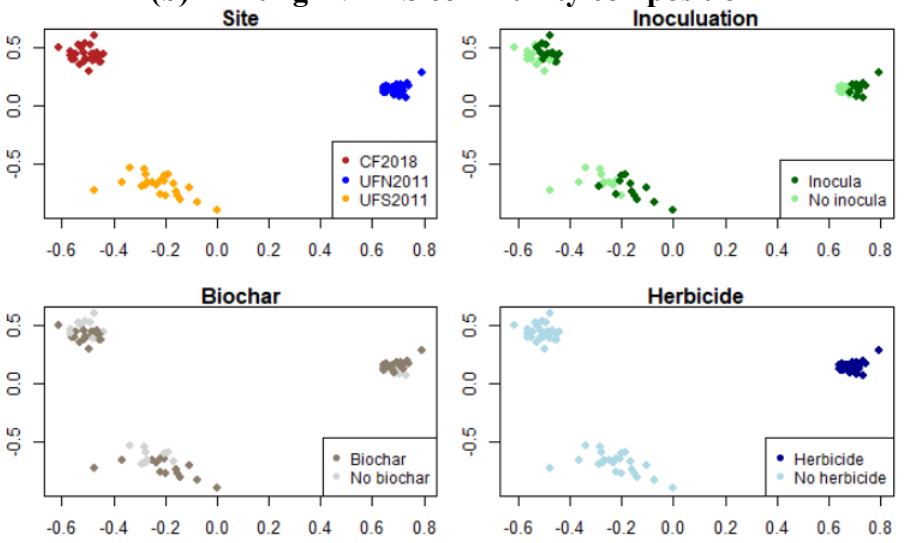


Fig 2.10 Correlation between AMF and DSE proportional abundances for all treatments (n=86) detected on sagebrush seedling roots at termination of the 3-month greenhouse study.

(a) Bacterial NMDS community composition



(b) All fungi NMDS community composition



(c) Only AMF community composition

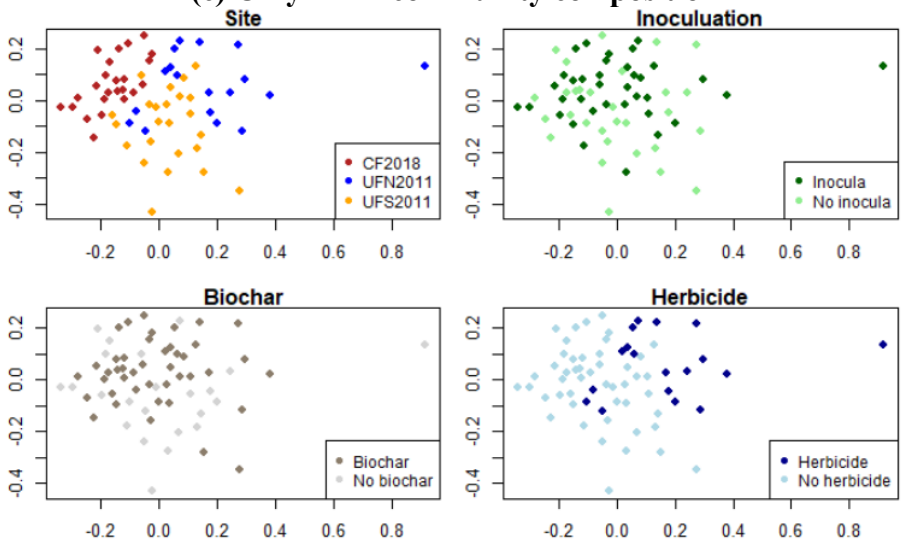


Figure 2.11 NMDS ordination results of bacteria (*a*), general fungi (*b*), and only AMF (*c*) by site (*top left*), inoculation (*top right*), biochar (*bottom left*), and herbicide (*bottom right*). For bacterial 16S and fungi ITS, all factors influenced composition. For only AMF FLR, all factors except inoculation influenced bacterial composition.

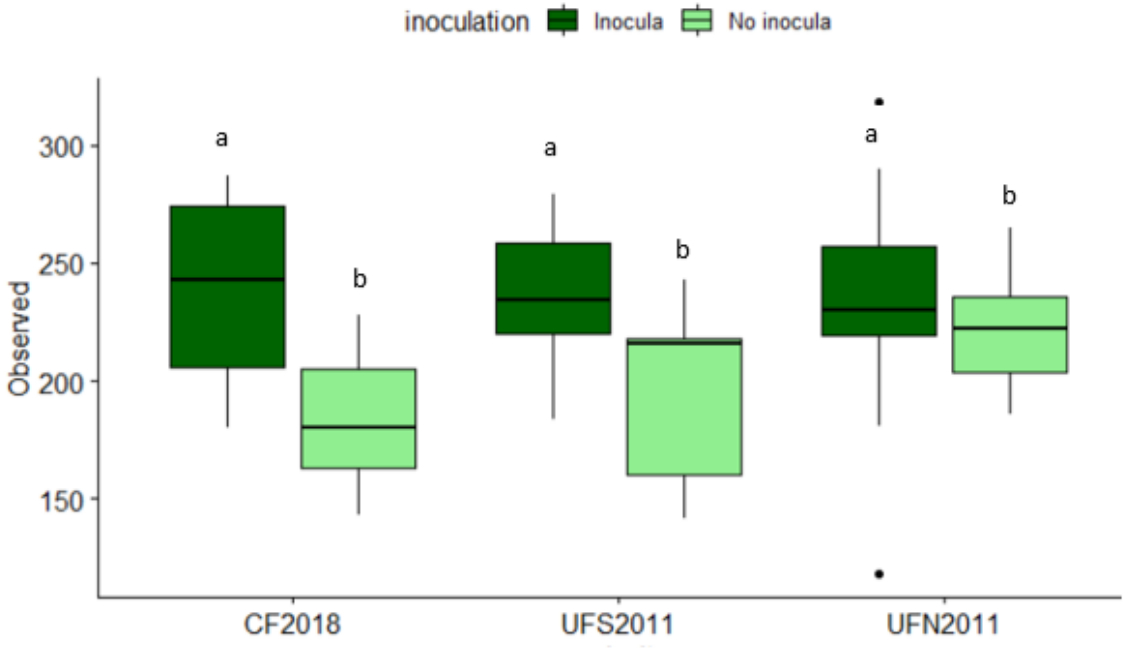


Figure 2.12 Site differences in fungal SV richness by inoculation. Lowercase letters represent significant differences between treatments ($p < 0.05$).

CHAPTER THREE: GENERAL CONCLUSIONS

The results of my study demonstrate that fire can significantly alter a variety of biogeochemical soil properties, and these changes catalyze a positive feedback loop between plants and soils that limits native plant recovery within sagebrush steppe ecosystems. Even years after fire with persisting non-native vegetation cover, my study supports the notion that soil properties will remain degraded without appropriate management strategies supporting restoration, but herbicide may damage efforts to restore soils that would support native plants. However, data from my second experiment indicate that biochar can be used as a soil amendment to enable soil recovery.

When assessing native microbial inoculum and biochar treatment impacts on sagebrush performance, results suggested that sagebrush would benefit from additions of biochar within a greenhouse setting, supporting my hypothesis that biochar addition enhances soil properties to benefit sagebrush performance. More research in the future should focus on incorporating various biochar amendments in sagebrush restoration projects in the field to observe and monitor the outcomes. In contrast, application of a native soil microbial inoculum did not promote sagebrush performance, despite a variety of impacts the inocula exhibited on soil microbial communities. Recent studies have used methods of extraction to obtain pure inocula (i.e., in vitro and in vivo), and this study reverts back to obtaining soil inocula from the source at rhizospheres, but is in need further refining. Although these treatments affected sagebrush performance in varying ways, future studies should encompass these, as well as explore other, soil amendments

to promote greater sagebrush performance in the midst of changing fire regimes, post-fire vegetation shifts, and current post-fire management.

Lastly, my study demonstrated that herbicide use can directly and indirectly contribute to soil property changes, with negative impacts for sagebrush growth. This contradicts the assumptions that herbicides like glyphosate and imazapic have relatively short residence times in soil, and that unintended impacts on native plant species are negligible. These effects can persist and affect future plant growth potential, as demonstrated in my greenhouse study, in which herbicide decreased plant biomass and significantly shifted the composition of the microbial community. This may be a result of increased soil moisture, as sagebrush seedlings were provided optimal conditions throughout the three-month growth period compared to field settings. Therefore, the microbial community was potentially granted a biological window of opportunity for maximum activity, which may have compounded with site-level effects of herbicide to yield these differences. This finding is important to consider because post-fire restoration could be compromised in areas that have been managed through herbicides. Future work should look into residual times of herbicide that will be applied on restoration sites, or potentially find alternative ways to contract so that the sagebrush and other native species may recover successfully.

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