

**EFFECTS OF PRESCRIBED FIRE ON SOIL BIOGEOCHEMISTRY IN A MIXED
GRASS PRAIRIE**

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By

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ABSTRACT

The northern mixed-grass prairies run from North Dakota to northern Nebraska in the U.S, and crosses Alberta, Saskatchewan, and southern Manitoba in Canada, covering an expanse of 270,000 km². The northern mixed-grass prairie ecoregion is arguably the most disturbed ecoregion of all grassland types; no intact patches remain, making ecosystem management and soil conservation critical to mixed grassland preservation. Grasslands have evolved with fire as a key ecosystem disturbance. Fire serves many objectives including the maintenance of biological diversity, regeneration of particular plant species, improvement in livestock nutrition, and prevention of woody plant encroachment. While the use of prescribed fire has been investigated in grassland vegetation worldwide, few studies have examined the effects of fire on soil biogeochemistry, especially in the mixed grass ecoregion. In this study, I examined the effects of grassland fire on soil chemical and microbial properties within two growing seasons post fire. Samples were collected during June, July and August of each growing season in both burn and control plots of a native and tame forage pastures. Results indicate that soils were largely resistant and resilient to disturbance by fire, showing only minor changes in microbial community composition and a significant increase in total fungi and the ratio of Gram-negative to Gram-positive bacteria within the tame forage pasture. Annual effects were stronger than burn effects; the tame forage pasture reported greater changes in microbial parameters, while the native forage pasture reported greater changes in chemical parameters. Variation in treatment responses were attributed to differences in vegetation structure between pastures, and the influence of vegetation response to fire, on soil. Overall, prescribed fire in the northern mixed grass prairies is amenable with soil conservation principles, as grassland soil appears highly resistant and resilient to disturbance by fire.

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LIST OF ABBREVIATIONS

AMF	arbuscular mycorrhiza fungi
AMP	adaptive multi-paddock
ANOVA	analysis of variance
AUM	animal unit month
C	carbon
cm	centimeter
CV	coefficient of variation
e.g.	for example
EC	electrical conductivity
FAME	fatty acid methyl esters
GN	Gram-negative bacteria
GNP	Grasslands National Park
GP	Gram-positive bacteria
ha	Hectares
KeC	Extractability factor; extractable part of microbial biomass C after fumigation
KeN	Extractability factor; extractable part of microbial biomass N after fumigation
km	kilometres
km/h	kilometres per hour
log	base 10 logarithm
ln	natural logarithm
mm	millimetres
m ²	square metre
N	nitrogen
MBC	microbial biomass carbon
MBN	microbial biomass nitrogen
NE	north east
NW	north west
NMDS	non-metric multidimensional scaling
N8	native prairie pasture in this study

OMB	Old Man on His Back Heritage and Conservation Area
PERMANOVA	permutational multivariate analysis of variance
PLFA	phospholipid fatty acid
SE	southeast
SK	Saskatchewan
SOC	soil organic carbon
SOM	soil organic matter
SW	southwest
S5	tame forage pasture in this study
U.S.A.	United States of America
%	percent
°C	degrees Celsius

1.0 INTRODUCTION

1.1 General Introduction

Grasslands are considered among the most endangered ecosystem on the planet, with temperate grasslands cited as the most altered, especially in the Great Plains of North America (Gauthier & Wiken, 2003; Henwood, 2010). Despite their vital importance, supplying a wide range of ecosystem services, conversion of native grasslands to cultivated cropland has been historically extensive and continues today. Only 4% of grasslands are under protected status—the lowest level of protection compared with the 14 major biomes of the world (Henwood, 2010).

Fire is a natural disturbance that plays a major role in grassland ecosystem function, having influenced soil properties and development since the advent of vegetation fires over 400 million years ago (Certini, 2014). Naturally, fire was a consequence of lightning and dry, combustion-prone weather conditions, however, fire was also deliberately used by Indigenous communities for vegetation management, soil fertilization and to influence the distribution of grazing herds (Gould, 1971; Mistry, Berardi, Andrade, Krahô, Krahô, & Leonardos, 2005). With the arrival of Europeans in the Americas however, fire was strongly suppressed as settlement increased. Currently, fire is mainly applied in prescriptions, only used on a subset of lands managed for conservation purposes.

During grassland prescribed fire, direct heat transfer through the soil is minimal, combusting aboveground litter while leaving belowground root structures and seedbanks intact (Keeley & Fotheringham, 2000). Even so, low severity fire constitutes an ecological disturbance, as indirect effects of fire also influence soil properties. Burning opens the vegetation canopy for competing species to emerge, and deposits charred biomass and mineral rich ash onto the surface soil. In productive years, soil properties and vegetation rapidly recover from fire disturbance. Under drought conditions however, vegetation may not recover from burning, and exposed soil may eventually become eroded (Snyman, 2002).

Fire is considered by many as a seventh soil forming factor (Certini, 2014), and in semi-arid regions such as North America, where high temperatures and low soil moisture limit nutrient cycling and vegetation growth, fire is critical to support soil nutrition and drive vegetation composition (Fultz et al., 2016). Soil carbon, soil nitrogen and the microfauna within the soil all play critical roles in supporting grassland productivity. Potentials for climate change mitigation are dependent upon grassland productivity (Yang, Tilman, Furey & Lehman, 2019), therefore it is

critical that grassland management practises ensure both the productivity and conservation of our remaining tracks of grassland.

Various studies have evaluated the effects of grassland fire on soil nutrient content (Úbeda, Lorca, Outeiro, Bernia & Castellnou, 2005; Augustine, Brewer, Blumenthal, Derner & von Fischer, 2014; Wang, Li, Ravi, Theiling & Sankey, 2019) and soil microbial communities (Grasso, Ripabelli, Sammarco & Mazzoleni, 1996; Viana et al., 2011; Dooley & Treseder, 2012) however, few studies have evaluated these parameters simultaneously. In addition, research investigating the effects of fire in the Northern mixed grass prairies, is largely limited to vegetation and aboveground changes (Grace, Smith, Grace, Collins & Stohlgren, 2000; Parker, Sullins, Haukos, Fricke & Hagen, 2022). Therefore, my research investigating the effects of prescribed fire on soil biogeochemistry during the two growing seasons following fire, is essential for an increased understanding of fire in the mixed grass prairies and its impact on soil chemical and microbial properties. Results will also be useful to land managers and conservationists, who utilize fire as an ecosystem management tool.

1.2 Research Objectives

The specific objectives of my thesis research were:

- 1) To understand the impacts of prescribed grassland fire on soil total carbon, total nitrogen, pH and electrical conductivity as soil chemical properties, and microbial biomass carbon, microbial biomass nitrogen as well as PLFA extraction, as soil microbial properties.
- 2) To evaluate these parameters at 2, 3 and 4-months following fire, marking the first growing season and 14,15- and 16-months following fire, marking the second growing season.

In my thesis I test the hypotheses that:

- 1) Direct effects of fire would primarily be observed in the first months (2, 3 and 4) following fire, whereas indirect effects of fire could be observed later, in the second growing season following fire.
- 2) Relative to a control plot, burned plots would show increases in all soil chemical parameters as a result of ash deposition into the top layers of soil.

- 3) Prescribed fire was expected to increase the absolute abundance of soil bacteria in the first growing season. Arbuscular mycorrhizal fungi were also expected to increase, while the prevalence of saprophytic fungi would be strongly reduced.
- 4) PLFA ratio of cy 17: 0 to 16:1 w7c, indicating microbial stress would be apparent in the first collections following fire (2 months).
- 5) Microbial community structure would be simplified in the first growing season following fire, with higher community diversity in the second growing season following fire.
- 6) Soil total carbon and pH would remain elevated in the second growing season following fire, while soil nitrogen and EC would more closely resemble control plots.
- 7) In the second growing season, microbial community parameters were expected to more closely resemble control plots, however, saprophytic fungi would remain suppressed in burned plots.

1.3 Thesis Organization

This thesis is comprised of four chapters. Chapter 1 (Introduction) provides a background overview of current information on the research topic and a broad introduction to, and justification for the present study. Chapter 2 (Literature Review) introduces the ecology of mixed grasslands focusing on soil processes and discusses pertinent literature relevant to ecological disturbances including fire and grazing. Chapter 3 is written as a stand-alone manuscript and presents the analysis from data I collected in the field during the 2018 and 2019 growing seasons. This chapter examines the effect of prescribed fire on soil chemical and microbial properties. Chapter 4 (General Discussion and Conclusion) provides a synthesis of the research as well as my suggestions for future research directions and conservation strategies for soils of the northern mixed grasslands.

2.0 LITERATURE REVIEW

2.1 Disturbance in grasslands

Disturbances constitute major forces that influence the structure and function of ecosystems (Connell, 1978); disturbances remove and alter biomass inducing a range of ecosystem responses at different levels of ecological organization (Parminter, 1998). Largely characterized by their type, magnitude, duration, spatial extent, frequency and timing (Lake, 2013), disturbances may result in positive (regenerative), neutral, or negative (degrading) responses (Battisti, Poeta & Fanelli, 2016). Three types of disturbance have been recognized in disturbance ecology characterized based on the temporal trend in the strength of the disturbing force: pulses, presses and ramps. Pulses are short term and sharply delineated disturbances, such as floods and many fires (Jentsch & White, 2019). Presses are disturbances that may arise sharply but then reach a constant level that is maintained (Battisti et al., 2016). Natural presses include sedimentation after landslide, volcanic eruption or ash deposition after large forest and bush fires (Battisti et al., 2016). Ramps are disturbances that steadily increase in time without an endpoint or that reach an asymptote after an extended period (Angeler & Rodrigo, 2004). Examples of ramps include the spread of an invasive species, drought or the continued sedimentation of a stream (Parasiewicz et al., 2019).

The response of an ecosystem to a disturbance can be characterized similar to disturbances themselves, with pulse, press and ramp disturbances exhibiting variations of pulse, press and ramp responses (Glasby & Underwood, 1996; Lake, 2013). Although a few specific traits (short life span, high dispersal capacity) are generally associated with responses to disturbance (Rusch, Pausas & Lepš, 2003), various studies have shown that there is usually more than one strategy for survival under any given condition (Cunningham et al., 1999; Westoby et al., 2002). Variation in disturbance response among species (response diversity) is a component of an ecosystem's functional trait distribution (Bond, Woodward & Midgley, 2005); the functional trait can determine how an ecosystem resists disturbance as well as post-disturbance shifts toward desirable ecosystem states (Elmqvist et al, 2003).

Resistance and resilience are qualities used to characterize disturbance responses. Resistance being a measure of the capacity of an ecosystem to withstand a disturbance, and resilience being a measure of the capacity of the system to recover from the disturbance (Lake &

Barmuta, 1986). These attributes, evaluated over spatio-temporal scales, assist in describing changes to ecological structure and function, and within the context of ecosystem management, may be strengthened by identifying factors through which they are influenced.

Grasslands are exposed to a variety of disturbances, pulses, presses and ramps. Drought, a ramp disturbance, is increasing in prevalence as shifts in climate occur (Humphries & Baldwin, 2003). Press disturbances are often initiated anthropogenically through fertilization and intensive grazing and can be driven by factors such as attempts to increase the grazing carrying capacity and economic return from grasslands (Catorci, Ottaviani, Ballelli & Cesaretti, 2011). Pulse disturbances in grasslands, such as fire, were historically common but are much reduced as fire is strongly suppressed in the Great Plains of North America. The result is reduced occurrence of short-term disturbances and an increased frequency of longer term, larger scale perturbations (Sinclair et al., 2007).

Fire is one of the major drivers of vegetation physiognomy and structure in grasslands (Bond & Keely, 2005). As fire disturbs the vegetation, community assembly processes are partially reset, reducing competition from dominant plant species and allowing non-dominant, functionally diverse and disturbance adapted species to establish (Overbeck, Müller, Pillar & Pfadenhauer, 2005; Grime, 2006). As a result of this process, pyrodiverse landscapes support a higher biodiversity of plants (Ponisio et al., 2016), soil biogeochemical networks (Grillo, Tabasso, Cravotto & Ree, 2020) and many terrestrial species (Bowman et al., 2016) of which an increasing number are classified as species at risk (Griffiths, Garnett, & Brook, 2015). Indeed, the decline of a range of taxa has been associated with the interruption of traditional burning practises (Davies et al., 2018).

2.2 Grassland fire

Fire is a natural part of ecological systems, with lightning the typical natural cause (Wright & Bailey, 1982). Other non-anthropogenic causes of fire include spontaneous combustion, occurrence of sparks when boulders roll down slopes, and volcanic eruption (Daubenmire, 1968; Fultz et al., 2016). The effect of fire is to injure plants to various degrees and remove surface litter and detritus. In a normal fire return interval, the plant community and surface soil then escape similar disturbance for sufficient time to allow restoration of its former composition and structure (Daubenmire, 1968). In grass and forb dominated vegetation, such as grasslands, fire is

characterized by a narrow zone of flames which advances across rather homogeneously dispersed fuel.

Natural fires have been occurring since the earliest appearance of vegetation on land (Daubenmire, 1968; Fultz et al., 2016). Fire was first used by man in early evolution to drive game animals to their destruction or initiate new succulent growth which attracted grazers intended for slaughter. Before European settlement, First Nations communities understood the principles of plant succession, and used fire to revert the successional sequence back to its early stages; areas suitable for coniferous forests or sagebrush and juniper were preserved as open prairie or for favored food plants such as wild berries, camas and other root vegetables (Boyd, 1999). Reports indicate that ash, as the result of intentional fire, was also used as a fertilizer to these soils in a run-off agroecosystem by First Nation's people (Sandor et al., 2007). After European establishment however, policies regarding fire suppression were enacted to protect settlement from destruction (Boyd, 1999; Driver, 1987). Expeditions of the 19th century who described their chosen settlement headquarters as "luxuriant lawns", "well-stocked parks" and "wide open spaces" were unaware that the Indigenous people of the area had not only managed the lands through fire, but actually created their very grandeur (Boyd, 1999).

In the Canadian prairies, fire has been largely suppressed for roughly 150 years (Driver, 1987). Over-grazed pastures in conjunction with large expansions to the road systems has also reduced the occurrence and spread of wildfire on the prairies. Only 30% of the Canadian prairie today remains as grassland (Samson & Knopf, 1994). Fire is now mainly used in small prescriptions on a subset of lands managed for conservation purposes and implemented by highly trained and experienced practitioners. Prescribed burning is setting fire to a selected area of vegetation under specific environmental conditions (e.g., windspeed, relative humidity, air temperature, fuel load) to allow practitioners a degree of control over fire spread and intensity to meet pre-determined land management objectives (Wright & Bailey, 1982). The effects of prescribed fire are intended for the prevention of largescale wildfire, disease, insect and weed control as well as to influence grazing distribution, grazer type and rejuvenate degraded rangelands (Toledo, Kreuter, Sorice & Taylor Jr, 2014). Fire suppression has simplified the structure, composition, and function of the prairie; therefore, an additional aim of prescribed fire is to increase the biological diversity through enhanced landscape heterogeneity, defined as spatial or temporal variation in a measured parameter (Wiens, 2000; Gross & Romo, 2010).

2.3 Fire grazing interaction

The grasslands of North America are a diverse assemblage of vegetation types ranging from the tallgrass prairie in the northeast, where annual rainfall may be in excess of 100 cm, to the semi-arid shortgrass prairie in the southwest, which can receive less than 25 cm of precipitation (Anderson, 1990). The origin of these grasslands can be traced back to the Miocene-Pliocene transition, which is associated with a drying trend, 7.5 million years before present. With the transition, the spread of grasslands occurred at the expense of forest vegetation, as grasses generally have greater drought tolerance compared to trees; grasses have the ability to die down to underground organs, leaving only dead shoot growth above ground (Anderson, 1990). This adaptation also prevents mortality from overgrazing or fire, as growth points beneath the soil may sprout after disturbance (Anderson, 1990).

In response to the worldwide spread of grass dominated vegetation, the late Cenozoic saw mammals evolve large size and hypsodont teeth to deal with abrasive silica rich grass and windblown dust (Jacobs, Kingston & Jacobs, 1999). In grasslands where climate supports a lower drought propensity, herbivory became an essential process affecting ecological processes at levels ranging from the physiology of individual plants, through plant population and community dynamics, to ecosystem processes and landscape patterns (Blair, Nippert & Briggs, 2014). Herbivory by microscopic invertebrates and burrowing rodents can also have a comparable impact on biomass, albeit belowground, with all grazers selectively removing palatable plant species (Hopkins, van Dam, & van Loon, 2009).

Selective biomass consumption by grazers leads to spatial and temporal patterns of vegetation succession throughout the landscape (Fuhlendorf & Engle, 2004; Vermeire, Mitchell, Fuhlendorf, & Gillen, 2004). However, patterns of grazing activity can be heavily influenced by other natural disturbances, such as fire. Fire creates high quality grazing areas that grazers are attracted to and repeatedly choose over unburned sites (Collins & Barber, 1985; Knapp et al., 1999; Limb et al., 2011). Concurrent defoliation by burning and grazing reduce plant biomass and the potential for additional combustion. Preferential grazing of these locations leaves portions of the surrounding matrix less utilized thereby permitting the buildup of biomass and litter which increases the potential for combustion by fire (Gross & Romo, 2010). As a result, fire and grazing in grasslands can be spatially and temporally dependent on each other with the processes regulating

one another with positive and negative feedbacks. The result is a dynamic mosaic of shifting patches within the landscape that vary in time since fire, grazing intensity, and fuel accumulation (Collins & Barber, 1985; Knapp et al., 1999; Allred, Fuhlendorf, Engle & Elmore, 2011)., 2011).The fire-grazing interaction which creates a shifting mosaic of plant succession on the landscape, has been referred to as pyric herbivory (Blair, Nippert & Briggs, 2014), and is the basis upon which the use of prescribed fire is intended. Pyric herbivory can prevent overgrazing and allows for cyclic rejuvenation of grassland species.

2.4 Grassland soil formation

Along with grazing and fire, soil forming factors also contribute to the differentiation of grassland types in the Northern Great Plains. The factors of soil formation: climate, topography, parent material, vegetation and time, are the principal factors affecting native grasslands (Van Breemen & Buurman, 2002). In the Canadian prairies, the advance of glacial ice sheets were the main determinant of the parent material mineralogy. The retreat of glacial ice sheets on the other hand, determined the location of landforms associated with glacial meltwaters and their sediments (Anderson & Cerkowski, 2010).

In Saskatchewan, advancing ice sheets pulverized underlying bedrock, mixing it into glacial deposits, and leaving behind a sediment called till upon retreat (Anderson, 1977). The direction of ice advance is central to understanding the mineralogy and texture of till at any location. The remnant hills of the Manitoba escarpment and the Missouri Coteau escarpment deflected and streamlined the ice mass as it moved from the NW to the SE of Saskatchewan (Anderson & Cerkowski, 2010). Thicker glacial sediments were left at the borders of the escarpments along with a smoother landscape. Once the ice sheets surmounted the Coteau, the ice sheet moved in the opposite direction (NE to SW) leaving thicker glacial sediments in the uplands, while the southwestern areas remained undisturbed. The Cypress Hills in Saskatchewan remained ice free throughout the Pleistocene (Sauchyn, 1990).

With the contrasting movement of ice sheets in Saskatchewan, soil chemistry and texture are reflective of their developmental differences. South of the Canadian shield in Saskatchewan to the Missouri Coteau escarpment, sedimentary rocks such as sandstones, limestones, and shales have been generously mixed into underlying bedrock creating a very fertile, loamy till soil (Anderson & Cerkowski, 2010). Southwestern Saskatchewan contains more salts inherited from

sedimentary rock and a large area of Bear Paw shale, which has contributed to the high concentrations of magnesium sulphates, chlorides; in places high concentrations of sodium are also present (Mermut & St. Arnaud, 1981). Outcrops from limestones found close to the Manitoba border also shared their high carbonate content with nearby tills containing CaCO_3 in excess of 40% (Mermut & St. Arnaud, 1981). In the Southwest corner of Saskatchewan, progressive dilution by other sediments reduced CaCO_3 content to approximately 5%. Soil maps of Southwestern Saskatchewan show interspersed areas of Solonchic soils, which have high-sodium B and C horizon, and Brown Chernozemic soils, which have a darker surface horizon due to surface and root inputs of organic matter (Pennock, McCann, Jong, & Lemmen 1999). Southwest Saskatchewan also tends to be high in clay, due to the lacustrine environment of the shale-origin parent material, which enabled the settling out of fine materials like silt and clay.

2.5 Soil chemical and biological properties of grasslands

Soils in most grasslands are characterized by thick, dark, organic matter-rich A horizons, as there are very significant belowground additions of organic matter through grass root systems. High belowground inputs coupled with the moisture-limited decomposition processes lead to a significant build-up of organic matter in the soil. In addition, bioturbation of the upper horizons from various species of burrowing animals helps create a relatively homogeneous biomantle (Johnson, 1989). Carbonate mineral accumulation in lower soil horizons is often due to frequent soil-moisture deficits of grasslands which limits the rate of mineral weathering within the soil (Kalev & Toor, 2018). In well-drained grassland soils, organic matter content increases with decreasing mean annual soil temperature, increasing the depth to secondary carbonates (Fontaine et al., 2007).

Fire impacts on soil carbon and soil nitrogen are of particular interest. After wetlands and boreal forests, grasslands are the third largest global store of carbon in soils and vegetation (Jobbagy & Jackson, 2000). In semi-arid climates grasslands have also been suggested as the most resilient C-sink as grassland responses to rising temperatures, drought and fire, coupled with preferential banking of C to belowground sinks, helps to preserve sequestered C and prevent it from re-entering the atmosphere (Dass, Houlton, Wang & Warlind, 2018). In basic terms, C sequestration refers to transferring of atmospheric CO_2 into long-lived pools and storing it securely, so it is not immediately reemitted (Lal, 2004). This helps offset climate change by

reducing the radiative forcing that takes place as a result of increases in atmospheric carbon dioxide concentration (Solomon, Plattner, Knutti & Friedlingstein, 2009). Climate change has been suggested to be largely irreversible for 1,000 years after emissions stop (Solomon et al., 2009)

Soil nitrogen has implications in both grassland productivity as well as climate change processes. While N mineralization can increase greenhouse gas (N₂O) emissions, N mineralized during litter decomposition (transformation of high C: N plant material to lower C: N soil organic matter) is critical to ecosystem productivity. Nitrogen is often the most limiting nutrient in temperate ecosystem biomass productivity (Schlesinger & Bernhardt, 2013), with C sequestration contingent upon biomass production (Jones & Donnelly, 2004).

While the cycling of both C and N are closely coupled, they are also dependent on the function and structure of soil microbial communities (Drissner, Blum, Tschirko, & Kandeler., 2007). However, the function and structure of soil microbial communities also depend on plant species composition and diversity, which regulates C and N additions to soil (De Deyn, Cornelissen & Bardgett, 2008; Johnson, Phoenix & Grime, 2008), resulting in complex feedbacks and symbioses within the ecosystem.

2.6 Effects of grazing on soil C and N

Through urination and defecation, large herbivores can directly modify soil properties by concentrating plant nutrients consumed in relatively large areas and depositing in small nutrient-enriched patches (Liu et al., 2016; Orwin et al., 2009). Indirect modification of soil properties also occurs from grazing applications via the effects of herbivores on the plants that make up their diet (Pastor et al., 1998). Changes to individual plants as well as changes to plant community structure, influence the soil microenvironment which includes soil nutrients, soil microbial diversity, and the activity of species in the rhizosphere (Tilman & Wedin, 1991; Stavi, Ungar, Lavee & Sarah, 2008). Based on forage preference, grazing patch size and grazing intensity, different herbivore species may have either additive or contrasting effects on the spatial pattern of plants, and therefore the spatial heterogeneity of soil (Augustine & Frank, 2001; Lin et al., 2010). Due to these confounding factors, the response of belowground biogeochemistry to herbivory is unclear, with studies reporting conflicting results (Tracy & Frank, 1988; Olf & Ritchie, 1998).

The direction of the belowground response to grazing is frequently unclear and unpredictable as several mechanisms are often involved, in addition to the already complex nature

of soil-food- web interactions (Bardgett, Wardle & Yeates, 1998). Belowground C and N cycles have received the most attention, largely attributed to their importance in plant productivity and climate change mitigation. Grazing intensity, grazing duration and climatic conditions may be responsible for the contradictory responses of belowground C and N processes (McSherry & Ritchie, 2013). However, McNaughton, Ruess & Seagle (1988) and Manley, Schuman, Reeder & Hart (1995) suggest grazing intensity to be the major driving factor in belowground C and N processes, as it is the most affective modifier of plant community structure.

Contrasting results of various studies portray the inconsistency in soil response to grazing. Two meta- analyses have reported negative results of grazing on soil nutrients. McSherry & Ritchie (2013) found that grazing decreased SOC in C₃-dominated grasslands only, while Zouh et al. (2017) found that grazing decreased the C and N pools in soils, belowground plant organs (roots, rhizomes and bulbs), microbes, and litters. In contrast, Schuman, Reeder, Manley, Hart & Manley (1999) found that neither total plant biomass nor soil C and N pools were significantly changed with heavy nor light grazing applications. Finally, Wei, Hai-Zhou, Zhi-Nan, & Gao-Lin, (2011) reported significant positive effects of grazing on SOC, total nitrogen and available nitrogen, with positive effects significantly increasing with higher grazing pressures.

2.7. Effects of grazing on soil microbial properties

Fungi and bacteria govern most organic matter transformations in the soil, as well as the ensuing long- term storage of organic C (Bailey, Smith & Bolton, 2002). Favored degradation pathways differ between the two major groups of microorganisms. Fungi have hyphae that allow them to move, colonize, and degrade surface litters where soil bacteria may have no contact. Fungi also tend to have higher C assimilation efficiencies, and so store more of the C they metabolize, compared to bacterial counterparts (Rousk et al., 2010). Native systems are characterized by higher F:B ratios compared to managed systems (Bardgett & McAlister, 1999, Zeller, Bardgett & Tappeiner, 2001), with absolute fungal biomass also more sensitive than microbial biomass to grassland management techniques (Zeller et al., 2001).

Gram-positive and Gram-negative bacteria are types of bacteria with different phospholipid fatty acids in their membrane. Gram-positive bacteria are generally characterized by saturated and branched PLFAs (Kramer & Gleixner, 2008). Monounsaturated and cyclopropyl-substituted PLFAs are signature fatty acids for Gram-negative bacteria (Kramer & Gleixner, 2008). Previous

analyses have indicated that available carbon substrates are not used equally by bacteria, as microbial groups have preferred C sources, which emphasizes the distinct functionalities of different microbial groups (Griffiths, Ritz, Ebbelwhite & Dobson, 1998). Gram-positive bacteria can prefer more recalcitrant C substrates while Gram-negative bacteria prefer more labile sources of C (Fanin, Hättenschwiler & Fromin, 2014).

Grazing has been shown to also affect soil microbial properties in grasslands. Specifically at high grazing intensities, negative effects on soil biological properties are observed (Yan et al., 2016; Qu et al., 2016, Zhong et al., 2016, Zhao et al., 2017). Knops, Bradley & Wedin (2002) found that changes in litter and root biomass induced by grazing are responsible for decreases in the abundance of soil microbial communities, as well as microbial biomass C and N pools (Hosseini Bai, Blumfield, Reverchon & Amini, 2015). However, various intensive grassland management techniques have shown potential toward alleviating the negative effects of grazing on soil microbial communities. Adaptive multi-paddock (AMP) grazing for example, where cattle are stocked at high densities for short periods of time, can cause total PFLA biomass to be higher compared to ungrazed controls (KC et al., 2018). Similarly, different microbial communities such as Gram-positive, Gram-negative, actinobacteria and fungi were higher in AMP vs. non-AMP grazing systems. However, ratios of fungal to bacterial biomass and Gram-positive to Gram-negative bacterial biomass were similar in both grazing systems (KC et al., 2018).

2.8 Effects of fire on soil chemical properties including nutrient C and N

A typical grassland fire creates a mosaic pattern leaving areas burned and unburned across a landscape ranging from low to high severity. Regardless of the ecosystem, fire typically impacts vegetation structure, composition, functional dynamics, and biodiversity. Interactions in the fire regime (e.g. frequency and intensity), and organismal fire response traits (Janzen & Tobin-Janzen, 2008) impact the severity of the fire's effect, and the rate of plant and soil recovery (Keeley, 2008). The combustion of organic material leaves portions of the soil surface bare or covered in ash and can reduce soil organic matter (SOM) (Gimeno-García, Andreu & Rubio, 2000). Exposed ground alters soil radiative forcing (shifts in both temperature and light), evapotranspiration, and the preferential regrowth of vegetation adapted to respond quickly to these conditions (Cerdá & Robichaud, 2009).

Soil chemical properties are affected by fire both directly during heating and combustion and indirectly, through the changes to its vegetation cover and enhanced redistribution of soil through accelerated post-fire erosion (Santin & Doer, 2016). The magnitude of soil changes are driven not only by temperature, but other fire parameters, such as heating duration and oxygen availability (Bryant, Doerr & Helbig, 2005). Soil characteristics such as mineral composition and moisture content also influence fire behavior and heat transfer through soils (Satin & Doer, 2016). Any destruction by heat of the soil organic matter leads to physical, chemical and biological changes to any soil parameter dependent on organic matter and its transformations (Wells, 1979). However, higher temperatures (above 200°C) are required for organic matter and chemical nutrient destruction. High temperature fires also often result in the generation of pyrogenic organic compounds, cause increases in soil pH, as well as alterations in water repellency and aggregate stability (Pereira, Cerdà, Úbeda, Mataix-Solera, & Rein, 2019).

The post fire period often experiences some of the most consequential changes to soil, although occurring both indirectly and gradually. Enhanced erosion and thinning of soils on hillslopes are the most studied post fire effect, with erosion occurring by both wind and water (Larsen et al., 2009; Shakesby, 2011). Erosion, however, may only be problematic during the first couple months post fire, and is decreased at larger spatial scales due to redeposition within hillslopes or catchments. Beyond sediment redistribution, less acknowledged are the inputs of new material to the soil that occur after fire, such as ash, mineral materials, and charred organic components (Santín, Doerr, Preston & Bryant, 2013). Ash is typically rich in nutrients and hence enhances soil fertility (Bodi et al., 2014). Unburnt vegetation killed by fire, charcoal and other types of pyrogenic organic matter which have enhanced resistance to degradation, allows them to survive in soils for centuries to millennia, and hence can act as long- or medium-term carbon sinks (Satin & Doer, 2016).

The effects of fire on soil carbon and nitrogen have not been studied in depth, with the available reports on SOM alterations due to fire often leading to divergent conclusions (Knicker, 2007). Contrasting understandings makes it difficult to postulate generalized models about the lasting effects of fire on soil nutrients (Knicker, 2007). Úbeda et al., (2005) found soil C and N to be elevated immediately post fire in a Mediterranean grassland of Spain. One year later, soil N remained elevated while C returned to baseline levels (Úbeda et al., 2005). Contrasting these results, in the Great Plains of central U.S.A, decreases in soil nitrogen, nitrogen availability and

higher C:N ratios in organic matter have been observed as a result of prescribed fire (Neary & Leonard, 2020). In review of the effects of prescribed fire on soil properties, Alcañiz, Outeiro, Francos & Úbeda (2018), suggest the main determinants regarding the variation in the response of soils to fire are the initial characteristics of soil, vegetation type and fire severity.

2.9 Effects of fire on soil chemical properties including pH and EC

Soil pH has an enormous influence on soil biogeochemical processes. For this reason, it is often described as the “master soil variable” as it relays information about the soil condition and the expected direction of many soil processes (Neina, 2019). Changes in soil pH affect the availability of all plant nutrients, and of particular note is its effect on the solubility of organic C (Andersson, Nilsson & Saetre, 2000) and nitrification (Schmidt, 1982). The size, activity, composition and community structure of soil micro-organisms are also influenced by soil pH (Marstop, Guan & Gong, 2000). Wardle (1992) suggested that soil pH is at least as important as soil C and N concentrations on many soil microbial processes.

Soil pH is one of the most commonly reported variables by authors working on fire affected soil (Blank, Chambers & Zamudio, 2003; Marcos, Tárrega, & Luis, 2007). Fire related changes in soil pH are largely attributed to its effects on soil organic matter, with the formation of oxides (Canti & Linford, 2000) and denaturation of organic acids (Certini, 2005) which occur when soil temperatures exceed 100°C (Giovannini & Lucchesi, 1997). The incorporation of ash into the soil profile can also influence soil pH (Raison & McGarity, 1980), however, fire intensity and the composition of combustible fuels is a large determinant of this effect. During grassland fire, soil temperatures are typically not affected (Marcos, Villalón, Calvo & Luis-Calabuig, 2009), as the majority of heat energy is lost to the atmosphere (Grier, 1975). Therefore, changes in soil pH are mainly the result of ash deposition and shifts in vegetation community structure and function.

Ash produced in grassland fires is not as well documented as ash produced in forest fires. However, the simplest model on combustion would assume that the end products of biomass burning are the minerals contained in the ash, along with H₂O and CO₂ (González-Pérez, González-Vila, Almendros, & Knicker, 2004). Under environmental conditions, oxygen availability during the combustion process generally does not occur, therefore combustion is often incomplete resulting in a complex range of pyrolysis products such hydrocarbons and particulate OM fractions, as well as CO and CH₄ (Cofer, Koutzenogii, Kokorin & Ezcurra, 1997; Gonzalez-

Perez et al., 2004). Vegetation fires, therefore, result in the production of a range of newly formed C arrangements, in addition to minor thermal modification of previously existing C structures (Gonzalez-Perez et al., 2004). Ash that is high in pyrogenic C is normally black in colour and poses little influence on soil pH (Bodi et al., 2014). Ash that is white in colour, is found to be highly basic, as high combustion temperatures volatilize C, leaving behind a complex of minerals to be incorporated into soil (Bodi et al., 2014).

Soil electrical conductivity can also be influenced by the incorporation of ash into burned soils. Soil electrical conductivity provides a measure of the available nutrients in soil, and the ability of soil to conduct an electrical current (Howard, 1993). Saline soils are high in electrical conductivity, and where pH is increased, greater solubility of major cations such as calcium, magnesium, sodium and potassium also occur, along with decreased solubility of minor elements such as aluminium, manganese, iron and zinc (Ponder, Tadros & Loewenstein, 2009; Pereira et al., 2017). Soil pH and EC are frequently evaluated simultaneously and often observe temporary modifications immediately after fire. In low severity fires, pH and EC often return to pre-fire levels within one year of burning (Granged, Zavala, Jordán & Bárcenas-Moreno, 2011). However, while Munoz-Rojas, Erickson, Martini, Dixon & Merritt, (2016), observed reversion of post fire soil pH increase within one year of burning, EC was found to remain elevated for up to 14 years after fire. Reductions in soil EC may also occur as the result of fire. Fonseca, de Figueiredo, Nogueira & Queiros, (2017), noted a decrease in soil EC with temporary soil pH increases, with the lowest EC levels noted 36 months after fire.

2.10 Effects of fire on soil microbial populations

Soil microorganisms are the driving agents which facilitate ecosystem regeneration following fire. Bacteria and fungi regulate the transfer of carbon from terrestrial ecosystems to the atmosphere via the decomposition of organic material in soil (Swift, Heal & Anderson, 1979), and are themselves affected by fire through combustion, cell death and denaturation of enzymes (Fortúrbel et al., 2012, Gutknecht, Henry & Balser., 2010). Due to the natural variability within an ecosystem, and differences in fire behavior, studies from different fire-prone systems report various effects on biological soil properties and the time scale required for restoration (O'Bryan, Prober, Lunt & Eldridge, 2009; Dangi, Stahl, Pendall, Cleary & Buyer, 2010).

Following most fire occurrences, soil microbial populations generally tend to be reduced (O'Bryan et al., 2009) and show less diversity. However, recovery can occur quickly and completely, as observed by Neary, Klopatek, DeBano & Ffolliott (1999), 6 months after grassland prescribed fire. Fultz et al., 2016 also found total microbial biomass to be unaffected by fire and noted no change to underground productivity. Affected PLFA lipid biomarkers (Gram-positive and Gram-negative bacteria) initially reduced by fire, showed indiscernible effects 33 months post fire (Fultz et al., 2016).

The driving force for recovery of the soil microbial system post fire may be entirely dependent on plant productivity. Dangi et al. (2010), investigated the effects of prescribed fire in a sagebrush-grassland ecosystem finding that with an increase in plant productivity, microbial diversity increased rapidly as time progressed, peaking at some critical stage and then declining thereafter. Bai et al. (2007) also observed this trend in a European steppe unaffected by fire; microbial species diversity increases at low levels of productivity and decreases at high levels of productivity. Wardle (2002) suggests that the strong links between plant functional groups, plant species and soil microbial communities may indicate that changes in vegetative community structure in the years following fire are potentially more dominant of a factor influencing soil microbial community dynamics compared to the direct impact of fire disturbance itself (Hart, DeLuca, Newman, MacKenzie & Boyle, 2005).

2.11 Seasonal changes in soil properties

Seasonal changes in soil properties, both biotic and abiotic are important to consider as they may be able to provide a framework of favorable response parameters for predicting longer term soil changes. In addition, understanding temporal changes may also encourage increased awareness of grassland management practices based on temporal timescales. At both small and large scales, soils are characterized by heterogeneity of vegetation, microclimate, land-use history and underlying geology (Regan et al., 2017).

Plants can be considered as both architects and drivers of spatial and temporal heterogeneity, where growing roots change the biogeochemical environment (Regan et al., 2017). In grasslands, the entire upper 10cm of soil can be altered as the result of intensive root growth and plant derived exudates early in the growing season (Mueller, Tilman, Fornara & Hobbie, 2013) As plants senesce, exudates cease, leaving plant litter and decaying root material as the most prolific supply

of nutrients (Kuzyakov & Blagodatskaya, 2015). As described, the amount and quality of nutrients from litter and exudates substantially vary over the season, and this variation strongly influences the soil biogeochemical environment (Kuzyakov & Xu, 2013).

A large majority of soil spatial biogeochemical studies following disturbance have been carried out a single time point. Studies which have combined spatial and temporal approaches have yielded conflicting results (Zak, Holmes, White, Peacock & Tilman, 2003; Grayston, Griffith, Mawdsley, Campbell & Bardgett, 2001; Regan et al., 2017), or focused solely on microbial dynamics. Bardgett, Lovell, Hobbs, & Jarvis, (1999), observed a spring peak in fungal to bacterial ratios following fire, which was attributed to a proliferation of decomposer fungal species and the availability of charred surface residues. However, a higher prevalence of bacteria compared to fungi in spring has also been reported (Lovell, Jarvis & Bardgett, 1995), where PLFA analyses showed the strongest seasonal effect after fire.

2.12 Current state of research on grassland prescribed fire and soil

Fire and grazing are interrelated with their co-occurrence having a synergistic effect on plant communities and soil properties. The effects depend on the type of vegetation, the phenological state of the plants, season, frequency and intensity of burning, grazing pressure, physical structure of the landscape, soil moisture content, soil organic matter, soil bulk density and climatic conditions (Coughenour, 1991). Previous research has been conducted on the interaction effect of fire and grazing on soils, however, due to the multitude of factors that influence ecological effects, results have reduced transferability across types of grassland ecosystems. Examples of recent fire-grazing interaction studies include research by Anderson, Fuhlendorf & Engle (2006), who found soil nitrogen to increase under the fire grazing interaction in a tallgrass prairie, similar to the results of Johnson and Matchet (2001), who noted increased N availability and N cycling. Johnson and Matchet (2001) found the fire grazing interaction to improve root tissue quality, which promoted the faster cycling of N, however, total root biomass was decreased, which resulted in a 50% reduction in N mineralization. Teague et al., 2010, also evaluated soil C and N following patch burning and rotational grazing in the semi-arid region of Texas. Results indicate that under rotational grazing soil C and N increased compared to soils subject to fire and continuous grazing. Soil bulk density, aggregate stability and water infiltration were also unchanged with either treatment (Teague et al., 2010). In the paramo environments of the northern Andes, the interaction

of fire and grazing was shown by Hofsteade et al. (1995) and Suárez & Medina (2001) to affect soil physical but not chemical properties. While both soil organic matter content and available P remained unchanged, soil compaction was observed to increase while soil moisture decreased. Changes in vegetation were also apparent and related to the changes in soil properties. Where soil compaction was extreme, vegetation was sparse, with erosion expected to occur with continued grazing. Vermerie et al. (2004) measured wind erosion and water holding capacity in soils that were subjected to prescribed fire and continuous grazing; results showed that burned patches experienced twice the erosion in both spring and fall, compared to unburned patches (Vermerie et al., 2004).

Further research on fire impacts on soils are required to support the use of fire in mixed grasslands. The inconsistent results of past studies show the need for confirmation that prescribed fire practises are amenable with soil conservation, especially when combined with grazing applications. Currently, few recommendations regarding soil management are available to land managers on the use fire and grazing in grasslands, therefore, this research will be beneficial for those seeking to manage grasslands with soil conservation considerations.

3.0 EFFECTS OF PRESCRIBED FIRE ON SOIL BIOGEOCHEMISTRY IN A MIXED GRASS PRAIRIE

3.1 Abstract

Limited information exists regarding the effects of prescribed fire on soil biogeochemistry in the mixed grasslands of North America. This study investigated the effects of prescribed grassland fire on soil biogeochemistry over two growing seasons in the mixed grass prairies of Southern Saskatchewan. Spring burning was conducted in continuously grazed native and tame pastures. Soils were sampled (0-10 cm) 2, 3, 4 and 15, 16, 17 months after fire in burned and adjacent control plots. Investigation of soil biogeochemical changes include the analysis of soil total carbon (C), nitrogen (N), microbial biomass C (MBC), microbial biomass N (MBN), C stock, pH, electrical conductivity (EC) and characterization of the microbial community through phospholipid fatty acid biomarker extraction. Results indicate that the mixed grasslands in southern Saskatchewan are largely resistant and resilient to the effects of disturbance by fire. Slight changes in the microbial community structure were observed in both pastures; burning increased the homogeneity in microbial community composition. Attributed to a post fire nutrient flush, the tame forage pasture had an increase in soil fungi 2-4 months following fire and an increase in the ratio of Gram-negative bacteria to Gram-positive bacteria throughout the duration of the study. Temporal effects on soil biogeochemistry were stronger than fire effects. Pastures responded differently over time, likely due to differences in vegetation composition and abundance as well as land use history. This research shows that the use of prescribed fire is compatible with soil conservation principles, as negative effects on soil biogeochemistry were not observed. Reducing barriers to the use of prescribed fire in grassland management is important for the preservation and productivity of remnant grassland parcels, and the ecosystem services grasslands provide.

3.2 Introduction

Approximately 7-10% of the world's grasslands are in the North American Great Plains, with the Northern Mixed Grasslands covering roughly 270,000 km² in the semi-arid, central region. Among all grassland ecoregions, the mixed grasslands have experienced the most disturbance; none of the remaining patches are considered to be intact (World Wildlife Fund, unpublished data, 2022). Biodiversity has declined substantially (Hoekstra et al., 2020) leaving only remnant bird and wildlife populations from what was once considered some of the world's greatest assemblages (Henwood, 2010). Despite significant species and ecosystem area loss, the remaining intact grasslands provide a large range of ecosystem services. Grasslands sequester and store large amounts of C in addition to conserving genetic diversity, acting as water catchment sites, religious and spiritual sites, providing recreation and tourism, wild food, and natural medicine sources (Henwood, 2010). To ensure the productivity and conservation of the remaining mixed-grass grasslands, it is essential that the remaining parcels are managed not only to maintain ecological function and biodiversity, but to enhance resilience.

Fire was historically commonly used by Indigenous communities on the Great Plains as part of the dynamic ecosystem process that shaped grassland plant communities (Neary & Leonard, 2020). Since the settlement of the Great Plains, fire has been strongly suppressed. A resurgence in the use of prescribed fire is a part of efforts to include disturbance as a part of ecosystem management and restoration ecology (Neary & Leonard, 2020). In grasslands, prescribed fire is commonly implemented by land managers and conservationists to shift plant community structure, enhance species diversity and biomass production, manage grazing patterns, and to reintroduce heterogeneity into the landscape. Heterogeneity is an integral feature of grassland ecosystems as variation in micro and macrohabitats accommodate a larger diversity of terrestrial species. While the effects of fire on the above ground components of grasslands are well studied (Fuhlendorf & Engle, 2001; Archibald & Bond, 2004; Huang, Wang, Deng, Sun, & Zeng, 2018), research investigating the effects of fire on soil is scarce (Ruprecht, Fenesi, Fodor & Kuhn, 2013; Pereira et al., 2013; Végvári et al., 2016). It is important that the effects of fire on soil are well understood to ensure fire prescriptions are compatible with soil conservation and ecosystem resilience.

The objectives of my study were to evaluate the effects of prescribed fire on soil chemical and microbial properties during two growing seasons following the spring burning of a seasonally grazed, northern mixed-grass grassland. Soil total carbon, total nitrogen, pH and electrical

conductivity were evaluated as soil chemical properties, and microbial biomass carbon, microbial biomass nitrogen as well as PLFA extraction, were evaluated as soil microbial properties. Initial measurements two months post-fire capture the grassland's capacity to resist disturbance, whereas longer term measurements reflect the resilience of the ecosystem to fire. Soil was evaluated 2,3 and 4 months following fire, marking the first growing season, and 14,15 and 16 months following fire, marking the second growing season. Fire was hypothesized to positively affect soil properties, showing increases in total nutrient content, supporting an increase in microbial abundance and diversity. Soil pH and EC were also hypothesized to increase post fire, due to nutrient ash deposition post fire. This research provides critical information to guide the environmentally sustainable management of grasslands using prescribed fire.

3.4 Materials and Methods

3.4.1 Field site

The experimental site is located at Nature Conservancy Canada's (NCC) Old Man on His Back Heritage and Conservation Area (OMB) property in southwestern Saskatchewan ($49^{\circ}12'$ N, $109^{\circ}33'$ W, elevation 976 m); a terminal moraine escarpment with a meltwater channel along the south and east sides. OMB is within the Mixed Prairie Ecoregion, 15 km west of Claydon, SK, 65 km east of the Alberta border and 23 km north of the U.S.A. border. OMB has a mean annual temperature of 4.7°C and a mean annual precipitation of 385.0 mm (Government of Canada, 2022) (Fig. 3.1). Soils are Solonchic clay-loams and Chernozems, and are within the Brown soil zone of Saskatchewan (Saskatchewan Soil Survey, 1992).

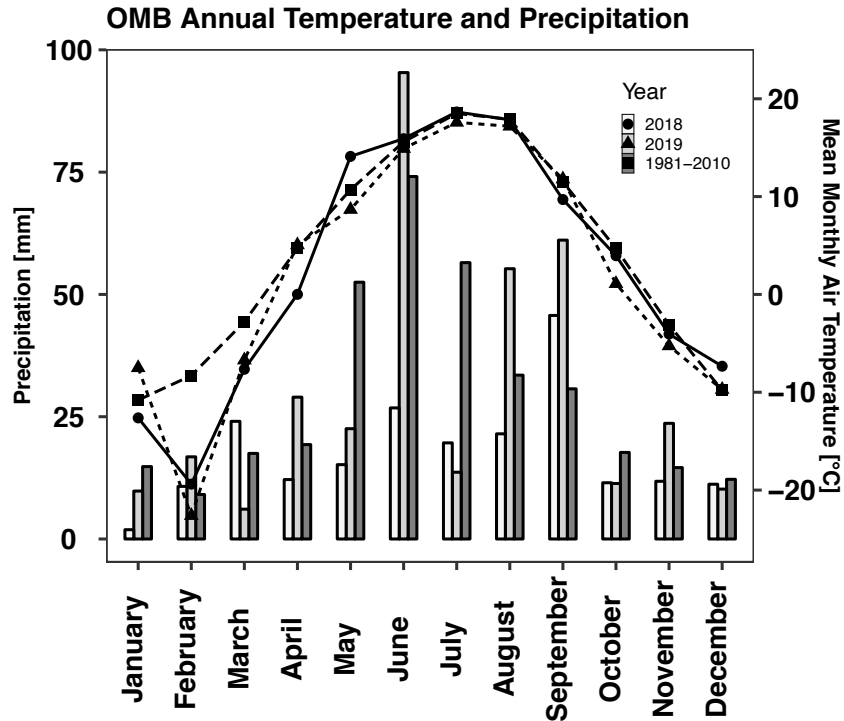


Fig. 3.1. Mean monthly temperature and total monthly precipitation in 2018–2019, and long-term averages (1981–2010). Line graph represents mean monthly air temperature (right axis), with circles indicating 2018, triangles indicating 2019 and squares indicating 1981–2010. Bars indicate total monthly precipitation (left axis). White bars indicate 2018, light grey indicates 2019 and dark grey indicates 1981–2010. Weather data collected and averaged from Eastend SK (51 km from OMB), and Val Marie, SK (70 km from OMB) weather stations (Government of Canada, 2019)

Typical of the semi-arid Mixed Prairie Ecoregion, the upland vegetation of OMB is dominated by Junegrass (*Koeleria macrantha*), Northern Wheatgrass (*Elymus lanceolatus* ssp. *lanceolatus*) and Blue Grama (*Bouteloua gracilis*), while the meltwater channel contains sagebrush dominated communities, common on alluvial deposits in southwest Saskatchewan (Hilger, 2020). The 5300-hectare property is divided up into two pastures for year-round bison grazing, and several pastures for cattle grazing (Fig. 3.2). Most of the property remains as native prairie; however, some of the pastures were historically cultivated and seeded to tame forage. Since the early 1900’s, the OMB property has been grazed by cattle, and no fires have been recorded within recent memory.

This study focuses on two pastures: S5, 45 ha of seeded tame forage and N8, 151 ha of unbroken native grassland (Fig. 3.2, 3.3). The tame forage pasture (S5) is dominated by non-native crested wheatgrass (*Agropyron cristatum*) and alfalfa (*Medicago sativa*), with non-dominant

native species including June grass (*Koeleria macrantha*) and broomweed (*Gutierrezia sarothrae*) (Hilger et al., 2020). The pastures are grazed each year by 45 heifers and 2 bulls on a deferred-rotation plan. Grazing pressure is managed by the NCC and adjusted based on plant community productivity. In general, from June to July, the tame forage pasture (S5) is grazed by cattle, followed by rotation to the native forage pasture (N8) from July to October. In 2018 and 2019, S5 was grazed with a stocking density of 0.8 AUMs/ha and 0.9 AMUs/ha and N8 was grazed with a stocking density of 0.7 AUMs/ha and 0.8 AMUs/ha respectively. Salt and mineral supplements were placed next to water sources.

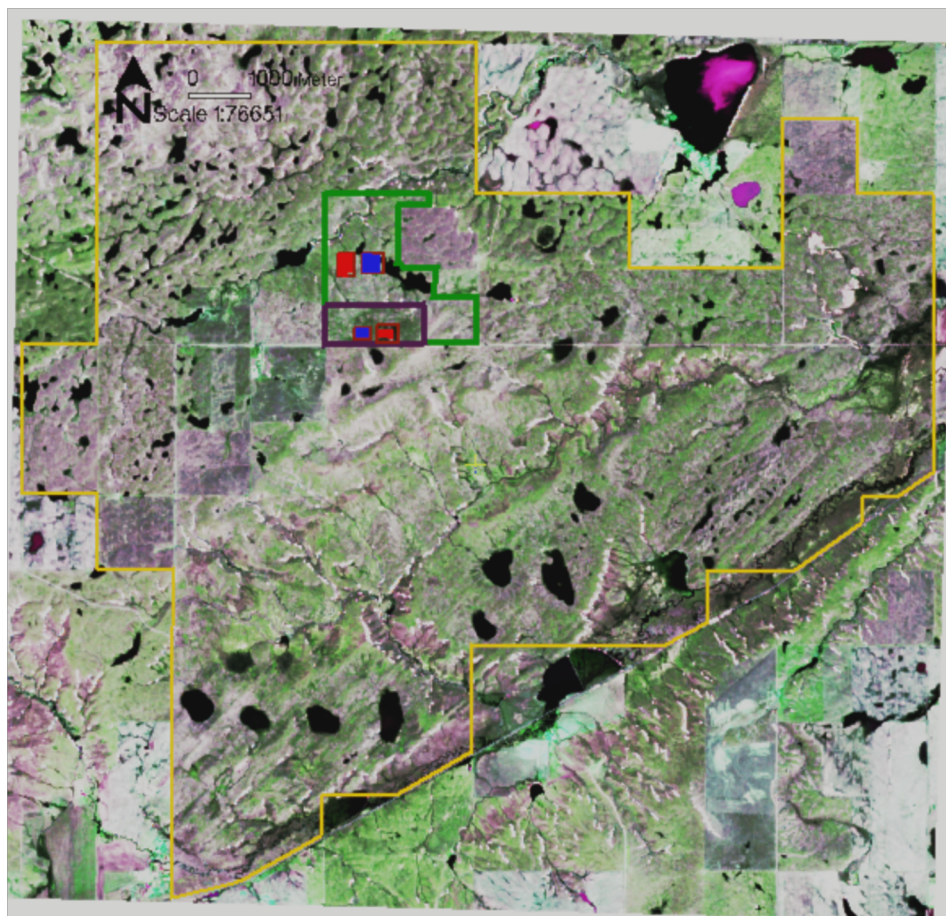


Fig. 3.2. OMB grassland boundary (yellow) showing native (green) and tame forage (purple) pastures, containing burn (red) and control (blue) treatment areas.

3.4.2 Prescribed fire

Prescribed fire was implemented in one treatment block of N8 (9 ha) and S5 (5.2 ha) in April 2018. S5 was burned over 2.5 hours on the morning of April 26, 2018, with atmospheric temperature between 5.8-15.1°C, average windspeed between 1.6-10 kmh⁻¹ and relative humidity between 33-42%. N8 was burned over April 27 and 28, 2018, with average windspeed varying between 1.4-21.3 km/h, relative humidity ranging between 18.4-60.5%, and temperatures between 5.1-22.5°C during the burning period.

3.4.3 Experimental Design

Each of the studied pastures contain two treatment blocks, chosen based on consistency in terrain. In the tame forage pasture, each treatment block is 5.2 ha in size, while in the native forage pasture, treatment blocks are each 9 ha in size. Of the two treatment blocks, one was chosen for burn treatment and the other designated as control (Fig. 3.3). Plots allocated to burn treatment were chosen based on ease of access, to reduce motor vehicle disturbance within the prairie.

Within each treatment block, permanent sample plots were designated every 50 m, in a grid pattern (Fig 3.3). Due to the size differences of the tame and native forage pastures, there were 6 permanent 4.0 m² sample plots in each of the treatment and control blocks of the tame forage pasture, and 14 and 15 permanent 4.0 m² sample plots in each of the treatment and control blocks of the native forage pasture, respectively (Fig. 3.3). Within the 4.0 m² sample plots, there were four 0.25 m² subplots used for biomass collection and soil sampling, and one 1.0 m² permanent subplot for recording plant canopy cover. Sampling within the four 0.25 m² biomass subplots was systematic to avoid re-clipping vegetation over time. Soil and vegetation biomass were sampled simultaneously for later correlation. Biomass and plant community data from these sites are reported by Hilger (2020).

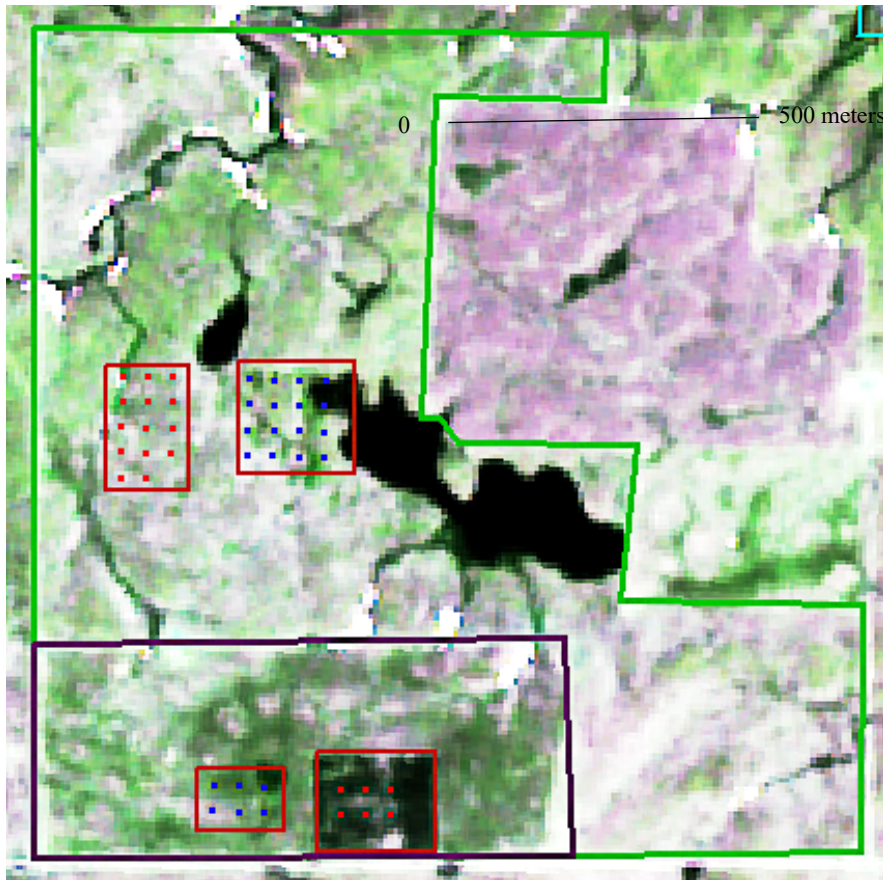


Fig. 3.3. Treatment plots within the native (green boundary) and tame forage pastures (purple). Red dots represent sample plots within burned treatment areas and blue dots represent sample plots within control areas. Landscape image was captured April 26th, 2018, coinciding with burn treatment in the tame forage pasture.

3.4.5 Soil sampling

Soil sampling was carried out in June, July, and August of 2018 and 2019. At each time point, a 3.2 cm diameter JMC Backsaver soil probe (JMC Soil Samplers, Newton, IA) was used to collect soil samples from within the 0.25m² subplots to a depth of 0-10 cm. Two samples were collected from each subplot, one of which was transported in a cooler on ice and then stored at -20 °C for later microbial analysis. The other sample was air dried and stored at room temperature for chemical analyses.. In July of 2018, a soil bulk density sample was collected using a 5 cm diameter by 5 cm deep soil core from each plot.

All soil samples were processed by sieving through a 4mm sieve. Microbial samples were processed over ice and stored again at -20°C. Dried samples were further ground with a mortar

and pestle for NIR C and N, pH and EC analyses. Soil gravimetric moisture contents were determined by placing 2 g of fresh soil in a conventional oven for 24 hr at 105°C

3.4.6 Soil analyses

3.4.6.1 Microbial biomass analysis

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined by the chloroform fumigation extraction method as described by Voroney, Brookes & Beyaert, (2008). Briefly, two 10 g portions of sieved (< 2mm) field moist soil were weighed and incubated at 65% field capacity for 14 days. After incubation, one portion was fumigated under vacuum with ethanol free chloroform for 24 hours and then extracted with 0.5M K₂S₀₄ (1:4 soil: extractant ratio). The other sample portion was extracted immediately after fumigation was initiated. Total organic C and N from both fumigated and unfumigated soil extracts were analyzed using a CN analyzer (TOC-Vcph-TN, Shimadzu). Values reported from non-fumigated samples were subtracted from the fumigated samples. Both MBC and MBN were calculated using Kec factor of 0.35 for MBC and Kec factor of 0.5 for MBN (Voroney et al., 2008). Samples were corrected for soil dry weight using prior gravimetric moisture determination.

3.4.6.2 Phospholipid fatty acid analysis (PLFA)

Phospholipid fatty acid analysis was conducted following the method adopted by Helgason, Walley & Germida (2010a) from White, Davis, Nickels, King & Bobbie (1979) and Bligh and Dyer (1959). Soils were prepared for analysis by freeze drying for 72 h and stored at -20°C until further use. Prior to analysis, freeze dried samples were pulverized using a mortar and pestle. PLFA extraction occurred with 4 g of lyophilized soil using 19 mL of phosphate buffered extractant solution (CH₃OH:CHC₁₃: Phosphate buffer at a ratio of 2:1:0.8). Phospholipids were separated using 500 mg silicon fractionation columns (Bond Elut, Agilent Technologies, Santa Clara, CA) then methylated for gas chromatograph (GC) analysis. Methyl nonadecanoate (19:0) was used as an internal standard. Fatty acid methyl-esters (FAMES) were quantified on a Bruker 436 GC flame ionization detector (FID, Bruker Corporation, Billerica, MA) then identified by comparing peak retention times against a library of known FAME retention indices. Specific biomarkers were used to assess the relative abundance of microbial functional groups (Table 3.1). The ratio of Gram negative to Gram positive bacteria (GN:GP) and the ratio of fungi to

bacteria (F: B) was used as an indicator of changes in the relative abundance of these microbial groups (Bardgett, Hobbs, & Frostegård, 1996; Bailey et al., 2002). Microbial stress was determined by the ratio of PLFA's cyclo 17:0 to 16:1 w7c. Higher ratio values represent greater stress (Kaur, Chaudhary, Kaur, Choudhary & Kaushik, 2005).

Table 3.1. Biomarkers used in the calculation of microbial functional groups and stress.

Functional Group	Biomarker	Reference
Bacteria	i14:0, i15:0, a15:0, i16:0, 16:1 ω 7c, 10Me16:0, i17:0, a17:0, cy17:0, 10Me17:0, 18:1 ω 7, 10Me18:0, cy19:0	(Helgason et al., 2010a) (Bååth and Anderson, 2003)
Fungi	18:2w6,9	(Bååth & Anderson, 2003)
GP^a bacteria	i14:0, i15:0, a15:0, i16:0, i17:0, a17:0	(Helgason, Walley & Germinda., 2010b) (Hedrick, Peacock & White., 2005)
GN bacteria	16:1 ω 7t, 16:1 ω 9c, 16:1 ω 7c, 18:1 ω 7c, 18:1 ω 9c, cy17:0, cy19:0	(Helgason et al., 2010b) (Hedrick et al., 2005)
AMF	16:1w5c	(Helgason et al., 2010b)
Actinobacteria	16:0 10-methyl, 17:0 10-methyl, 18:0 10-methyl	(Zelles, 1999)
Stress	cy 17: 0 / 16:1 w7c	(Grogan & Cronan, 1997) (Kaur et al., 2005)

^aGP = Gram-positive bacteria, GN = Gram-negative bacteria, AMF = arbuscular mycorrhiza fungi

3.4.6.3 Soil bulk density

Bulk density was determined on July 2018 soil samples. Collected soil was weighed and oven-dried at 105 °C for 24 h to determine soil moisture content of bulk density samples. The bulk density was calculated as the dried soil mass divided by the soil core volume. Bulk density measurements determined in July of 2018 were used to estimate soil C stocks in all 2018 and 2019 samples, assuming that bulk density remained relatively constant from spring 2018 to fall 2019.

3.4.6.4 Soil carbon and nitrogen analyses

Air dried soil was prepared for chemical analysis by sieving (< 2mm) and pulverizing with a mortar and pestle. Pulverized soil was subsampled and analyzed for total carbon and total nitrogen with NIR spectrography (FOSS NIR XD). Wavelength spectral data was exported from the internal FOSS NIR software and converted to plain text using SpectraGryph software (Menges,

2018). Spectra files were merged with PLSR model data for the estimation of soil total carbon and soil total nitrogen. Briefly, the Partial Least Squares Regression calibration model (pls, Mevik, Wehrens & Liland, 2018; R Development Core Team, 2018) uses a canonical Gamma link function, and builds a matrix with spectral and calibration data to predict total soil carbon and nitrogen based on regression analysis. Calibration data was obtained through analyzing 160 samples from OMB by dry combustion using a LECO C632 carbon combustion analyzer (LECO® Corporation, St. Joseph, MI, USA) on soil samples ground to a fine powder (<250µm). Combustion occurs at 1350°C for 90 s in the LECO C632 ((LECO® Corporation, St. Joseph, MI, USA) combustion analyzer.

3.4.6.5 Soil carbon stock

Soil total carbon stock was calculated using the fixed depth/fixed volume approach (Ellert, Janzen & Entz, 2002), Eq. 1.0. The entire topsoil (Ah horizon) was contained within the 0-10 cm profile sampled for soil carbon, therefore using the equivalent mass approach should not be necessary, as little soil carbon is left below the sampled area (VandenBygaart & Angers, 2005). Sampling the whole topsoil accounts for any changes in bulk density affecting soil carbon storage calculations. (VandenBygaart & Angers, 2005). Bulk density was assessed using the core analysis method (Ellert, Janzen, VandenBygaart & Bremer, 2008).

$$\text{Mass}_{\text{SC}} = \text{conc}_{\text{SC}} \times \rho_b \times T \times 10,000 \text{ m}^2 \text{ ha}^{-1} \times 0.001 \text{ Mg kg}^{-1} \quad (\text{Eq. 1.0})$$

where Mass_{SC} = total SC mass per unit area (Mg C ha^{-1}); conc_{SC} = total SC concentration (kg C Mg^{-1}); ρ_b = field bulk density (Mg m^{-3}); T = thickness of soil layer (m); 0.001 Mg kg^{-1} = conversion factor.

3.4.6.6 Soil pH and electrical conductivity

Soil pH and electrical conductivity (EC) were analyzed in duplicate, using deionized water at a 1:2 of soil to water extraction paste (Gavlak, Horneck, Miller & Kotuby-Amacher, 2003). Samples were filtered (No.1, Whatman Inc., Piscataway, NJ) after shaking for 1 hour, prior to pH and EC measurement. The extract was read for pH using an Accumet AB150 pH meter and EC

was determined using a digital EC meter (PC450 pH/mV/conductivity, Oakton, Vernon Hills, IL, USA) (Miller & Curtin, 2008).

3.4.7 Statistical analysis

3.4.7.1 Univariate analysis

The effect of prescribed fire on soil total carbon and nitrogen, carbon to nitrogen ratio, microbial biomass carbon and nitrogen, ratio of MBC to MBN, pH, EC, carbon stock, total PLFA biomass, total bacteria, total fungi, fungal to bacterial ratio, Gram-negative and Gram-positive bacteria, the ratio of Gram-negative to Gram-positive bacteria, actinobacteria, arbuscular mycorrhizal fungi and the ratio of cy 17: 0 / 16:1 w7c (stress) were examined using linear mixed effect models. Due to differences in species composition, pasture size and grazing timing and intensity, the tame forage (S5) and native (N8) pastures were analyzed separately. The model compared the seasonal and yearly effects of burn treatment to the adjacent control block. The fixed effects were treatment (burn or control), sample month (June, July, or August) and year (2018 or 2019), and the interaction between treatment, month, and year. To account for the repeat sampling of individual sample plots, a random factor (sample plot ID) was used in the model. Model residuals were checked for normality with normal qq plots and by comparing the raw versus fitted values. The mixed effects models were fit using R statistical software and the *lmer* function from the *lme4* package (Bates, Maechler, Bolker, & Walker, 2015; R Development Core Team, 2019). All statistical analyses were done using R (R Core Team, 2014) with significance determined using $\alpha = 0.05$.

3.4.7.2 Multivariate analysis

Non-metric multidimensional scaling (NMDS) was used to visualize the microbial community structure and compositional (PLFA) changes induced by burn treatment and throughout each sampling month (June, July, August) and year (2018, 2019). Three separate ordinations were used to display 1) correlation of soil variables with microbial community structure, 2) treatment effects and, 3) seasonal effects (month or year). Multiple ordinations were used to ensure factors influencing the microbial community structure were clearly represented. All ordinations were performed with relative abundance data calculated by absolute abundance of each biomarker divided by total abundance of all biomarkers, multiplied by 100 to reach a percentage

value, for each sample. Relative abundance data was ln transformed prior to analysis and PLFAs that were present in less than 5% of samples were omitted. Relative abundance data was used as it more suitably reflects the distribution of microbial biomarkers between samples for the purpose of multivariate analysis.

To examine the correlations between soil variables and the ordination, vectors were added using the *envfit* function. Vectors indicate the direction of the most rapid change in environmental variable and are proportional to the magnitude of the correlation between ordination and environmental variable. Regression analyses between soil variables and ordination axes were used to evaluate the strength of the vector correlation (Appendix C). Only significantly correlated variables ($p < 0.05$) were plotted.

Multivariate analysis of variance (PerMANOVA) using the *adonis* function was used to determine the significance of month, year, and treatment effects on microbial community structure. Ellipses represent 95% confidence intervals of the microbial community structure and were used to indicate significant effects of treatment, month and year in separate ordinations. NMDS models for the tame (S5) and native (N8) pastures were fit separately due to differences in pasture plant community composition. The final stress value of each ordination indicates the consistency of the final ordination in relation to the dissimilarities within the dataset. Lower stress values indicate better ordination of the data. Multivariate analyses were completed with the R package ‘vegan’ (Oksanen et al., 2013; R Development Core Team, 2019).

3.5 Results

3.5.1. Prescribed fire

Visual observations after prescribed fire in both the tame and native forage pastures showed areas of complete, partial and unburned biomass, interspersed amongst one another in a shifting mosaic pattern. Herbaceous stubble was visible above a relatively unaffected surface soil; charring of the organic soil layer was undetected. No apparent discolouration of the soil was visible; however, charred biomass and black coloured ash covered the herbaceous stubble and patches of bare soil. Areas beneath smoldering cow patties appeared to burn biomass more completely; fuels were completely consumed, and herbaceous stubble was burned.

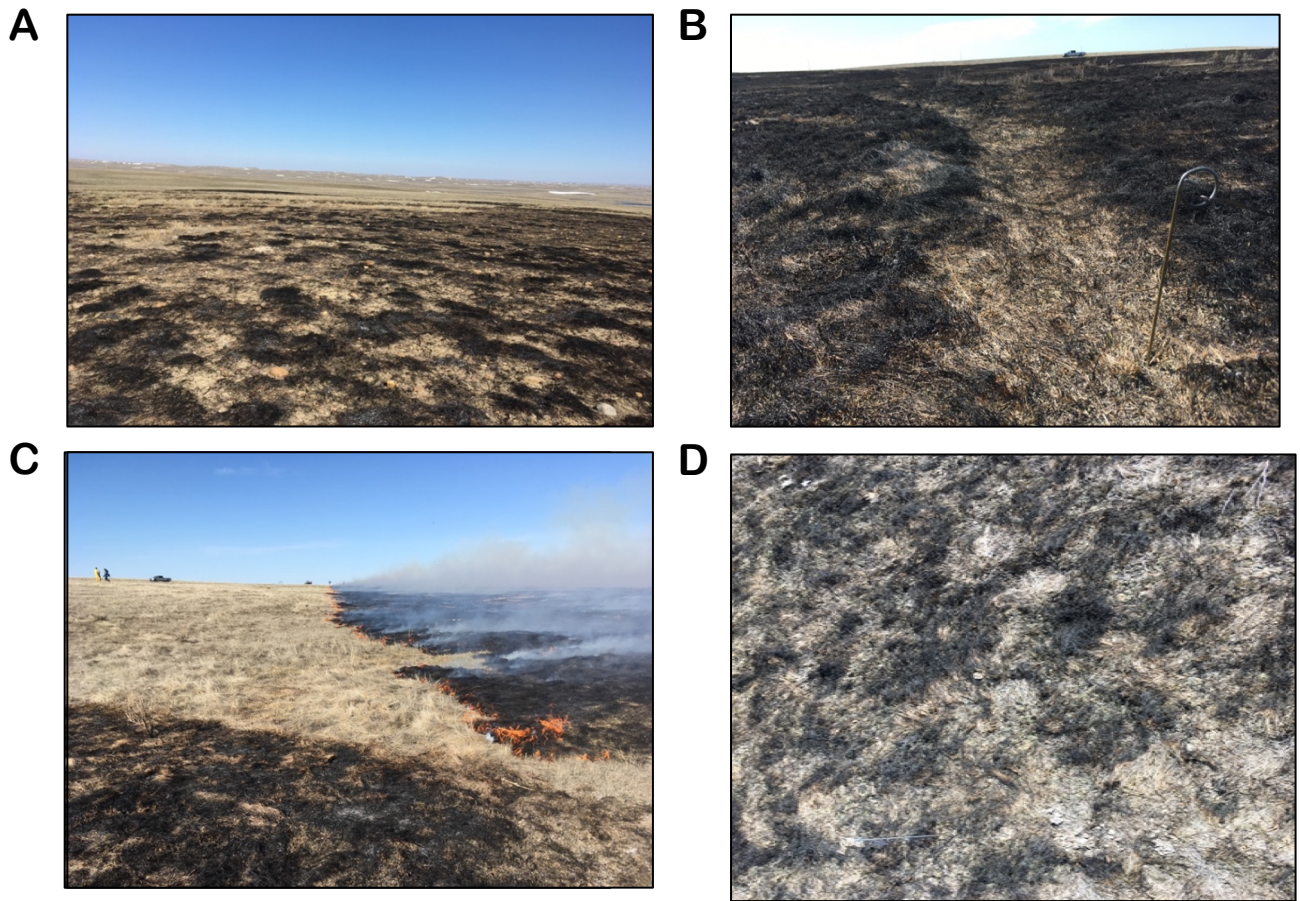


Fig. 3.4. Images captured during prescribed fire and immediately after burning, indicating low intensity fire and low burn severity in pastures S5, tame forage, (Fig. A, B) and N8, native forage, (Fig. C, D).

3.5.2 Tame forage pasture (S5)

Soil microbial properties

Prescribed fire did not significantly affect any of the soil biogeochemical parameters in the tame pasture with the exception of fungi and GN:GP, which were both significantly higher in the burn treatment compared to the control (Table 3.2). For fungi, the treatment effect was only present in 2018, noted by the treatment by year interaction (Fig. 3.6), whereas the treatment effect for the GN:GP was significant in 2018 and 2019 (Fig. 3.7). There were significant year to year differences in all microbial parameters apart from GN:GP (Table 3.2 Fig. 3.6, 3.7). All significant year to year differences involved declines in the respective parameters from 2018 to 2019.

The two-dimensional NMDS model for the tame forage pasture had a final stress of 0.209 (Fig. 3.8, Fig. 3.9). Absolute abundance PLFA groups showed stronger correlation with the

microbial community structure, compared to remaining soil variables (Fig. 3.8). PerMANOVA indicated that prescribed fire and sampling year significantly influenced the microbial community structure (Table 3.3, Fig 3.9). Prescribed fire homogenized microbial community composition as indicated by the clustering of burn plots in the ordinations (Fig. 3.9).

Soil chemical properties

Soil pH was significantly affected by years (Fig. 3.5), with 2019 resulting in slightly higher soil pH than 2018. Monthly changes in the tame forage pasture produced a significant effect in soil EC, with July reporting higher EC compared to June and August (Table 3.2, Fig. 3.5).

Table 3.2. Linear mixed effect model comparing burn treatment, seasonal (month) and year effects on soil biogeochemical properties from the tame forage pasture (S5). Bolded terms denote significant ANOVA effect of model factor $p \leq 0.05$.

Model Factor (s)	Treatment ^a	Month	Year	Treatment:Month	Treatment:Year	Month:Year	Treatment:Month:Year
Total Carbon	F=1.634, p=0.227	F=1.566, p=0.220	F=0.477, p=0.493	F=0.856, p=0.300	F=1.097, p=0.300	F=0.160, p=0.852	F=0.199, p=0.820
Total Nitrogen	F=2.604, p=0.135	F=1.194, p=0.311	F=0.036, p=0.851	F=0.578, p=0.565	F=0.310, p=0.581	F=0.291, p=0.748	F=0.166, p=0.847
C : N	F=1.795, p=0.207	F=1.789, p=0.178	F=1.209, p=0.277	F=0.411, p=0.666	F=0.663, p=0.420	F=0.284, p=0.754	F=0.642, p=0.531
Carbon Stock	F=0.443, p=0.519	F=1.780, p=0.179	F=0.711, p=0.403	F=0.986, p=0.380	F=1.603, p=0.211	F=0.296, p=0.745	F=0.291, p=0.749
pH	F=0.400, p=0.542	F=0.820, p=0.445	F=0.760, p=0.009	F=0.001, p=0.997	F=0.002, p=0.877	F=0.180, p=0.832	F=0.340, p=0.717
EC	F=0.738, p=0.409	F=4.033, p=0.024	F=0.814, p=0.371	F=1.199, p=0.310	F=3.269, p=0.077	F=1.731, p=0.188	F=0.650, p=0.526
MBC	F=1.008, p=0.337	F=0.624, p=0.540	F=0.318, p=0.576	F=1.709, p=0.191	F=2.696, p=0.107	F=0.579, p=0.564	F=1.211, p=0.307
MBN	F=0.731, p=0.411	F=0.102, p=0.903	F=1.071, p=0.306	F=1.451, p=0.244	F=1.634, p=0.207	F=0.951, p=0.393	F=1.053, p=0.356
MBC : MBN	F=0.239, p=0.635	F=0.388, p=0.681	F=0.0001, p=0.999	F=1.141, p=0.328	F=1.716, p=0.196	F=1.432, p=0.249	F=0.620, p=0.542
Total PLFAs	F=0.053, p=0.822	F=0.634, p=0.534	F=17.413, p=0.001	F=1.889, p=0.161	F=0.100, p=0.753	F=0.358, p=0.701	F=1.476, p=0.238

Table 3.2 continued.

Model Factor (s)	Treatment ^a	Month	Year	Treatment:Month	Treatment:Year	Month:Year	Treatment:Month:Year
Bacteria	F=0.0001, p=0.991	F=0.852, p=0.433	F=14.691, p=0.0004	F=2.191, p=0.122	F=0.019, p=0.889	F=0.472, p=0.626	F=1.476, p=0.238
Fungi	F=5.239, p=0.042	F=0.823, p=0.445	F=27.329, p<0.0001	F=1.459, p=0.242	F=4.042, p=0.049	F=0.164, p=0.849	F=0.630, p=0.536
F : B	F=1.581, p=0.234	F=0.542, p=0.584	F=17.543, p=0.0001	F=0.403, p=0.669	F=2.882, p=0.095	F=1.436, p=0.248	F=0.132, p=0.877
GN	F=0.464, p=0.509	F=1.575, p=0.217	F=18.282, p=0.0001	F=2.321, p=0.108	F=0.063, p=0.431	F=0.294, p=0.746	F=1.329, p=0.273
GP	F=0.026, p=0.874	F=0.740, p=0.482	F=13.411, p=0.0006	F=1.990, p=0.147	F=0.008, p=0.927	F=0.513, p=0.601	F=1.587, p=0.214
GN : GP	F=8.733, p=0.013	F=2.550, p=0.088	F=3.751, p=0.058	F=0.197, p=0.822	F=2.114, p=0.152	F=0.941, p=0.397	F=0.078, p=0.925
AMF	F=0.553, p=0.473	F=0.354, p=0.703	F=7.109, p=0.010	F=1.641, p=0.204	F=0.245, p=0.622	F=0.133, p=0.875	F=1.871, p=0.165
Actinobacteria	F=0.0005, p=0.981	F=0.872, p=0.424	F=14.048, p=0.0005	F=1.788, p=0.177	F=0.044, p=0.835	F=0.432, p=0.652	F=1.535, p=0.225
Stress	F=4.305, p=0.062	F=3.074, p=0.055	F=0.1571, p=0.694	F=1.436, p=0.248	F=0.0072, p=0.933	F=2.415, p=0.100	F=0.871, p=0.4249

^a dF, Treatment =28, dF, Month = 139, dF, Year =50. C: N = carbon to nitrogen ratio, Carbon Stock = soil total carbon stock, EC = electrical conductivity, MBC = microbial biomass carbon, MBN = microbial biomass nitrogen, MBC:MBN = ratio of microbial biomass carbon to microbial biomass nitrogen, F:B = ratio of fungi to bacteria PLFA counts, GN = Gram-negative bacteria, GP = Gram-positive bacteria, GN:GP = ratio of Gram-negative to Gram-positive bacteria PLFA counts, AMF = arbuscular mycorrhiza fungi, Stress = ratio of cy 17: 0 / 16:1 w7c PLFAs.

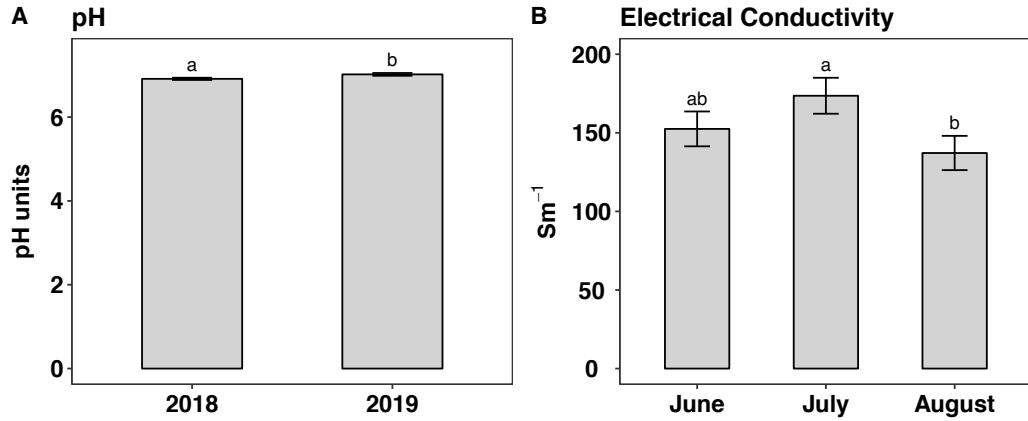


Fig. 3.5. Effects of A) year on pH, and B) month on electrical conductivity, in the tame forage pasture (S5). Means and standard error are reported. Letters over bars indicate significant differences detected in linear mixed effects models.

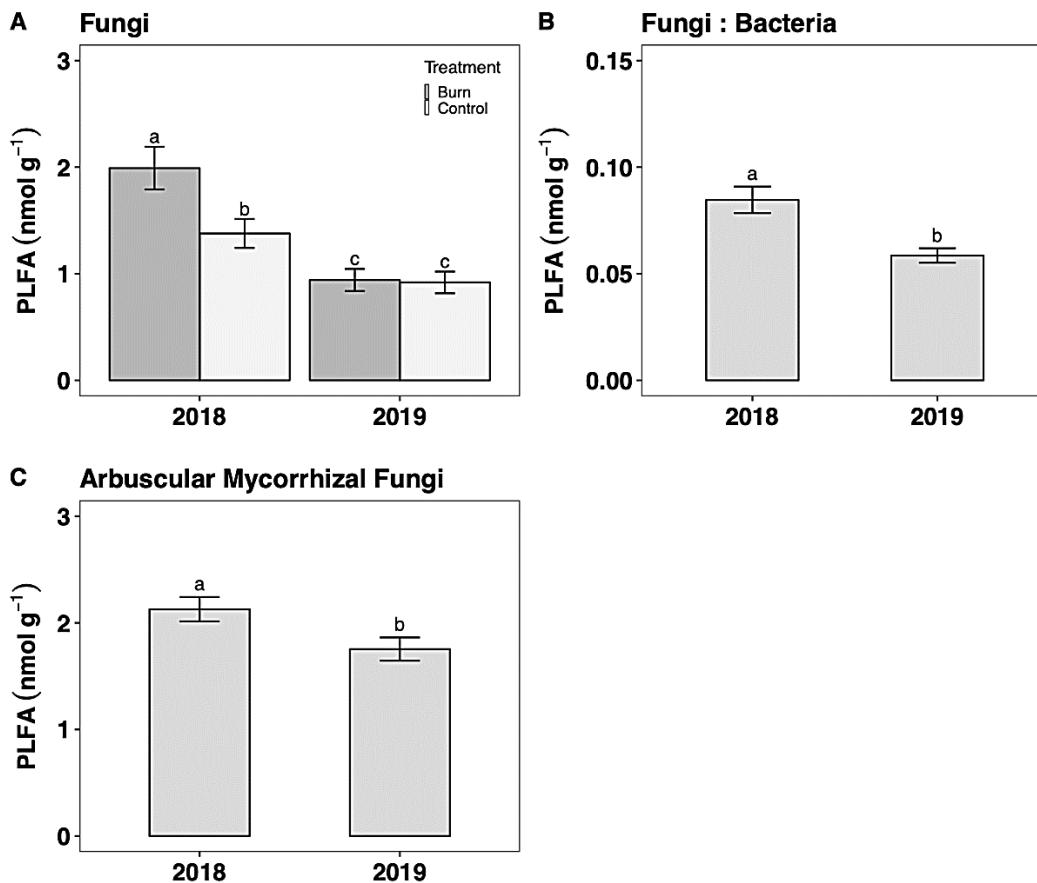


Fig. 3.6. Effects of A) treatment and year on soil fungi B) year on fungal to bacterial ratio, C) year on arbuscular mycorrhizal fungi. Means and standard error are reported. Letters over bars indicate significant differences detected in linear mixed effects models.

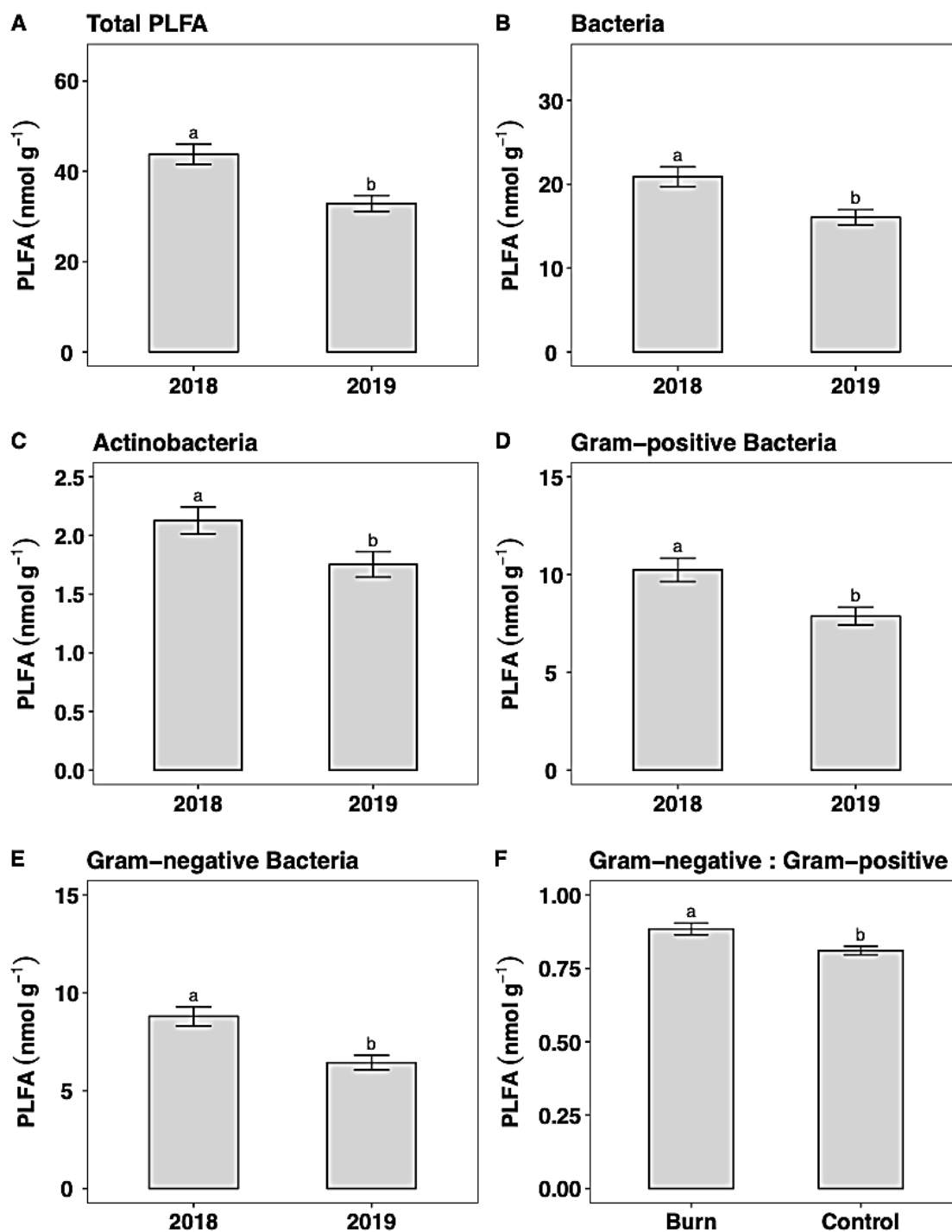


Fig 3.7. Effects of year on A) total PLFA abundance B) bacterial abundance C) actinobacteria D) Gram -positive bacteria E) Gram-negative bacteria F) the ratio of Gram-negative to Gram-positive bacteria in the tame forage pasture (S5). Means and standard error are reported. Letters over bars indicate significant differences detected in linear mixed effects models.

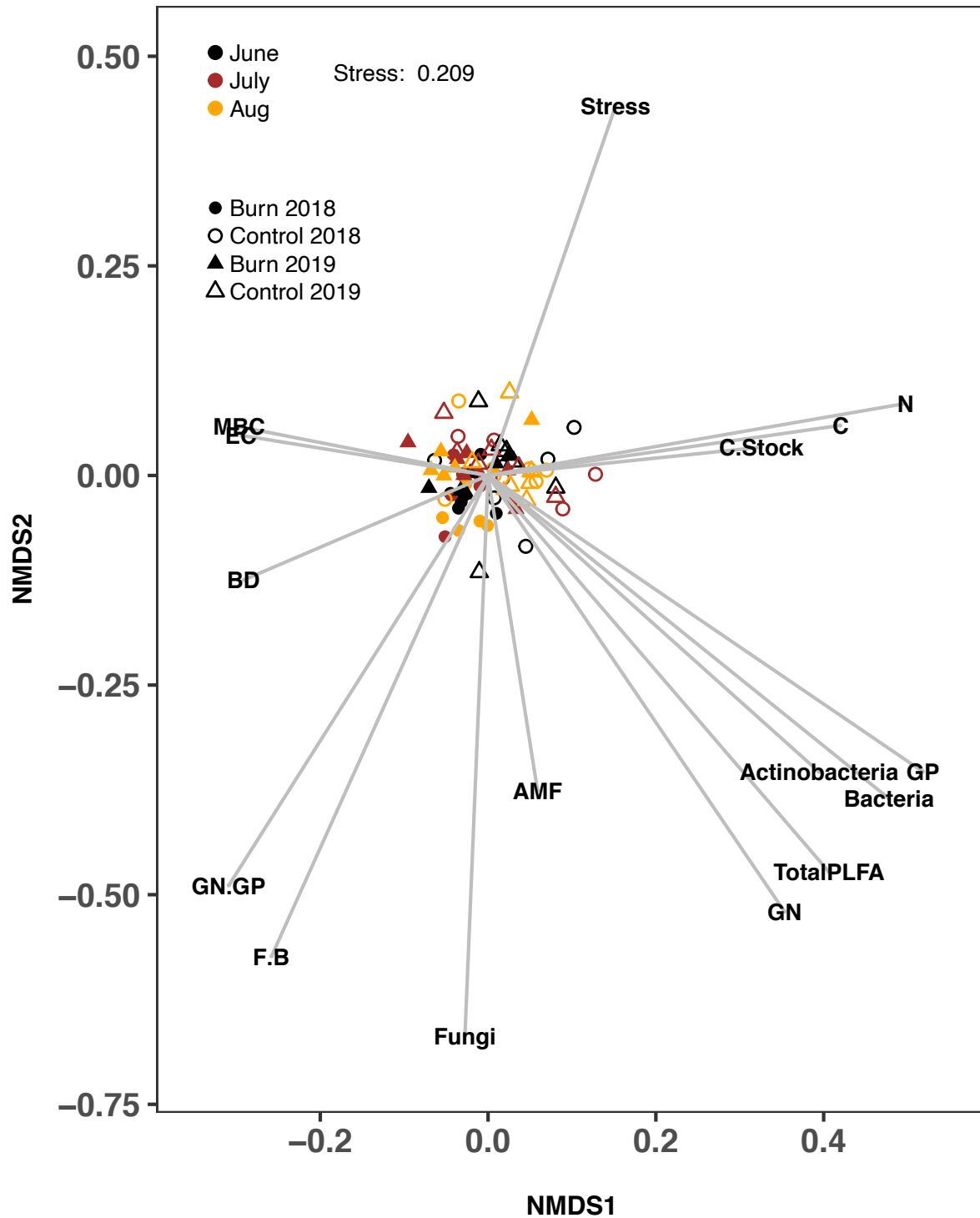


Fig. 3.8. NMDS ordination of 2018 and 2019 PLFA community composition in the tame forage pasture. Site scores of PLFA biomarkers collected from the tame forage pasture (S5) in June (black), July (brown), and August (orange), in the burn treatment block (filled circles, filled triangles) and control treatment block (open circles). Vectors represent gradients associated with the ordination, with vector length proportional to the correlation between ordination and soil variable.

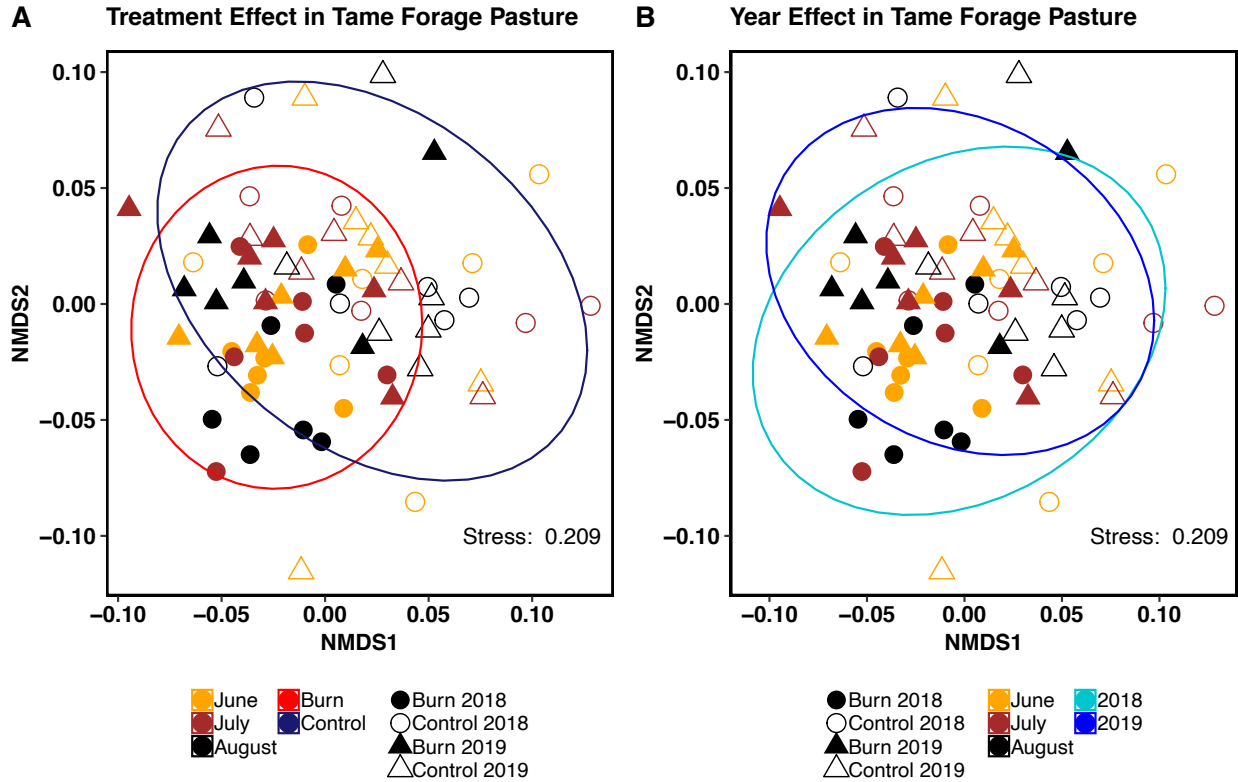


Fig. 3.9. NMDS ordinations of PLFA community composition in the tame forage pasture (S5). Site scores of PLFA biomarkers collected in June (orange), July (brown) and August (black) of 2018 (circles) and 2019 (triangles). Burn treatment is indicated by filled circles (2018) and filled triangles (2019). Ellipses represent 95% confidence intervals of microbial communities. Red ellipse in Figure A indicates burn treatment plots, black ellipse indicates control plots. Ellipse in figure B correspond to sampling years: turquoise ellipse indicates 2018, dark blue ellipse indicates 2019.

Table 3.3. PERMANOVA results based on Bray-Curtis dissimilarities using soil microbial community composition data from the tame forage pasture (S5) in 2018 and 2019.

Model Factor (s)	DF	Sum Sq	F Model	r ²	P
Treatment	1	0.025	0.082	6.187	0.001^a
Month	2	0.007	0.023	0.885	0.608
Year	1	0.009	0.030	2.247	0.026
Treatment:Month	2	0.005	0.017	0.642	0.891
Treatment:Year	1	0.003	0.010	0.789	0.630
Month:Year	2	0.007	0.022	0.844	0.662
Treatment:Month:Year	2	0.006	0.021	0.812	0.709

^abolded terms denote significance, $p \leq 0.05$.

3.5.3 Native forage pasture (N8)

Soil microbial properties

In the native forage pasture, N8, no significant treatment effects were observed on soil variables. Microbial biomass carbon and GN:GP declined from 2018 to 2019 (Table 3.4, Fig. 3.10, Fig. 3.11). Microbial stress and GN:GP significantly changed over sampling months (Table 3.4, Fig. 3.11). Stress was significantly higher in August than in June or July of both 2018 and 2019 (Fig. 3.11). The same pattern was observed for soil fungi, which was significantly affected by sampling months (Table 3.4, Fig. 3.11). GN:GP was significantly higher in June compared to July and August (Fig. 3.11).

The two-dimensional NMDS model for the native forage pasture had a final stress of 0.226 (Fig. 3.12, Fig. 3.13). Absolute abundance PLFA groups showed stronger correlation with the microbial community structure, compared to remaining soil variables (Fig. 3.12). PerMANOVA indicated that prescribed fire and sampling month significantly influenced the microbial community structure (Table 3.5). Prescribed fire homogenized microbial community composition as indicated by the clustering of burn plots in the ordinations (Fig. 3.13). The microbial community composition was more similar in June, compared to July and August (Fig. 3.13).

Soil chemical properties

Soil pH was affected by a month by year interaction, and soil total nitrogen, total carbon and carbon stock declined from 2018 to 2019 (Table 3.4, Fig. 3.10).

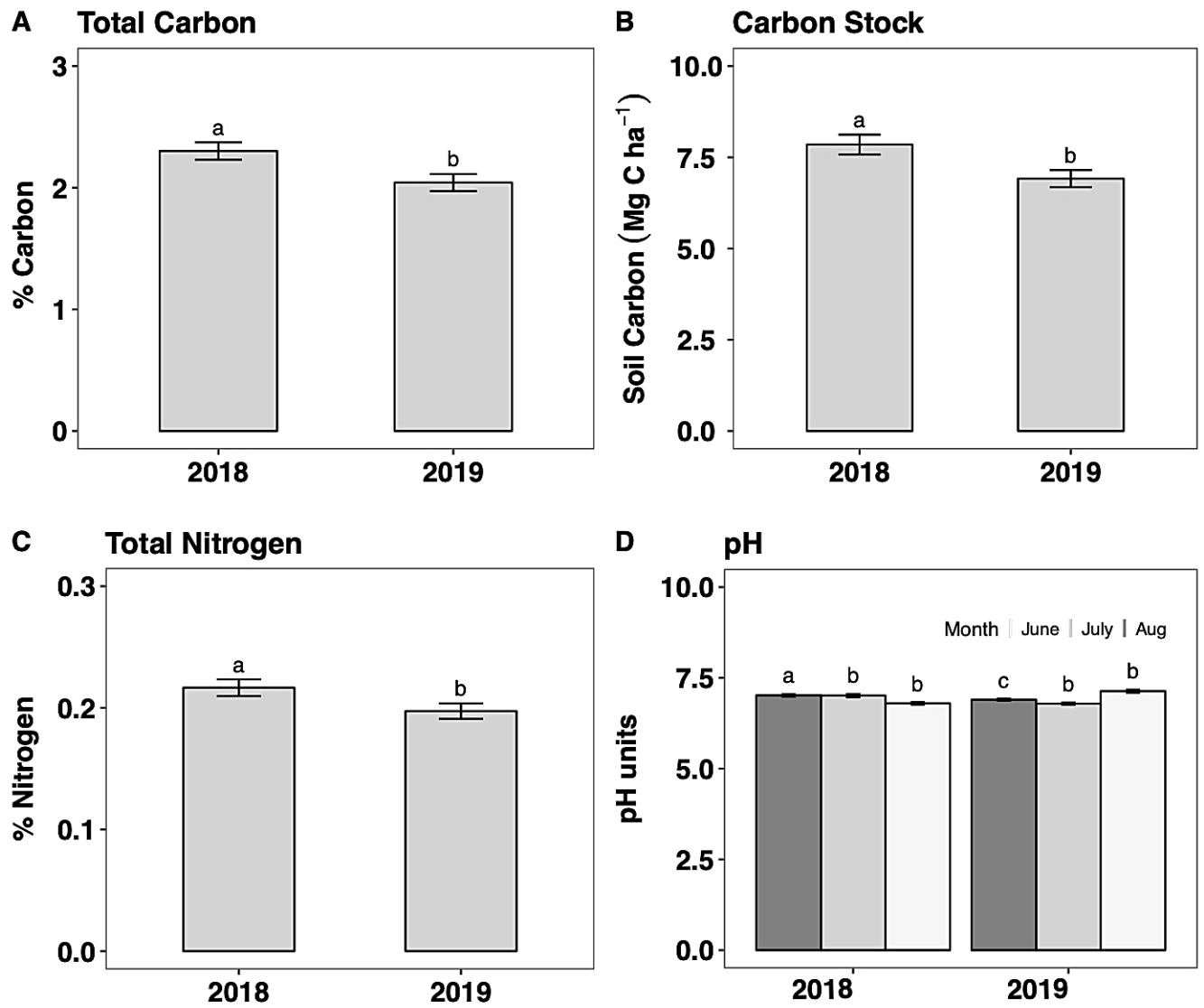
Table 3.4. Linear mixed effect model comparing burn treatment, seasonal (month) and year effects on soil biogeochemical properties from the native forage pasture (N8). Bolded terms denote significant ANOVA effect of model factor $p \leq 0.05$.

Model Factor (s)	Treatment ^a	Month	Year	Treatment:Month	Treatment:Year	Month:Year	Treatment:Month:Year
Total Carbon	F=1.490, p=0.232	F=1.348, p=0.263	F=10.242, p=0.002	F=0.611, p=0.544	F=0.003, p=0.955	F=1.687, p=0.189	F=1.150, p=0.320
Total Nitrogen	F=2.552, p=0.121	F=0.285, p=0.752	F=7.324, p=0.008	F=0.847, p=0.431	F=0.346, p=0.557	F=1.046, p=0.354	F=0.736, p=0.480
C : N	F=0.624, p=0.436	F=1.939, p=0.148	F=1.589, p=0.210	F=0.177, p=0.838	F=0.442, p=0.507	F=0.443, p=0.643	F=0.692, p=0.502
Carbon Stock	F=1.729, p=0.199	F=1.916, p=0.151	F=10.915, p=0.001	F=0.594, p=0.554	F=0.118, p=0.732	F=0.514, p=0.224	F=0.722, p=0.488
pH	F=0.560, p=0.461	F=2.20, p=0.115	F=0.010, p=0.908	F=0.910, p=0.404	F=0.350, p=0.557	F=37.48, p=<0.0001	F=0.64, p=0.527
EC	F=2.025, p=0.166	F=0.046, p=0.955	F=0.137, p=0.712	F=1.042, p=0.355	F=1.060, p=0.305	F=1.035, p=0.357	F=0.447, p=0.640
MBC	F=0.537, p=0.467	F=0.764, p=0.468	F=8.848, p=0.004	F=1.750, p=0.178	F=0.018, p=0.895	F=2.185, p=0.116	F=0.335, p=0.716
MBN	F=0.040, p=0.843	F=1.704, p=0.186	F=1.833, p=0.178	F=1.086, p=0.340	F=1.157, p=0.284	F=0.231, p=0.793	F=0.608, p=0.546
MBC : MBN	F=0.563, p=0.459	F=0.273, p=0.761	F=1.538, p=0.217	F=0.793, p=0.455	F=0.464, p=0.497	F=2.204, p=0.114	F=0.229, p=0.796
Total PLFAs	F=0.437, p=0.514	F=0.795, p=0.454	F=1.213, p=0.273	F=0.616, p=0.541	F=1.474, p=0.227	F=1.009, p=0.367	F=0.891, p=0.413

Table 3.4. continued.

Model Factor (s)	Treatment	Month	Year	Treatment:Month	Treatment:Year	Month:Year	Treatment:Month:Year
Bacteria	F=0.757, p=0.393	F=0.133, p=0.876	F=0.680, p=0.411	F=0.767, p=0.465	F=2.130, p=0.147	F=0.629, p=0.535	F=0.988, p=0.375
Fungi	F=0.888, p=0.354	F=8.970, p=0.0002	F=3.244, p=0.074	F=0.013, p=0.987	F=0.777, p=0.380	F=1.772, p=0.174	F=0.322, p=0.725
F : B	F=1.581, p=0.234	F=0.542, p=0.584	F=17.543, p=0.0001	F=0.403, p=0.669	F=2.882, p=0.095	F=1.436, p=0.248	F=0.131, p=0.877
GN	F=0.424, p=0.520	F=0.315, p=0.730	F=1.812, p=0.180	F=0.787, p=0.457	F=1.749, p=0.188	F=0.700, p=0.498	F=1.068, p=0.346
GP	F=0.537, p=0.470	F=0.562, p=0.578	F=0.368, p=0.545	F=0.862, p=0.424	F=1.632, p=0.204	F=0.464, p=0.630	F=1.133, p=0.325
GN : GP	F=0.061, p=0.806	F=10.523, p=0.0001	F=4.077, p=0.045	F=0.554, p=0.5758	F=0.239, p=0.626	F=0.488, p=0.695	F=0.364, p=0.695
AMF	F=0.007, p=0.935	F=0.323, p=0.724	F=0.309, p=0.579	F=0.716, p=0.491	F=0.229, p=0.633	F=0.223, p=0.801	F=1.494, p=0.228
Actinobacteria	F=2.002, p=0.168	F=0.065, p=0.937	F=0.691, p=0.407	F=0.773, p=0.463	F=2.89, p=0.091	F=1.261, p=0.287	F=0.358, p=0.700
Stress	F=0.620, p=0.0.438	F=4.464, p=0.0.013	F=0.010, p=0.922	F=0.734, p=0.482	F=1.375, p=0.243	F=0.445, p=0.642	F=2.765, p=0.067

^a dF, Treatment=28, dF, Month= 139, dF, Year=50. C:N = carbon to nitrogen ratio, EC = electrical conductivity, MBC = microbial biomass carbon, MBN = microbial biomass nitrogen, MBC:MBN = ratio of microbial biomass carbon to microbial biomass nitrogen, F:B = ratio of fungi to bacteria PLFA counts, GN = Gram-negative bacteria, GP = Gram-positive bacteria, GN:GP = ratio of Gram-negative to Gram-positive bacteria PLFA counts, AMF = arbuscular mycorrhiza fungi, Stress = ratio of cy 17: 0 / 16:1 w7c PLFAs.



c

Figure 3.10. Effects of year on A) Total carbon, B) Carbon Stock, C) Total nitrogen, and effect of month and year on D) pH in the native forage pasture (N8). Means and standard error are reported. Letters over bars indicate significant differences detected in linear mixed effects models.

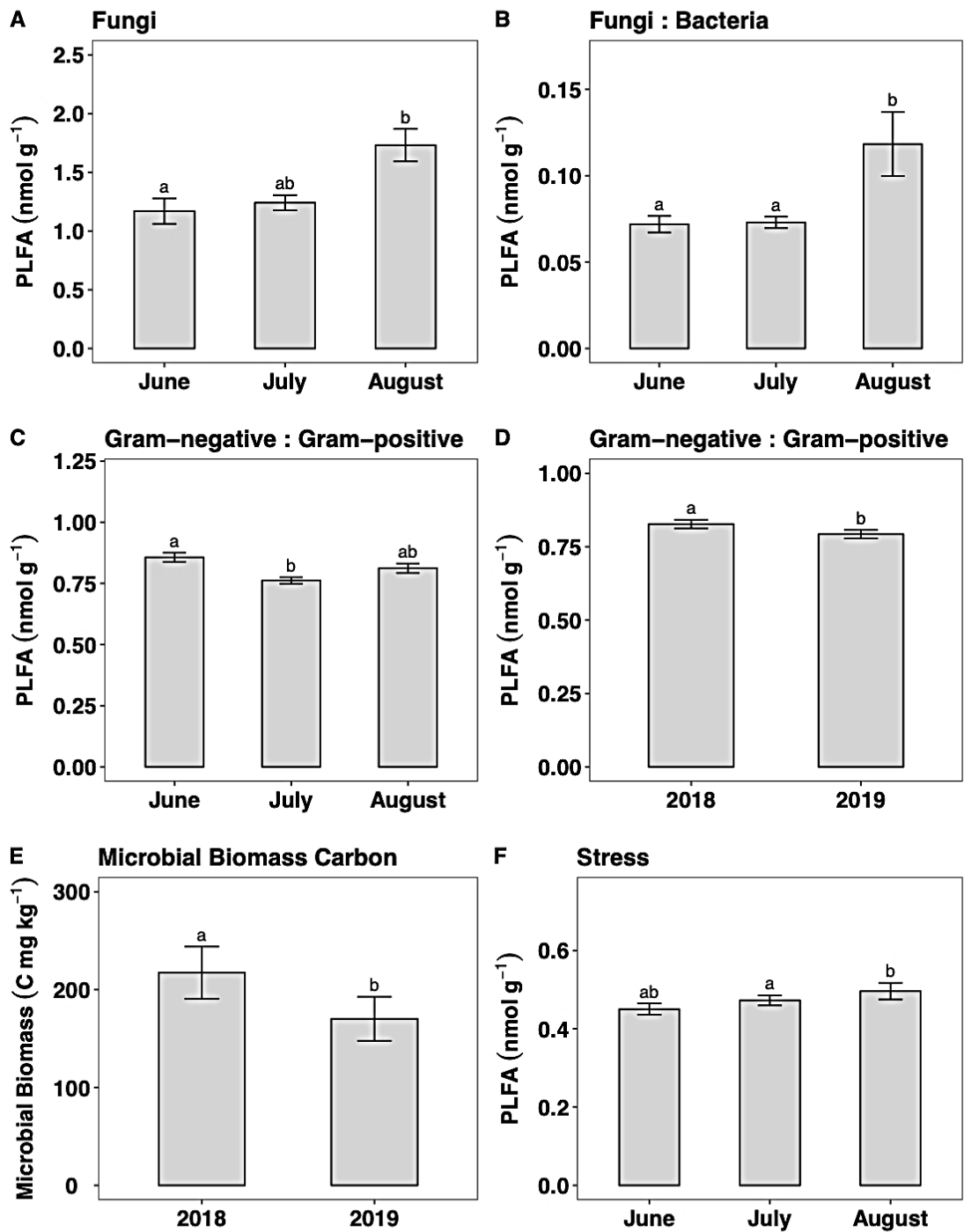


Fig. 3.11. Effects of month on A) Fungi, B) Fungal to bacterial ratio, C) Ratio of Gram negative bacteria to Gram Positive bacteria, F) Stress, and effect of year on D) Ratio of Gram negative bacteria to Gram Positive bacteria and E) Microbial biomass carbon in the native forage pasture (N8). Means and standard error are reported. Letters over bars indicate significant differences detected in linear mixed effects models.

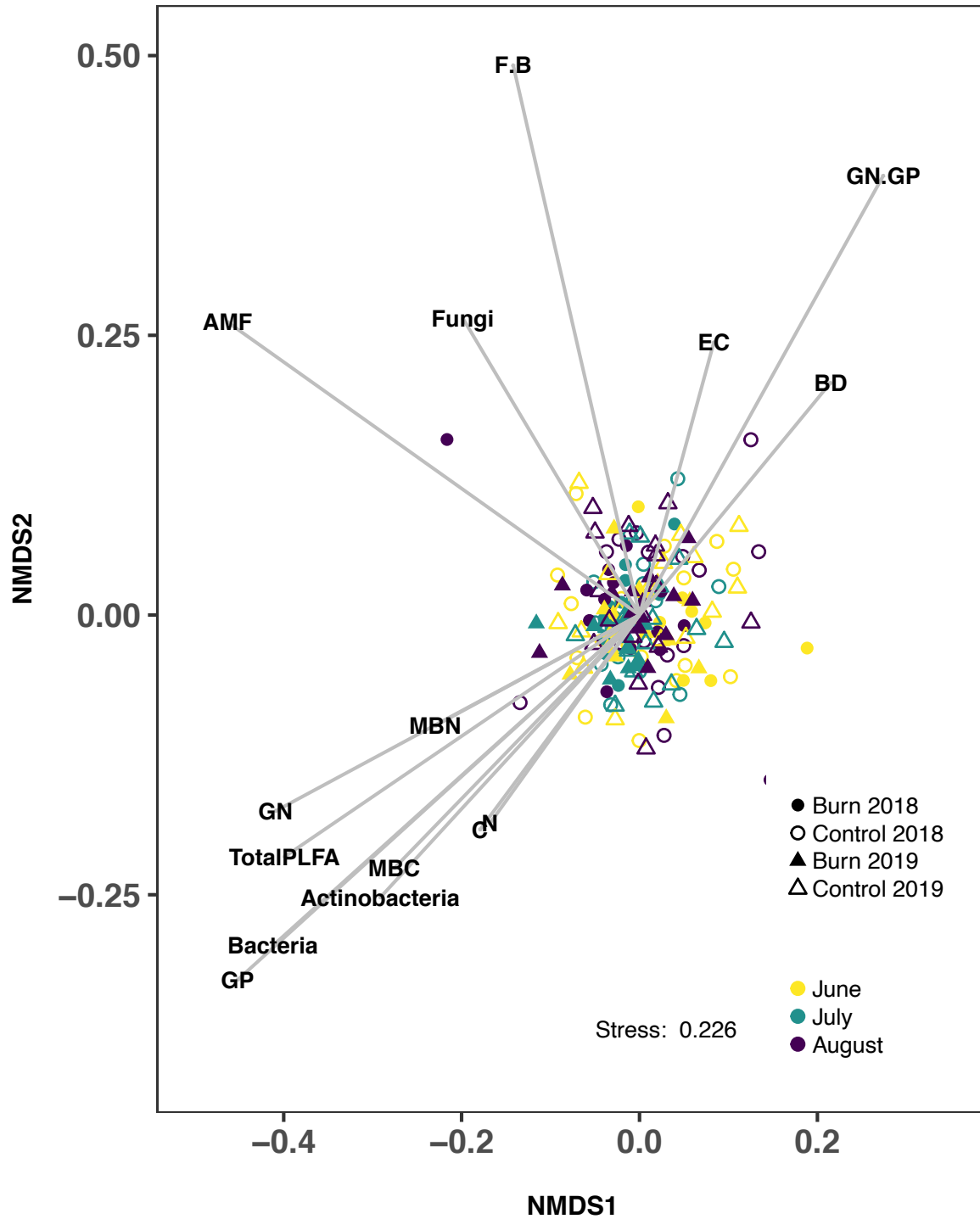


Fig 3.12 NMDS ordination of 2018 and 2019 PLFA community composition. Site scores of PLFA biomarkers collected from the native forage pasture (N8) in June (yellow), July (green), and August (brown), in the burn treatment block (filled circles) and control treatment block (open circles). Circles signify year 2018, triangles signify year 2019. Vectors represent gradients associated with the ordination. Vector length is proportional to the correlation between ordination and soil variable.

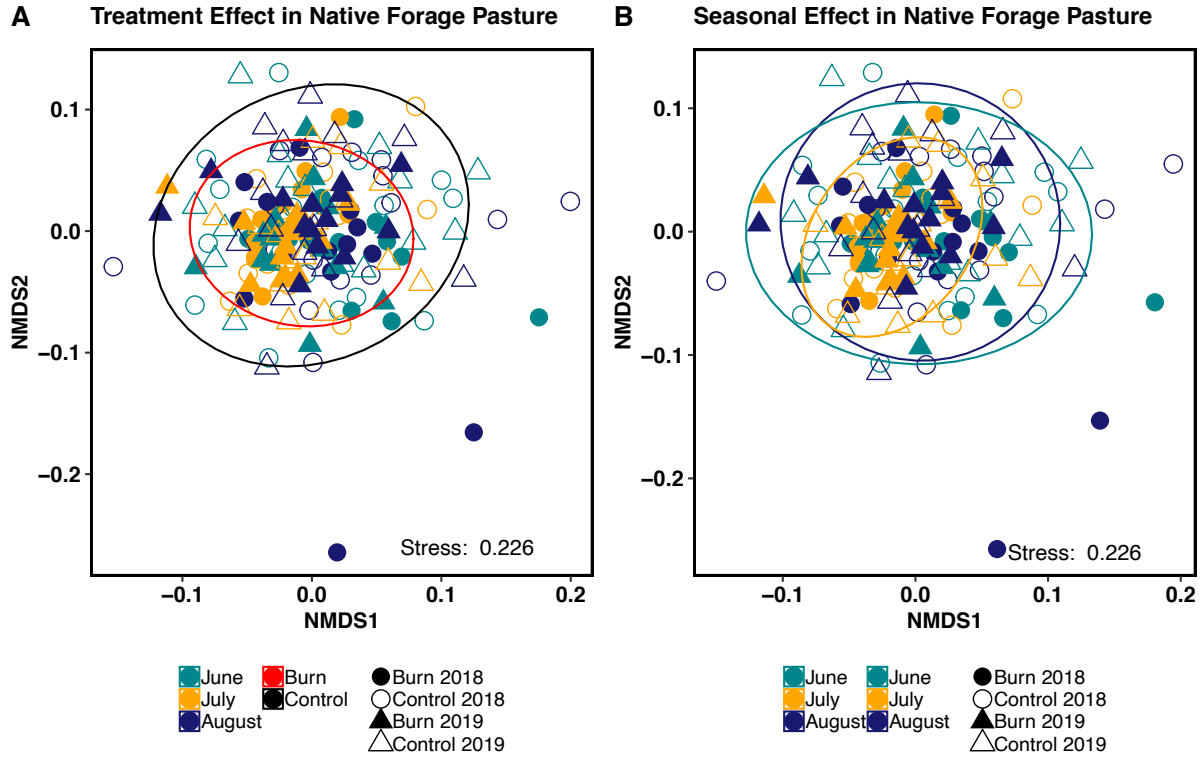


Fig. 3.13. NMDS ordinations of PLFA community composition in the native forage pasture (N8). Site scores of PLFA biomarkers collected in June (turquoise), July (yellow) and August (dark blue) of 2018 (circles) and 2019 (triangles). Burn treatment is indicated by filled circles (2018) and filled triangles (2019). Ellipses represent 95% confidence intervals of microbial communities. Red ellipse (Figure A) indicates burn treatment plots, black ellipse indicates control plots. Ellipses in Figure B correspond to sampling months: turquoise ellipse indicates June, yellow indicates July, dark blue indicates August.

Table 3.5. PERMANOVA results based on Bray-Curtis dissimilarities using soil microbial community composition data from the native forage pasture (N8) in 2018 and 2019.

Model Factor (s)	DF	Sum Sq	F Model	r ²	P
Treatment	1	0.035	0.020	3.730	0.002^a
Month	2	0.056	0.032	2.954	0.001
Year	1	0.010	0.006	1.097	0.365
Treatment:Month	2	0.013	0.008	0.690	0.806
Treatment:Year	1	0.003	0.001	0.268	0.987
Month:Year	2	0.024	0.014	1.252	0.218
Treatment:Month:Year	2	0.012	0.007	0.629	0.881

^abolded terms denote significance, $p \leq 0.05$.

3.6 Discussion

Understanding the responses of soil biogeochemistry to prescribed fire is important for conservationists and land managers using prescribed fire for mixed grass prairie management. Successful management is essential to ensure that the ecosystem services provided by the remaining grasslands are sustainable and have high productivity. Questions about fire impacts on soils and soil carbon storage are frequently raised when prescribed fires are proposed. Grasslands are a highly endangered ecosystem (Carbutt, 2020) with relatively little federal and international protection (Carbutt, Henwood & Gilfedder, 2017); reducing barriers to the use of fire in grasslands is important to maintain and enhance grassland ecosystem integrity.

The effects of prescribed grassland fire on plant diversity (White, 1965; Romo, 2003), species regeneration (Komarek, 1962), wildlife habitat (Daubenmire, 1968, Wright, 1974), forage quality (Vogl, 1965), range management (Humphrey, 1959, Duvall, 1962) and sociologic factors (Jackson, 1965, Glascock, 1966) are well understood. However, the effects of grassland fire on soils have been rarely studied. To understand whether fire prescriptions are compatible with soil conservation goals, my research aimed to evaluate how prescribed fire could alter soil chemical and microbial properties in a mixed grassland. In the discussion below I will consider 1) fire severity and effects on grassland soil, and 2) temporal heterogeneity in these soils. Overall, I show that low severity prescribed fire does not cause broad or consistent changes to soil properties within the two growing seasons following fire, and the changes that did occur were small relative to climate driven differences between growing seasons (2018, 2019).

3.6.2 Low severity prescribed fire had minimal impacts on soil

Fire is considered a soil forming factor (Certini, 2014), and an important driver of vegetation composition and nutrient supply, especially in semi-arid regions such as the North American Great Plains, where high temperatures and low soil moisture conditions limit nutrient cycling and plant productivity (Fultz et al., 2016). The impacts of fire on soil properties depend on a variety of factors, including the type of soil and vegetation affected, burn temperature and intensity, the type of ash produced, and the severity of burn effects (Brook & Wittenberg, 2016; Unzué-Belmonte et al., 2016; Pereira et al., 2017). Burn severity, which integrates soil temperature change and burning duration (Drooger, 2009), largely depends on fuel spatial distribution, consumption rate,

combustion residence time, and soil moisture at the time of burning (Hamman, Burke, & Stromberger, 2007).

Prescribed fires in this study were performed before bud break in spring and resulted in patchy areas of complete, partial, and unburned biomass, depending on fuel continuity. Burns conducted in early spring compared to fall typically have moister fuels (James & M'Closkey, 2003; Knapp, Estes & Skinner, 2009) and as a result, often consume less of the available fuel, are patchier and less severe (Perrakis & Agee 2006; Monsanto & Agee 2008; Knapp et al., 2009). In the North American Great Plains, weather conditions are often most consistently amenable to safe burning shortly after snow melt when soil moisture is typically at its peak. Consequently, soils are largely protected from excess heating and increased burn severity, even under high fuel loading conditions (Iverson & Hutchinson, 2002). Substantial energy is required (temperatures above 100°C) to drive off moisture before the temperature of soil is significantly affected (Kanpp et al., 2009).

The pastures selected for burning in this study have been seasonally grazed throughout recent history, preventing high fuel loading conditions and causing spatial heterogeneity of fuels. Typical of low severity burns (Neary & Leonard, 2020), approximately 65% of the herbaceous fuels were consumed and much of the remaining fuel was charred. Herbaceous stubble was visible above the surface soil, and larger plants such as sage brush were still discernable, with rootstocks typically not deeply burned. Changes in soil colour were not observed during or after burning and charring of the mineral soil was not visually detected. Ignited materials were charred to a black colour and remained as a thin blanket of ash on the herbaceous stubble at the soil surface. Cow pies were found to smolder for hours post fire, likely instigating small patches of deeper burns. Deep burns result in the complete consumption of fuel and may cause charring of the mineral soil within the first centimeter of the surface (Neary & Leonard, 2020). Woody plant crowns and ant hills were also associated with increased burn severity.

In both pastures, fire behavior observations suggested short combustion residence times as the fine textured, grassy litter allowed rapid combustion of fuels within a narrow fire front (Heyward, 1938; Anderson, 1963; McArthur, 1966). Manual ignition was required in areas where fuels were not continuous as flame lengths and burning duration were not sufficient to ignite neighboring resources. The duration of burning is important in regard to the effects of fire on soil, as the rate at which energy can be transmitted through the soil is limited by the soil's thermal properties (Campbell, Jungbauer Jr, Bristow & Hungerford, 1995). The Chernozemic soils at OMB

have a high bulk density and mineral content making them poor conductors of heat, likely requiring long combustion residence times before soil temperatures are affected.

The combination of on-site fire behavior observations and burn severity indicators suggested that the prescribed fires at OMB were low in severity; therefore, minimal effects of fire on soil properties could be expected. Low severity fires are common in grasslands, as only about 5% of the heat energy released by fire is partitioned to the soil (Grier, 1975). Direct effects of fire on soil properties are dependent upon heat transfer through the soil; temperatures must exceed 47°C before plant roots, seed germination, soil fauna and microorganisms are affected (Neary & Leonard, 2020). Water repellency occurs at temperatures greater than 175°C (Debano, 1981), and slightly higher temperatures (~200°C) cause the loss of soil nutrients (Zabowski, Thies, Hatten & Ogden, 2007). Heat transfer through grassland soils has been measured by Morgan (1999) and Drooger (2009), who reported maximum soil temperatures (<10 mm) of 10 and 27°C, respectively, even when surface temperatures exceeded 350 and 104°C. Studies on prescribed fire in forest soils have also observed similar results, with a maximum absolute difference of 2.4°C between burn and control soils, while temperatures 10 cm above the forest floor were > 200 °C during burning (Iverson & Hutchinson, 2002).

Soils collected at OMB 2, 3, 4 and 14, 15,16 months post fire also showed minimal effects of fire on biogeochemical properties, supporting on-site observations that the prescribed fires were low severity, causing little direct disturbance to the soil. Treatment effects reporting an increase in soil fungi and GN:GP within the tame forage pasture were observed in samples collected 2-4 months following fire for fungi, and during all sampling months for GN:GP. The native forage pasture did not experience any significant effects of fire, possibly due to greater fire tolerance of the native vegetation species and more diverse vegetation cover.

Consistent with Certini (2005) and Hopkins (2021), our results suggest that fire related shifts in fungal community abundance reflect a post-fire nutrient flush. Most fungal species prefer substrates that are highly recalcitrant and low in nitrogen (Six, Frey, Thiet & Batten, 2006), typical of charred plant residues resulting from fire (Knicker, Hilscher, González-Vila & Almendros, 2008). Ash that is generated by temperatures less than 450°C is normally black in colour and composed mainly of pyrogenic organic C (Bodi et al., 2014). Combustion temperatures beyond 450°C, however, result in white coloured mineral ash due to the volatilization of carbon (Bodi et al. 2014) As fire application at OMB was low in severity, resulting ash likely favored fungal

proliferation, noted by the thin black charcoal layer that replaced combustible fuels on the surface soil, believed to be high in pyrogenic C.

Fungi that thrive in post fire environments are commonly referred to as pyrophilous fungi (Seaver, 1909). They are believed to be part of a rapid successional sequence, fruiting only in post fire environments (Bruns, Chung, Carver & Glassman, 2020). Although no obvious fruiting structures were observed in vegetation plots throughout my study, many post fire fungi are microscopic and thus unlikely to be observed in *in situ* visual surveys (Claridge, Trappe & Hansen, 2009.) The fungal genus *Pyronema* has been found to be among the first to emerge from burned soil, doing so within weeks to months after burning in many post fire environments (Adamczyk, Kruk, Penczak & Minter, 2012; Bruns et al., 2020; Hughes et al., 2020; Fischer et al., 2021). Bruns et al., 2020 showed that *Pyronema* fungal reads in a recent ITS amplicon community analysis made up less than 1% of reads prior to fire yet achieved a post fire relative abundance of 60%. Further, in *Pyronema* species, Fischer et al. (2021) used RNA sequencing to observe the comprehensive induction of genes that involve the mineralization and metabolism of aromatic compounds commonly found in pyrogenic organic matter (Fischer et al., 2021).

Areas of bare ground, exposed by the removal of litter during burning, benefit from, and are primarily colonized by soil fungi as their filaments and hyphal growth form allow fungi to more readily access spatially separated resources (Helgason, Walley & Germida, 2009) and translocate nutrients from microsites where resources are abundant to sites where they are limiting (Strickland & Rousk, 2010; Furuno et al., 2012). The increase in post-fire soil fungi in the tame forage pasture also coincided with a significant increase in bare ground at this site (Hilger, 2020). The higher percentage of bare ground cover was expected in the tame forage pasture after burning, as the soil had previously been cultivated and the protective biological soil crust, comprised of moss and lichen, was then lost (Weber, Bowker, Zhang & Belnap, 2016; Hilger, 2020). Biological soil crusts in the native pasture remained intact, functioning to stabilize the soil surface and retain soil nutrients and moisture. Consequently, soil fungi in the tame forage pasture may be serving, in part, to stabilize and advance the succession on bare soils which includes the development of biological soil crusts.

An increase in the ratio of Gram-negative to Gram-positive bacteria was observed in the burn treatment plot in the tame forage pasture for two years post fire. Gram-negative bacteria prefer more labile sources of carbon compared to Gram-positive bacteria (Fannin et al., 2019). The higher

ratio of GN:GP may indicate increased plant C exudation, a labile carbonaceous substrate (Zhao, Long, et al., 2019) as a product of rapid vegetation recovery post fire. This effect may be accentuated by seedling establishment in areas where litter residues previously impeded vegetation growth prior to burning (Tyler & D'Antonio, 1995). Gram negative bacteria have also been shown to be present much earlier in microbial community succession than Gram-positive bacteria (Konstantin & Lubov, 2014). Gram-negative prevalence is encouraged by the availability of chitin (Konstantin & Lubov, 2014), a major structural component of fungal cell walls. Therefore, the response of Gram-negative bacteria to fire may have been due to the chitinolytic activity of these bacteria and the extensive fungal colonization of soils affected by fire. In the course of the succession, when the fungal mycelium begins to die off, Gram-positive bacteria replace Gram-negative, thereby decreasing GN:GP.

Resilience of the native forage pasture soils towards the effects of fire, in all chemical and microbial properties, persisted for the duration of the 2018 and 2019 growing seasons. The slightly greater resistance of the native pasture to disturbance may be attributed to a higher diversity of plant species and less bare ground exposure compared to the tame forage pasture (Hilger, 2020). Biodiverse systems have a greater potential to minimize dynamic fluctuations and defy changes after disturbance (McCann, 2000) as they support a wider range of functional traits and ecological processes. In addition, a large number of experimental studies show that biological diversity is crucial to maintaining soil ecological processes (Loreau et al., 2001; Lyons, Brigham, Traut & Schwartz, 2005; Cadotte, Carscadden & Mirotchnick, 2011). Diversity in the composition and abundance of plant species within the native forage pasture may have produced a greater diversity in burn patterns, burn severity, and the type of ash nutrient deposited as the result of fire. The combination of these factors may have created greater heterogeneity of effects on soil such that the magnitude of fire perturbation was not greater for any one soil parameter; disturbance effects were absorbed over a greater variety of soil processes leading to increased probability of resilience.

Although soil chemical properties and the absolute abundance of microbial groups showed high resilience toward the effects of prescribed fire at OMB, the microbial community composition was significantly affected by fire in both the tame and native forage pastures. In both pastures, fire treatment appeared to reduce the heterogeneity of the microbial community composition, as indicated by greater homogenization of burned samples compared to unburned samples within the NMDS ordination space (Fig. 3.8, Fig. 3.12). In the tame forage pasture, a small shift in the

microbial community structure of burned sites away from the microbial community structure of unburned sites was apparent. However, this effect was relatively minor, with the majority of burned and unburned sites overlapping in ordination space. In the native forage pasture, microbial community structures between the burned and control plots were grouped within the same ordination space (Fig. 3.12); however, microbial communities within the burned sites were more similar. The microbial community structure of both the tame and native forage pastures were strongly correlated with PLFA groups and less correlated with soil chemical variables, as indicated by vector length (Fig. 3.9, Fig. 3.13). Overall, burn and control plots showed wide compositional diversity; however, burning reduced microbial community heterogeneity and shifted the structural arrangement of microbial communities within treatment plots.

Spatial variation in the structure and composition of soil microbial communities is common in grasslands (Smith, Facelli & Cavagnaro, 2018), often attributed to complex soil-plant interactions and local abiotic conditions (Bezemer et al., 2006; Rúa et al., 2010). Small scale niche differentiation and species selection also supports a high number of very unique microbial species in grasslands (Yang et al., 2019); however, species level diversity cannot be determined through PLFA analyses as biomarkers are only associated with family level taxa and higher (Kirk et al., 2004). Plant data recorded from treatment plots at OMB reported significant homogenization of species as the result of fire (Hilger, 2020). Burning also had a homogenizing effect on soil microbial community structure and is likely associated with the fire-induced changes in plant communities, and concurrent plant soil interactions. Various studies have also reported an influence of fire on microbial community structure (D'Ascoli, Rutigliano, De Pascale, Gentile & De Santo, 2005; Mubyana-John, Wutor, Ringrose & Yeboah, 2007; Zhang et al., 2013)

3.6.3 Temporal variability in grassland soils

Temporal changes in microbial communities were much stronger than burn effects. Compared to the native forage pasture, the tame forage pasture showed more temporal fluctuations in microbial parameters; total PLFA biomass, bacteria, fungi, F: B, actinobacteria, arbuscular mycorrhizal fungi, Gram-positive bacteria, Gram-negative bacteria and the ratio of GN:GP were all higher in 2018 compared to 2019. The native forage pasture had an increase in microbial biomass carbon in 2018 compared to 2019, an increase in the ratio of GN:GP in June of 2018, as well as higher fungi, the ratio of fungi to bacteria and microbial stress in August of both 2018 and

2019. The microbial community composition within both the tame and native forage pastures were also influenced temporally. The tame forage pasture had significant changes between years, while the native forage pasture had significant seasonal changes. Temporal changes in microbial parameters, within both pastures, are likely to have been stimulated by seasonal plant community development and climatic factors.

Differences in plant species composition between the native and tame forage pastures may have contributed to differences in temporal soil microbial attributes. The tame forage pasture is dominated by crested wheatgrass (*Agropyron cristatum*), a C₄ species, while the native pasture is dominated by C₃ grasses and hosts a greater diversity of native forbs. The structure and function of the soil microbial communities is strongly influenced by plants (Bardgett 2005; Horner-Devine et al. 2004). Within rhizospheres, differences in root secretions between photosystems have been observed to cause variation in bacterial diversity (Berg & Steinberger, 2010; Epron et al., 2011; Sivaram, 2020). Similarly, plant species richness and evenness can structure the diversity of soil microbial communities (Bartelt-Ryser, Joshi, Schmid, Brandl & Balsler, 2005; Loranger-Merciris, Barthes, Gastine & Leadley, 2006; Lamb, Kennedy & Siciliano, 2011; Sun, Wang, Shen, He & Ge, 2019). A plant community with greater species evenness, characterized by co-dominant species rather than a dominant-subordinate relationship, allows for more efficient resource use within soil communities, through niche complementarity (Lamb et al., 2011). The native forage pasture, with greater species richness and evenness is therefore less likely to experience significant temporal fluctuations in soil microbial communities, as patterns of root exudation are likely more even throughout growing seasons. The presence of a dominant plant species may control patterns of nutrient availability as well as soil microbial activity (Vinton & Burke, 1995), thus temporal differences in microbial communities as observed in the tame forage pasture can be expected. Crested wheat grass, the dominant plant species in the tame forage pasture has fast growth early in the season (Vaness & Williams, 2007), compared to later developing native plants. As precipitation was very low in the early 2018 growing season, the higher relative abundance observed in PLFA biomarkers of the tame forage pasture may also reflect an attraction of microorganisms to carbon in the form of mucilage and border cells, released as lubrication for crested wheatgrass root growth through dry soils early in the growing season (Henry, Doucette, Norton, & Bugbee, 2007).

Grazing also occurs earlier in the tame forage pasture, coinciding with the early growth patterns of crested wheatgrass. Grazing is commonly reported to increase root exudation, belowground C allocation and influence the microbial composition within soils (Wilson, Strickland, Hutchings, Bianchi, & Flory, 2018). Early grazing (in June and July), under low biomass conditions (Hilger, 2020) may have increased substrate availability for proliferating microbial populations (Guitian & Bardgett, 2000, Hamilton & Frank, 2021). In addition, grazer excrement has a facilitative effect on soil processes, including the growth of microbial communities (Hamilton & Frank, 2021). Under low biomass conditions, grazing effects can be more severe as standing biomass is removed more completely. High grazing intensities have been shown to increase certain fungal and bacterial species (Esch, Hernández, Pasari, Kantor & Selmants, 2013; Wang, Pei, Cao, Guo & Du, 2022), as root exudation from grazed plants can surpass ungrazed levels during vegetation recovery (Sun et al., 2017). Cattle grazing of the burned plot was also higher than in the control plot in the tame forage pasture (Hilger, 2020), possibly contributing to the abundance of GN bacteria, which prefer labile C sources such as root exudates. Accordingly, differences in grazing intensities between the tame and native forage pastures may have also contributed to temporal differences in soil microbial attributes within each pasture.

The microbial community of the native forage pasture showed greater temporal stability throughout the duration of the study; very little variation between months or years was observed in the majority of soil microbial parameters, in terms of their absolute abundance. Compositionally, however, the microbial community was significantly different between sampling months although changes in ordination space were extremely minor. Native prairies, having never been tilled, typically are observed to have a highly stable microbial system that is resilient when challenged with various disturbances, such as herbicide treatment (Udawatta, 2010), foreign plant invasion (Shivega & Aldrich-Wolfe, 2017), moisture perturbations (Roy Chowdhury et al., 2019), as well as prescribed burns (Veum, Lorenz, & Kremer, 2018).

With a more complex vegetation-soil system, and later timing of growth, the native forage pasture soil would also likely respond differently to precipitation events than the tame forage pasture. In 2018, precipitation was only 58% of normal annual precipitation; however, some of the largest rainfall events during the growing season occurred just prior to, and on the days of, sampling in June and July of 2018. Rainfall-induced pulses of resource availability (Carol Adair & Burke, 2010), stemming from the reduction of water limitation for plant and microbial

processes, has been observed in various studies (Austin et al., 2004, Huxman et al, 2004, Ford, Cookson, Adams & Grierson, 2007). Even small events (3 mm) have been shown to stimulate microbial activity (Schwinning & Sala, 2004) and may have contributed to the significantly higher absolute abundance of many PLFA biomarkers observed in the tame forage pasture in 2018 compared to 2019.

In the mixed grass prairies, August can represent a shift in plant-soil dynamics, as many plants begin to senesce, especially when moisture is limiting. As senescence occurs, plant litter inputs increase, providing a preferred substrate to soil fungal communities, the primary decomposers of surface litter (Vivelo & Bhatnagar, 2019; Francioli et al., 2021). High or increasing F:B ratios are also frequently associated with greater soil C storage (Malik et al., 2016; Bailey et al., 2002). Fungi and their ratio to bacteria were more prevalent during August within the native forage pasture, along with a higher marker of microbial stress. Higher stress ratios are commonly reported under high temperature conditions (Yokobe, Hyodo, & Tokuchi, 2018), consistent with seasonal temperature peaks of 2018 and 2019 at the OMB grasslands.

Annual variation in soil chemical properties are commonly reported (Gutknecht et al., 2010; Wieder et al., 2018), with temperature and precipitation being the main factors affecting soil chemistry (Knoepp, See, Vose, Miniati, & Clark, 2018). Nutrient concentrations often have temporally inconsistent variation patterns, which has been observed in other grassland soils (Rychnovska, 1993; Giese, Gao, Lin & Brueck, 2001; Leimer et al., 2016). In 2017 (the year prior to the start of this study) and in 2018, the study area experienced higher than normal temperatures and received only half of the average yearly precipitation. Soil organic carbon decreases with increasing temperatures as a result of accelerated mineralization (Schimel, Parton, Kittel, Ojima & Cole, 1990; Jackson, 1980; Zhao, Ge, et al., 2019). Soil organic carbon storage is regulated by the balance of C inputs from plant production and outputs from mineralization. Low precipitation most often results in low plant productivity, the key input for SOC accumulation. Coupled with grazing and unfavorable climate conditions, 2019 likely represents the combined stress of concurrent annual poor SOC accumulation conditions, as soil carbon was observed to significantly decrease in the native forage pasture from 2018 to 2019. Decreasing SOC under higher temperature conditions has previously been observed in the grasslands of both North America (Burke et al., 1989) and South America (Alvarez & Lavado, 1998; Paruelo et al. 2010).

Soil nitrogen is also highly influenced by climate, following the same trends as soil carbon regarding temperature and precipitation (Risch et al., 2019). Standford, Carter & Smith (1972) demonstrated that net nitrogen mineralization follows first order kinetics, with the rate of N mineralization doubling for each 10°C increase in temperature. Rustad et al. (2001) reported that temperature increases of 0.3-6°C significantly accelerates the net N mineralization rate by 30-64%. The dry conditions in 2017 and 2018 likely stimulated the mineralization of soil nitrogen to support plant productivity. While nitrogen is returned to the soil through the decomposition of plant residues, decomposition is strongly reduced under drought conditions (Sanaullah, Rumpel, Charrier & Chabbi, 2012) and may have contributed to the reduced soil N content observed in 2019 from 2018 levels. Additionally, populations of denitrifier bacteria in grasslands have shown increased activity under drought and warming conditions (Cantarel et al., 2012; Keil et al., 2015), which increases N₂ losses to the environment, decreasing soil N content.

While climatic parameters were consistent between pastures at OMB, soil nutrients were not consistently affected, with the native forage pasture reporting the only statistically significant decreases in soil carbon and nitrogen in 2019 from 2018 levels. Although this result was unexpected, it may be explained by the differences in vegetation composition between pastures. The native forage pasture hosts higher total plant biomass and thus requires increased nutrient access to support productivity. During temperature peaks in August, the microbial community of the native forage pasture shifted towards a higher prevalence of drought tolerant species (i.e., soil fungi) (Gehring, Stultz, Flores-Rentería, Whipple & Whitham, 2017), and bacterial membranes altered their composition to protect against stress as observed through the higher ratio of cy 17: 0 to 16:1 w7c (Table 3.4). In this case, microbial stress was likely induced by heat due to seasonally warm, and dry conditions. Under environmental stress, microbial respiration has been shown to increase (Li et al., 2020; Qui et al., 2018). Evidence of increased microbial activity was also observed through significantly higher microbial biomass C in 2018 compared to 2019 in the native forage pasture. Higher microbial biomass carbon may have been the result of increased nutrient capture during SOM mining. Nutrient mining is the process of soil organic matter decomposition when microorganisms experience nutrient limitation (Mori, Lu, Aoyagi & Mo, 2018). Nutrient mining can significantly increase soil respiration (Ghimire, Ghimire, VanLeeuwen & Mesbah, 2017; Zhu et al., 2019), thereby decreasing soil nutrient levels.

Changes within the microbial community under drought conditions may not solely reflect nutrient mining. When water is scarce, plants are observed to invest more biomass toward roots in comparison to shoots (Bloom, Chapin III, & Mooney, 1985; Poorter & Sack, 2012). Even under drought conditions, roots release appreciable quantities of organic materials which are utilized by soil microbial communities for biosynthesis and energy supply (Van Veen, Merckx & Van de Geijn, 1989). Thus, the observed increase in 2018 microbial biomass carbon may reflect increased root growth and rhizodeposition in response to low precipitation. This result was more likely in the native forage pasture due to the vegetation diversity at the site. Under drought conditions MBC has been observed to increase, compared to optimum conditions, by 20 and 36% in soils under a mixture of grassland plant species in comparison to monocultures (Sanaullah, Blagodatskaya, Chabbi, Rumpel, & Kuzyakov, 2011). Other studies have also observed resistance of the microbial biomass to drought under diverse grassland species (Mackie, Zeiter, Bloor, & Stampfli, 2019; Thakur et al., 2015).

4.0 GENERAL DISCUSSION AND CONCLUSIONS

Following rapid settlement of the Great Plains of North America, landscapes were largely managed based on a paradigm of uniformity (Fuhlendorf et al., 2006), resulting in widescale suppression of natural disturbances such as fire. Only a fraction of the former extent of grasslands now exists, with very little formal protection for the remaining grassland parcels (Lark, 2020). As such, it is imperative that management practises aiming to restore and maintain the ecological functions of grasslands are utilized within the remnant areas to prevent further losses to biodiversity (Hilger, 2020). Patchwork applications of prescribed fire is one method through which natural landscape disturbances are reintroduced, and the spatial and temporal patterns of natural ecosystem structures are restored. Heterogeneity created through spatio-temporal variation is a precursor to biological diversity, and the basis for grassland ecosystem management conservation (Fuhlendorf et al., 2006).

In grasslands, the effects of fire on aboveground vegetation have been extensively studied, while less is known concerning the response of soil biogeochemistry to grassland fire. Soil is variously integrated with other ecosystem components, providing water, nutrient, and subsistence to plants, as well as habitat to assorted fauna. It is critical that the response of soil to grassland fire is evaluated to ensure fire prescriptions are compatible with soil conservation and maintaining resilience of an integrated ecosystem.

I conducted my research in southwest Saskatchewan on Nature Conservancy Canada's Old Man on His Back Heritage and Conservation Area property of the northern mixed grass prairies. Through prescriptions, fire was reintroduced to the landscape within a tame forage and native forage pasture. Burned plots (5 and 9 ha) were compared to control plots (5 and 9 ha) within the tame forage and native forage pastures, respectively, where samples were collected every 50 m, covering a wide range of grassland spatio-temporal heterogeneity. I examined the seasonal and annual effects of fire prescription on soil chemical and microbial properties to assess soil's resistance and resilience to prescribed fire disturbance. I found that soil chemical properties (total carbon, total nitrogen, pH and EC) were unaffected by fire within 0-10 cm of the surface soil. Similarly, the absolute abundance of soil microbial groups remained relatively stable throughout the two growing seasons post-fire. A transient increase in total fungi (in 2018 only) and GN:GP (2018 and 2019) was observed only in the tame forage pasture. Composition and structure of the

soil microbial community was significantly influenced temporally and by fire treatment. Fire treatment and temporal effects resulted in a more homogenous microbial community composition; however, temporal effects were relatively minor. Fire response of the microbial community may be temporary as over time a more diverse community structure develops. Year to year changes in soil properties were stronger than treatment effects in this study. Annual variations produced changes in the chemical properties of the native pasture while the tame pasture showed more significant effects in microbial parameters.

My study demonstrates that the Chernozemic grassland soils at OMB have a high capacity to resist and absorb fire disturbance, as soils showed only few variations in soil parameters compared to control plots. Due to seasonal grazing, fuel build-up as litter was minimal, and rapid fuel consumption during burning prevented significant heat transfer to the soil (Hill, Bakker & Dunwiddie, 2017). Compositional changes in the soil microbial community were largely attributed to the effects of fire on plant communities and concurrent plant–soil interactions, rather than direct heat-induced changes of fire to soil. The resistance and resilience of grassland soils to fire disturbance has been documented in other grasslands, corroborating the results of this work (Úbeda et al., 2005.; Augustine, Derner, & Milchunas, 2010; Alcañiz et al., 2018; White & Loftin, 2000; Wang et al., 2019).

4.1 Study limitations

Limitations to my study include the analysis of only a single burn in each pasture type (Hilger, 2020). Replicating burns within the same, or alternate, tame and native forage pastures at OMB may have assisted in minimizing the topo-edaphic effects of vegetation composition and grazing intensity on the response of soil to grassland fire, while also increasing the statistical power of my study. Replicate fires were planned for this study, however, burning could not be implemented due to inclement conditions within narrow safe fire prescription weather windows (Hilger, 2020).

Lack of fire replication is a common drawback for large-scale studies on prescribed fire. Conducting prescribed fire can be challenging as the seasonal windows for fire application are narrow, and fire behavior within these windows can also be quite variable (Yurkonis, Dillon, McGranahan, Toledo & Goodwin, 2019). Without predictability in fire behavior, fire managers must be generous when ascribing risk mitigation by ensuring an abundance of equipment and

trained personnel are present. Training requires prior instruction and observation during burns where competent personnel are active. Investments required for safety are therefore quite high, and in addition to the cost of other essentials such as water, fire retardants, and their transportation, which in remote locations like grasslands, are often considerable distances away from the fire site.

Apart from the direct obligations required of burning, prescribed fire also requires considerable desktop planning, preparation and administration costs as well as pre- and post-fire input such as mowing fire containment lines and mop up for hours to days after the initial ignition (Florec, Burton, Pannell, Kelso & Milne, 2019; Russell-Smith, Edwards, Sangha, Yates, & Gardener, 2019). In areas where fire crews are not continuously positioned, organizing burns takes extensive collaboration of rural municipalities, local fire departments, community organization, conservation agencies, First Nations communities and societal agreement that burning can help to preserve endangered land bases and mitigate wildfire risks (Hilger, 2020). Costs therefore exceed the monetary value of fire line equipment and resources, of which few studies have provided precise estimates (Elliott, Venn, Lewis, Farrar & Srivastava, 2021).

Additional limitations of my study include conservation considerations in the OMB grasslands, which precludes digging large soil pits and limits the volume of soil removed. Increasing the amount of soil collected at each plot would have allowed for replication of analyses, particularly the microbial analyses. Replicate samples taken within plots, may have helped to reduce between-plot variation. The coefficient of variation (CV) for microbial biomass analyses, for example, was 110% in my study. While other studies have reported MBC with a CV of 10 to 90% (Wardle, 1998), replicate analyses would help to confirm accuracy. Various authors have reported CVs of around 50% for many biochemical properties (Debosz, Rasmussen & Pedersen, 1999; Waldrop & Firestone, 2006; Šnajdr, et al., 2008; Dong, Kou, Yang, Chen & Xu, 2018).

Environmental variables can have a strong influence on soil biogeochemistry (Schimel et al., 1990; Evans & Wallenstein, 2012; Neilsen & Ball, 2015; Orem, Newman, Osborne & Reddy, 2015). Collecting environmental data throughout the site may have assisted in the explanation of seasonal and annual variations to soil microbial and chemical parameters. Future work may include the use of soil temperature and moisture probes as they greater reflect the impact of environmental factors (precipitation and ambient temperature) on soil properties (Su, 2011).

Surface, organic, and mineral soil temperatures collected while burning would also benefit interpretations on the effects of prescribed fire. Temperature measurements relate to fire intensity

and temperature fluctuation patterns can indicate heterogeneity within the burn. However, installing thermocouple probes into soil prior to burning is a delicate and time-consuming operation (Sackett, 1992), and measurement interference often occurs. Other modalities, such as automatic temperature sensors lead to vague conclusions as analyses are based on trigger point temperatures. There is a wide variation among techniques in cost, labor, accuracy, and level of measurement detail, making fire-initiated soil temperature hard to standardize amongst studies. Simple observations of fire behavior during burns and post burn observation of fire severity indicators are inexpensive to estimate and still reveal useful differences among fires (Kennard, Outcalt, Jones & O'Brien, 2005).

Grazing exclusion areas within the burn and control plots would have allowed for comparisons to be made between undisturbed soil, soil where vegetation was grazed and soil where vegetation was burnt. However, logistical challenges in designing and implementing large-scale fire and grazing studies limited these comparisons (Hilger, 2020). Future research in grassland studies utilizing fire and grazing as disturbances should focus on manipulating burn frequency, timing, fuel loads and the size of burn patches, as these parameters may have been limiting factors in capturing burn effects (Hilger, 2020). Increasing the frequency of sampling with soil collections immediately post fire and at smaller increments after burning may also provide greater clarity on responses of soil to fire.

4.2 Future work

The results of my study have provided a comprehensive basis for future work relating to the effects of grassland fire on soil, specifically in Chernozems of semi-arid environments. While fire has not been part of the landscape in the documented history of OMB, fire and grazing are the key evolutionary disturbances northern grasslands have adapted to. Analyzing the historic pyrogenic carbon present in these grassland soils may be useful as an indicator of historic fire frequency and plant biomass in the pre-documented grasslands. This analysis would be beneficial for determining fire return frequencies as well as understanding the topo-edaphic influence of pyrogenic carbon on plant and soil microbial communities. Radiocarbon dating and isotopic carbon composition has been applied widely across a range of disciplines for obtaining environmental and paleoenvironmental information (Bird & Ascough, 2012).

In the future, greenhouse studies providing grass with isotopically labelled carbon as a carbon dioxide source could be followed throughout the growth cycle to pyrolysis after senescence and burning. Determining the mechanisms by which fire influences soil carbon and carbon sequestration by following the entire cycle of carbon through to its incorporation into soil organic matter will provide much needed information on accelerated nutrient turn over by fire and fully ensure goals in soil conservation are obtained. Additions of pyrogenic organic matter have been shown to both increase and decrease mineralization of native soil organic carbon (Santín, Doerr, Preston & González-Rodríguez, 2015; Maestrini, Nannipieri and Abiven, 2015, DeCiucies, Whitman, Woolf, Enders & Lehmann, 2018; Schmit et al., 2019). Mechanistic information is vital as climate change presents increasing variability (Lal, 2013; Evans & Wallenstein, 2014; Naidu & Bagchi, 2021).

As the soil microbial community has great influence over soil organic matter storage, laboratory analyses incorporating pyrogenic organic matter may be useful. Microbial biomass represents the living fraction of organic matter, controlling decomposition and virtually every reaction in the soil C and N cycles (Whalen, Thomas & Sharifi, 2019). Microbial biomass is measured to give an indication of the response of soil microbiota to management, environmental change, site disturbance and pollution (Kandeler, 2007). Adding pyrogenic organic matter to microbial biomass incubations may provide clarity on biological responses to charred biomass from fire. Alternatively, sampling and analyzing only the organic layer of soils (0-1cm depth in some cases), where ash and pyrolyzed matter has been deposited following burning, may be useful in elucidating the responses of microbes directly affected by fire. Further microbial characterizations of the organic soil layers, or soil incubated with pyrolyzed matter, should include DNA fingerprinting and enzyme activities. The combination of analyses may provide a more comprehensive understanding of the microbial species amenable to post fire environments.

As climate change begins to pose a stronger influence over semi-arid environments, there is increased importance in understanding the disturbance responses of soil biogeochemistry under climate extremes. Longer term studies, that incorporate both wet and dry cycles may be needed to fully determine the effects of fire and grazing on soil properties in the northern mixed grass prairie. The nearby Grasslands National Park (GNP), 97,000 ha, utilizes prescribed fire to obtain certain ecosystem objectives, burning at least 75 ha per year. Due to its proximity to OMB, this may be an ideal place to build upon current work, and fully differentiate the response of soil to burning,

grazing and the combination of fire and grazing throughout a wide expanse of spatio-temporal heterogeneity.

4.3 Management implications

My study shows that prescribed fire in the mixed grass prairie aligns with soil conservation objectives as fire did not cause detrimental changes in soil chemical or microbial properties. During prescribed fire planning, questions regarding soil effects of burning are often asked by managers and the public (Lamb, personal communication, 2022), and this research provides evidence that unintended soil effects of fire are not likely to be a concern. The Nature Conservancy Canada manages the grasslands at OMB with the primary objective to maintain and create habitat for species at risk. As a grassland management tool at OMB, prescribed fire is useful for obtaining NCC's objective in promoting habitat for species at risk by creating spatio-temporal diversity in biomass structures (Hilger, 2020) without negatively affecting soil ecology. Further, as healthy grasslands are increasingly counted upon to mitigate anthropogenic carbon emissions by sequestering C within the soil, the lack of impact of prescribed fire on soil C storage is important as it demonstrates that prescribed fire is aligned to global objectives on atmospheric CO₂ mitigation.

My results are specific to small patch spring burns in mixed grass pastures under deferred grazing systems. Burning in spring may be an important management consideration, not only for fire escape safety, but to prevent heat transfer through the soil and potential root and shoot mortality. Spring burns are often less severe and more heterogenic, due to the moisture content of fuels and the surface soil. My study applied a low severity burn which resulted in high pyrodiveristy throughout the burned area. Pyrodiversity in burning may have also mitigated the intensity of disturbance effects, resuting in little disruption to soil properties. Comparison to fall fires and to high-intensity wildfire is needed to refine these recommendations.

The fire treatment plots in this study comprised only a small percentage (< 10%) of total pasture area. Small patch burning as such may be optimal to prevent overgrazing of recovering biomass. Additionally, grazers were allowed access to the total pasture area 2 months after fire, when regrowth was well established. Cattle did increase useage of burned areas relative to adjacent unburned areas (Hilger 2020), demonstrating that fire can be used to manage grazing intensity to some degree. Managing grasslands with prescribed fire takes careful consideration of vegetation

growth patterns, often interacting with environmental variables. Land managers will benefit from extensive planning and consideration of fire objectives.

4.4 Conclusion

Soil conservation requires that ecological systems are capable of remaining stable when faced with various disturbances. Stability can encompass the resistance of an ecosystem to change and the resilience of an ecosystem to adapt and recover ecological function. This project demonstrates that spring burning within the mixed grass prairies is compatible with soil conservation and may be utilized in deferred rotational grazing systems without negatively impacting soil properties. Additionally, prescribed fire may be suitable for grasslands that experience fluctuations in environmental conditions. Native prairies, comprised of a diversity of vegetation species may have greater resistance to disturbance. Although fire may increase homogeneity at smaller scales, larger scale heterogeneity within grasslands can be achieved. This is a critical goal, as large-scale heterogeneity is regarded as the precursor to biological diversity and the basis for grassland ecosystem management and conservation (Fuhlendorf et al., 2006).

The completion of this project also demonstrates that the public fear of fire can be overcome by communication and vast interagency collaboration. The prescribed fire in this study brought together community members and local fire departments, rural municipalities and First Nations communities, exposing a wide variety of people to prescribed fire and fire training (Hilger, 2020). The contribution of many groups towards the conservation management of the mixed grass prairies is a tremendous step in the direction of their protection, and their ability to continue providing the essential ecosystem services that we have depended on for hundreds of years.

5.0 LITERATURE CITED

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APPENDIX A. SUPPLEMENTAL FIGURES: SOIL CHEMICAL AND MICROBIAL PROPERTIES FOR ALL BURN TREATMENT AND SAMPLING TIME COMBINATIONS.

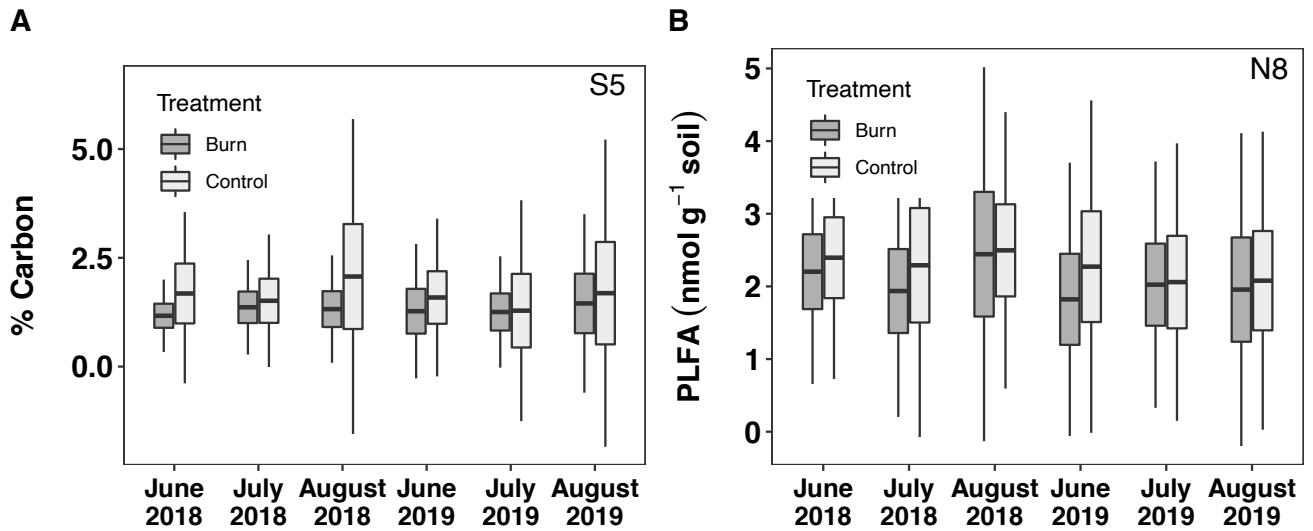


Fig. A-1. Mean and standard deviation of soil total carbon in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

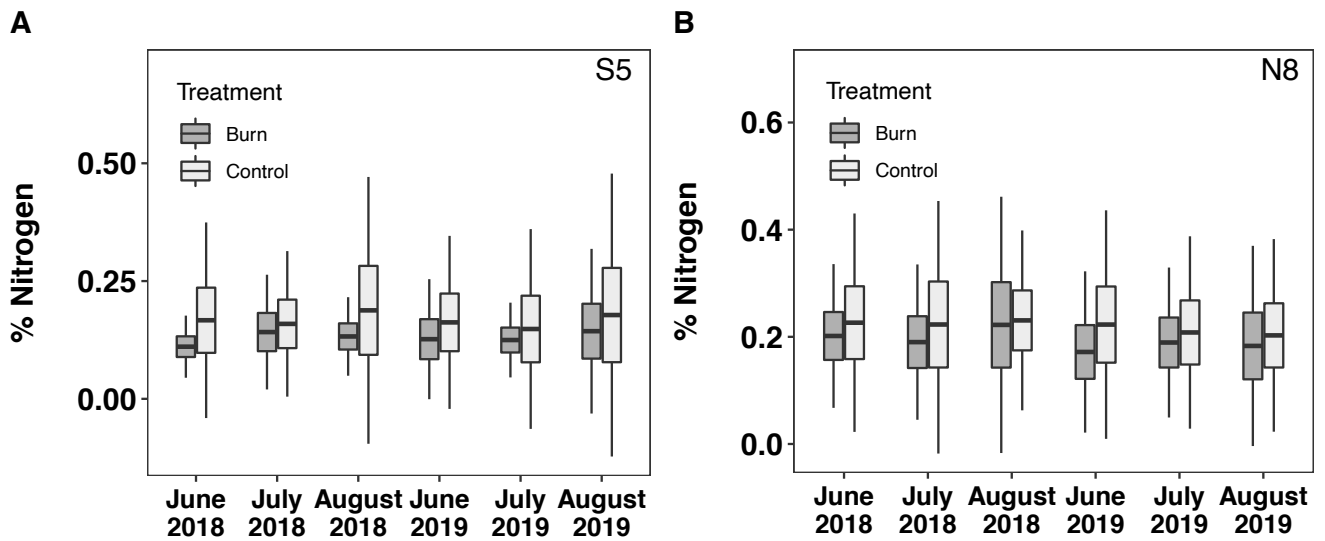


Fig. A-2. Mean and standard deviation of soil total nitrogen in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

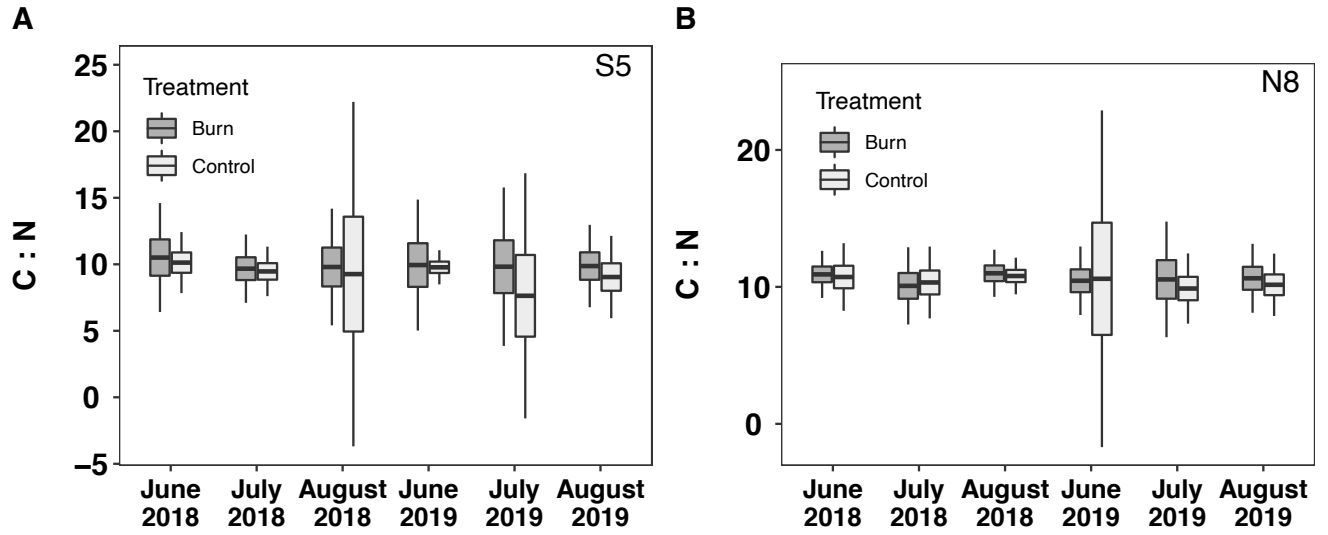


Fig. A-3. Mean and standard deviation the soil total carbon to total nitrogen ratio in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

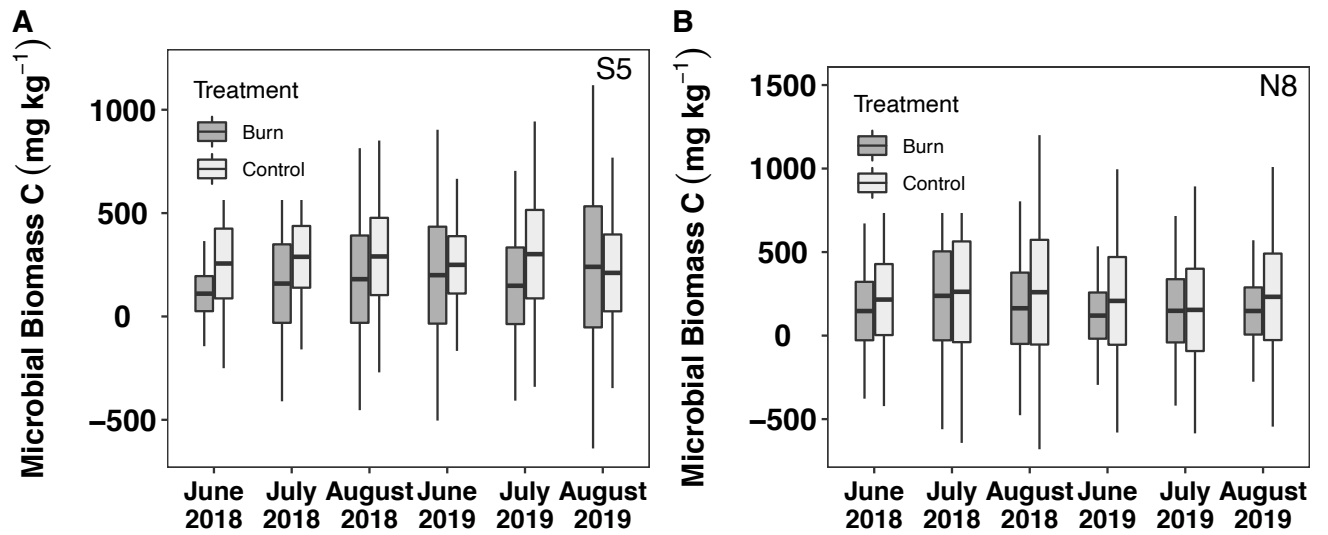


Fig. A-4. Mean and standard deviation of microbial biomass carbon in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

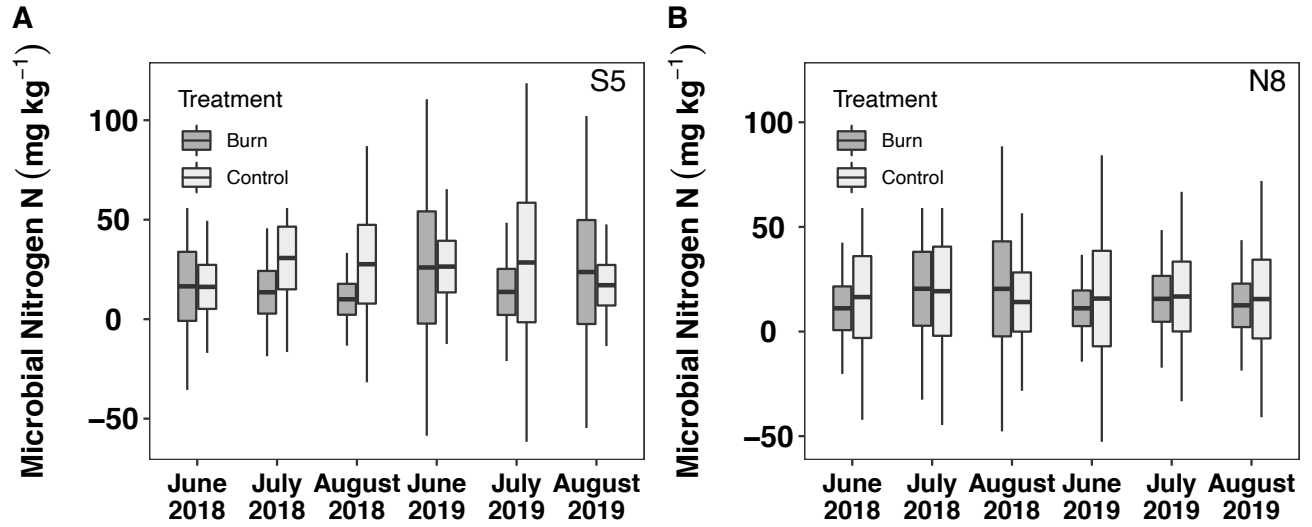


Fig. A-5. Mean and standard deviation of microbial biomass nitrogen in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

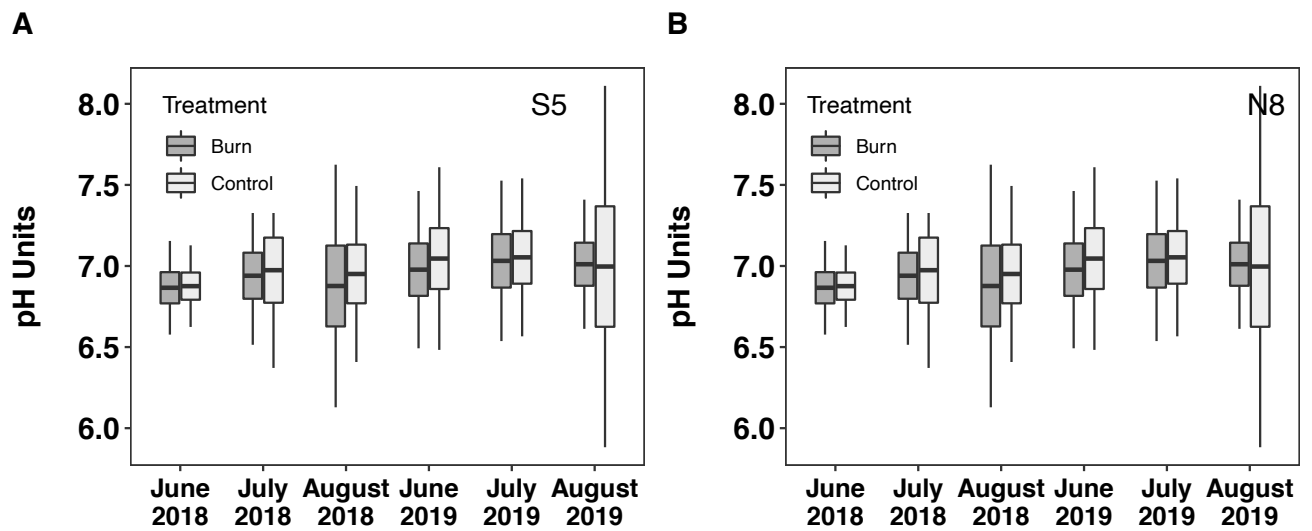


Fig. A-6. Mean and standard deviation of soil pH in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

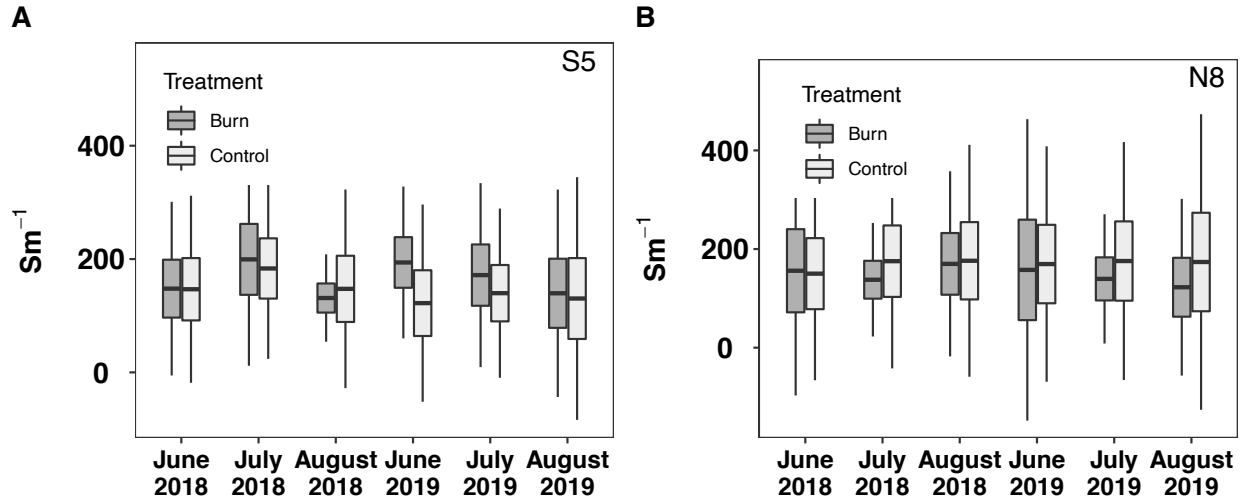


Fig. A-7. Mean and standard deviation of soil electrical conductivity in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

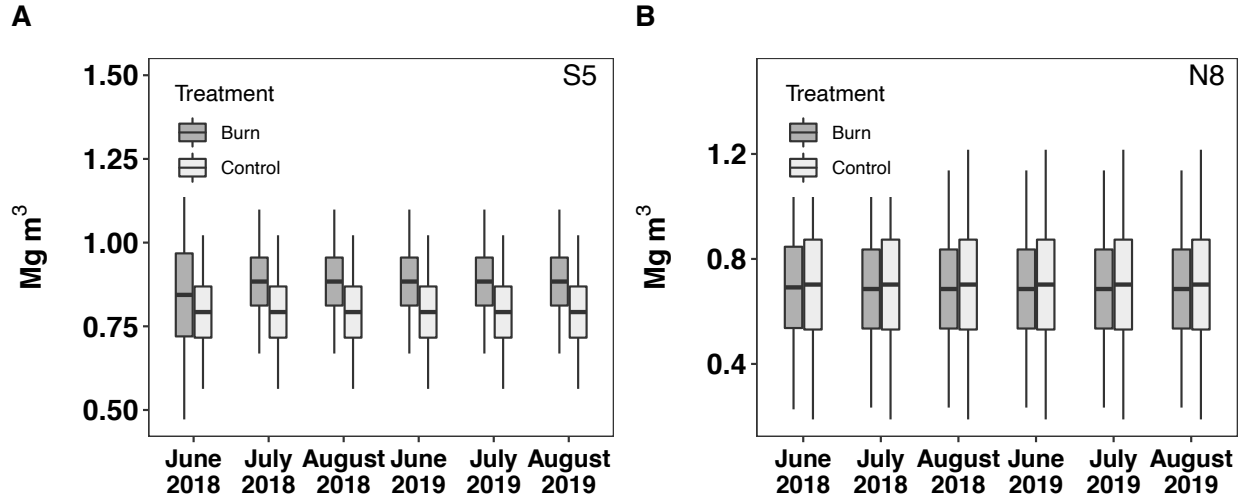


Fig. A-8. Means and standard deviation of soil bulk density in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

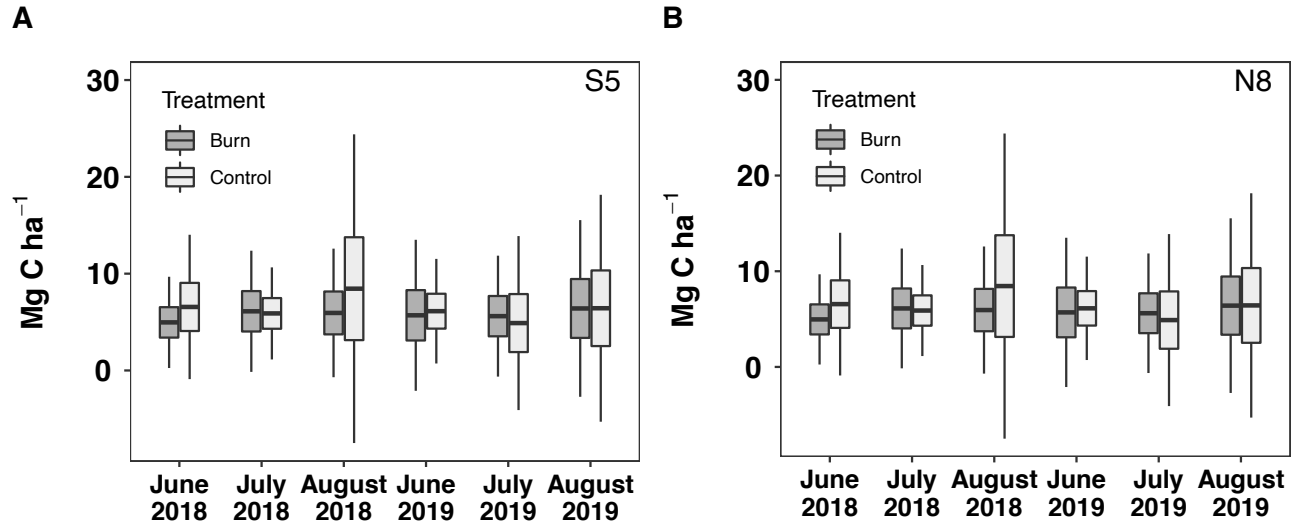


Fig. A-9. Mean and standard deviation of soil carbon stock in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

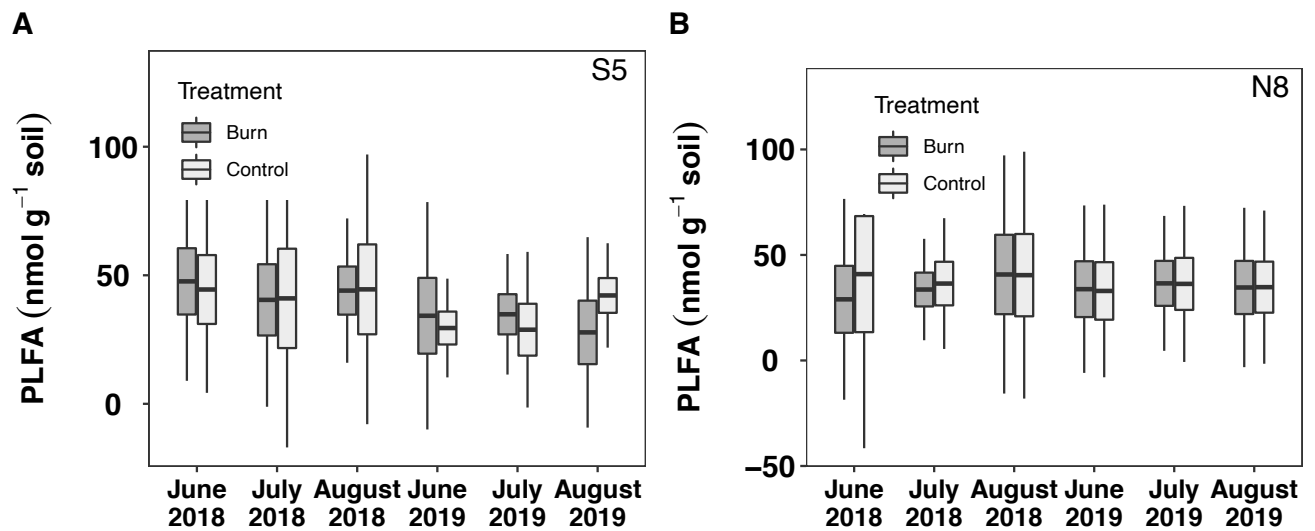


Fig. A-10. Mean and standard deviation of total PLA abundance in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

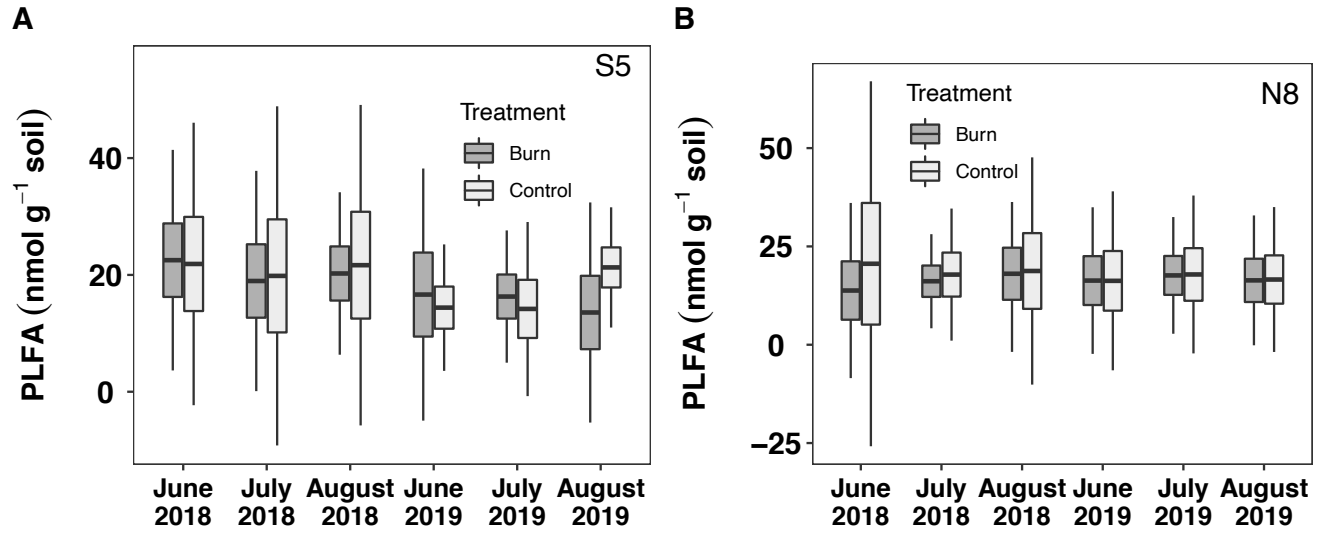


Fig. A-11. Mean and standard deviation of total bacterial abundance in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

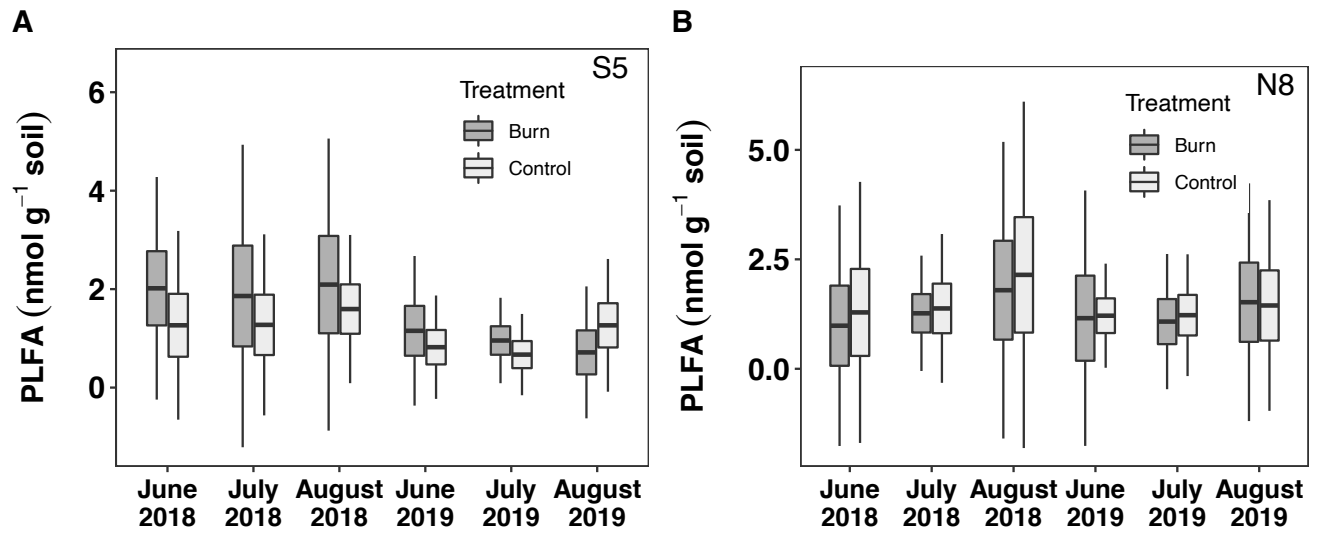


Fig. A-12. Means and standard deviation of total fungal abundance in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

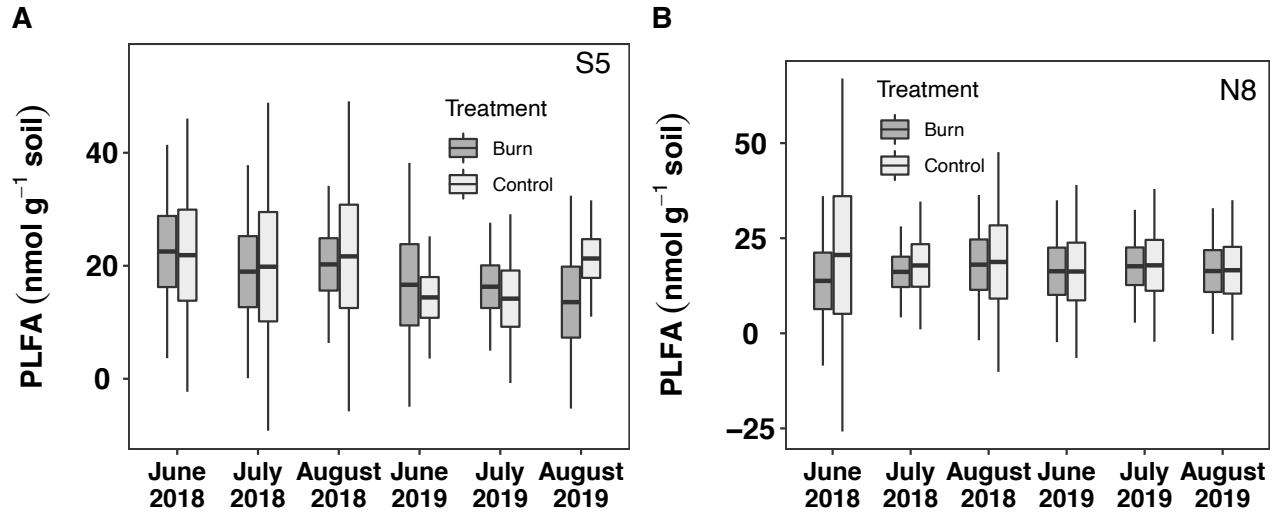


Fig. A-13. Mean and standard deviation of the ratio of fungal to bacterial PLFA counts in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

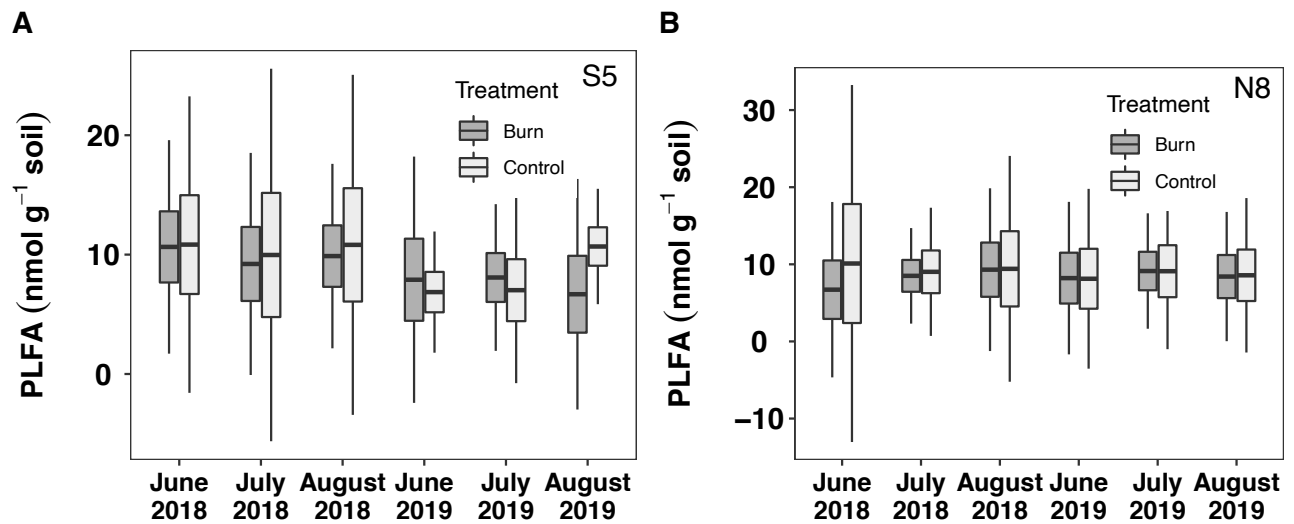


Fig. A-14. Mean and standard deviation of Gram-positive bacteria PLFA counts in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

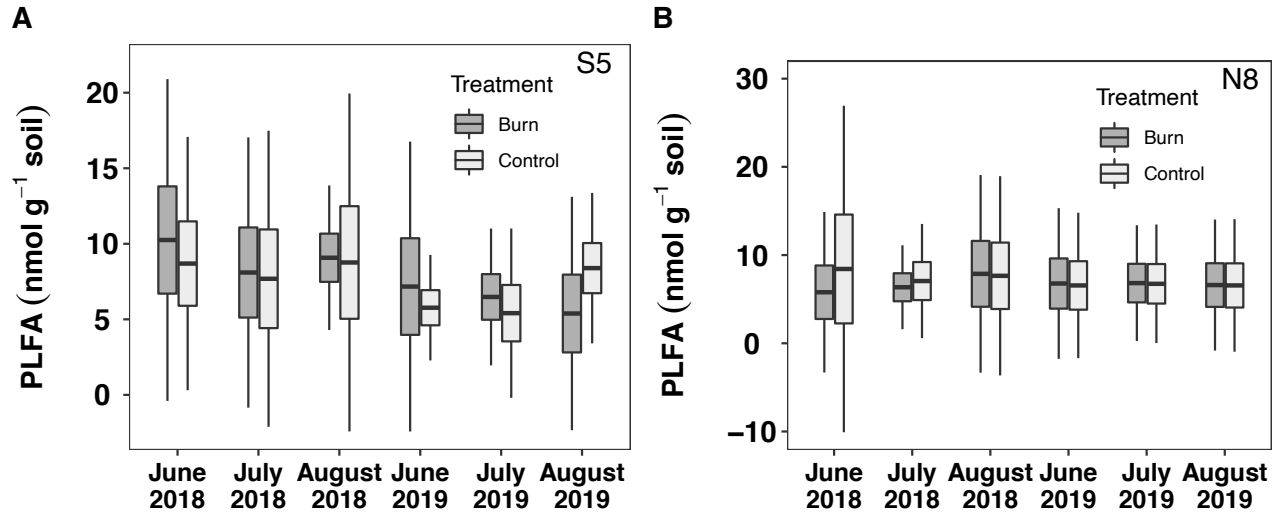


Fig. A-15. Mean and standard deviation of Gram-negative bacteria PLFA counts in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

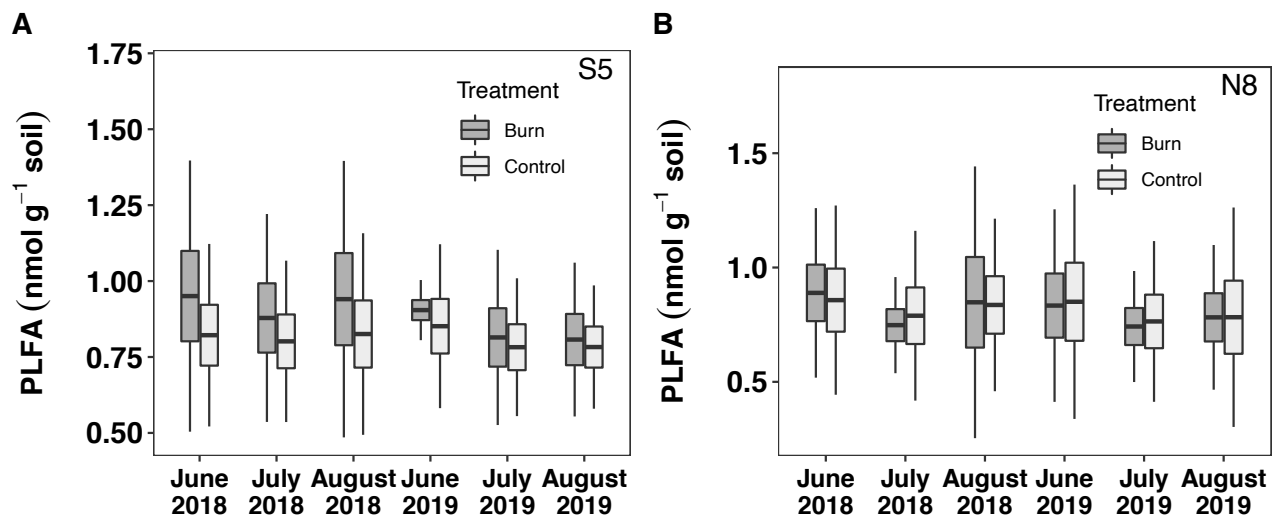


Fig. A-16. Mean and standard deviation of the ratio of Gram-negative to Gram-positive bacteria PLFA counts in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

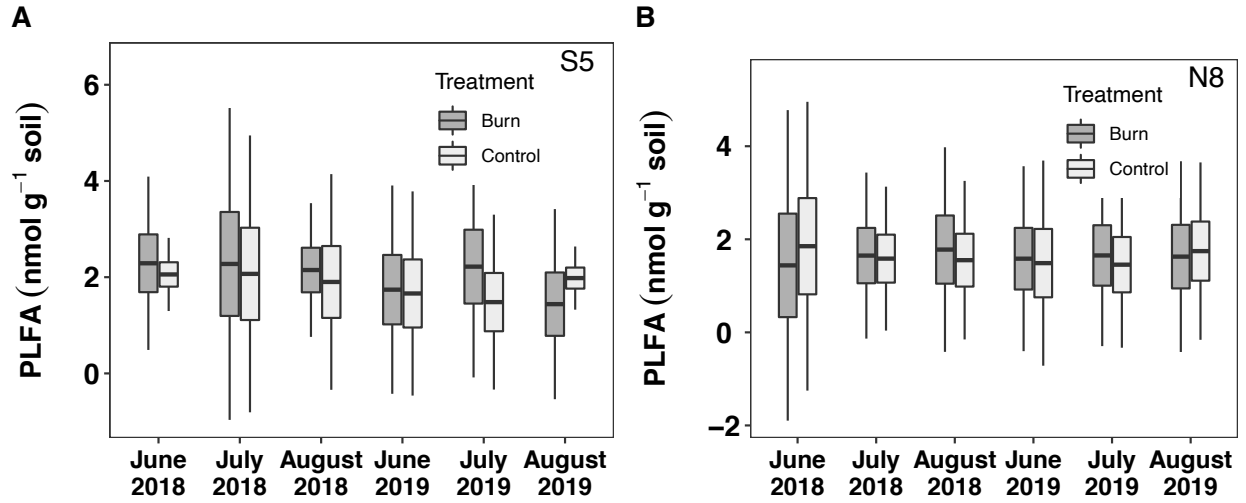


Fig. A-17. Mean and standard deviation of arbuscular mycorrhizal fungal PLFA counts in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

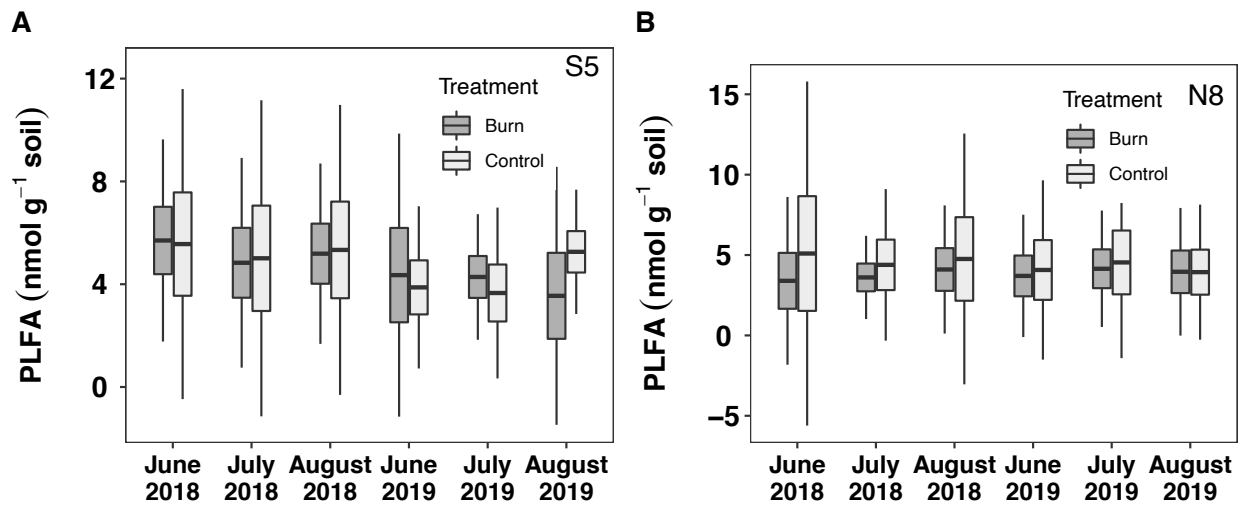


Fig. A-18. Mean and standard deviation of actinobacteria PLFA counts in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

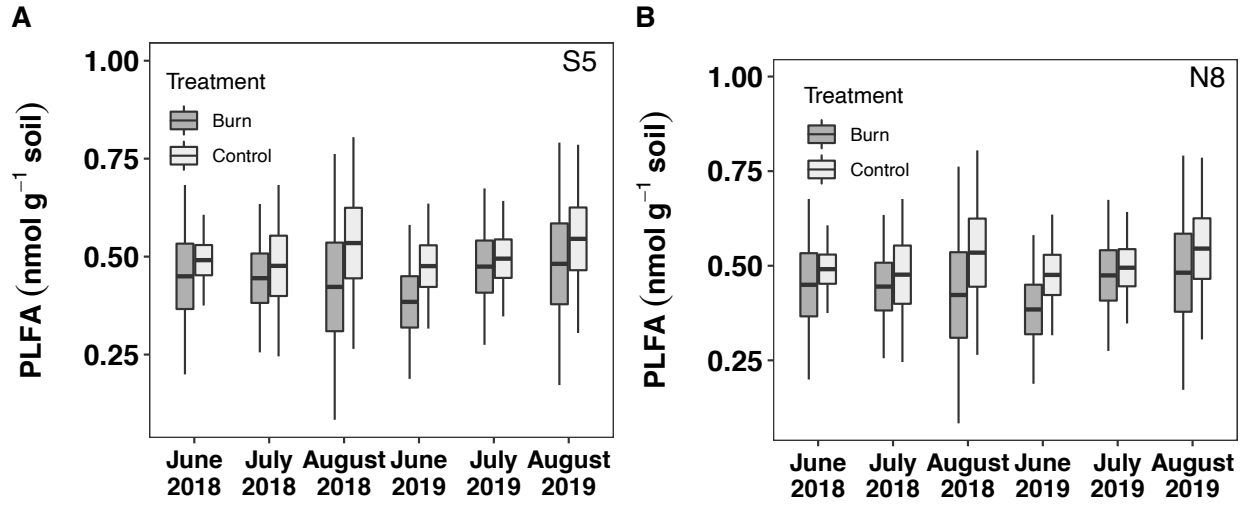


Fig. A-19. Mean and standard deviation of microbial stress in pasture A) S5 and B) N8. Boxes represent 50% of the central data.

APPENDIX B. SUPPLEMENTAL TABLES: AXIS LOADING FOR PLFA ISOMERS FOR EACH NMDS ORDINATION AXIS

Table B-1. Axis loadings for each PLFA, for each axis on the tame forage pasture (S5) ordination.

PLFA	NMDS1	NMDS2
C12:0 2OH	0.15714781	-0.1225855
C14:0 iso	0.0636969	-0.0003227
C14:1 w7c	-0.2326265	-0.1172443
C14:1 w5c	0.20526703	-0.158534
C14:0	0.10124114	0.01688146
C14:1 w7c DMA	0.19962699	-0.1503044
C15:1 iso w9c	-0.0381691	-0.1437151
C15:1 iso w6c	0.05595906	0.00340043
C15:4 w3c	0.01434128	-0.0583539
C15:1 anteiso w9c	-0.0088782	0.00743489
C15:0 iso	0.02825766	0.0027543
C15:0 anteiso	0.02775252	0.00759391
C15:1 w8c	0.03501076	-0.2427352
C15:1 w6c	0.32027698	-0.0882184
C15:0	0.03487011	0.00247326
C15:0 iso DMA	-0.0591448	0.0093446
C16:1 w7c alcohol	0.02898112	-0.0659193
C15:0 DMA	-0.0106143	0.01110888
C16:0 N alcohol	0.10532949	0.02182071
C16:3 w6c	-0.2543121	-0.1416748
C16:0 iso	-0.0007702	0.01087134
C16:0 anteiso	-0.0067699	-0.0117866
C16:1 w9c	0.01310242	-0.0064729
C16:1 w7c	0.01828858	-0.0055984
C16:1 w5c	-0.0208046	0.00466753
C16:0	0.02321843	-0.0027752
C16:2 DMA	0.37479709	0.17703839
C16:1 w7c DMA	0.00855062	-0.079064
C16:0 10 methyl	-0.0008374	0.00706524
C16:0 DMA	0.01698022	-0.3079379

Table B-1. Continued

PLFA	NMDS1	NMDS2
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C17:1 iso w9c	-0.1209844	-0.3869032
C17:1 anteiso w7c	-0.199036	-0.089116
C17:0 iso	-0.0118597	0.01065679
C17:0 anteiso	-0.0181017	0.00469192
C17:1 w8c	-0.0016507	0.00447655
C17:0 cyclo w7c	0.03216215	0.01209101
C17:1 w3c	-0.0947323	0.01862258
C17:0	0.01106844	0.00941416
C17:1 w7c 10 methyl	-0.095276	0.02053235
C16:0 2OH	0.1836324	-0.1142219
C17:0 10 methyl	0.03759196	0.02105749
C17:0 DMA	0.07652778	0.04610162
C18:3 w6c	-0.0610902	0.09643883
C18:0 iso	-0.0242244	0.03197812
C18:2 w6c	-0.0242006	-0.0508993
C18:1 w9c	-0.0071659	-0.010153
C18:1 w6c	-0.0531168	0.02034699
C18:0	0.00652816	-0.0018328
C18:1 w7c 10 methyl	0.01351489	-0.028908
C18:2 DMA	0.07692527	-0.0045307
C18:1 w9c DMA	0.21759703	-0.2746538
C18:1 w7c DMA	-0.0036537	-0.0217129
C18:0 10 methyl	-0.0161612	0.02406517
C18:0 DMA	-0.0703717	0.0498993
C19:4 w6c	-0.2907726	-0.1587541
C19:0 iso	-0.1005106	-0.0631776
C19:1 w9c	-0.0141756	0.03489677
C19:1 w7c	-0.0938286	-0.035766
C19:0 cyclo w9c	0.03921626	0.04459694
C19:0 cyclo w7c	-0.0252881	-0.0048151
C19:0	-0.0359888	0.04959361
C19:1 w7c 10 methyl	0.11483242	0.05645361
C20:4 w6c	-0.0377337	-0.0596978
C20:5 w3c	-0.0957791	-0.1246145
C20:3 w6c	-0.0732735	-0.0241572
C20:2 w6c	0.1833819	-0.2562358

Table B-1. Continued

PLFA	NMDS1	NMDS2
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C20:1 w9c	-0.0617355	-0.0169195
C20:1 w8c	-0.0087803	-0.0258794
C20:0	0.02630756	-0.0320217

Table B-2. Axis loadings for each PLFA, for each axis on the native forage pasture (N8) ordination.

PLFA	NMDS1	NMDS2
C10:0 2OH	-0.18104	-0.03029
C13:0 iso	-0.30643	-0.01885
C13:0 anteiso	-0.37391	-0.07340
C13:0	-0.50898	-0.20460
C12:0 2OH	-0.32510	-0.25731
C14:0 iso	-0.15137	0.01128
C14:1 w9c	-0.43667	-0.45330
C14:1 w7c	-0.39264	-0.09673
C14:1 w5c	-0.35780	-0.21144
C14:0	-0.08966	-0.04607
C14:0 iso 3OH	-0.07180	-0.60125
C14:1 w7c DMA	-0.38987	-0.10532
X15 1 iso w9c	-0.22521	0.06534
C15:1 iso w6c	-0.08430	0.02863
C15 4 w3c	-0.09915	-0.05464
C15:1 anteiso w9c	-0.05755	0.08927
C15 :0 iso	-0.03158	-0.01682
C15:0 anteiso	-0.03778	-0.01386
C15:1 w8c	-0.09842	-0.23334
C15:1 w6c	-0.27289	-0.02176
C15:0	-0.03818	0.00495
C15:0 iso DMA	-0.11400	-0.20348
C16:1 w7c alcohol	-0.09660	0.12118
C15:0 DMA	-0.00924	0.01571
C16:0 N alcohol	0.04896	-0.13929

Table B-2. Continued.

PLFA	NMDS1	NMDS2
C16:3 w6c	0.06035	0.41755
C16:0 iso	-0.00351	-0.00868
C16:0 anteiso	-0.01410	0.01521
C16:1 w9c	-0.01289	0.00773
C16:1 w7c	-0.01483	-0.00379
C16:1 w5c	-0.00022	0.04180
C16:0	-0.00393	-0.01009
C16:2 DMA	-0.03908	-0.29559
C16:1 w7c DMA	-0.07638	0.09304
C16:0 10 methyl	0.00012	-0.00249
C16:0 DMA	0.30061	-0.05543
C17:1 iso w9c	-0.10272	-0.01408
C17:1 anteiso w7c	0.00361	0.16696
C17:0 iso	0.01099	-0.00217
C17:0 anteiso	0.01744	0.01049
C17:1 w8c	0.01140	0.02381
C17:0 cyclo w7c	0.00122	-0.01709
C17:1 w3c	0.02386	0.16473
C17.0	0.03341	-0.01591
C17:1 w7c 10. methyl	0.04375	0.05728
C16:0 2OH	-0.03810	-0.10819
C17:0 10 methyl	0.01561	-0.03775
C17:0 DMA	0.02386	-0.05200
C18:3 w6c	0.12455	0.01912
C18:0 iso	0.01969	0.03329
C18:2 w6c	0.01494	0.07549
C18:1 w9c	0.00710	0.01367
C18:1 w6c	0.15095	0.04698
C18:0	0.02201	-0.02581
C18:1 w7c 10 methyl	-0.00740	-0.01354
C18:2 DMA	-0.00859	0.02759
C18:1 w9c DMA	-0.06168	-0.09111
C18:1 w7c DMA	0.04726	-0.10097
C18:0 10 methyl	0.02173	0.00173

Table B-2. Continued.

PLFA	PLFA	PLFA
C18:0 DMA	0.09075	-0.15407
C19:4 w6c	-0.16228	0.20292
C19:3 w6c	0.08636	-0.23490
C19:0 iso	-0.09946	-0.04870
C19:1 w9c	0.06368	0.13110
C19:1 w7c	0.12792	0.16983
C19:0 cyclo w9c	0.02054	-0.07896
C19:0 cyclo w7c	0.00019	-0.00508
C19:0	0.06812	0.00271
C19:1 w7c 10 methyl	-0.01498	-0.14000
C20:4 w6c	0.04799	0.01042
C20:5 w3c	0.10289	0.06242
C20:3 w6c	0.02929	0.06442
C20:2 w6c	-0.06829	-0.27106
C20:1 w9c	-0.00018	0.04302
C20:1 w8c	-0.08455	-0.02760
C20:0	0.00886	-0.01825

**APPENDIX C: SUPPLEMENTAL TABLES: REGRESSION VALUES FROM VECTORS IN
NMDS ANALYSIS.**

Table C-1. Regression of soil variables to ordination axes tested by permutational analysis in the tame forage pasture (S5).

Variable	NMDS1	NMDS2	r ²	Pr(>r)	
MBC	-0.97259	0.23253	0.0904	0.04	*
MBN	-0.84391	0.53648	0.0623	0.113	
Carbon	0.99359	0.11302	0.1845	0.003	**
Nitrogen	0.98987	0.14201	0.259	0.001	***
C: N	-0.48943	-0.87204	0.0147	0.578	
Bulk density	-0.93125	-0.36439	0.0911	0.045	*
Carbon stock	0.99665	0.08174	0.1133	0.014	*
pH	-0.62342	0.78189	0.0297	0.334	
EC	-0.96591	0.25887	0.0973	0.025	*
Total PLFAs	0.64773	-0.76187	0.3777	0.001	***
Bacteria	0.77351	-0.63379	0.3658	0.001	***
Fungi	-0.0334	-0.99944	0.4201	0.001	***
F:B	-0.39481	-0.91876	0.3595	0.001	***
GP	0.82275	-0.5684	0.3817	0.001	***
GN	0.55805	-0.82981	0.3836	0.001	***
GN:GP	-0.53229	-0.84656	0.3193	0.001	***
AMF	0.11938	-0.99285	0.1328	0.009	**
Actinobacteria	0.73911	-0.67358	0.2711	0.001	***
Stress	0.36846	0.92965	0.2164	0.002	**

*, **, and *** indicate significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively. C:N = carbon to nitrogen ratio, EC = electrical conductivity, MBC = microbial biomass carbon, MBN = microbial biomass nitrogen, MBC:MBN = ratio of microbial biomass carbon to microbial biomass nitrogen, F:B = ratio of fungi to bacteria PLFA counts, GN = Gram-negative bacteria, GP = Gram-positive bacteria, GN:GP = ratio of Gram-negative to Gram-positive bacteria PLFA counts, AMF = arbuscular mycorrhizal fungi, Stress = ratio of cy 17: 0 / 16:1 w7c PLFAs

Table C.2. Regression of soil variables to ordination axes tested by permutational analysis in the native forage pasture (N8).

Variable	NMDS1	NMDS2	r2	Pr(>r)	
MBC	-0.99619	0.08717	0.1019	0.001	***
MBN	-0.92844	0.37148	0.0375	0.027	*
Carbon	-0.97742	-0.21132	0.0402	0.035	*
Nitrogen	-0.98357	-0.18055	0.04	0.032	*
C:N	-0.93849	-0.34532	0.005	0.595	
Bulk density	0.99862	0.05251	0.0702	0.002	**
Carbon stock	-0.18353	-0.98301	0.0022	0.82	
pH	0.99986	0.01649	0.0122	0.35	
EC	0.88935	0.45722	0.0737	0.003	**
Total PLFAs	-0.96398	0.26599	0.1558	0.001	***
Bacteria	-0.98831	0.15245	0.2353	0.001	***
Fungi	0.38279	0.92384	0.0766	0.001	***
F.B	0.71078	0.70342	0.3351	0.001	***
GP	-0.98749	0.15771	0.2969	0.001	***
GN	-0.93032	0.36675	0.1388	0.001	***
GN.GP	0.99944	0.03345	0.3679	0.001	***
AMF	-0.26302	0.96479	0.2259	0.001	***
Actinobacteria	-0.99834	0.05767	0.1404	0.001	***
Stress	0.98348	-0.18099	0.0223	0.122	

*, **, and *** indicate significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively. C:N = carbon to nitrogen ratio, EC = electrical conductivity, MBC = microbial biomass carbon, MBN = microbial biomass nitrogen, MBC:MBN = ratio of microbial biomass carbon to microbial biomass nitrogen, F:B = ratio of fungi to bacteria PLFA counts, GN = Gram-negative bacteria, GP = Gram-positive bacteria, GN:GP = ratio of Gram-negative to Gram-positive bacteria PLFA counts, AMF = arbuscular mycorrhiza fungi, Stress = ratio of cy 17: 0 / 16:1 w7c PLFAs