THE REPONSE OF SOIL MICROBIAL COMMUNITIES TO THE INTERACTIVE EFFECTS

OF DROUGHT AND FIRE IN A SEMI-ARID SAVANNA

A Thesis

By

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ABSTRACT

The frequency and severity of both drought events and unintentional wildfires are expected to increase within the next century. In addition to having profound effects on vegetation structure and composition, this is expected to disrupt key nutrient cycling processes within the soil. Soil microbes play a fundamental role in degrading soil organic matter as well as transporting nutrients to plants. Changes in microbial biomass or composition induced by fire and drought may decrease nutrient cycling efficiency. Despite numerous studies assessing the individual impacts of drought and fire on soils, few studies have analyzed their combined effects. Our objective was to assess the impacts of drought, fire, and their combined impacts on the soil microbial community, the abundance of the microbes that compose the microbial community, and extracellular enzyme activity. At the Texas A&M AgriLife research station in Sonora, Texas, a full-factorial randomized plot experiment was established to assess the effects of drought and fire on soil microbial community composition and activity in a semi-arid savanna. Our results show that a semi-arid savanna experiences no negative impacts on the measured biological properties in response to drought, fire, or a combination of drought and fire. We also observed the presence of bacteria and fungi that are associated with drought tolerance, which may explain why there was no observed response to disturbance treatments in this study. We can conclude that fire remains a viable management option for the maintenance of herbaceous vegetation in a semi-arid savanna without concerns of negatively impacting soil biological properties even in the event of a short-term, extreme drought.

DEDICATION

To my parents for instilling in me a sense of wonder for the natural world and to every science educator I have ever had that has made learning both a challenging yet rewarding endeavor of the mind.

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**Contributors**

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Samples were gathered and pre-processed at Texas A&M University by Dr. Yamina Pressler of the Natural Resources Management and Environmental Sciences Department at California Polytechnic State University. Bioinformatics processing was completed by John Blazier at the Texas A&M Institute for Genome Sciences and Society. All other work conducted for the thesis was completed by the M.S. student.

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CHAPTER I INTRODUCTION AND LITERATURE REVIEW

1: Introduction

Drought and fire severity are expected to increase in the near future (Moritz et al., 2012; Cook et al., 2015), and the impact of these disturbances will undoubtedly cause large ecosystem and economic changes (Bodner and Robles, 2017). Drought results in roughly nine billion dollars in annual losses (NOAA) and as of 2020, wildfires resulted in an estimated 130-150 billion dollars direct and indirect damages across the United States (Roman et al., 2020). As drought and fire frequency and severity are expected to increase, annual losses can also be expected to increase. With climate change causing concerns about carbon storage, it is pertinent to understand how disturbances such as drought and fire impact its largest sink: soils. Soils hold an estimated 1500 petagrams (Pg) of organic carbon in the top 1 m  (Smith et al., 2015b) with plants acting as the primary vehicle moving carbon into soil (Bonkowski et al., 2000). In addition to these inputs, soil microorganisms also play a large role in key nutrient cycles such as carbon (C) and nitrogen (N) through the regulation of organic matter turnover, which in turn influences plant growth (Fultz et al., 2016). With 20% of Earth's surface consisting of grassland and savanna ecosystems (Lieth and Whittaker, 1975) storing an estimated 30% of all soil organic carbon (Field et al., 1998), it is crucial to understand the influence of abiotic disturbances in these environments.

Semi-arid savanna ecosystems are dependent on disturbances for the maintenance of herbaceous vegetation (Veldman et al., 2015), but the response varies based on the type and severity of any given disturbance (Vetter, 2009; Taylor et al., 2012). Fire has been an important natural disturbance in semi-arid savannas that drives long-term soil carbon (C) and nitrogen (N)

stocks (Soong and Cotrufo, 2015; Pellegrini et al., 2018) and is still often used for the

maintenance of these ecosystems. Fire immediately changes aboveground vegetation, but also

maintains vegetative diversity in semi-arid savannas (Bond and Keeley, 2005). Not often thought

about as much in the event of a fire, the soil environment also experiences immediate changes,

but the response to fire in the microbial community varies (Fultz et al., 2016; Pressler et al.,

2019). Fire causes a reduction in soil microbial biomass, enzyme activity, and volatilizes C and

N upon combustion but only influences surface soils (Tilman et al., 2000; Hinojosa et al., 2016).

Fire can immediately reduce microbial community composition and biomass during combustion,

but both can rapidly restore themselves within a year of the fire (Sheik et al., 2011; Fultz et al.,

2016). Microorganisms residing in surface soils are more susceptible to the effects of fire than

microbes in subsoils (Pressler et al., 2019) Yet, fast, low-intensity fires can also have little

impact on the microbial community in surface soils (Neary et al., 1999). Like drought, soils

subjected to fire have shown a reduction in extracellular enzyme activity (Fultz et al., 2016;

Hinojosa et al., 2016) and depending on the severity of the fire, microbial biomass can either

increase or decrease (Andersson et al., 2004) or show no response (Dunn et al., 1985),

highlighting the need for more research on the mechanisms that results in such conflicting

results.

Semi-arid savanna ecosystems are also susceptible to extreme drought (Breshears et al.,

2016) and tend to exhibit negative responses in aboveground vegetation and C and N stocks over

long periods of time (Vicente-Serrano et al., 2013; de Vries et al., 2016). The slow response in

both vegetation and C and N stocks to drought in semi-arid savannas would suggest that the

impacts of drought on soil communities may not be immediately detectable, whereas fire can

elicit an immediate change to the soil environment, even if that change is often restricted to the

top five centimeters of the soil (Raison, 1979; Andersson et al., 2004). Drought can cause long-term soil moisture limitations that negatively impacts soil microbial communities by reducing access to nutrients (Schimel, 2018). As drought severity increases, extracellular enzyme activity can decrease, further limiting nutrient availability to microbes (Sardans and Peñuelas, 2005). Extracellular enzyme activity is often a sensitive indicator of soil microbial community responses to various environmental disturbances such as drought and fire (Aon and Colaneri, 2001; Leinweber and Schlichting, 2003; Sanaullah et al., 2011). As drought persists, there have been observed increases (Conant et al., 2011) and decreases (Burns et al., 2013) in extracellular enzyme activity, which suggests that there are soil specific responses to abiotic disturbances. Whether experiencing drought or fire disturbances, exposure to water stress in these semi-arid savannas is likely to impact both microbial and plant communities through the interruption of key nutrient cycles, such as C and N (Sheik et al., 2011).

Surprisingly, drought and fire have been largely studied independent of each other, and to the best of our knowledge, few studies have assessed their combined impact on soil microbes and extracellular enzyme activity (but see Hinojosa et al., 2016; Hinojosa et al., 2019). The segregation of environmental variables in research is not exclusive to understanding drought and fire, and the current body of research investigating the combined impacts of drought and fire on ecosystems is limited (Holden et al., 2012; Luyssaert et al., 2014; Schlesinger et al., 2016). As research needs to meet the complex interactions of the natural world with complex research itself the need to assess multiple environmental variables on ecosystem functions will only become more important. We will only begin to properly understand how to mitigate the risks from climate change through research studying the interactive effects of various disturbances such as drought and fire. Disturbance ecology is already thoroughly researched for plant community

ecology, but research into soil responses to disturbances is not as intensely investigated (Coyle et al., 2017). With the influence soil communities have on ecosystems, it is necessary to start investigating the impact of various perturbations on soil environments.

In this review, I will explore the impacts that drought and fire have on the soil environment and I aim to answer the following two questions: 1) What are the independent impacts of drought and fire on soil microbial communities and extracellular enzyme activity? 2) How does the combination of drought and fire impact alter soil microbial communities and extracellular enzyme activity? After exploring the independent impacts of drought and fire, I will compare the independent influences of drought and fire to their combined impact. Subsequently, I will highlight common findings as well as knowledge gaps that pertain to soil microbial communities, extracellular enzyme activity, and their responses to abiotic disturbances.

1.1: Drought's impact on soil microbial communities and extracellular enzyme activity:

Since the definition of drought is not concrete and varies based on discipline, the definition I will be following is that of an ecological drought as defined by (Crausbay et al., 2017): "an episodic deficit in water availability that drives ecosystems beyond thresholds of vulnerability, impacts ecosystem services, and triggers feedbacks in natural and/or human systems." Using this definition provides the framework at which we can identify the impacts of drought on soils and soil microbial communities.

Soils are largely impacted and influenced by the vegetation present in its given environment (Sanaullah et al., 2011), and numerous studies show that plant-microbe interactions largely influence nutrient cycling belowground (Reynolds et al., 1999; Smith et al., 2015b). However, with an expected increase in climate change induced droughts, microbial communities

may be impacted (Neary et al., 1999; Bonkowski et al., 2000; Smith et al., 2015b). Drought can limit microbial resource acquisition and transport as water becomes scarce (Schimel, 2018). Drought can also reduce the amount of carbon inputs into soil from plants (Canarini et al., 2018), which may result in an overall decrease in microbial decomposition.

The main impact of drought on soils is the limited diffusion and supply of resources to soil organisms. However, drought can also directly result in the selection of more resistant microbial groups (Fuchslueger et al., 2014). Water is the primary resource that transports nutrients through soils (Schimel, 2018). Therefore, as drought induces water stress, there is reduced access to resources which likely will result in a reduction of microbial activity (Schimel, 2018). With a decline in microbial activity, extracellular enzyme activity is likely to be directly impacted. Soil extracellular enzyme activity reflects microbial community functions and their response to disturbances (Sanaullah et al., 2011). Research has shown that drought causes sensitivities in extracellular enzyme activity (Sardans and Peñuelas, 2005), though the effects may differ depending on plant community composition (Sanaullah et al., 2011). If drought results in a decline in vegetative cover (Bodner and Robles, 2017), then there may be a decrease in nutrient transport from microbes to plants based on reduced symbiotic relationships. Sardans et al. (2005) found enzyme activity to be highly sensitive to slight decreases in water availability and that as drought increased, enzyme activity decreased, which may reduce nutrient availability to plants in the long-term (Sardans and Peñuelas, 2005).

During periods of drought, plant growth is limited due to a reduction in microbial activity restricting the transfer of nutrients to plants. If drought conditions persist, vegetation will senesce resulting in a temporary increase in litter production  (Sardans et al., 2008). These added inputs of litter may initially excite microbial activity, but over time as soil moisture decreases and

microbial activity declines the overall decomposition of organic material will be reduced. This long-term impact on ecosystems may ultimately inhibit carbon storage potentials through the combined impact of an overall decrease in vegetation and the subsequent slowing of microbial activities that prove to be fundamental for carbon storage and organic matter turnover (Fuchslueger et al., 2014). A simple yet effective way to portray the impact drought may have on environments is as Schimel et al. (2018) states: "water is the ultimate resource for life". Without water, many environments will be significantly altered in structure and limited in function.

1.2: Fire's impact on soil microbial communities and extracellular enzyme activity:

Despite fire being a relatively short-lived phenomenon, there are lasting impacts of fire on soils. Fires alter many soil components such as nutrient concentrations and physical properties, which can negatively impact soil microbes (Holden et al., 2012). While fire is a common disturbance in many ecosystems and can alter soil C and N concentrations (Neary et al., 1999; Holden et al., 2012; Fultz et al., 2016), it also is known that soil microbes have an important role in ecosystem recovery (Li et al., 2019). Since soil microorganisms play a crucial role in soil organic matter decomposition (Fultz et al., 2016), one would think that fire may largely interfere with this process. However, Fultz et al. (2016) states that while microbial populations are typically reduced immediately after a fire, they quickly recover, which may be attributed to the importance of soil moisture. Previous studies have shown that soil moisture is a major influence on soil microbial communities and microbial activity (Hart et al., 2005; Fultz et al., 2016). Fire may vaporize water in the upper centimeters of soils, but may also only impact microbial communities in surface soils (Andersson et al., 2004). Research has shown that soil moisture is impacted by fire, but soil moisture recovers, even increases, within six months after burning (Fultz et al., 2016). Even in event of a severe wildfire that evaporates much of the water

in surface soils, soil moisture recovers a year after the fire event (Holden et al., 2012). Research has also observed a change in the microbial community following a fire and note that both above- and belowground responses to fire drive ecosystem structure, both vegetative and microbial (Fultz et al., 2016). Although microbial communities have exhibited a degree of plasticity in response to fire, their overall response to ecosystem disturbances will be indicative of the speed at which ecosystems may or may not be able to recover (Hart et al., 2005; Fultz et al., 2016; Li et al., 2019; Pressler et al., 2019).

Bacteria have been shown to be more resistant to fire than fungi, which is attributable to fungal networks often residing in the uppermost layers of soil due to their associations with root networks (Pressler et al., 2019). Although more resistant, bacteria experience the greatest impact from fire in surface soils than in subsoils. This is due to microbes being more abundant in surface soils than in subsoils that are more insulated (Hart et al., 2005), which results in decreased bacterial biomass (Pressler et al., 2019). This claim is further supported by Tilman et al. (2000), which states that there are no detectable effects of fire frequency on belowground C in the first 20 cm of soil (Tilman et al., 2000). While some findings state there is no impact of fire on soils many surface soils are often rich in organic matter, which is likely to burn. If fire does not perturb soil communities and they are found to recover within a year, it raises the question about what may additionally drive soil communities in response to fire. Given that microbial biomass in the rhizosphere of plants is considerably different than that of the surrounding bulk soil environment (Bonkowski et al., 2000), studies have expressed caution to believing that responses seen in the microbial community are due to the disturbance alone. In a study about natural disturbances on soil communities, Coyle et al. (2017) concluded with saying that changes in the

soil community may not be directly attributable to the disturbance, but indirectly from changes in the plant community over time during recovery from the disturbance (Coyle et al., 2017).

Although fire alters the microbial community through changes in aboveground vegetation, understanding how fire influences soil nutrients and resources may show the indirect impact of fire on extracellular enzyme activity. In response to fire, extracellular enzyme activity has shown to be reduced (Hinojosa et al., 2016), which may simply be attributed to decreases in microbial biomass (Pressler et al., 2019). In addition to finding reduced enzyme activity in response to fire, it has been found that boreal systems have shown long-term effects on extracellular enzyme activity, which may alter litter decay rates and soil C dynamics (Holden et al., 2012). Other long-term changes in response to fire are vegetative shifts which subsequently alter the soil microbial community (Coyle et al., 2017). Additionally, fire can change the dynamics of litter decay rates based on the reduction of extracellular enzyme activity. However, since fire does not appear to directly induce long-term reductions in microbial communities, the impact on soil functions may not be as negative as the long-term impact of drought, despite shifts in community structure. Although drought and fire are different disturbances, they share one common theme that impacts soil microbial communities, and that is soil moisture (Dunn et al., 1985).

1.3: Combined impact of drought and fire on soil microbial communities and extracellular enzyme activity:

There are studies that state that hotter and drier environments can enable more intense wildfire (Breshears et al., 2016). With drier environments being frequently linked with drought events, but now appearing to coincide with increased fire risk and frequency, it is perplexing as

to why few studies have approached studying the impact of both drought and fire on soil environments. Research has shown that small changes in soil moisture impact the efficiency of soil microbial communities to transport and breakdown soil nutrients (Schimel, 2018), which is a common result in a drought prone environment. Therefore drought, over fire, is the major controlling factor on soil microbial communities.

Fire influences soil moisture and can impact microbial community structure, but it does not induce such limiting constraints that drought does (Dunn et al., 1985; Acosta-Martínez et al., 2014; Schimel, 2018). There have been a handful of experiments that have investigated drought and fire, which indicate that drier environments limit the extent to which nutrients are available in a post-fire habitat (Hinojosa et al., 2012; Potts et al., 2012). Microbial communities in environments that have experienced fire are often resilient in their recovery (Fultz et al., 2016), but once drought occurs in that same ecosystem, the level of microbial community recovery is stinted and limited resulting in reduced enzyme activities (Hinojosa et al., 2016). In a study on a Mediterranean environment under experimental drought post-fire, Hinojosa et al. (2016) found most soil environment properties were decreased if they were both burned and under drought. Extracellular enzyme activity and overall microbial biomass were shown to have decreased. However, it has been shown that prescribed fires do not have lasting impacts on soil nutrients (Fultz et al., 2016).

With soil moisture appearing to be the fundamental process dictating the efficiency of microbial activity (Schimel, 2018), and recurrent drought significantly impacting ecosystem fire frequencies (Clark et al., 2002) through the increased fuel flammability (Andersson et al., 2004), it appears that as drought frequency increases, fire frequency is almost certain to follow suit. Since fire intensity often correlates with fuel load (Brando et al., 2014), and drought can increase

litter production as plants senesce (Sardans et al., 2008), it would be reasonable to assume that an increase in drought will cause a spike in fire frequency.

1.4: Future needs, directions, and predictions:

The majority of studies that have investigated the combined impact of drought and fire are either in the Mediterranean or of a Mediterranean climate since they are strongly influenced by fire (Potts et al., 2012; Hinojosa et al., 2016). Mediterranean environments are an ideal place to start understanding the combined impacts of drought and fire on ecosystem functioning. However, as drought is predicted to increase mostly in higher northern latitudes (Moritz et al., 2012), there needs to be an expansion of studying the combined impact of drought and fire on a wider range of ecosystems. Due to the expansion of arid zones (Seager et al., 2018), it is pertinent that future research assesses both drought and fire at a large scale.

Many studies discussed in this review also spent a lot of time looking at microbial communities quantitatively which provides insight into how these communities react to disturbances. However, without a qualitative look at where these communities are shifting towards, we may not be able to understand why certain ecosystems are experiencing greater success in recovery compared to others. Through quantifying the changes in the microbial community and identifiying what taxa remain after disturbance we can infer why certain ecosystems may respond better to drought and fire as previous research has done (Schimel et al., 2007; Bachar et al., 2010; Acosta-Martínez et al., 2014). Knowing the function of the microbial community after disturbances may help explain increases or decreases in certain extracellular enzymes over others. Additionally, if plants have a strong influence on microbial communities via rhizodeposition, it would be wise to understand how microbial communities respond to

disturbances. Understanding the changes in the microbial communities post-disturbance could help determine which taxa are no longer present that may aid in ecosystem recovery rates.

Drought and fire impact soil moisture, and since soil moisture appears to have the greatest impact on soil microbial communities, drought and fire disturbance may have far-reaching impacts on microbial communities aside from direct effects. If future fire frequency appears to be a correlated with soil moisture present (Moritz et al., 2012), I would make the following predictions: 1) As drought severity increases we will begin seeing initial spikes in fire frequency as there is more fuel to be burned however, 2) as fires expend the given fuel load in an environment undergoing severe drought, fires will decrease as ecosystems become increasingly arid. 3) If areas under extreme drought become arid, soil microbial communities will become less active, but maintain a level of diversity as some microbes are more resistant to drought over others. With reduced microbial activity, it will take longer for environments to recover if they are not already completely shifted into a new steady state.

In conclusion, we can suggest that both drought and fire have their respective impacts on soil microbial communities and extracellular enzyme activity. However, given they are closely correlated in nature, it would be wise to increase investigations of the combined impacts of these abiotic disturbances. It is time we start conducting multi-faceted experiments to answer the more complicated questions in landscape ecology as these disturbances will likely become more devastating in the near future.

CHAPTER II MICROBIAL RESPONSE TO DROUGHT AND FIRE

2: Introduction

Drought severity and fire frequency are expected to increase in the next 10 to 20 years (Moritz et al., 2012), which is anticipated to impair soil carbon and nutrient cycling processes (Schimel, 2018). Soils hold an estimated 1500 Pg of organic carbon in the top 1m of soil (Smith et al., 2015b) and soil organic matter (SOM) represents a significant fraction of the organic carbon (C) that is held in soil (Powlson et al., 2001). Soil microorganisms play a key role in soil organic matter (SOM) turnover and nutrient cycling processes (Sardans et al., 2008; Smith et al., 2015b), but a change in drought and fire regimes may alter microbial abundance, activity, and their role in SOM stabilization (Fultz et al., 2016). Therefore, if microbial communities are altered due to disturbances, there may be severe implications for the fate of C storage.

Grassland and savanna ecosystems cover 20% of Earth's surface (Lieth and Whittaker, 1975) and store an estimated 30% of global soil carbon stocks (Field et al., 1998); therefore, it is crucial to understand the influence of abiotic disturbances on these environments. Historically, fire has been a natural disturbance in the United States with the majority of landscapes experiencing an average return interval of 1-12 years (Frost, 1998), but given its suppression during European settlement (Baker, 1992) fire has only recently been re-implemented as a management practice to suppress woody shrub encroachment (Lohmann et al., 2014). While fire is both historically present and recommended for management practices in semi-arid savannas, these ecosystems are more susceptible to changes as a result of extreme drought (Breshears et al., 2016) than they are to fire.

Despite being more susceptible to changes from drought, drought is a disturbance with impacts that manifest over longer periods of time (Vicente-Serrano et al., 2013). Soil moisture is a key driver that influences microbial biomass (Fultz et al., 2016). In drought conditions microbial biomass decreases, but reaches a stable state as more drought tolerant taxa persist in environments with low soil mositure (Schimel, 2018). In response to drought and fire the microbial community tends to shift towards more stress tolerant taxa (Bachar et al., 2010) and the proprotions of Gram + to Gram – groups of microbes can further inform us about an ecosystems tolerance to stress (Acosta-Martínez et al., 2014). Therefore, knowing which taxa are tolerant to limited soil moisture can offer insight about the responses seen in our study site (Mohammadipanah and Wink, 2016). Exposure to water stress in these ecosystems is likely to disturb both microbial and plant communities by interrupting key nutrient cycles (Sheik et al., 2011). Soil moisture is a limiting factor on soil microbial biomass and microbial extracellular enzyme activity (Sheik et al., 2011). Extracellular enzymes serve as the primary mechanism that soil microbes use to mineralize nutrients and degrade soil organic matter (Fultz et al., 2016). Extracellular enzyme activity (EEA) in soils reflect microbial community function and are used to measure the effect of various environmental stressors on carbon and nutrient cycling in soils (Sanaullah et al., 2011). In a semi-arid system, a reduction in soil moisture can decrease organic matter decomposition (Hinojosa et al., 2016). As a result, fuel loads may increase due to persistent drought which increases the probability of fire (Littell et al., 2016). In the event of fire, microbial populations tend to be reduced immediately but can quickly recover (Fultz et al., 2016). There is also evidence that burned soils under the influence of drought have lower extracellular enzyme activities than soils being impacted by drought or fire independently (Hinojosa et al., 2016). However, enzymes do not show consistent responses to changes in soil

moisture (Sardans et al., 2008; Alster et al., 2013).Due to the uncertainties of enzymatic response to the combined impact of drought and fire, further research is necessary to understand observed responses.

Drought and fire have been largely studied independent of each other, but to the best of our knowledge, few studies have assessed their combined impact on soil microbial biomass, composition, and extracellular enzyme activity. To understand how experimental drought, fire, and the combination drought and fire affects the microbial community and EEA in a semi-arid savanna soil, we measured total organic carbon, ran extracellular enzyme assays, and used 16S and ITS DNA sequencing. Through these measurements we investigated the microbial community's response to experimental drought and prescribed fire. We hypothesized that prescribed fire would reduce extracellular enzyme activity as a result of a decrease in microbial biomass due to an immediate loss of soil moisture on combustion. Conversely, we hypothesized that experimental drought would maintain levels of extracellular enzyme activity due to sustained microbial biomass carbon. We also hypothesized that prescribed fire would increase microbial community species richness by attracting species to the newly available burned biomass, but that experimental drought would be associated with greater microbial community abundance through sustained diversity over time.

2.1: Methods

2.1.1 Study site

The study site is located at the Texas A&M AgriLife Sonora Research Station roughly 56 km south of Sonora, Texas (30°16'N, 100°33'W). In accordance with the Drought-Net protocol ([www.drought-net.colstate.edu](www.drought-net.colstate.edu)), thirty-two 5 x 5 m plots were established in a wildlife and

livestock-exclusion fence. This site is dominated by herbaceous plants, and livestock had been

excluded from grazing for more than five years while non-native axis deer (*Axis* axis) and other

wildlife regularly grazed the site prior to fence construction. The soil (Valera Series) is a well-

drained, slowly permeable silty clay found primarily in rangelands, and it is classified as a fine,

smectitic, thermic Petrocalcic Calciustoll with an alkaline pH. This site is traditionally a

rangeland with grasses and forbs as the predominant vegetation and tallgrass prairie as the

climax community ((https://soilseries.sc.egov.usda.gov/OSD_Docs/V/VALERA.html) accessed

January 21, 2021). The mean annual precipitation at this location is approximately 567 mm, and

the mean annual temperature is 18.79°C (Western Regional Climate Center data from 2018-

2020).

2.1.2 Experimental Design

Four treatments were set up across thirty-two 5 x 5 m plots to assess the impacts of

drought, fire, drought + fire, and control conditions. Experimental drought conditions were

induced using rainout shelters. Rainout shelters covered a 3 x 3 m area and were constructed

with a clear polycarbonate roof attached to PVC framing; they were installed on March 3, 2018

and later reconstructed in 2019 with a wood frame. The rainout shelters diverted precipitation

away from the plots to simulate a $1^{st}$ percentile drought extreme (175.65 mm/year) based on the

Drought-Net protocol (www.drought-net.colstate.edu). Ring fires were prescribed and

administered on March 1 and 2, 2018, and again on August 15, 2019. A propane vapor torch was

used to compensate for areas low in fuel. The experimental drought + prescribed fire conditions

were established with a combination of ring fires and rainout shelters. Control plots maintained

ambient conditions. Preliminary vegetation responses to these treatments can be seen in

Hannusch et al. (2020).

2.1.3 Soil Sampling

Two types of sampling were conducted in each plot: composite cores to assess spatial

variability in each plot and bulk density cores. The composite cores consisted of 8 cores per plot

taken at a depth of 0-5 cm which were then composited into 1 sample per plot, for a total of 32

samples. These samples were used to measure soil pH, soil moisture, extracellular enzyme

activity, and microbial biomass as well as extract DNA to sequence the microbial community.

Bulk density cores consisted of 1 sample per plot at a depth of 0-5 cm for a total of 32 samples

and were used to measure bulk density. Enzyme subsamples were passed through a 4.75 mm

sieve and subsampled to 3 g and subsequently freeze dried and stored in a -80°C freezer. DNA

subsamples were also passed through a 4.75 mm sieve but were subsampled to 5 g and stored in

a -20°C freezer.

2.1.4 Physical and Chemical Properties

Soil from the bulk density core was passed through a 2 mm sieve and oven dried at 50°C.

Bulk density was determined by dividing the dry mass of the soil sieved by the volume of the

soil expressed as $g/cm^3$. Soil moisture samples were passed through a 4.75 mm sieve and were

oven dried at 50°C. Gravimetric water content of the soil samples was determined by calculating

the difference between wet and oven dried soil, which reflects the moisture of our samples on

August 15, 2019. Soil pH was measured using a Fisher Scientific accumet electrode (Waltham,

MA).

To quantify organic C in our samples we calculated the difference between total C and

inorganic C. Total C was measured via combustion using an elemental analyzer (Elementar vario

EL cube CHNS Langenselbold, Germany), and inorganic carbon was determined by pressure

calcimetery  (Sherrod et al., 2002). Stocks of soil organic C were calculated using percent C and bulk density measurements (Lee et al., 2009).

2.1.5 Microbial Properties

2.1.5.1 Microbial Biomass Carbon Measurements

Microbial biomass carbon (MBC) values were determined via chloroform ($CHCl_3$) fumigation and extraction (Beck et al., 1997), and each sample was run in duplicate (non-fumigated and fumigated). Non-fumigated samples were prepared using 10 g (oven dry soil equivalent, ODE) in 50 mL of 0.5 M $K_2SO_4$, shaken for 1 h, filtered through Whatman No. 1 filter paper, and stored frozen until measured for total dissolved organic carbon (DOC) analyses. Fumigated samples, 10 g ODE, were fumigated with ethanol-free $CHCl_3$ for three days in the dark, prior to DOC extractions with $K_2SO_4$.DOC and total dissolved nitrogen (TDN) were quantified using high temperature platinum-catalyzed combustion with a TOC-VCSH and TNM-1 (Shimadzu Corp., Houston, TX, USA) (Potter and Wimsatt, 2012). MBC was calculated as *C = EC/kEC* where EC is the chloroform-labile pool, which is proportional to microbial biomass C (C) and kEC is soil specific, but is often estimated as 0.45 (Beck et al., 1997).

2.1.5.2 Extracellular Enzyme Assay

Extracellular enzyme activity was quantified using 4-methylumbelliferone (MUB fluorescent linked substrates. I tested five enzymes associated with the microbial acquisition of C, N, and P using a modified method (Sinsabaugh et al., 2008; Smith et al., 2015a): β-glucosidase, Cellobiohydrolase, Xylosidase, N-acetylglucosaminidase (NAG), and Acid Phosphatase. One gram of each sample was mixed in a blender with 100 ml of 50 mM Tris Hydroxymethyl Aminomethane Hydrochloride (1M Tris) buffer and 200 µl was dispensed into

each column of the 96-well plates for both the one-hour and three-hour incubations. After the respective incubation periods, 10 µl of 1M NaOH was added to end the reaction for two to three minutes prior to taking a reading. Extracellular enzyme activity was measured using a fluorometric plate reader and values reported were used to calculate extracellular enzyme activity in µmolg$^{-1}$h$^{-1}$ using the following equation modified from (German et al., 2011; Smith et al., 2015a) (Equation 1).

Equation 1.

$$Activity \ (\mu mol \ g^{-1}h^{-1}) = \frac{Net \ Fluorescence \times Buffer \ Volume \ (mL)}{Emission \ coefficient \times Homogenate \ Volume \ (mL) \times Time \ (h) \times Soil \ mass \ (g)}$$

2.1.5.3 Microbial Community Composition

DNA was extracted from 250 mg of each soil sample using a Qiagen DNeasy Powersoil kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. DNA concentration was determined using a Qubit fluorometer and later quantified using a KAPA Library Quantification Kit (Illumina® platforms). PCR amplification of regions V1-V9 of the bacterial 16S rRNA gene and the fungal ITS1 and ITS2 genes was performed using the protocol developed by Swift Biosciences (https://swiftbiosci.com/) and used gene-specific primers to cover the designated regions of both the 16S gene (Table 1) and ITS gene (Table 2).

Amplicons were sequenced on an Illumina MiSeq using the 500 cycle MiSeq Reagent Kit v2 and the 600 cycle MiSeq Reagent kit v3 (http://www.illumina.com/) according to manufacturer's instructions. Amplicon data was analyzed using the qiime2 pipeline version 2020.2. Primers were removed using Cutadapt and read pairs merged using Vsearch; merged and unmerged reads were imported into qiime2 and denoised using DADA2; taxonomy was assigned using the qiime2 consensus BLAST classifier with the SILVA database version 138. Features were filtered to remove low-confidence data – features not present in at least three samples were removed as well as features with fewer than 50 counts. In addition, mitochondrial and chloroplast features were removed. Finally, features without at least a class-level taxonomic identification were removed. After collapsing the amplicon sequence variants (ASVs) to the species level, 507 bacterial species-level features and 71 fungal were identified.

2.1.6 Data Analysis:

All analyses, aside from initial sequence filtering, were completed in RStudio (RStudio Team, 2020). All data were checked for normality via histograms and qqplots prior to any needed transformations or further assessment. To calculate the relative abundance of 16S and ITS amplicon sequences, ASV counts were measured in each sample and then divided by the total ASV counts within their respective sample. Alpha diversity was measured as Shannon diversity, and Beta diversity was calculated using a Bray-Curtis dissimilarity measure. To test for treatment effects on beta diversity, I performed an analysis of similarity (ANOSIM) using the Vegan package in R (Oksanen et al., 2019). An ANOSIM is similar to and ANOVA but uses the dissimilarity matrix as its input rather than the raw data to determine if the differences between two or more groups is significant, which makes an ANOSIM test a nice compliment to an NMDS plot. To determine unique taxa within each treatment, we further filtered ASVs by

discarding taxa that were not present in five of eight plots in each treatment since they may not accurately represent the microbial community present in these plots. ASV counts were transformed to binary to determine taxa on a presence absence basis and then used the VennDiagram package in R. After quantifying the number of unique microbial taxa within each treatment, we were able to locate those specific taxa among all identified taxa based on their individually assigned taxonomic unit. This allowed us to further determine the role these unique microbes may play in this environment in response to the induced disturbances. All other microbial, physical, and chemical property measurements were statistically analyzed using a two-way analysis of variance (ANOVA). We used an ANOVA test to evaluate the effect of our four treatement groups: control, fire, drought, and fire + drought. Correlation analyses were run between MBC and pH, soil moisture, and bulk density. All analyses were done in R (4.0.3) and significance was set at 0.05.

2.2. Results

2.2.1 Soil physical and chemical responses to experimental drought and prescribed fire.

Soil moisture was not significantly different among the treatments with an average of $3.48 \pm 0.85$ percent soil moisture across all samples (Table 3). We also found that pH was not significantly different among the plots in response to the different treatments with an average pH of $8.1 \pm 0.09$ (Table 3). Bulk density also showed no significant change in response to treatments across all plots with a mean of $0.79$ g/cm$^3$ $\pm 0.10$ g/cm$^3$ (Table 3). Percent organic C showed no significant differences among treatments (Figure 3). However, organic C had a significantly positive correlation with soil moisture (Figure 4), and a significantly negative correlation with pH and bulk density (Figure 5 & 6).

2.2.2 Soil biologic responses to experimental drought and prescribed fire.

There was no effect of drought or fire on C cycling related enzymes (β-glucosidase, Cellobiohydrolase, and Xylosidase) (Table 4). Similarly, the extracellular enzyme activity measured in relation to phosphorus acquisition (Phosphatase) and N cycling (N-acetyl glucosaminidase) showed no response to drought or fire (Table 4). Across all our treatments we observed no significant response in MBC (Figure 1). When comparing MBC to each extracellular enzyme to determine if there were any correlations, MBC was significantly correlated with xylosidase ($R^2 = 0.25$, $p < 0.05$) (Figure 2).

2.2.3 Microbial community composition in response to experimental drought and prescribed fire

Drought, fire, and the interaction of drought and fire had no effect on bacterial community composition at the phyla level (Figure 7). Of the 507 identified bacterial ASVs, the dominant bacteria phyla included Proteobacteria (34.7%), Actinobacteria (27.2%), Chloroflexi (8.6%), and Bacteroidetes (5.5%). Despite Proteobacteria having a greater number of identified ASVs, Actinobacteria had a greater relative abundance across all treatment plots (Figure 7). Actinobacteria composed 56 percent of the relative abundance of bacterial taxa across all treatments compared to Proteobacteria composing 19 percent of the relative abundance of identified bacterial taxa (Figure 7).

There was also no effect of drought or fire as an interaction or main effects on Shannon diversity (*H'*) (Figure 8). For our beta-diversity measurement, a nonmetric multi-dimensional scaling (NMDS) using Bray-Curtis dissimilarity of ASVs measured by 16S rRNA gene amplicon sequencing showed that there was no separation of the bacterial communities in response to treatment plots (Figure 9). Using an ANOSIM test to determine the similarity of the bacterial

communities across treatments we found that there was no significant difference in the bacterial community in response to treatments ($p > 0.05$) (Figure 9).

Of the 367 identified bacterial ASVs present in at least five of eight plots of at least one treatment, 4 were observed unique to the control treatment, 7 were observed unique to the drought + fire treatment, 9 were observed unique to the fire treatment, and 24 were observed unique to the drought treatment (Figure 10). Since the drought treatment and the fire treatment had the most unique taxa, the subsequent analyses of the microbial community will be focusing only on these two treatments. The drought treatment not only had more overall unique taxa compared to the fire treatment (Table 5), but the composition of those unique taxa was different (Figure 11). When looking at the distribution of Gram + and Gram - bacteria in the drought treatment we observed 16 of the 24 taxa to be Gram - and the remaining eight of 24 as Gram +.

There was no effect of drought or fire in interaction or as main effects on fungal diversity. Of the 71 identified fungal ASVs, the dominant fungal phyla included Ascomycota (63.3%), Basidiomycota (18.3%), and Glomeromycota (11.2%). When analyzing for unique taxa, three were observed unique to the control treatment and four were observed unique to the drought treatment of the 13 identified fungal ASV (Table 6) (Figure 12). Based on the overall low abundance of unique fungal taxa, we chose to compare the control and drought treatments since they had the only unique taxa. The fungi in the drought and control treatment plots were identical (Figure 13) and nearly identical at the class level (Table 6).

## 2.3 Discussion

Our findings show that the prescribed fire and experimental drought did not alter most soil physical, chemical, and biological properties measured immediately after a burn, suggesting

that land managers of semi-arid savannas can continue using prescribed fires without concerns of impairing soil microbial biomass or enzymatic activity immediately post-fire. We hypothesized that organic carbon would be lower in the fire treatment because of the combustion of soil organic matter, whereas drought would show an increase in organic matter due to reduced extracellular enzyme activity. Neither prediction was supported, given that amounts of organic carbon did not differ significantly among any of the treatments (Figure 3). Since organic carbon is largely influenced by the degradation of organic matter via extracellular enzymatic activity, our results demonstrating no change in extracellular enzyme activity agree with our soil organic carbon results. We also hypothesized that fire would reduce extracellular enzyme activity by reducing microbial biomass carbon via combustion. However, our findings show neither a loss in microbial biomass carbon nor a reduction in extracellular enzyme activity (Figure 1, Table 4). This response may be due to a combination of factors. One factor may be that our study took place in a semi-arid savanna. Although fire directly heats and dries soil, and thus increases microbial mortality (Sherrod et al., 2002; Cairney and Bastias, 2007), previous studies have shown that grassland and semi-arid savanna ecosystems are less susceptible than temperate environments are to loss of microbial biomass in response to fire (Andersson et al., 2004; Novara et al., 2013; Fultz et al., 2016; Pressler et al., 2019). Previous studies have concluded that prescribed fires may change aboveground vegetation (Collins, 1992; Brockway and Lewis, 1997; Govender et al., 2006), but they do not have any major impact on soil enzyme activity (Boerner et al., 2000). Also, previous studies have found that wildfires cause greater losses of microbial biomass than prescribed fires (Dooley and Treseder, 2011) and a prescribed fire may not achieve the same amount of intense heat that a wildfire might. From these observations we can suggest that because our study took place in a semi-arid savanna and the prescribed fire was likely short

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lived and of a low intensity, microbial biomass and enzymatic activity remain stable even in the event of a disturbance.

Microbial biomass and extracellular enzyme activity can be resistant to periods of drought (Schimel, 2018). There was no observed change in extracellular enzyme activity or microbial biomass carbon across all treatments, which suggests that the microbial community in this environment may already be drought-tolerant (Figure 1, Table 4). The observed lack of response in microbial biomass carbon and extracellular enzyme activity upholds our hypothesis that drought would maintain levels of extracellular enzyme activity. Drought is often defined as a period of insufficient moisture compared with the environment's normal annual precipitation level (McKee et al., 1993). Microbial biomass has been shown to correlate with soil moisture (Dunn et al., 1985; Fultz et al., 2016), and enzyme activity is directly influenced by microbial biomass (Sanaullah et al., 2011; Hinojosa et al., 2016). We observed no significant correlation between soil moisture and microbial biomass, which may explain why we only found one significant correlation between microbial biomass and our five measured enzymes (xylosidase) (Figure 2). We found that soil moisture did not significantly differ in the drought treatment from that in other treatments. While our results are only a snapshot in time reflecting the environmental conditions at the specific moment of sampling, observing no response in soil moisture to drought and fire further suggests the microbial community is drought tolerant. Similar environments have shown soil microbes exhibit a high sensitivity to changes in soil moisture levels (Sardans and Peñuelas, 2005), but our results suggest that the microbial communities at our study site are more resistant (Castro et al., 2010). Like drought and fire individually, a combination of drought and prescribed fire had no effect on soil microbial biomass or extracellular enzyme activity (Figure 1, Table 4). However, to further affirm that

what is being reflected in the microbial biomass and extracellular enzyme activity is indicative of the microbial community present in these plots, we further examined the community composition.

Disturbance from drought and prescribed fire alone does not influence soil microbial abundance and diversity in a semi-arid savanna. We hypothesized that microbial communities in the fire treatment would have lower abundance, but greater beta-diversity while microbial communities in the drought treatment would have lower beta-diversity. However, we found no differences in relative abundance of identified taxa (Figure 7) or in beta-diversity, which is a measure of overall species richness (Figure 9). Studies have shown that fire increases microbial diversity, which would typically result in an increase in beta-diversity (Fontúrbel et al., 2012). However, this phenomenon was not seen in our study environment. Despite not seeing a distinct difference in microbial diversity between the treatments, there are some copiotrophs identified in our treatment plots. We observed one unique Bacilli (Firmicutes) taxa found only in the fire treatment, but all treatments shared two Bacilli taxa. What this suggests is that this environment has copiotroph taxa that are indicative of biocrust communities present at this site that are known to be drought tolerant and are occasionally present in recently burned sites (Aanderud et al., 2019). Finding these taxa in our drought and fire treatment plots provides initial signs that the microbial community exhibits tolerance to these disturbances. While Firmicutes can play an important role in the microbial community in response to disturbance (Bachar et al., 2010), they only constituted 0.7% of the relative abundance whereas 56% of the relative abundance of bacterial taxa across all treatments was in the phyla Actinobacteria.

Semi-arid savanna tolerance to disturbances, such as drought and fire, are reflected in the microbial community. While eight of the 24 unique bacteria in the drought treatment were

Actinobacteria, observing a majority relative abundance of this taxa indicates that regardless of

treatment, these plots already contain microbes that exhibit drought tolerance. Actinobacteria are

notably used for the production of many antibiotics but also exhibit a high degree of adaptability

to different environmental stressors and often form symbiotic relationships with plants to provide

protection from pathogens (van Bergeijk et al., 2020). Actinobacteria are also known to be

tolerant of desiccation (Mohammadipanah and Wink, 2016). We observed Actinobacteria to

have greatest relative abundance in the bacterial community, which suggests that the microbial

community is already tolerant to disturbances such as drought and fire. Notable Actinobacteria

found across all treatment plots are the genera Blastococcus and Modestobacter, which have

been found to thrive in arid regions that experience low water availability (Mohammadipanah

and Wink, 2016). Additional Actinobacteria that were observed in our treatment plots were the

genera Micromonospora and Actinomadura, which are known thermotolerant bacteria often

found in desert soils (Mohammadipanah and Wink, 2016). Seeing a widespread presence of

Actinobacteria across all treatments further suggests that semi-arid savannas can withstand

drought and fire without negatively impacting microbes in the soil. Previous research has shown

Acidobacteria to be more abundant and Chloroflexi to be less abundant in semi-arid sites (Bachar

et al., 2010). Our data shows no Acidobacteria, but Chloroflexi in the drought treatment (Table

5). This may likely be due to processes of rewetting and the uncharacteristically high amount of

rainfall in the summer of 2019 at this site and microbial communities are resilient and any added

moisture to the system can result in rapid community recovery (Schimel et al., 2007; Fuchslueger

et al., 2014).

The fungal community observed in our study included drought-tolerant taxa. The primary

taxa that were present in the treatment plots were Ascomycota, and the unique Ascomycota

present in both the drought and control treatment plots reflect drought tolerance. Dothideomycetes and Sordariomycetes are two Ascomycete fungal classes that have both been found to colonize plant roots to aid the plant in growing in stressed environments (Porras-Alfaro et al., 2008). For plants to persist in drought-prone environments they frequently form associations with microorganisms to aid in nutrient uptake in addition to stress tolerance (Porras-Alfaro et al., 2008; Acosta-Martínez et al., 2014).

2.4 Conclusions

The microbial community in a West Texas semi-arid savanna exhibits drought resistance, which allowed for sustained enzymatic activity and microbial biomass under drought conditions. Additionally, seeing no response in any of our measurements to prescribed fire suggests that fire may remain a viable management option in semi-arid savannas without concerns of negatively impacting soil microbial communities even during periods of drought. Our snapshot of how a semi-arid savanna in West Texas responds to prescribed fire suggests that fire may not impair soil microbes and the described taxa in our drought treatment further suggests the microbes in this environment may be resistant to drought. Therefore, we can suggest that a combination of drought and fire in semi-arid savannas has minimal impact on chemical, physical, and biological properties in the first 5 cm of soil. Future studies should investigate plant-associated microbes following drought and fire to gather a greater understanding of how vegetation changes may affect belowground communities in response to these disturbances.

REFERENCES

Aanderud, Z.T., Bahr, J., Robinson, D.M., Belnap, J., Campbell, T.P., Gill, R.A., McMillian, B., St. Clair, S., 2019. The Burning of Biocrusts Facilitates the Emergence of a Bare Soil Community of Poorly-Connected Chemoheterotrophic Bacteria With Depressed Ecosystem Services. Frontiers in Ecology and Evolution 7.

Acosta-Martínez, V., Cotton, J., Gardner, T., Moore-Kucera, J., Zak, J., Wester, D., Cox, S., 2014. Predominant bacterial and fungal assemblages in agricultural soils during a record drought/heat wave and linkages to enzyme activities of biogeochemical cycling. Applied Soil Ecology 84, 69-82.

Alster, C.J., German, D.P., Lu, Y., Allison, S.D., 2013. Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. Soil Biology and Biochemistry 64, 68-79.

Andersson, M., Michelsen, A., Jensen, M., Kjøller, A., 2004. Tropical savannah woodland: effects of experimental fire on soil microorganisms and soil emissions of carbon dioxide. Soil Biology and Biochemistry 36, 849-858.

Aon, M.A., Colaneri, A.C., 2001. II. Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. Applied Soil Ecology 18, 16.

Bachar, A., Al-Ashhab, A., Soares, M.I., Sklarz, M.Y., Angel, R., Ungar, E.D., Gillor, O., 2010. Soil microbial abundance and diversity along a low precipitation gradient. Microb Ecol 60, 453-461.

Baker, W.L., 1992. EFFECTS OF SETTLEMENT AND FIRE SUPPRESSION ON LANDSCAPE STRUCTURE1. Ecology 73.

Beck, T., Joergensen, R.G., Kandeler, E., Makeschin, F., Nuss, E., Oberholzer, H.R., Scheu, S., 1997. An Inter-Laboratory Comparison of Ten Different Ways of Measuring Soil Microbial Biomass C. Soil Biology and Biochemistry 29, 10.

Bodner, G.S., Robles, M.D., 2017. Enduring a decade of drought: Patterns and drivers of vegetation change in a semi-arid grassland. Journal of Arid Environments 136, 1-14.

Boerner, R.E.J., Decker, K.L.M., Sutherland, E.K., 2000. Prescribed burning e€ects on soil enzyme activity in a southern Ohio hardwood forest: a landscape-scale analysis. Soil Biology and Biochemistry 32.

Bond, W.J., Keeley, J.E., 2005. Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. Trends Ecol Evol 20, 387-394.

Bonkowski, M., Chengb, W., Griffithsc, B.S., Alpheid, J., Scheua, S., 2000. Microbial-faunal interactions in the rhizosphere and effects on plant growth. European Journal of Soil Biology 36, 135-147.

Brando, P.M., Balch, J.K., Nepstad, D.C., Morton, D.C., Putz, F.E., Coe, M.T., Silverio, D., Macedo, M.N., Davidson, E.A., Nobrega, C.C., Alencar, A., Soares-Filho, B.S., 2014. Abrupt increases in Amazonian tree mortality due to drought-fire interactions. Proc Natl Acad Sci U S A 111, 6347-6352.

Breshears, D.D., Knapp, A.K., Law, D.J., Smith, M.D., Twidwell, D., Wonkka, C.L., 2016. Rangeland Responses to Predicted Increases in Drought Extremity. Rangelands 38, 191-196.

Brockway, D.G., Lewis, C.E., 1997. Long-term effects of dormant-season prescribed fire on plant community diversity, structure and productivity in a longleaf pine wiregrass ecosystem. Forest Ecology & Management 96.

Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biology and Biochemistry 58, 216-234.

Cairney, J.W.G., Bastias, B.A., 2007. Influences of fire on forest soil fungal communitiesThis article is one of a selection of papers published in the Special Forum on Towards Sustainable Forestry — The Living Soil: Soil Biodiversity and Ecosystem Function. Canadian Journal of Forest Research 37, 207-215.

Canarini, A., Mariotte, P., Ingram, L., Merchant, A., Dijkstra, F.A., 2018. Mineral-Associated Soil Carbon is Resistant to Drought but Sensitive to Legumes and Microbial Biomass in an Australian Grassland. Ecosystems 21, 349-359.

Castro, H.F., Classen, A.T., Austin, E.E., Norby, R.J., Schadt, C.W., 2010. Soil Microbial Community Responses to Multiple Experimental Climate Change Drivers. Applied and Environmental Microbiology 76, 999-1007.

Clark, J.S., Grimm, E.C., Donovan, J.J., Fritz, S.C., Engstrom, D.R., Almendinger, J.E., 2002. Drought Cycles and Landscape Responses to Past Aridity on Prairies of the Northern Great Plains, USA. Ecology 83, 6.

Collins, S.L., 1992. Fire Frequency and Community Heterogeneity in Tallgrass Prairie Vegetation. Ecology 73, 6.

Conant, R.T., Ryan, M.G., Ågren, G.I., Birge, H.E., Davidson, E.A., Eliasson, P.E., Evans, S.E., Frey, S.D., Giardina, C.P., Hopkins, F.M., Hyvönen, R., Kirschbaum, M.U.F., Lavallee, J.M., Leifeld, J., Parton, W.J., Megan Steinweg, J., Wallenstein, M.D., Martin Wetterstedt, J.Å., Bradford, M.A., 2011. Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. Global Change Biology 17, 3392-3404.

Cook, B.I., Ault, T.R., Smerdon, J.E., 2015. Unprecedented 21st century drought risk in the American Southwest and Central Plains. Science Advancements, 8.

Coyle, D.R., Nagendra, U.J., Taylor, M.K., Campbell, J.H., Cunard, C.E., Joslin, A.H., Mundepi, A., Phillips, C.A., Callaham, M.A., 2017. Soil fauna responses to natural disturbances, invasive species, and global climate change: Current state of the science and a call to action. Soil Biology and Biochemistry 110, 116-133.

Crausbay, S.D., Ramirez, A.R., Carter, S.L., Cross, M.S., Hall, K.R., Bathke, D.J., Betancourt, J.L., Colt, S., Cravens, A.E., Dalton, M.S., Dunham, J.B., Hay, L.E., Hayes, M.J., McEvoy, J., McNutt, C.A., Moritz, M.A., Nislow, K.H., Raheem, N., Sanford, T., 2017. Defining Ecological Drought for the Twenty-First Century. Bulletin of the American Meteorological Society 98, 2543-2550.

de Vries, F.T., Brown, C., Stevens, C.J., 2016. Grassland species root response to drought: consequences for soil carbon and nitrogen availability. Plant and Soil 409, 297-312.

Dooley, S.R., Treseder, K.K., 2011. The effect of fire on microbial biomass: a meta-analysis of field studies. Biogeochemistry 109, 49-61.

Dunn, P.H., Barro, S.C., Poth, M., 1985. Soil Moisture Affects Survival of Microorganisms in Heated Chaparral Soil. Soil Biology and Biochemistry 17, 6.

Field, C.B., Behrenfeld, M.J., Randerson, J.T., Falkowski, P., 1998. Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. Science 281, 5.

Fontúrbel, M.T., Barreiro, A., Vega, J.A., Martín, A., Jiménez, E., Carballas, T., Fernández, C., Díaz-Raviña, M., 2012. Effects of an experimental fire and post-fire stabilization treatments on soil microbial communities. Geoderma 191, 51-60.

Frost, C.C., 1998. PRESETTLEMENT FIRE FREQUENCY REGIMES OF THE UNITED STATES: A FIRST APPROXIMATION, Tall Timbers Fire Ecology Conference Proceedings, No. 20. , Tallahassee, FL.

Fuchslueger, L., Bahn, M., Fritz, K., Hasibeder, R., Richter, A., 2014. Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow. New Phytol 201, 916-927.

Fultz, L.M., Moore-Kucera, J., Dathe, J., Davinic, M., Perry, G., Wester, D., Schwilk, D.W., Rideout-Hanzak, S., 2016. Forest wildfire and grassland prescribed fire effects on soil biogeochemical processes and microbial communities: Two case studies in the semi-arid Southwest. Applied Soil Ecology 99, 118-128.

German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L., Allison, S.D., 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology and Biochemistry 43, 1387-1397.

Govender, N., Trollope, W.S.W., Van Wilgen, B.W., 2006. The effect of fire season, fire frequency, rainfall and management on fire intensity in savanna vegetation in South Africa. Journal of Applied Ecology 43, 748-758.

Hart, S.C., DeLuca, T.H., Newman, G.S., MacKenzie, M.D., Boyle, S.I., 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. Forest Ecology and Management 220, 166-184.

Hinojosa, M.B., Laudicina, V.A., Parra, A., Albert-Belda, E., Moreno, J.M., 2019. Drought and its legacy modulate the post-fire recovery of soil functionality and microbial community structure in a Mediterranean shrubland. Global Change Biology 25, 1409-1427.

Hinojosa, M.B., Parra, A., Laudicina, V.A., Moreno, J.M., 2016. Post-fire soil functionality and microbial community structure in a Mediterranean shrubland subjected to experimental drought. Sci Total Environ 573, 1178-1189.

Hinojosa, M.B., Parra, A., Ramírez, D.A., Carreira, J.A., García-Ruiz, R., Moreno, J.M., 2012. Effects of drought on soil phosphorus availability and fluxes in a burned Mediterranean shrubland. Geoderma 191, 61-69.

Holden, S.R., Gutierrez, A., Treseder, K.K., 2012. Changes in Soil Fungal Communities, Extracellular Enzyme Activities, and Litter Decomposition Across a Fire Chronosequence in Alaskan Boreal Forests. Ecosystems 16, 34-46.

Lee, J., Hopmans, J.W., Rolston, D.E., Baer, S.G., Six, J., 2009. Determining soil carbon stock changes: Simple bulk density corrections fail. Agriculture, Ecosystems & Environment 134, 251-256.

Leinweber, C.B.P., Schlichting, A., 2003. Effects of chemical conditions in re-wetted peats on temporal variation in microbial biomass and acid phosphatase activity within the growing season. Applied Soil Ecology 22, 7.

Li, W., Niu, S., Liu, X., Wang, J., 2019. Short-term response of the soil bacterial community to differing wildfire severity in Pinus tabulaeformis stands. Sci Rep 9, 1148.

Lieth, H., Whittaker, R.H., 1975. Modeling the primary productivity of the world. Springer-Verlag, New York.

Littell, J.S., Peterson, D.L., Riley, K.L., Liu, Y., Luce, C.H., 2016. A review of the relationships between drought and forest fire in the United States. Global Change Biology 22, 17.

Lohmann, D., Tietjen, B., Blaum, N., Joubert, D.F., Jeltsch, F., 2014. Prescribed fire as a tool for managing shrub encroachment in semi-arid savanna rangelands. Journal of Arid Environments 107, 49-56.

Luyssaert, S., Jammet, M., Stoy, P.C., Estel, S., Pongratz, J., Ceschia, E., Churkina, G., Don, A., Erb, K., Ferlicoq, M., Gielen, B., Grünwald, T., Houghton, R.A., Klumpp, K., Knohl, A., Kolb, T., Kuemmerle, T., Laurila, T., Lohila, A., Loustau, D., McGrath, M.J., Meyfroidt, P., Moors, E.J., Naudts, K., Novick, K., Otto, J., Pilegaard, K., Pio, C.A., Rambal, S., Rebmann, C., Ryder, J., Suyker, A.E., Varlagin, A., Wattenbach, M., Dolman, A.J., 2014. Land management and land-cover change have impacts of similar magnitude on surface temperature. Nature Climate Change 4, 389-393.

McKee, T.B., Doesken, N.J., Kleist, J., 1993. THE RELATIONSHIP OF DROUGHT FREQUENCY AND DURATION TO TIME SCALES, Eigth Conference on Applied Climatology,, Anaheim, California.

Mohammadipanah, F., Wink, J., 2016. Actinobacteria from Arid and Desert Habitats: Diversity and Biological Activity. Frontiers in Microbiology 6.

Moritz, M.A., Parisien, M.-A., Batllori, E., Krawchuk, M.A., Van Dorn, J., Ganz, D.J., Hayhoe, K., 2012. Climate change and disruptions to global fire activity. Ecosphere 3, 22.

Neary, D.G., Klopatek, C.C., DeBano, L.F., Ffolliott, P.F., 1999. Fire effects on belowground sustainability: a review and synthesis. Forest Ecology & Management 122, 21.

NOAA, DROUGHT: Monitoring Economic, Environmental, and Social Impacts.

Novara, A., Gristina, L., Rühl, J., Pasta, S., amp, apos, Angelo, G., La Mantia, T., Pereira, P., 2013. Grassland fire effect on soil organic carbon reservoirs in semiarid environment. Solid Earth Discussions 5, 883-895.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. vegan: Community Ecology Package.

Pellegrini, A.F.A., Ahlstrom, A., Hobbie, S.E., Reich, P.B., Nieradzik, L.P., Staver, A.C., Scharenbroch, B.C., Jumpponen, A., Anderegg, W.R.L., Randerson, J.T., Jackson, R.B., 2018. Fire frequency drives decadal changes in soil carbon and nitrogen and ecosystem productivity. Nature 553, 194-198.

Porras-Alfaro, A., Herrera, J., Sinsabaugh, R.L., Odenbach, K.J., Lowrey, T., Natvig, D.O., 2008. Novel root fungal consortium associated with a dominant desert grass. Appl Environ Microbiol 74, 2805-2813.

Potter, B.B., Wimsatt, J.C., 2012. USEPA method 415.3: Quantifying TOC, DOC, and SUVA. Journal - American Water Works Association 104, E358-E369.

Potts, D.L., Suding, K.N., Winston, G.C., Rocha, A.V., Goulden, M.L., 2012. Ecological effects of experimental drought and prescribed fire in a southern California coastal grassland. Journal of Arid Environments 81, 59-66.

Powlson, D.S., Hirsch, P.R., Brookes, P.C., 2001. The role of soil microorganisms in soil organic matter conservation in the tropics. Nutrient Cycling in Agroecosystems 64, 11.

Pressler, Y., Moore, J.C., Cotrufo, M.F., 2019. Belowground community responses to fire: meta-analysis reveals contrasting responses of soil microorganisms and mesofauna. Oikos 128, 309-327.

Raison, R.J., 1979. Modification of the Soil Environment by Vegetation Fires, With Particular Reference to Nitrogen Transformations: A Review. Plant and Soil 51, 36.

Reynolds, J.F., Virginia, R.A., Kemp, P.R., De Soyza, A.G., Tremmel, D.C., 1999. Impact of Drought on Desert Shrubs: Effects of Seasonality and Degree of Resource Island Development. Ecological Monographs 69, 38.

Roman, J., Verzoni, A., Sutherland, S., 2020. Greetings from the 2020 Wildfire Season, NFPA Journal.

Sanaullah, M., Blagodatskaya, E., Chabbi, A., Rumpel, C., Kuzyakov, Y., 2011. Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant community composition. Applied Soil Ecology 48, 38-44.

Sardans, J., Peñuelas, J., 2005. Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. forest. Soil Biology and Biochemistry 37, 455-461.

Sardans, J., Peñuelas, J., Estiarte, M., 2008. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. Applied Soil Ecology 39, 223-235.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial Stress-Response Physiology and its Implications for Ecosystem Function. Ecology 88, 9.

Schimel, J.P., 2018. Life in Dry Soils: Effects of Drought on Soil Microbial Communities and Processes. Annual Review of Ecology, Evolution, and Systematics 49, 409-432.

Schlesinger, W.H., Dietze, M.C., Jackson, R.B., Phillips, R.P., Rhoades, C.C., Rustad, L.E., Vose, J.M., 2016. Forest biogeochemistry in response to drought. Glob Chang Biol 22, 2318-2328.

Seager, R., Lis, N., Feldman, J., Ting, M., Williams, A.P., Nakamura, J., Liu, H., Henderson, N., 2018. Whither the 100th Meridian? The Once and Future Physical and Human Geography of America's Arid–Humid Divide. Part I: The Story So Far. Earth Interactions 22, 1-22.

Sheik, C.S., Beasley, W.H., Elshahed, M.S., Zhou, X., Luo, Y., Krumholz, L.R., 2011. Effect of warming and drought on grassland microbial communities. ISME J 5, 1692-1700.

Sherrod, L.A., Dunn, G., Peterson, G.A., Kolberg, R.L., 2002. Inorganic Carbon Analysis by Modified Pressure-Calcimeter Method. Soil Science Society of America Journal 66.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol Lett 11, 1252-1264.

Smith, A.P., Marin-Spiotta, E., Balser, T., 2015a. Successional and seasonal variations in soil and litter microbial community structure and function during tropical postagricultural forest regeneration: a multiyear study. Glob Chang Biol 21, 3532-3547.

Smith, P., Cotrufo, M.F., Rumpel, C., Paustian, K., Kuikman, P.J., Elliott, J.A., McDowell, R., Griffiths, R.I., Asakawa, S., Bustamante, M., House, J.I., Sobocká, J., Harper, R., Pan, G., West, P.C., Gerber, J.S., Clark, J.M., Adhya, T., Scholes, R.J., Scholes, M.C., 2015b. Biogeochemical cycles and biodiversity as key drivers of ecosystem services provided by soils. SOIL 1, 665-685.

Soong, J.L., Cotrufo, M.F., 2015. Annual burning of a tallgrass prairie inhibits C and N cycling in soil, increasing recalcitrant pyrogenic organic matter storage while reducing N availability. Glob Chang Biol 21, 2321-2333.

Taylor, C.A., Twidwell, D.r.c., Garza, N.E., Rosser, C.l.n., Hoffman, J.K., Brooks, T.D., 2012. Long-Term Effects of Fire, Livestock Herbivory Removal, and Weather Variability in Texas Semiarid Savanna. Rangeland Ecology & Management 65, 21-30.

Tilman, D., Reich, P., Phillips, H., Menton, M., Patel, A., Vos, E., Peterson, D., Knops, J., 2000. Fire Suppression and Ecosystem Carbon Storage. Ecology 81, 5.

van Bergeijk, D.A., Terlouw, B.R., Medema, M.H., van Wezel, G.P., 2020. Ecology and genomics of Actinobacteria: new concepts for natural product discovery. Nature Reviews Microbiology 18, 546-558.

Veldman, J.W., Buisson, E., Durigan, G., Fernandes, G.W., Le Stradic, S., Mahy, G., Negreiros, D., Overbeck, G.E., Veldman, R.G., Zaloumis, N.P., Putz, F.E., Bond, W.J., 2015. Toward an old-growth concept for grasslands, savannas, and woodlands. Frontiers in Ecology and the Environment 13, 154-162.

Vetter, S., 2009. Drought, change and resilience in South Africa's arid and semi-arid rangelands. South African Journal of Science 105, 5.

Vicente-Serrano, S.M., Gouveia, C., Camarero, J.J., Begueria, S., Trigo, R., Lopez-Moreno, J.I., Azorin-Molina, C., Pasho, E., Lorenzo-Lacruz, J., Revuelto, J., Moran-Tejeda, E., Sanchez-Lorenzo, A., 2013. Response of vegetation to drought time-scales across global land biomes. Proc Natl Acad Sci U S A 110, 52-57.

**Table 1.** Swift Amplicon 16S Primers

| Gene | Vregions | Primer Name | Primer Name |
|------|----------|-------------|-------------|
| 16S rRNA | 1 to 4 | S-D-Bact-0009-b-S-19 | S-D-Bact-0785-a-A-21 |
| | 3 to 4 | S-D-Bact-0341-b-S-17 | S-D-Bact-0785-a-A-21 |
| | 4 to 5 | S-D-Bact-0517-a-S-17 | S-D-Bact-0907-a-A-20 |
| | 7 to 8 | S-D-Bact-1099-a-S-16 | S-*-Univ-1390-a-A-18 |
| | 6 to 9 | S-D-Bact-1055-a-S-16 | S-D-Bact-1492-a-A-16 |

**Table 2.** Swift Amplicon ITS Primers

| Gene | Vregions | Primer Name | Primer Name |
|------|----------|-------------|-------------|
| ITS1 | ITS1 | Forward | Reverse |
| | | | - |
| | | | - |
| | | | - |
| ITS2 | ITS2 | 5.8S-FUN | ITS4-FUN |

**Table 3.** Mean percent soil moisture, pH and bulk density (g/cm$^3$)

| | Mean |
|---|------|
| Soil Moisture (%) | $3.48 \pm 0.85$ |
| pH | $8.12 \pm 0.09$ |
| Bulk Density (g/cm$^3$) | $0.79 \pm 0.10$ |

**Table 4.** Mean extracellular enzyme activity of all five measured enzymes show no significant difference based on treatment ($\mu$mol g$^{-1}$ h$^{-1}$).

| Enzyme | Mean ($\mu$mol g$^{-1}$ h$^{-1}$) |
|---|---|
| $\beta$-glucosidase | 2526.95 ± 1229.00 |
| N-acetylglucosaminidase | 137.82 ± 70.12 |
| Phosphatase | 816.39 ± 457.03 |
| Cellobiohydrolase | 76.23 ± 40.08 |
| Xylosidase | 222.96 ± 107.76 |

**Table 5.** Unique bacterial taxa in the drought treatment and fire treatments

| Treatment | Domain | Phylum | Class | Order |
|---|---|---|---|---|
| Drought | Bacteria | Actinobacteria | Actinobacteria | Frankiales |
| | | | | Micrococcales |
| | | | | Micromonosporales |
| | | | | Propionibacteriales |
| | | | | Pseudonocardiales |
| | | Bacteroidetes | Bacteroidia | Chitinophagales |
| | | | | Cytophagales |
| | | Chloroflexi | Anaerolineae | Caldilineales |
| | | | Gitt-GS-136 | |
| | | Proteobacteria | Alphaproteobacteria | Rhizobiales |
| | | | | Sphingomonadales |
| | | | Deltaproteobacteria | Myxococcales |
| | | | Gammaproteobacteria | Betaproteobacteriales |
| Fire | Bacteria | Actinobacteria | Acidimicrobiia | Microtrichales |
| | | | Actinobacteria | Propionibacteriales |
| | | Cyanobacteria | Oxyphotobacteria | Nostocales |
| | | Entotheonellaeota | Entotheonellia | Entotheonellales |
| | | Firmicutes | Bacilli | Bacillales |
| | | Proteobacteria | Alphaproteobacteria | Caulobacterales |
| | | | | Rhizobiales |

**Table 6.** Unique fungal taxa in the drought and control treatments

| Treatment | Kingdom | Phylum | Class | Order |
|---|---|---|---|---|
| Drought | Fungi | Ascomycota | Dothideomycetes | Capnodiales |
| | | | | Pleosporales |
| | | | Sordariomycetes | |
| | | | | Sordariales |
| Control | Fungi | Ascomycota | Dothideomycetes | Capnodiales |
| | | | Sordariomycetes | Hypocreales |
| | | | Xylonomycetes | Symbiotaphrinales |
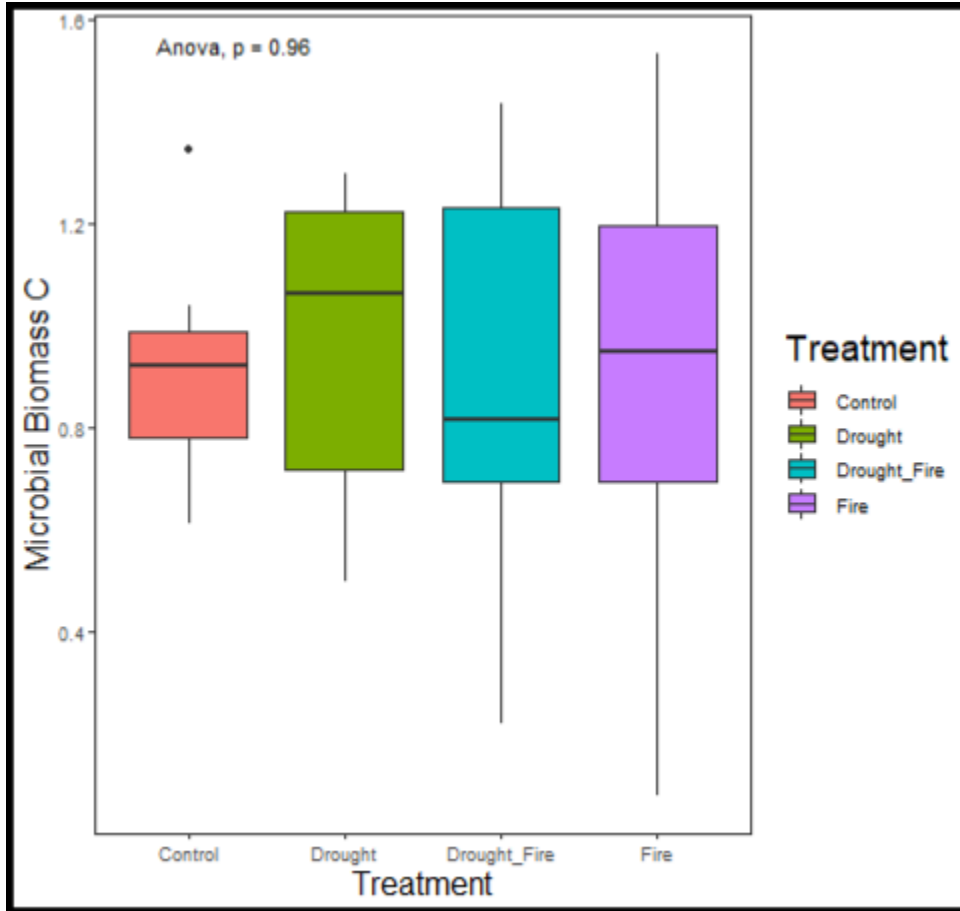
**Figure 1.** Microbial biomass carbon does not differ based on treatment. Box and whisker plots represent the interquartile range and the standard error within each treatment (p > 0.05)
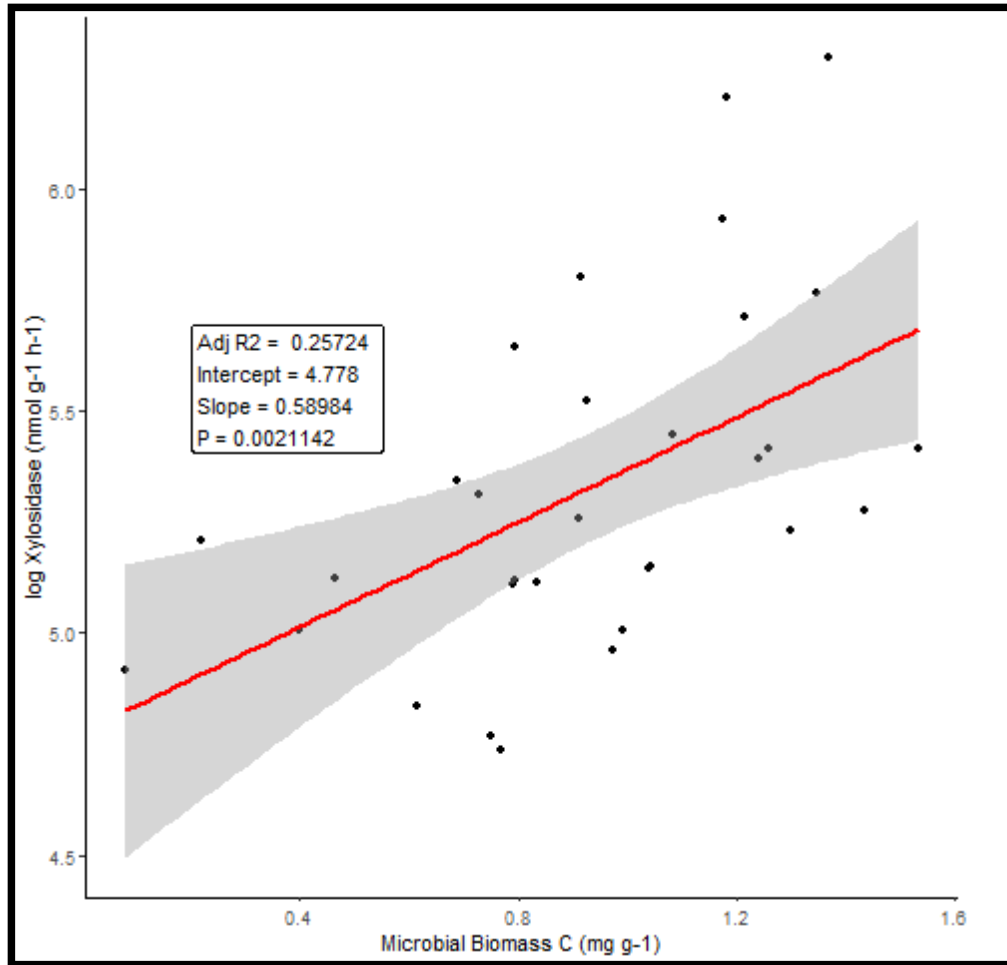
**Figure 2.** Microbial biomass carbon is positively correlated with xylosidase ($p < 0.05$)
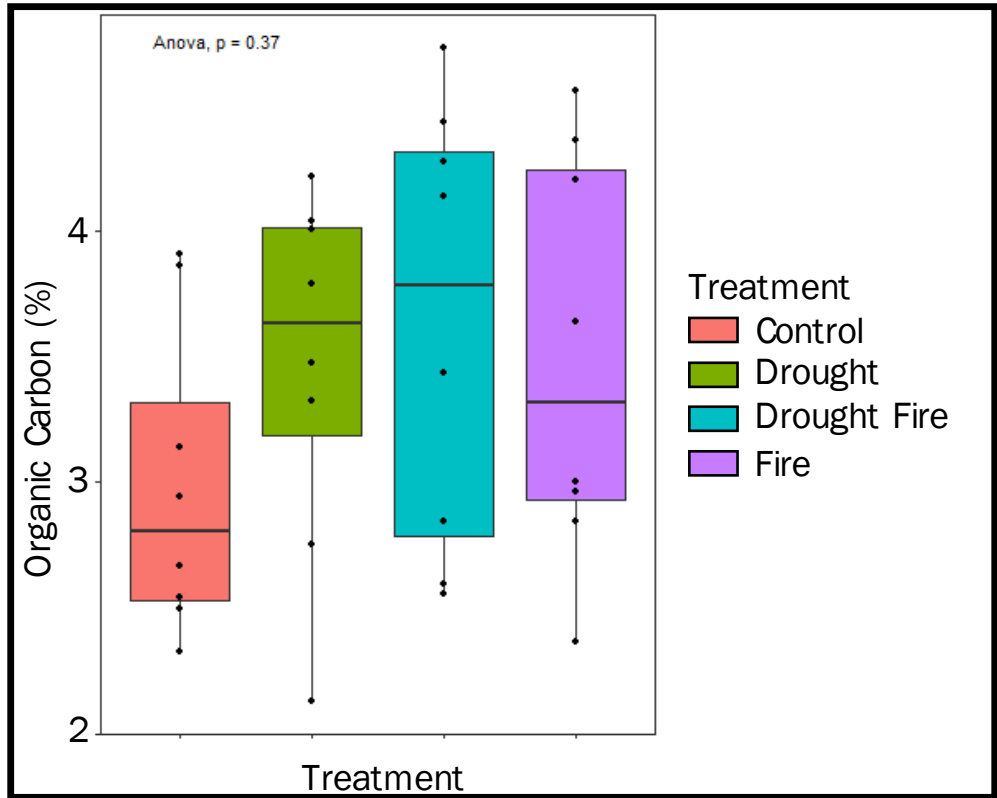
**Figure 3.** Organic carbon shows no change in response to treatment (p > 0.05)
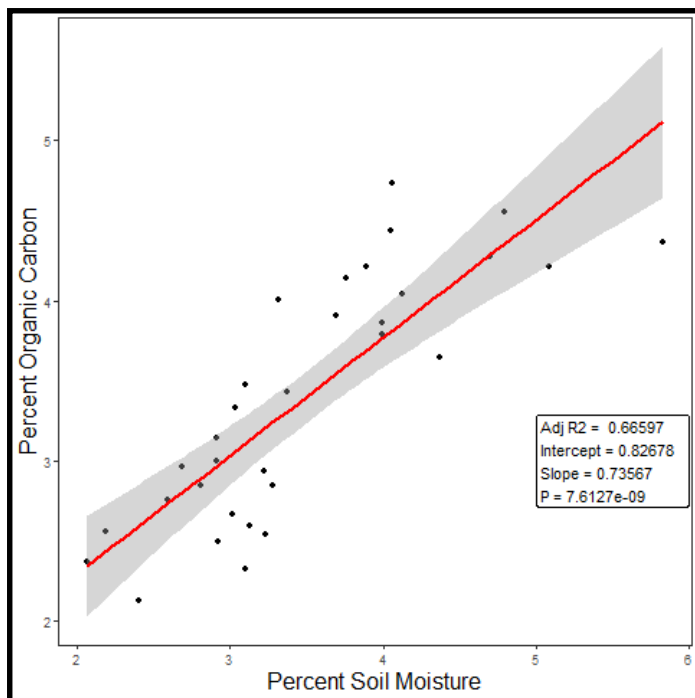
**Figure 4.** Percent organic carbon is positively correlated with soil moisture ($p < 0.05$)
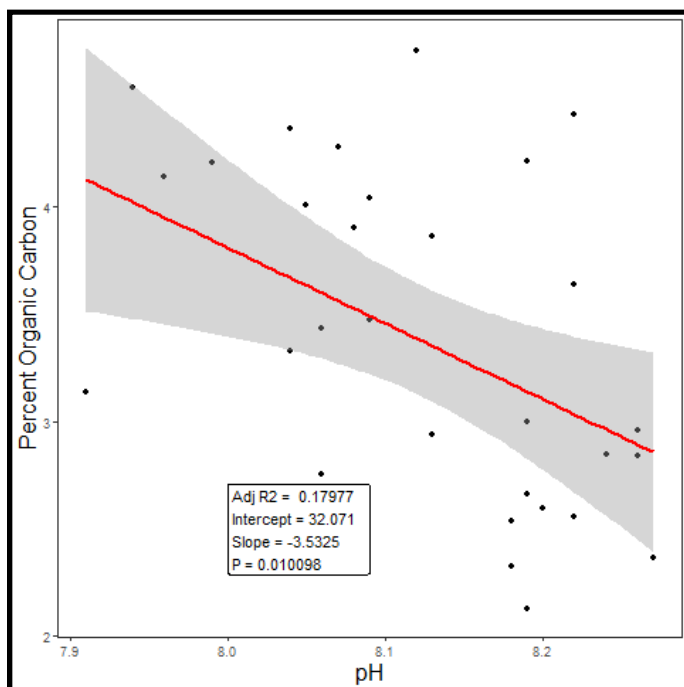


**Figure 5.** Percent organic carbon is negatively correlated with pH ($p < 0.05$)
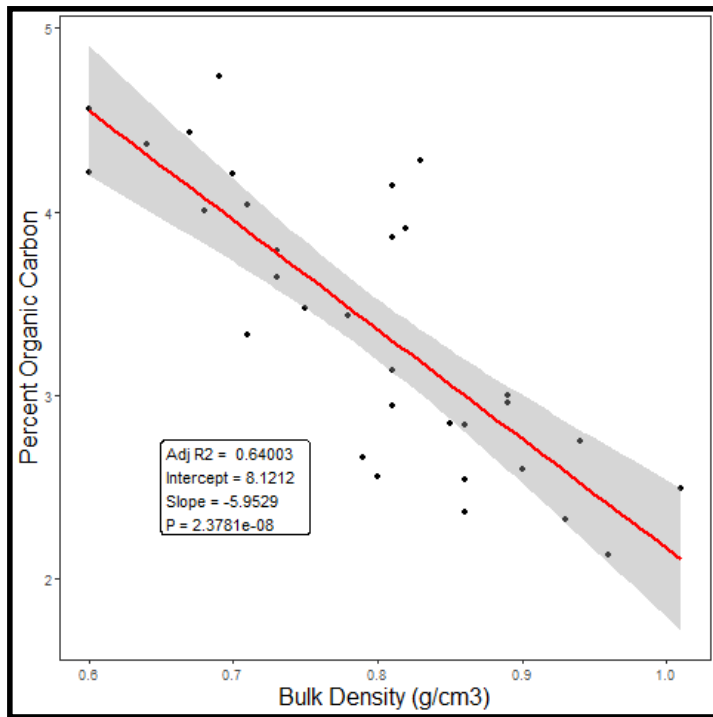
**Figure 6.** Percent organic carbon is negatively correlated with bulk density (p < 0.05)
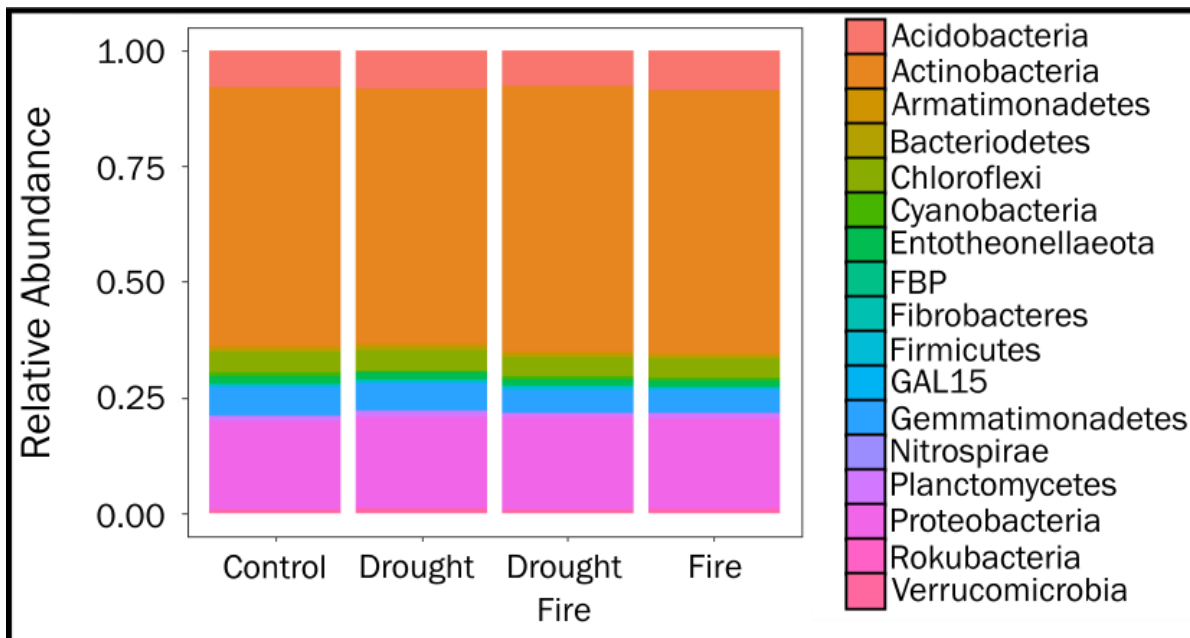


**Figure 7.** The relative abundance of bacterial ASVs is similar across all treatments
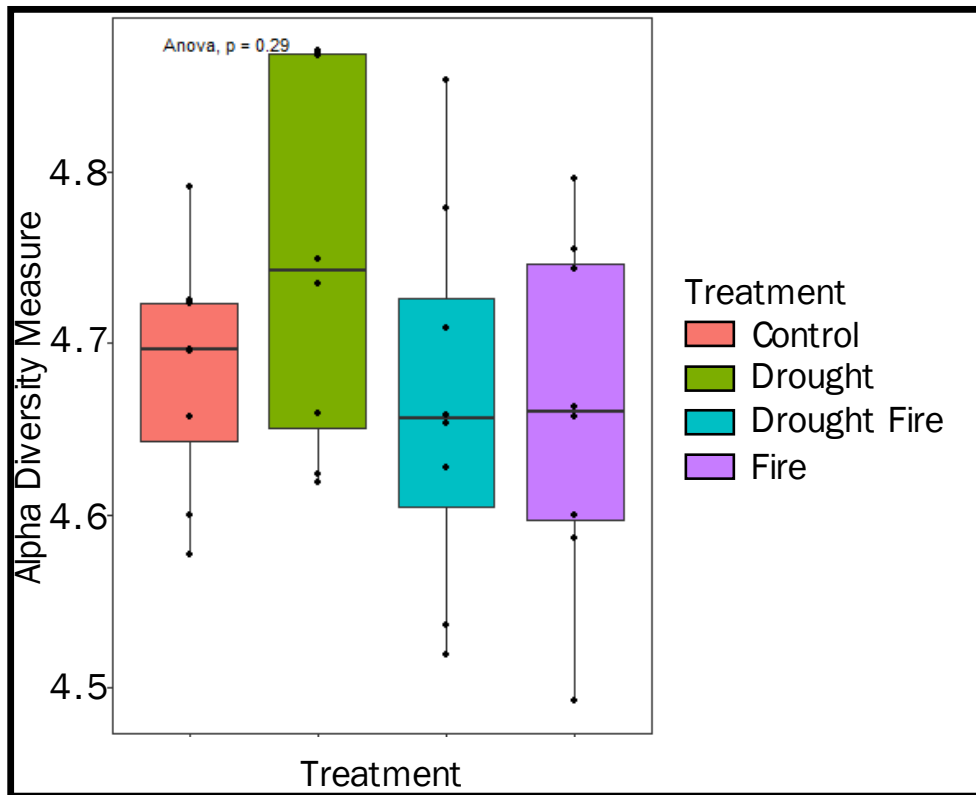
**Figure 8.** Alpha diversity shows no change in response to treatment (p > 0.05)
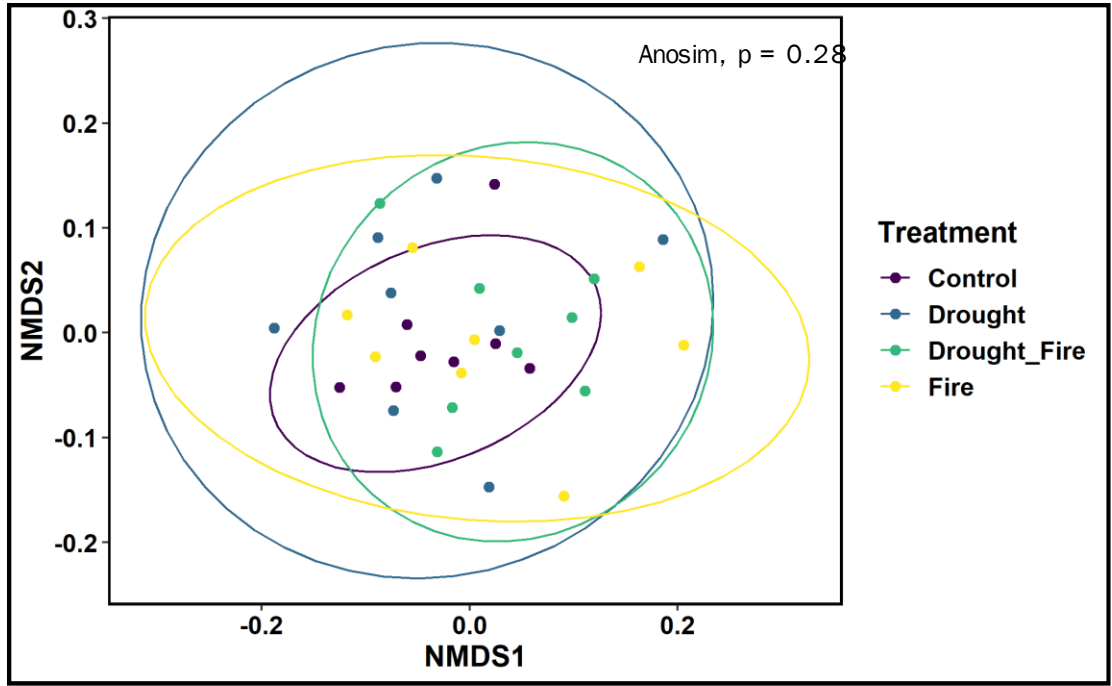
**Figure 9.** Beta diversity shows no change in response to treatment (p > 0.05)
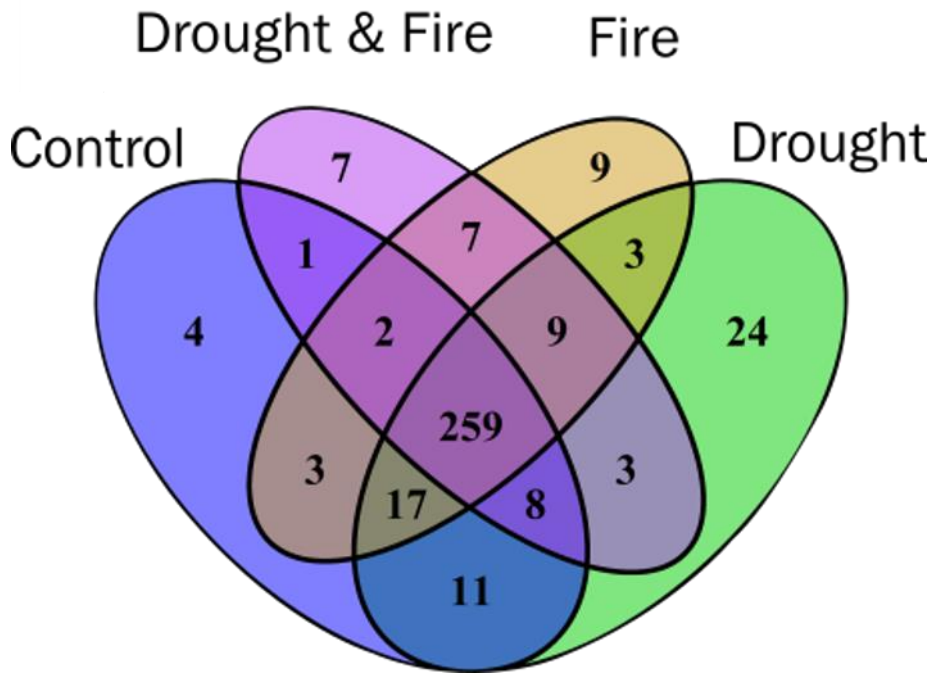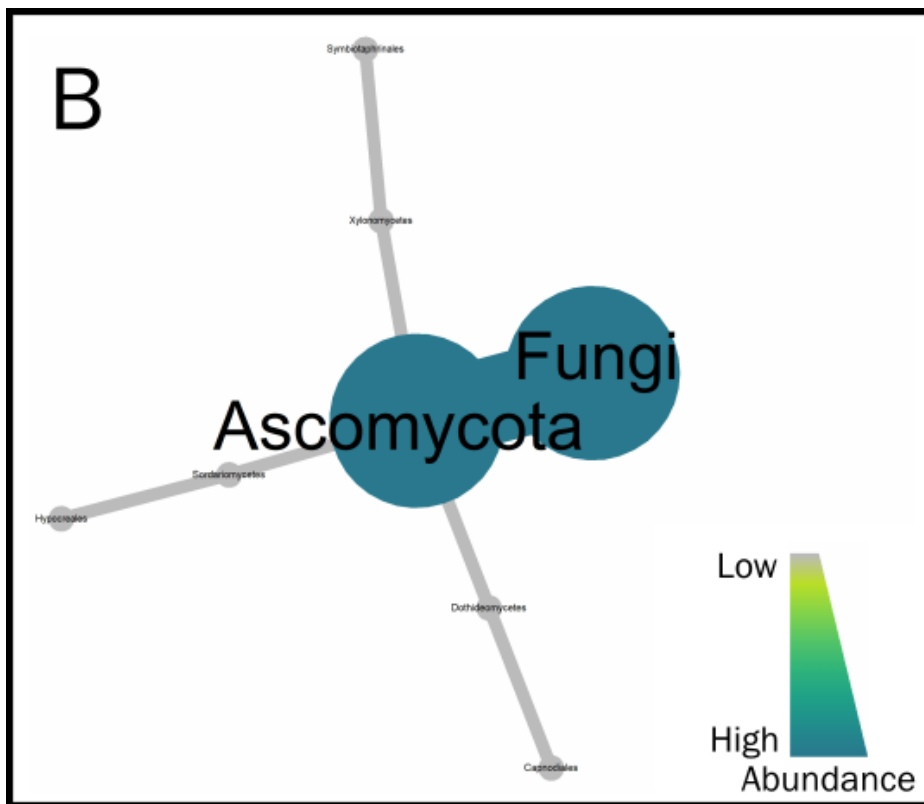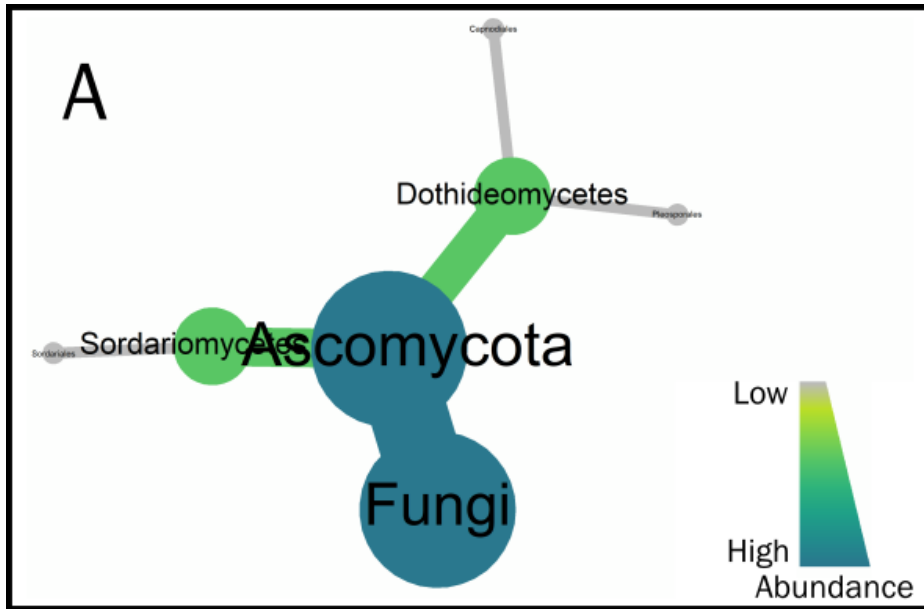


**Figure 10.** 4-way Venn Diagram showing the distribution of bacterial taxa among treatments
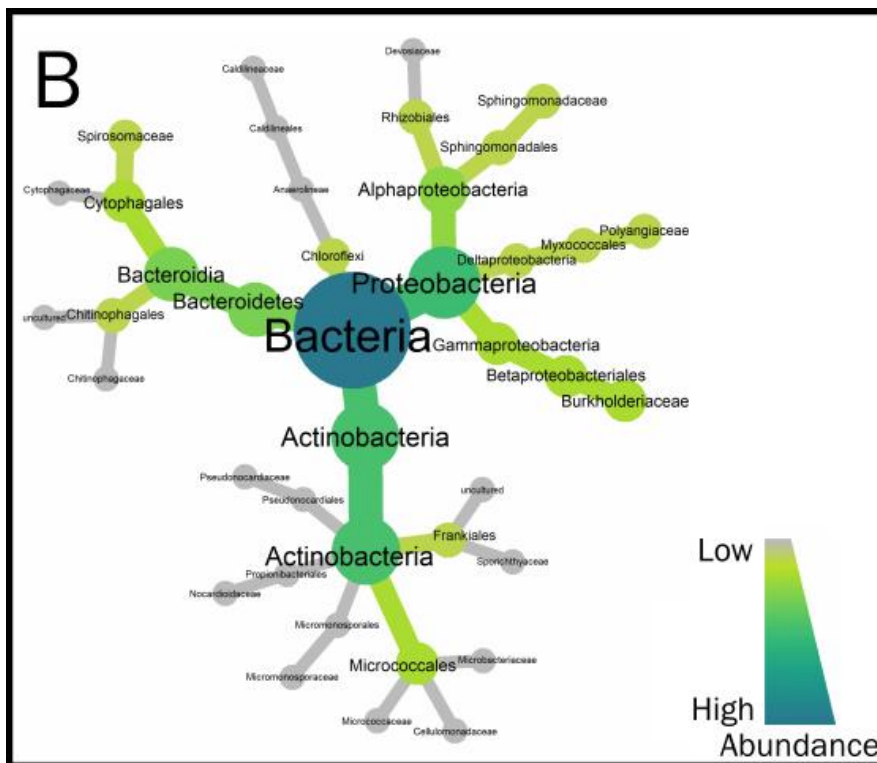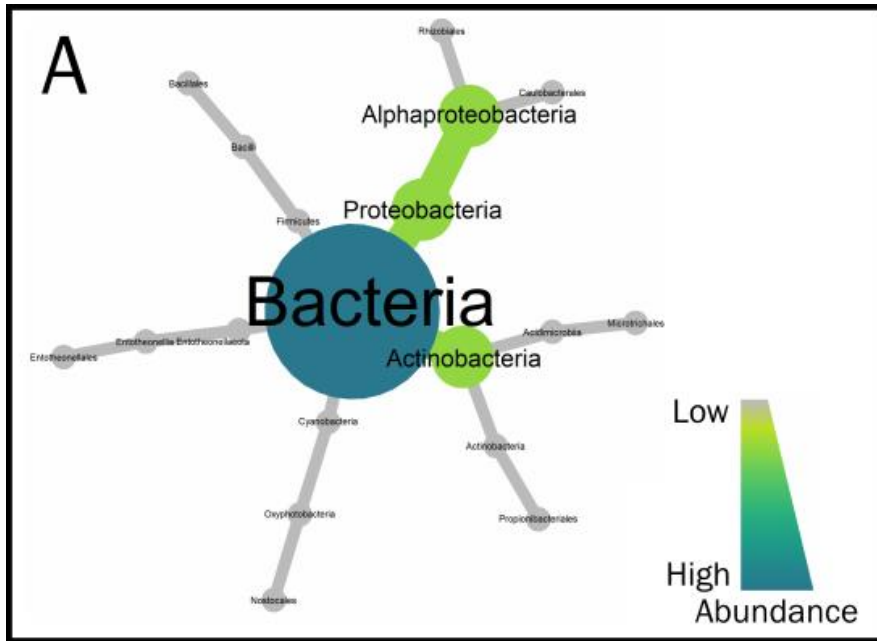
**Figure 11.** Unique fungal taxa in the (A) fire treatment and the (B) drought treatment. Node size is determined by the abundance of the unique taxa
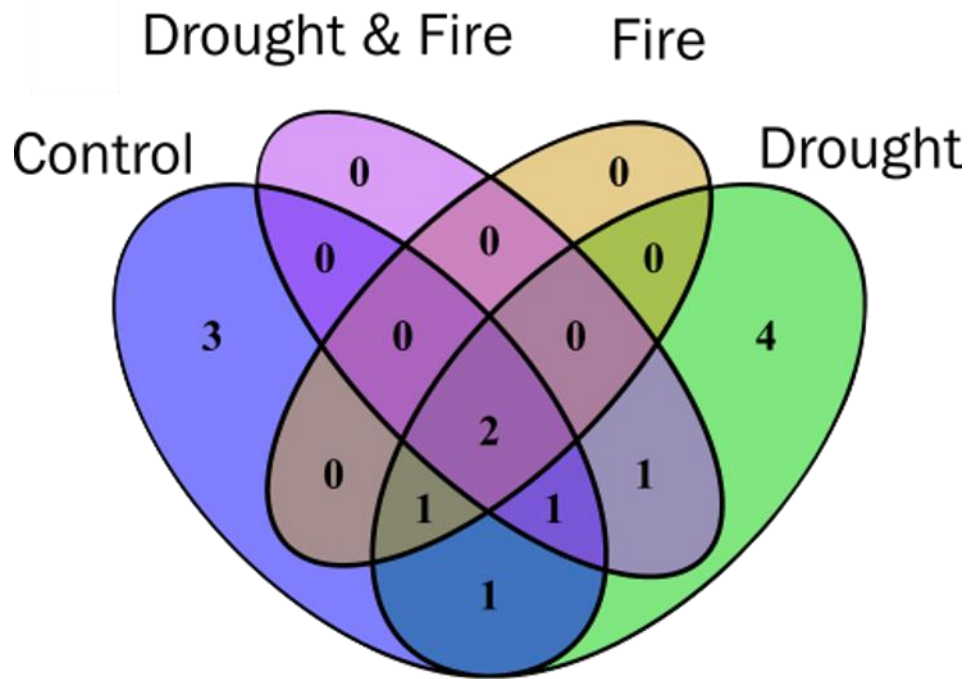
**Figure 12.** 4-way Venn Diagram showing the distribution of fungal taxa among treatments

**Figure 13.** Unique fungal taxa in the (A) drought treatment and the (B) control treatment. Node size is determined by the abundance of the unique taxa