Extracellular enzyme activity, greenhouse gas emissions and soil microbial communities responses to drought and defoliation in northern temperate grasslands

by

Ahsan Mir Rajper

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in
Rangeland and Wildlife Resources

Department of Agricultural, Food and Nutritional Science
University of Alberta

Abstract

Grassland ecosystems cover more than 40% of the global land area and provide many ecological goods and services, therefore, it is important to sustain these terrestrial ecosystems. One of the ecological services they provide is their potential to act as a carbon (C) sink. However, northern temperate grasslands, which are arid/semi-arid in nature, are specifically vulnerable to climate change (e.g. drought) and natural as well as anthropogenic disturbances such as overgrazing. This study was conducted, at seven different locations across a climate gradient, to test the effects of drought (45% rainfall reduction using rainout shelters) and defoliation on different grassland soil components to understand how these disturbances affect C and nutrient cycling. Defoliation was applied by clipping experimental plots either once or twice a year at two variable heights (3 and 7 cm). A combination of five defoliation treatments was applied including control (no-defoliation). In the first study, I tested the effect of drought and defoliation on soil extracellular enzyme activity. Five enzymes were selected based on their role in C (β-glucosidase, β-cellobiosidase and β-xylosidase), N (N-acetyl-β-glucosaminidase) and P (Acid phosphatase) cycling. I found that the activity of all enzymes decreased with drought, except that β-xylosidase increased with drought conditions, suggesting a shift in biogeochemical processes of these soils under future drought. Furthermore, β-glucosidase activity was reduced under intermediate defoliation. In the second study, I tested the effects of drought and defoliation on greenhouse gas (CO_2, N_2O and CH_4) emissions over two growing seasons (April to September in 2017 and 2018). I found that intermediate defoliation (i.e., defoliation once a year) reduced CO_2 emissions as compared to heavy defoliation (i.e. defoliation twice a year). No treatment effects were observed on N_2O and CH_4 fluxes. Furthermore, I found that defoliation frequency, not timing, was the driving factor for CO_2 emissions. Soil temperature and

extracellular enzyme activity were the best predictors for greenhouse gas emission rates. Findings suggest that annual single-event defoliation could reduce CO_2 emissions in future drought conditions in northern temperate grasslands. In the third study, I tested the effects of drought and defoliation on soil microbial communities in two contrasting grasslands (Kinsella vs Mattheis). I found that drought affected beta diversity at Kinsella (wetter site) where there was a significant effect of drought on beta diversity between as well as within groups (ambient vs drought). Soil bacterial communities were affected by drought; however, soil fungi showed resistant and/or even favored drought conditions. At Kinsella, xylanolytic bacteria (involved in breakdown of xylan) were increased under drought conditions. Overall, the findings of this thesis imply that drought effects were more consistent on enzymes, greenhouse gas emissions and soil microbial communities, while defoliation effects were limited and dependent upon drought treatment and local climate. Results from this thesis suggest that single annual defoliation (specifically in early season) have the potential to reduce CO_2 emissions and increase C stocks in northern temperate grassland soils under future drought conditions.

Preface

This thesis is an original work by Ahsan Mir Rajper

Chapter 2 of this thesis has been submitted (under review) to the Journal of Arid Environments as

> Rajper, Ahsan; Benjamin P. Willing; James F. Cahill; Edward W. Bork; Scott X. Chang; Cameron N. Carlyle, 2023. "Drought and defoliation affect soil extracellular enzyme activity in northern temperate grasslands". I was responsible for experimental setup, sample collection and processing, data analysis and manuscript writing. Cameron Carlyle, Scott Chang, Edward Bork, James Cahill and Benjamin Willing participated in experimental design, funding acquisition, data interpretation and editing of the manuscript.

Chapter 3 of this thesis will be submitted to Agriculture, Ecosystems and Environment as

> Rajper, Ahsan; Benjamin P. Willing; James F. Cahill; Edward W. Bork; Scott X. Chang; Cameron N. Carlyle, 2023. "Soil CO_2 emissions were reduced by single annual defoliation under drought conditions in northern temperate grasslands". I was responsible for experimental setup, sample collection and processing, data analysis and manuscript writing. Cameron Carlyle, Scott Chang, Edward Bork, James Cahill and Benjamin Willing participated in experimental design, funding acquisition, data interpretation and editing of the manuscript.

Chapter 4 of this thesis is intended to be submitted for publication as Rajper, Ahsan; Benjamin P. Willing; James F. Cahill; Edward W. Bork; Scott X. Chang; Cameron N. Carlyle, 2023. "Soil microbial communities' response to drought and defoliation in northern temperate grasslands". I was responsible for experimental setup, sample collection and processing,

data analysis and manuscript writing. Cameron Carlyle, Scott Chang, Edward Bork, James Cahill and Benjamin Willing participated in experimental design, funding acquisition, data interpretation and editing of the manuscript.

Dedication

I would like to dedicate this thesis to my loving Mother, who always supported me at every stage of life!

Acknowledgment

I would like to thank my supervisors Dr. Cameron Carlyle and Dr. Scott Chang. I made it through to this point because of their continuous support throughout my program. They were always present and willing to help. I have learned a lot from them to increase my knowledge and improve myself. I would also like to thank my supervisory committee members Dr. Edward Bork and Dr. Benjamin Willing. Their suggestions and feedbacks throughout the program were very important for my improvement, especially in manuscript writing. I would also like to thank Dr. James Cahill for his support and timely suggestions on my writing.

Throughout my program, I have learned and received immense support from many other students, lab mates and staff at the University of Alberta, including Batbaatar Amgaa, Upama K C, Xiaozhu (Sean) Chuan, Ekaterina Stolnikova, Sara Barszczewski, Brendan Bischoff, Kelvin Lien, Prem Pokharel, Jinhyeob Kwak, Tingting Ju, Jessica Roberts, Ashton Smith, Brittany Onysyk, Nick Brown, and Lisa Raatz.

Many thanks to Dr. Ghulam Murtaza Jamro, who has always been on my side as my teacher, mentor and a big brother. Not only during my PhD program but throughout my life, he always has been a big support.

Back home, I'd like to thank my friends Muhammad Khan (DLife), Mubashar Channa, Sagar Soomro and Sajjad Sial for their support. Thanks to my brothers (Sohail, Parvaiz, Shakeel, Sagheer, Aamir and late Tofique) and uncle (Asghar Rajper) for their continuous support throughout my studies.

Lastly, I would like to thank my mother (Sakina) and my wife (Aisha), what I am able to achieve today is because of these two women who were always with me through the ups and downs, and my loving kids (Nigarish and Mustafa).

Table of Contents

List of tables

List of figures

1. Chapter 1 – Introduction

1.1 Introduction

Grasslands are one of the most widely distributed terrestrial ecosystems and cover more than 40% of the global land area (Dixon et al., 2014; White et al., 2000). They provide ecological goods and services such as providing food, fiber, energy and other products with economic value, and wildlife habitat, supporting biodiversity, and mitigating climate change (Havstad et al., 2007; Sala et al., 2017). Grasslands are known to be a potential carbon (C) sink when managed properly (Follet et al., 2001; Havstad et al., 2007). However, the functioning and productivity of grassland ecosystems are changing as they are affected by climate change and anthropogenic activities such as land disturbance and intensified grazing (Mitchell, 2010). In particular, temperate grasslands of the northern Great Plains are at greater risk since they are arid in nature and largely rely on soil microorganisms to supply nutrients for plant growth (Parry et al., 2007; Macdonald et al., 2004; Parton et al., 1994; Tisdale et al., 1975). Climate change models suggest that precipitation in northern temperate grasslands could increase up to 36% in the next 60 years with increased interannual changes in precipitation events and prolonged drought periods (Jiang et al., 2017; Sherwood and Fu, 2014). This may increase the primary productivity of grasslands but can change species composition, and enhance organic matter decomposition and causing a change in overall C balance of arid and semi-arid grasslands, subsequently shifting soil microbial communities (Bargett et al., 2008; Barnard et al., 2014; Jonas et al., 2015). Due to the positioning of these grasslands, they are prone to extreme events such as drought, blizzards, heat and cold waves, and floods which makes these ecosystems

highly vulnerable (Wuebbles et al., 2017). Furthermore, landuse and changes in management have increased soil degradation and C loss due to the conversion to croplands (Tan et al., 2005).

Droughts directly affect soil microorganisms by reducing soil moisture content in their immediate environment and indirectly by limiting the substrate supply for microorganisms via dissolution and diffusion, which could alter C cycling and plant productivity (Schimel, 2018). Furthermore, overgrazing makes temperate grasslands more vulnerable to future drought conditions because the excessive removal of vegetation and litter cover can exacerbate drought effects (Ruppert et al., 2015). Drought and grazing could alter their ecosystem functioning (such as substrate limitation for soil microorganisms and C cycling; Schimel et al., 2007). The main factors affecting soil C storage could be divided into environmental (such as mean annual precipitation and soil type) and biotic factors (i.e., plant species composition, grazing frequency and intensity; McSherry and Ritchie, 2013).

Grazing intensity can play role in soil structure and capacity to storge organic C thereby changing soil C strocks (Cui et al., 2005). Soil organic C has a significant influence on ecological goods and services provided by grasslands, therefore, changes in soil C stocks can ultimately affect soil productivity, especially these affects could be magnified due to climate change such as drought (Lal, 20009; Rounsevell et al., 1999). High grazing intensity has been known to alter species composition by reducing soil moisture content, consequently reducing net primary production (Pineiro et al., 2010). On the other hand, some studies found that high grazing intensity may increase soil C sequestration when mean annual precipitation is less than 600 mm (Derner and Schuman, 2007; McSherry and Ritchie, 2013), which suggest that the relationship between grazing and soil C is usually non linear, because of various factors involved. For example, another study found that higher grazing intensity increased C in C4 and

mixed grasslands but decreased C3 dominated grasslands (McSherry and Ritchie, 2013). There mixed results could be to due the fact that grazing interactions have been found with elevation, temperature, soil depth, livestock type and local climate conditions (Lu et al., 2017; Zhou et al., 2017). I applied a clipping treatment to similar different grazing regimes with different timing and intensity of defoliation allowing uniform application of treatments across various sites (Batbaatar et al., 2023; Filazzola and Cahill, 2021; Waterman et al., 2019). It should be noted that clipping is not the same as grazing, because it does not have secondary effects, which grazing have, such as animal selectivity, saliva-induced responses and trampling by animals. However, it was necessary to apply clipping as a simulation of grazing to allow the uniform application in a controlled environment and to allow for the rainout-shelters (simulated drought) which cannot be used in the presence of livestock.

Soil organic matter is the important fraction of the soil consisting plants, residues and animal tissues (biomass) which is decomposed in the soil over the time. It is divided into different types based on the physical fractions such as particulate organic matter (POM; >53mm) and mineral-associated organic matter (MAOM; <53mm; Panagos et al., 2022). Soil organic matter which is associated with mineral surfaces is known as MAOM while the POM is larger and lighter fraction of organic matter which is easily decomposable compared to MAOM (Lavallee et al., 2020). Larger part of persisting organic matter is made up of MAOM because when organic matter is associated with mineral surfaces, it becomes less accessible to decomposition by microbes (Derrien et al., 2023). However, that may not always be the case since studies have shown that the persistence of organic matter in the soil cannot be evaluated only by physical fraction or chemical composition of organic matter (Amelung et al., 2008). Environmental conditions such as soil temperature, pH, microbial activity and soil moisture

content are important factors that play role in the persistence of organic matter (Keiluweit et al., 2016). Additionally, organic matter is decomposed primarily by various enzymes which are released by soil microorganism and those enzymes are specific to breakdown certain moleculre. Therefore, to understand the persistence of organic matter in the soil, it is of crucial importance to test and evaluate the soil microbial communities including composition and diversity (Derrien et al., 2023).

In this thesis, I tested the effects of simulated drought and defoliation (mimicking grazing) regimes on different soil microbiological properties, greenhouse gas emissions and activity of soil enzymes to understand how a climate stressor (drought) interacts with anthropogenic disturbances (here defoliation) to impact the soil nutrient cycling and biogeochemical processes in northern temperate grasslands (Figure 1.1). Specifically, the thesis consists of three data chapters to cover the effects of drought and defoliation on i) soil extracellular enzyme activity, ii) greenhouse gas emissions, and iii) soil microbial communities (composition, diversity and ecological functions related to various taxa). I used simulated drought and defoliation (instead of grazing) in this study because defoliation provides a more controlled approach to mimic the grazing uniformly among experimental plots and reduce the livestock's selectivity, therefore providing a more uniform treatment application across various sites (Waterman et al., 2019; Amgaa, 2022).

1.1.1 Extracellular enzyme activity

Plants and particularly microorganisms release extracellular enzymes in the soil to break down organic matter and aid in obtaining nutrients (Baldrian, 2014). Enzymes in the soil are

widely used as a proxy for soil organic matter decomposition and nutrient cycling (Burns et al., 2013; Cheng et al., 2017; Luo et al., 2017). Enzyme activity in the soil is the primary mechanism by which soil microorganisms regulate nutrient cycling; enzyme activity in a given soil can reflect the active fraction of organic matter (Kelley et al., 2011; Logsdon et al., 2008). Enzymatic activity in soils may respond rapidly to biotic (grazing) and abiotic stressors (drought and heat) due to their sensitivity and substrate specificity (Bell and Henry, 2011; Hewins et al., 2016). Due to their critical role in belowground biogeochemical cycling and sensitivity, enzymatic activity can be used to understand the effect of precipitation and grazing on soil nutrient cycling. Soil extracellular enzyme activity has been known to reduce under drought conditions due to the altered soil microbial biomass, activity and community composition (Kwon et al., 2013; Piton et al., 2020; Sardans and Peñuelas, 2005; Steinweg et al., 2013; Xiao et al., 2018), however, some studies found the opposite (Ochoa-Hueso et al., 2018) or no effect of drought on enzyme activity due to the adaptability of soil microbial communities to stress, especially in arid regions (Kreyling et al., 2008).

Drought effects on enzyme activity were mainly found to be negative; grazing, on the other hand, had more variable effects on soil enzymatic activities. For example, rotational grazing may stimulate soil enzymatic activity and increase organic matter turnover (Garcia et al., 2011). On the other hand, a negative relationship between stocking rates and soil microbial and enzymatic activity was found in Canadian grasslands in Manitoba (Banerjee et al., 2000). Similarly, grazing decreased the activity of enzymes related to C cycling in the northern temperate grasslands of Alberta (Hewins et al., 2015), and another study of defoliation showed that frequent defoliation reduced enzymatic activity of enzymes related to C and P cycling (Hewins et al., 2016). Many other studies also observed mixed effects of grazing and/or drought

on enzyme activities (Allison et al., 2010; Allison and Treseder, 2008; Kardol et al., 2010). However, what is more, challenging is that it is very rare for a single stressor (biotic or abiotic) to occur in different ecosystems as these systems are generally concurrently exposed to a combination of biotic and abiotic factors, which suggests the need for experimental designs covering more than one stressor to address their interactions and related uncertainties (Crain et al., 2008; Ma et al., 2020). While a few studies have tested the effects of multiple stressors (such as precipitation and grazing), the next challenge for the scientific community is to find a way to generalize the findings from different studies (Hewins et al., 2016; Xiao et al., 2018). In addition, most studies focus more on aboveground productivity than biogeochemical processes (Esch et al., 2013). Therefore, a study spanning a climate gradient covering variable environmental conditions could potentially help understand the interactive effects of abiotic (drought) and biotic (vegetation removal, i.e., defoliation) on soil extracellular enzyme activity or soil microbial communities and nutrient cycling in general.

1.1.2 Greenhouse gas emissions

Terrestrial ecosystems are one of the main sources of greenhouse gas emissions (namely CO_2, N_2O and CH_4). Grassland ecosystems could potentially reduce greenhouse gas (GHG) emissions as these systems can become a net C sink (Allard et al., 2007; Chang et al., 2021). However, anthropogenic activities, such as livestock grazing, could increase grassland GHG emissions (Cardoso et al., 2017). The primary source of CO_2 emissions from grassland is soil microbial respiration, through which microbes break down organic matter to obtain energy and release CO_2 into the atmosphere (Conrad, 1996). Furthermore, plants ingested by livestock are respired back into the atmosphere to a large extent (Soussana et al., 2007). Nitrous oxide is

released from the soils when microorganisms transform nitrogen via nitrification and denitrification pathways (Bouwman, 1998; Wrage et al., 2001). The main sources of CH_4 emissions are livestock and anaerobic soils, but methane is mainly consumed in aerobic soils via microbial oxidation (Soussana et al., 2004). The exchange of GHG emissions between grasslands and the atmosphere is mainly dependent upon soil type, % vegetation cover and management practices (Derner et al., 2006; Oertel et al., 2016). Therefore, anthropogenic activities such as livestock grazing frequency and intensity could potentially regulate GHG emissions. On the other hand, arid grasslands are also affected by climate change, and warmer temperatures increase GHG emissions (IPCC, 2021). Combined, These two factors can alter soil nutrient cycling and thus could make grasslands a net source of GHG emissions (Zhu et al., 2016).

Various studies have tested the effects of drought or grazing on GHG emissions as individual treatments (Bai et al., 2015; Liu et al., 2015; Schwalm et al., 2012; Tian et al., 2016; Yan et al., 2018). However, studies covering their interactive effects are limited and often provide inconsistent results due to the complexity of grassland ecosystems and different methodologies (Yue et al., 2017; Zhou et al., 2014). Inconsistent findings are often the result of variations in treatments, such as different grazing regimes or simulations of precipitation at different rates (Zhu et al., 2015; Zhou et al., 2019). Therefore, an experimental design with the same defoliation intensities and frequencies and simulated drought levels across various sites has the potential to provide a more standardized evaluation of C and nutrient cycling in these arid and semi-arid grassland ecosystems.

1.1.3 Soil microbial communities

Soil microorganisms are vital for the stability of grassland ecosystems as they transform and decompose organic matter to provide nutrients for plants in the soil, especially in those systems where fertilization is not common (Tibbett et al., 2019; Yang et al., 2013). Therefore, underlying soil microbial communities directly affect plant productivity in these ecosystems (Kardol and Wardle, 2010). Previous studies suggest a decline in soil microbial biomass, activity and respiration rates under drought conditions (Carbone et al., 2011; Wu et al., 2011; Wu and Brookes, 2005). However, soil microbial composition is relatively more resistant to drought (Barnard et al., 2015; Kakumanu et al., 2013). As soils become dry, they can reduce the resource use efficiency of microorganisms as well as limit the resources due to altered diffusion (Schimel, 2018). This could cause a shift in the community as not all microorganisms behave the same in the soil. In such circumstances, some microbial communities may adapt to a severe drought by dormancy while others may die, providing more nutrients for the surviving community (Bogati and Walczak, 2022). On a broader level, the general consensus is that soil fungal communities are more resistant to drought than soil bacterial communities (de Vries et al., 2018). Within bacterial communities, because of their thick cell wall, gram-positive bacteria are relatively more resistant to drought as compared to gram-negative bacteria (Bogati and Walczak, 2022).

Effects of defoliation on soil microbial communities are not widely studied and are often inconsistent among different ecosystems. For example, Ma et al. (2018) found that defoliation reduced alpha and beta diversity, while Attaeian (2010) found no effect of defoliation on either microbial composition or diversity. But how soil microbial communities would be affected by defoliation and grazing regime together in grassland ecosystems under future drought conditions is unclear because the majority of the studies focus more on either effect of extreme natural events (such as drought) on soil microorganisms (Hartmann et al., 2017; Hueso et al., 2012;

Preece et al., 2019) or only on anthropogenic disturbances (Ford et al., 2013; Qi et al., 2011; Wakelin et al., 2009). In temperate grasslands, soil fungal communities were shown to be resistant to simulated drought as opposed to bacterial communities, which increased with increasing precipitation and decreased (in relative abundance) under drought (Li et al., 2022). Defoliation also tends to decrease soil microbial activity in northern temperate grasslands (Guitian and Bardgett, 2000). However, how soil microbial communities would respond to these factors simultaneously (which is always the case in terrestrial ecosystems) is not well explored.

1.2 Thesis structure

The overall goal of this thesis was to test the effects of drought and defoliation on biogeochemical processes, soil microbial communities and GHG emissions across seven locations in temperate grasslands of Alberta, Canada.

The thesis was divided into five chapters based on the parameters tested. The first and fitfth chapters are introduction and conclusion of complete thesis respectively. The second chapter investigated the effects of drought and defoliation on soil extracellular enzyme activity. The specific objectives were to test i) how extracellular enzyme activity responds to drought (reduced rainfall) and modified defoliation regimes and their interaction at seven locations across temperate grasslands in northern Great Plains, ii) how the patterns of enzymatic activity among different treatments change with environmental factors (rainfall, soil temperature). The third chapter (Chapter 3) investigated the effects of drought and defoliation on greenhouse gas emissions (CO_2, N_2O and CH_4) across seven locations in northern temperate grasslands. Specific objectives of chapter 3 were to i) test if combinations of defoliation intensity and frequency

interact with drought to alter GHG emissions, ii) identify which treatment and environmental factors were most important in regulating grassland soil GHG emissions. The fourth chapter tested the effects of drought and defoliation regimes on soil microbial communities (microbial diversity, composition, and ecological functions) at two distinct sites (mesic vs xeric). Specific objectives of this chapter were to test i) community-level responses of soil bacterial and fungal communities to drought and defoliation, ii) taxa-level responses of soil bacterial and fungal communities to drought and defoliation, iii) responses of relative functions of soil microbial taxa to drought and defoliation (and their interactions) treatments, and iv) relationships between environmental variables (such as pH, soil moisture content, soil temperature) and biotic factors (extracellular enzyme activity) with soil microbial community's functions.

Figures

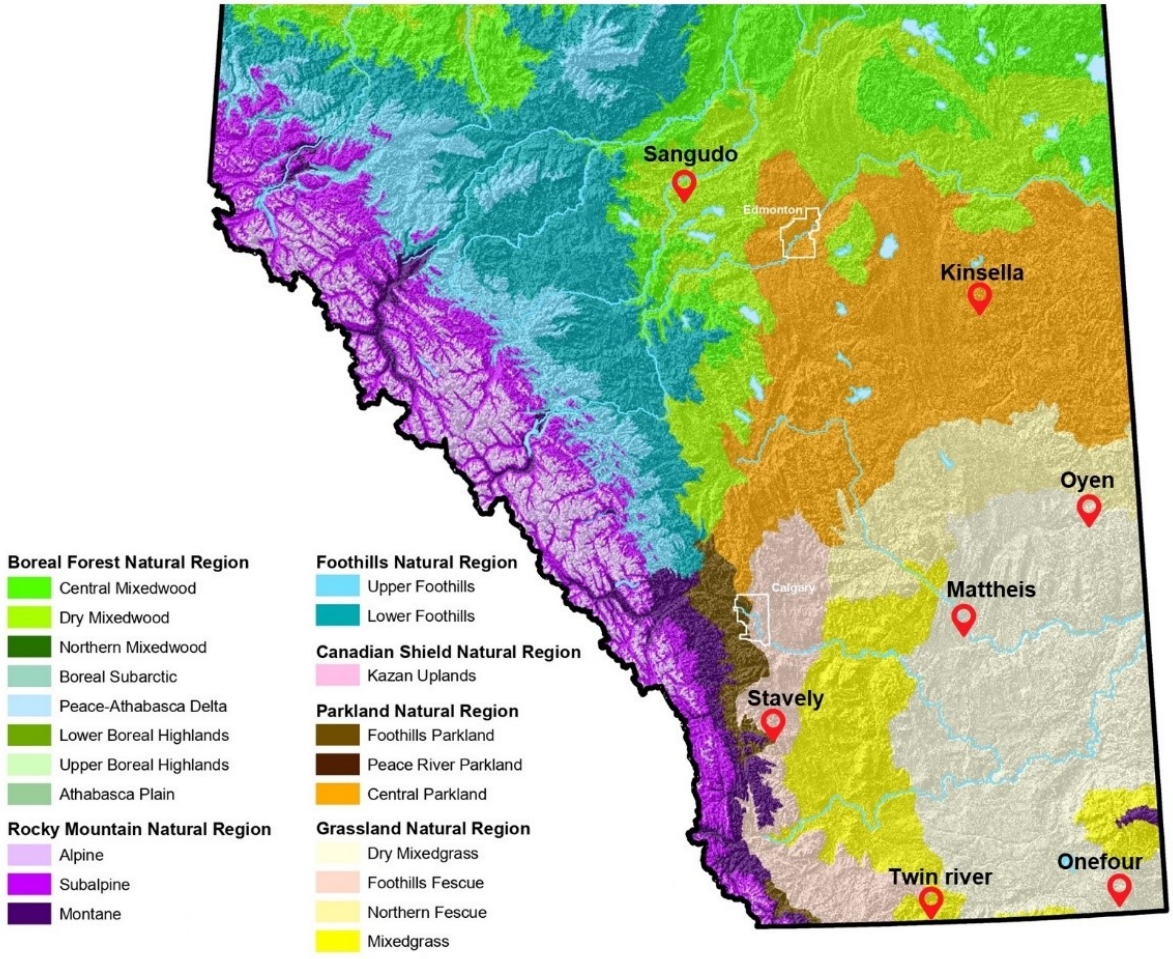


Figure 1.1 Map of southern Alberta showing the locations of seven sites (red pins) included in the experiment. Original map was obtained from Open Government Program of Alberta (www.open.alberta.ca)

2. Chapter 2 - Soil extracellular enzyme activity response to drought and defoliation in northern temperate grasslands

2.1 Abstract

Grassland ecosystem functions are affected by climate change (i.e. increases in temperature and altered precipitation patterns). Managed grazing, including the timing and intensity of defoliation, may interact with climate change to differentially affect processes related to soil organic matter decomposition. Soil extracellular enzyme activity (EEA) provides an integrated measure of soil microbial activity which affects soil nutrient cycling. However, the response of EEA to climate change and grazing may not be universal across different grassland ecosystems. This study examined soil EEAs in response to five experimentally-imposed defoliation regimes and drought (45% rainfall reduction using rainout shelters) at seven grassland locations across a climatic gradient in the Northern Great Plains of Alberta, Canada. All sites were dominated by perennial grasses and forbs, but differed in dominant plant species, climate, and soil characteristics. Soil samples were collected over 2 years (2017 and 2018) and analyzed for five EEAs involved in carbon (C), nitrogen (N) and phosphorus (P) biogeochemical cycling. Drought reduced activity of enzymes involved in C cycling, β-glucosidase and β-cellobiosidase by 16 and 17%, respectively, P cycling (acid phosphatase) by 11%, and N cycling (N-acetyl-β-glucosaminidase) by 12%. Non-metric multidimensional scaling revealed a positive association of β-xylosidase activity with drought suggesting a reduction in C turnover under future drought conditions. C-acquisition enzymes, and in particular β-glucosidase activity, were reduced by an intermediate defoliation regime relative to both control and heavy defoliation. Acid phosphatase and N-acetyl-β-glucosaminidase were affected by a three-way interaction of drought, defoliation and mean growing season precipitation, which highlights the complex mechanism underlying EEA responses to changes in the environment. Overall, the findings of

this study suggest that soil EEA was affected by drought, but defoliation effects were largely dependent upon rainfall treatment and local climate.

2.2 Introduction

Grassland ecosystems cover approximately one-third of the world's land, and store 20% of the global soil organic carbon (SOC) stock (Stockmann et al., 2013). Grasslands of the Northern Great Plains are used extensively for livestock grazing and are expected to experience more frequent drought because of climate change (Morgan et al., 2011). Climate change and livestock grazing may alter grassland soils and associated ecosystem functions, including the activity of soil microbial communities (Schimel et al., 2007; Xun et al., 2018), with implications for the ability of grasslands to mitigate climate change through carbon (C) storage. The activity of soil microbes is sensitive to changes in the environment (Dick and Tabatabai, 1992; Wang et al., 2018) and alterations to microbial activity can impact soil nutrient cycling (Gougoulias et al., 2014). Climate change is expected to increase interannual variation in temperature and precipitation, and in turn, levels of evapotranspiration (i.e., moisture stress) across the Great Plains (Meehl et al., 2007; Smith et al., 2014). In particular, even though mean annual rainfall is expected to increase (Parry et al., 2007), drought is likely to become more common because of less frequent but intense precipitation events (Eldridge et al., 2016; Török et al., 2016).

Soil microorganisms play a crucial role in ecosystem functioning, particularly in the cycling of C and other nutrients such as nitrogen (N) and phosphorus (P) by releasing extracellular enzymes that break down complex organic matter and plant residues to release nutrients back to the soil (Liang et al., 2017). Soil extracellular enzyme activity (EEA) is an

indicator of biological activity in soils, particularly that of the soil microbial community (Das and Varma, 2010). Enzyme activities in soils are known to be a rate-limiting step in microbial-mediated soil organic matter decomposition (Cheng et al., 2017; Das and Varma, 2010; Gomez et al., 2020; Sinsabaugh, 2010;), and are, therefore, an indicator of transformation rates within organic material (Burns et al., 2013). Levels of EEA are primarily regulated by temperature, moisture, substrate abundance and quality, and other soil properties (e.g., pH, texture, porosity) (Burns and Dick 2002). Consequently, EEA can provide insight as to how environmental changes, including land use activities, affect soil microbial activity, and, ultimately, soil nutrient cycling (Bell et al., 2013; Henry, 2012; Nannipieri et al., 2012). Enzyme assays are sensitive to management change and therefore provide an early indication of soil organic matter status and turnover as they degrade the major components of soil organic matter (Fansler et al., 2005; Schimel and Weintraub, 2003).

Water availability is an important driver of soil microbial biomass and activity (Clark et al., 2009; Hawkes et al., 2011; Hueso et al., 2012). Even though microbial biomass can remain stable or increase through extended dry periods lasting several months (Landesman and Dighton 2010; Parker and Schimel 2011; Schaeffer et al., 2017), EEA may decrease or remain stable (Alster et al., 2013; Sardans and Peñuelas, 2005). Many studies in the past have observed enzyme activity as a direct expression of organic matter transformation rates in response to climate change within different ecosystems (Bell et al., 2013; Chuan et al., 2020; Luo et al., 2017; Todd-Brown et al., 2012) and found that EEA generally decreases under drought conditions (Piton et al., 2020; Sardans and Peñuelas, 2005) but contradictory findings have also been observed (Ochoa-Hueso et al., 2018). Furthermore, Kreyling et al. (2008) found no change in EEAs in arid and semiarid environments due to the high adaptability of soil microbial

communities to drought stress. Moreover, most previous studies (e.g., Allison et al., 2010; Allison and Treseder, 2008; Kardol et al., 2010) include only one or a few study locations, making extrapolating results across different environmental conditions difficult.

The management of livestock grazing includes modifying the timing and intensity of defoliation, necessitating an understanding of whether and how different grazing practices alter the effects of drought on soil biogeochemical processes such as EEA. Changes to grazing management, e.g., stocking rates, are important for maintaining soil C pools and microbial processes such as decomposition and stabilization of organic matter (McSherry and Ritchie, 2013; Salvati and Carlucci, 2015) and reducing atmospheric carbon dioxide (Wang et al., 2014). Grazing may affect microbial communities and EEA activity through the removal of vegetation and subsequent effects on soil properties (Hewins et al., 2016; Ma et al., 2021). Additionally, defoliation can alter soil properties through the removal of foliage and litter that together insulates the soil (Deutsch et al., 2010). However, the effects of grazing on EEA may also depend on the timing of defoliation, potentially in concert with different intensities.

Given the uncertainty of grazing impacts on biogeochemical cycling processes, and the potential for the dependency of defoliation effects on environmental conditions, a study including various sites with different environmental conditions is needed to understand better the potential effects of defoliation and drought on EEA in grassland ecosystems. While we generally expect that drought will reduce EEA due to enzyme dependence on water availability (Sardans and Peñuelas, 2005), how drought interacts with different defoliation regimes is uncertain since there is very little information about how changes in vegetation and its interaction with drought affects soil EEA.

In this study, our objectives are to: 1) test whether EEA responds to reduced rainfall and modified defoliation regimes, at seven locations across the Northern Great Plains, in Alberta, Canada, and 2) test how the patterns of EEA depend on environmental changes (rainfall, soil temperature) associated with the treatments. Results from this study will provide insight into how drought and defoliation act to alter EEA, thereby enabling inference about the fate of grassland ecosystem function, including biogeochemical cycling and associated soil microbial activity, under future climate change and grazing management scenarios.

2.3 Materials and Methods

2.3.1 Study sites and experimental design

The experiment was conducted at seven grassland sites located across a precipitation and temperature gradient in Alberta, Canada. All sites were dominated by perennial grasses and forbs, with unique plant composition and soil characteristics (Table 2.1), with mean annual precipitation (MAP) ranging from 315 to 532 mm and mean annual temperature ranging from 2.3 to 5.2 °C. At each site, we established a fully factorial experiment in the summer of 2016 with two rainfall treatments and five defoliation treatments, and 4 or 5 replicates at each site. Defoliation treatments were applied via manual clipping and/or using a lawnmower to create combinations of varying frequency and intensity of defoliation. The frequency of defoliation included a single clipping done in either June or September and in both months for plots that were clipped twice. The intensity of defoliation included a heavy clipping that removed vegetation to a 3 cm stubble height, while a light clipping reduced vegetation to 7 cm. In total, we created five defoliation treatments: heavy-heavy, light-heavy, heavy-none, none-heavy and none-none (i.e., non-defoliated control), where the first intensity designates the June defoliation

and the second a September defoliation. Heavy-heavy and light-heavy plots were clipped two times each year, in June and September. Heavy-none plots were clipped once a year (June), and none-heavy plots were clipped once a year (September). Rainfall was reduced to 45% of the ambient using rainout shelters (Gherardi and Sala, 2013) in drought plots (Figure 2.1), while the other plots experienced ambient rainfall conditions. Most sites had 4 replicates of each treatment combination, while two sites, Kinsella and Mattheis, had 5 replicates, creating a total of 300 study plots, each plot was 2.5 by 2.5 m (the size of the rainout shelter). Treatments were applied in 2016 and continued through 2018.

2.3.2 Soil sampling and processing

In early August 2016, five soil samples (5 cm diameter) were randomly collected from the 0-15 cm layer at each site in order to describe site-level soil characteristics such as soil texture (Table 2.1). To assess treatment effects, soil samples were collected from each plot in early May, mid-June and mid-August of 2017 and 2018. Soil cores were collected using a 3.25 cm diameter soil corer to a depth of 15 cm. Five cores were collected from random points in each plot at each sampling time and mixed to form a composite sample. Samples were immediately placed in a cooler with dry ice and kept frozen during transport to the lab, and then stored at -20 °C until further analysis. All samples were sieved (2 mm) to remove coarse fragments and visible roots. Subsamples were taken from each sample to measure gravimetric soil water content and soil pH. Gravimetric soil water content was determined by drying 40 g of soil at 100 °C for 48 hr. Soil pH was determined using a 1:5 soil:deionized water suspension (Rayment and Higginson, 1992) using a Fisherbrand Accumet AB150 pH benchtop meter (Fisher Scientific, Waltham, MA, USA).

We selected and measured five EEAs; β-glucosidase (BG), β-D-cellobiosidase (CELLO), β-xylosidase (XYLO), acid phosphatase (AP) and N-acetyl-β-glucosaminidase (NAG) in each soil sample (Saiya-Cork et al., 2002) based on their significance and function in the soil, targeting the cycling of C, N and P (Appendix 1). Briefly, 1 g of soil was buffered in 50 mM sodium acetate buffer solution (Bell et al., 2013; German et al., 2011). The pH of the buffer solution was adjusted to the field pH (±0.2) of the sample (Chuan et al., 2020; Hewins et al., 2016) using acetic acid to stabilize and control the fluorescence intensity, which is highly pH dependent (Marx et al., 2001). Enzyme activity was measured in 96-well Costar black polystyrene microplates (Corning Inc., NY, USA). After the addition of substrate, microplates were incubated in the dark at room temperature for 4 hr. Microplates were read using a SpectraMax M3 microplate reader (Molecular Devices LLC., Sunnyvale, CA, USA) at 365 nm excitation and 450 nm emission. Final enzyme activities were calculated in nmol substrate converted per gram of dry soil per hour (Bell et al., 2013; German et al., 2011). Furthermore, the geometric mean of BG, CELLO and XYLO was calculated to examine the effects of treatments on the total activity of all three C-acquisition enzymes (CEEA) (Chuan et al., 2020).

2.3.3 Statistical analysis

The effect of treatments on the individual enzymes, and CEEA, were examined with linear mixed models using the lme4 package (Bates et al., 2015) in R (R Core Team, 2019). To observe the generalized main and interactive effects of rainfall and defoliation treatment, both treatments were considered fixed factors (with interactions), while site, plots, years and months were treated as random factors.

Furthermore, to assess the effects of rainfall and defoliation treatments across different climate conditions on average annual EEAs , a linear mixed model was used where the rainfall treatment, defoliation, and mean growing season precipitation (GSP) were considered as fixed factors (with interactions) and site and year were considered as random factors. Mean GSP from May to September for each site was obtained from ClimateNA (Wang et al., 2016) for both years (2017 and 2018). Average annual enzyme activity was calculated by adding EEA analyzed throughout the year from each plot and divided by number of times samples were collected.

A non-metric multidimensional scaling (NMDS) ordination was performed to examine the relationship among the EEAs (BG, CELLO, XYLO, AP, NAG), environmental factors (long term mean annual precipitation, soil moisture content, soil temperature, soil C and N content and soil pH), the 7 study sites and the two primary treatments (rainfall and defoliation) tested in the study. Environmental factors were fitted using the *envfit* function in the *vegan* package in R (Oksanen et al., 2019). A biplot was constructed to visualize the relationship of environmental factors (soil pH, soil moisture content, soil temperature, and soil C and N content) with EEAs relative to the various study sites and treatments, where the latter were plotted as centroids and sites were shown as ellipses (75% CI). To directly test for the effects of rainfall and defoliation treatments on collective EEAs, Permutational Multivariate Analysis of Variance (PerMANOVA) was performed with 999 permutations, where site, rainfall treatment and defoliation were treated as fixed factors on the distance matrix of enzymes using the *adonis2* function in the vegan package in R.

Finally, Pearson correlation coefficients (r) were calculated to observe the association between EEAs and environmental variables (soil moisture content, soil temperature, soil C, N and pH), which were plotted using the *ggscatter* package in R (Kassambara, 2020).

2.4 Results

2.4.1 Extracellular enzyme activity

Almost all EEAs were affected by the rainfall treatment, but only CEEA and BG activities were altered by defoliation (Table 2.2). The activities of BG, CELLO, AP, NAG, and CEEA were reduced by drought by 16, 17, 11, 12 and 11%, respectively, while XYLO was not affected (Figure 2.2).

The activity of CEEA and BG were affected by the defoliation treatments, but note the p-value for CEEA was slightly above our alpha value of 0.05 (Table 2.2); for both these enzymes, activity was greatest under heavy-heavy followed by none-none, light-heavy, none-heavy and heavy-none (Figure 2.3).

Mean GSP, rainfall treatment and defoliation had a significant three-way interaction that affected AP and NAG (the p-value for NAG was 0.069, but given the low sample size we are presenting these responses) (Table 2.3). More specifically, for both AP and NAG, the heavy-heavy and none-none defoliation treatments had larger differences between ambient and rainout shelter treatments with greater GSP, while under the other defoliation treatments, there was little difference in enzyme activity due to the rainfall treatments (Figure 2.4). Additionally, the only C cycling enzyme affected by defoliation, in an interaction with GSP, was BG, which notably had a p-value of 0.076, but the response has been presented due to the low sample size. Generally, EEA activity was greater at locations with greater GSP, with the exceptions of CEEA and XYLO that did not vary with GSP.

2.4.2 Multivariate EEA response to treatments and environmental variables

The non-metric multidimensional scaling (NMDS) resulted in an ordination with two dimensions (k = 2) and a stress value of 0.085. All tested environmental factors were significantly correlated with the ordination (Appendix 2). A biplot of the environmental factors on the NMDS suggested a negative association of XYLO with soil moisture content (MC), and soil C and N content (Figure 2.5). In contrast, BG and CELLO activities were positively associated with MC, MAP, soil C and N. Activity of NAG and AP were negatively associated with soil temperature and pH. Three distinct groups of sites can be observed in the NMDS biplot; Sangudo, Stavely, Kinsella and Oyen are grouped on the left, Twin river in the middle, and Mattheis and Onefour on the right, which corresponds to a general climatic gradient of increasing aridity. Plotting sites on the ordination produced distinct groups, where BG, CELLO and NAG activity were associated with wetter sites, while XYLO activity was more closely associated with the two drier sites. Furthermore, results from the perMANOVA showed that the collective activity of enzymes was significantly affected by rainfall treatment and site (Table 2.4), and the centroids of the treatment drought shifted towards the drier sites associated with XYLO. Defoliation had no effect on the multivariate EEA response. Pearson correlation coefficients revealed that activities of the C-cycling enzymes BG and CELLO were positively related to MC ($p < 0.001$) and C content ($p < 0.001$; Appendix 3). The activity of AP and NAG showed a negative relationship with soil pH ($p < 0.001$). The relationships of other EEA with soil moisture, soil C content and pH were examined, but were not significant and are not shown.

2.5 Discussion

Our experiment, which was repeated at multiple grassland sites, demonstrated that EEA, as expected, is generally reduced by a drought treatment and altered by the timing and intensity of plant defoliation. The responses to defoliation depended on the rainfall at the specific study location or on the rainfall treatment. Furthermore, the responses were specific to particular enzymes which were individually responsive to different environmental conditions.

Overall, the drought treatment reduced enzyme activity and shifted enzyme composition. The EEA reductions we observed are in agreement with numerous other studies reporting EEA reductions due to drought or reduced soil moisture content (Gao et al., 2021; Sardans and Peñuelas, 2005; Sardans and Peñuelas, 2010; Steinweg et al., 2012; Xiao et al., 2018). Drought can reduce EEA by reducing plant biomass and substrate availability resulting in suppressed soil microbial activity (Bardgett et al., 2008), or soil microbial communities can respond to stresses, such as drought, by investing less in enzyme production to survive under suboptimal conditions (Gao et al., 2021; Piton et al., 2020).

XYLO was the only EEA not affected by the rainfall treatment, and ordination revealed that there was a shift in C-cycling enzymes from BG and CELLO, under ambient conditions, to XYLO under drought. Similar patterns were observed within the study region, where neither the addition of water nor defoliation affected XYLO activity (Hewins et al., 2015; Hewins et al., 2016). Activity of XYLO is known to increase with warmer temperatures (Ylla et al., 2012), and our drought treatments may have universally increased soil temperature at all sites (Amgaa, 2022; Carlyle et al., 2011). Furthermore, XYLO has been observed to decrease with the addition of water (Gutknecht et al., 2010), possibly indicating that ambient conditions do not favor its production or activity (Breitkreuz et al., 2021; Mganga et al., 2019). This may be the result of the treatments having no effect on XYLO's primary substrate or the maintenance of its production

through changes in the soil microbial community. The majority of the extracellular XYLO is produced by filamentous fungi, while XYLO is produced by bacteria and archaea that largely remain within cells (Naraian and Gautam, 2018; Polizeli et al., 2005). Soil fungal communities have been observed to be more resilient to drought, changes in soil moisture content and altered plant community composition than bacterial communities in grasslands (de Vries et al., 2018; Deng et al., 2021; Sun et al., 2020). Together these results suggest that XYLO plays an important role in regulating C-cycling within these grasslands during periods of drought, but also within sites that are inherently more arid.

The defoliation regime altered the activity of C cycling enzymes and BG. While some studies have found effects of defoliation and grazing on EEA (Fterich et al., 2012; Xu et al., 2017), effects of defoliation or grazing alone have been largely inconsistent, varying across enzyme type, sites and defoliation treatments (Banerjee et al., 2000; Hewins et al., 2015). In this study, BG, was the only enzyme affected by the defoliation treatment across all sites, which was greater under the heavy-heavy and none-none (no defoliation). That such contrasting treatments lead to similar enzymatic activity raises questions about plant-soil feedback and the role of plants in contributing C sources for microbial activity. BG breaks down cellobiose, the structural unit of cellulose, into glucose; thus, we can speculate that grazing may be changing the availability of cellulose moving into the soil ecosystem. For example, non-defoliated systems may have greater cellulose inputs because of relatively more graminoid biomass and less eudicot biomass (Lyseng et al., 2018). Alternatively, changes in plant community composition can directly affect EEA as defoliation-tolerant plant species can exhibit greater EEA on their tissue (Chuan et al., 2020).

The average annual activities of NAG and AP were reduced by drought under none-none defoliation and the heavy-heavy defoliation only at sites with greater GSP, highlighting the

specificity of enzyme responses to conditions. Phosphatase activity was similarly found to be affected by defoliation at a mesic site but not a xeric site (Hewins et al., 2016) and NAG increased due to light grazing compared to no-grazing (Hewins et al., 2015). As previously highlighted, this pattern may be due to the effect of drought on soil microbial communities, particularly fungal communities, and/or plant community composition (Amgaa, 2022; Sun et al., 2020). Additionally, the activity of these two enzymes decreased with pH and water removal could potentially allow calcium to remain in upper soil layers, buffering pH levels and reducing their activity (Giel and Bojarczuk, 2011; Parham and Deng, 2000). Again, these results highlight the potentially complex mechanisms underlying EEA responses to changes in the environment as two highly contrasted treatments create similar outcomes for enzyme activity.

A general lack of direct response of enzymes (except for BG) to defoliation could be related to the relative stability of soil organic matter in these soils, limiting further microbial decomposition, thereby resulting in no large differences in the release of extracellular enzymes regardless of intensity or timing of defoliation. Within the region, a comparison of SOC revealed small differences in available SOC, but no differences in size fractions due to the long-term removal of livestock grazing (Hewins et al., 2018). Additionally, it is important to note that our treatments tested defoliation rather than actual grazing by livestock. Grazing by livestock includes many additional effects on the grassland, such as vegetation and soil trampling, urine and dung addition and incorporation into the soil, as well as the ongoing selection of plant species over time. The latter alone can influence EEA activity due to differences in EEA associated with litter derived from different grass species (Chuan et al., 2020), which in turn can alter litter decomposition rates (Chuan et al., 2018). The relationships between environmental variables, drought treatment and EEA across sites suggest that drought and local climate and soil

factors have greater control over EEA, at least during the study period, relative to the defoliation, as there was clear differentiation among enzymes at the site level but not within sites. Multiple interactions of defoliation with rainfall treatment and climate on annual average EEA suggest that defoliation effects on EEA are largely contingent upon drought and local climate, or may be the result of defoliation on microclimate.

2.6 Conclusion

Results suggest that drought suppressed EEAs involved in nutrient cycling (including C, N and P) except XYLO which showed a positive relationship with drought treatment, suggesting an increase of XYLO activity under future drought conditions. Direct effects of defoliation were limited to the C-acquisition enzyme BG. Interactions of defoliation with drought and GSP suggests that defoliation effects are contingent upon precipitation and local climatic conditions. Average annual EEA affected by the interactions of rainfall treatment and defoliation with mean GSP suggests that differences between ambient and drought conditions were more prominent at mesic sites. In particular, the converging effects of no defoliation and intense defoliation treatments for some enzyme activities require further mechanistic studies to elucidate their controls. They also highlight the potentially complex controls on enzyme activity.

Tables

Table 2.1 Soil and environmental characteristics, and dominant vegetation at the study sites. Long-term mean annual precipitation and air temperature data were obtained from ClimateNA (Wang et al., 2016). MAP: mean annual precipitation, MAT: mean annual temperature.

Site	MAP (mm)	MAT (°C)	Soil texture	pH (1:2)	Dominant species
Stavely	531.9	3.7	Silty clay loam	5.96	*Festuca campestris, Danthonia parryi, Poa pratensis*
Sangudo	501.4	2.6	Loam	6.71	*Elymus repens, Trifolium repens*
Kinsella	405.9	2.3	Clay	5.73	*Poa pratensis, Agropyron dasystachyum, Pascopyrum smithii*
Twinriver	361.4	5.1	Clay loam	6.51	*Agropyron dasystachyum, Artemisia frigida, Festuca idahoensis*
Oyen	328.6	3	Clay loam	5.57	*Agropyron dasystachyum, Avenula hookeri, Hesperostipa curtiseta*
Onefour	323.9	5.2	Clay loam	6.47	*Agropyron dasystachyum, Hesperostipa comata, Artemisia frigida*
Matthews	315.8	3.9	Sandy loam	6.21	*Hesperostipa comata, Artemisia frigida, Koeleria macrantha*

Table 2.2 ANOVA results (F and P values) for extracellular enzyme activity across seven sites where site was used as a random factor. Significant p-values are in bold ($p < 0.05$). CEEA (C-acquisition enzymes: BG+CELLO+XYLO), BG: β-glucosidase, CELLO: β-Cellobiosidase, XYLO: β-xylosidase, AP: acid phosphatase and NAG: N-acetyl-β-glucosaminidase)

Treatment	F	p	F	p
	CEEA		BG	
Rainfall	15.20	**<0.001**	14.16	**<0.001**
Defoliation	2.36	0.054	3.01	**0.019**
Rainfall x Defoliation	2.24	0.065	1.83	0.123
	CELLO		XYLO	
Rainfall	15.27	**<0.001**	0.11	0.736
Defoliation	0.86	0.489	0.43	0.785
Rainfall x Defoliation	0.84	0.502	1.20	0.309
	AP		NAG	
Rainfall	4.87	**0.028**	8.42	**0.004**
Defoliation	0.91	0.457	1.96	0.100
Rainfall x Defoliation	0.23	0.919	0.82	0.511

Table 2.3 ANOVA results (F and P values) of mean annual extracellular enzyme activity from a linear mixed model where rainfall and defoliation treatments and mean growing season precipitation (GSP) were used as main effects, site as random effects and mean annual enzyme activity was used as a response variable. Significant p-values are in bold ($p < 0.05$). CEEA (C-acquisition enzymes: BG+CELLO+XYLO), BG: β-glucosidase, CELLO: β-Cellobiosidase, XYLO: β-xylosidase, AP: acid phosphatase, and NAG: N-acetyl-β-glucosaminidase), GSP: mean growing season precipitation.

Treatment	F	p	F	p
	CEEA		BG	
Rainfall	1.56	0.213	0.35	0.555
Defoliation	0.81	0.519	1.07	0.368
GSP	0.48	0.490	3.55	0.060
Rainfall x Defoliation	1.36	0.247	1.34	0.253
Rainfall x GSP	0.03	0.870	0.73	0.394
Defoliation x GSP	1.50	0.203	2.12	0.076
Rainfall x Defoliation x GSP	1.43	0.224	1.92	0.106
	CELLO		XYLO	
Rainfall	6.18	**0.013**	0.49	0.485
Defoliation	1.15	0.333	0.89	0.470
GSP	6.53	**0.011**	1.33	0.249
Rainfall x Defoliation	1.27	0.281	0.64	0.632
Rainfall x GSP	0.87	0.352	0.30	0.583
Defoliation x GSP	1.37	0.243	0.57	0.683
Rainfall x Defoliation x GSP	1.36	0.246	0.40	0.811
	AP		NAG	
Rainfall	1.68	0.195	0.00	0.971
Defoliation	2.35	0.053	1.63	0.165
GSP	4.53	**0.034**	4.62	**0.032**
Rainfall x Defoliation	3.21	**0.013**	1.59	0.175
Rainfall x GSP	5.43	**0.020**	1.43	0.232

Defoliation x GSP	2.88	**0.022**	3.62	**0.006**
Rainfall x Defoliation x GSP	3.66	**0.006**	2.19	0.069

Table 2.4 Statistical results of Permutational multivariate analysis of variance (PERMANOVA) analysis testing the main effects of site, rainfall and defoliation treatment on the distance matrix of extracellular enzyme activity. Significant values are in bold ($p < 0.05$).

	Df	SS	R^2	F	p
Site	6	26.1	0.66	592.3	**< 0.01**
Rainfall	1	0.32	0.01	44.3	**< 0.01**
Defoliation	4	0.03	0.00	1.16	0.31
Residual	1782	13.11	0.33		

Figures

Figure 2.1 Rainout shelter to reduce rainfall (by 45%) for simulating drought conditions in the field.

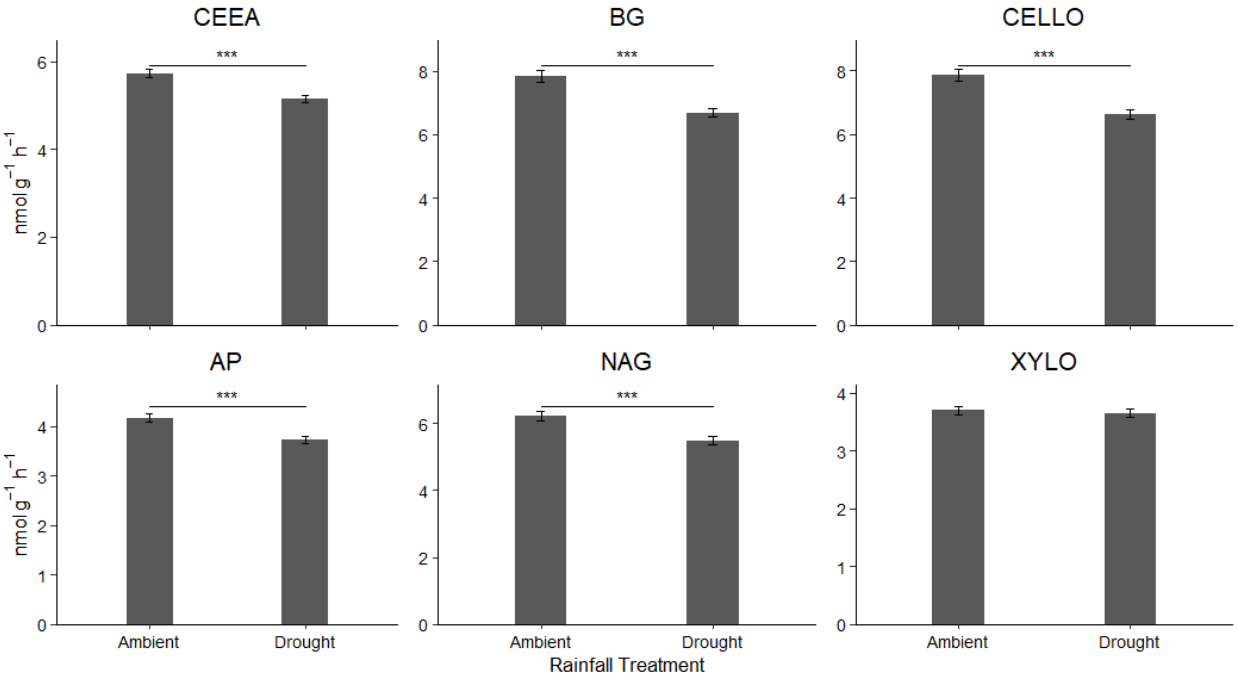


Figure 2.2 Main effects of rainfall treatment on mean extracellular enzyme activity (CEEA: C-acquisition enzymes: BG+CELLO+XYLO), BG: β-glucosidase, CELLO: β-Cellobiosidase, AP: acid phosphatase, NAG: N-acetyl- β-glucosaminidase and XYLO: β-xylosidase). Plots with asterisks "*" differ significantly between rainfall treatments ($p < 0.05$).

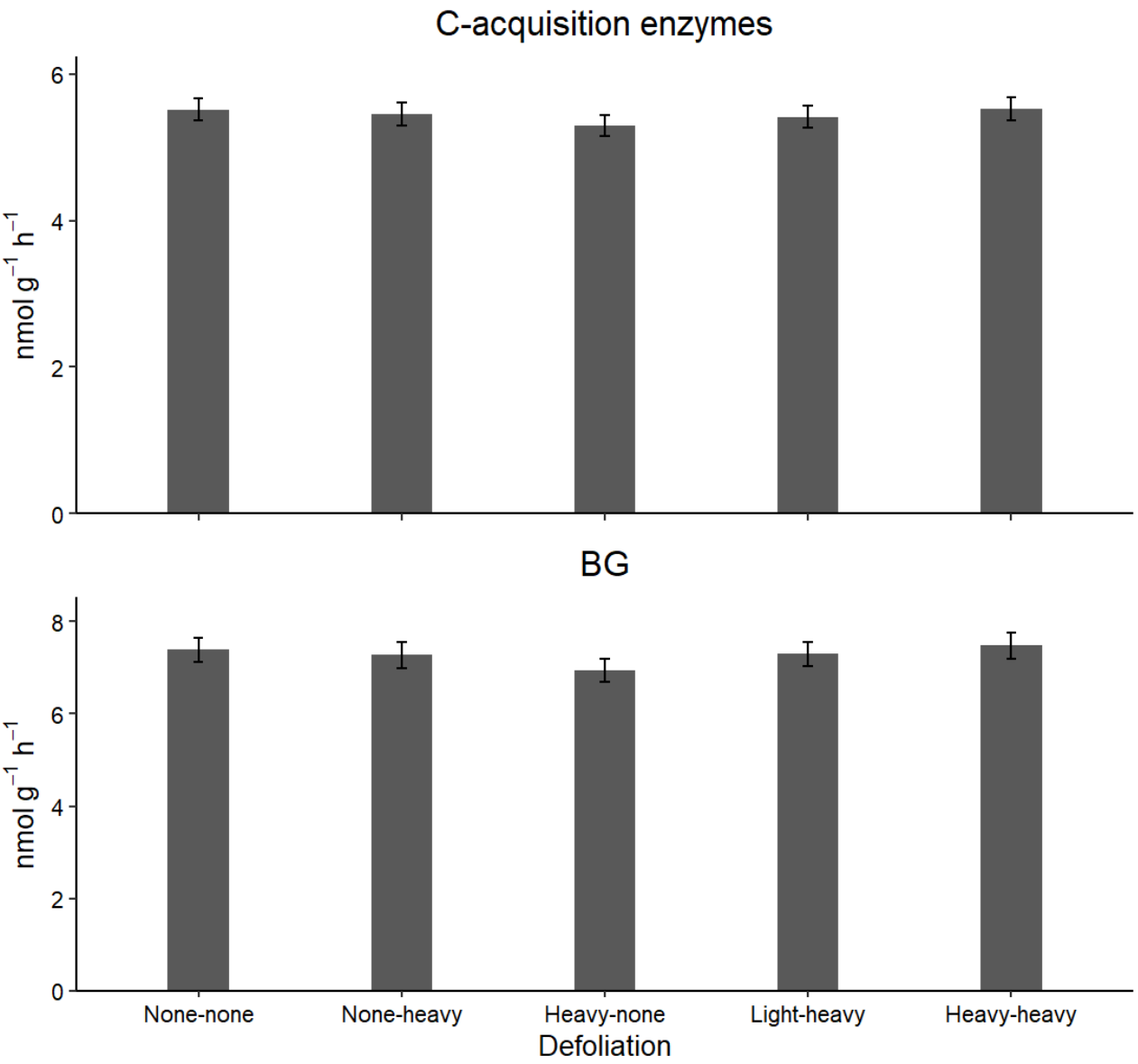


Figure 2.3 Main effects of defoliation treatment on mean extracellular enzyme activity of CEEA (C-acquisition enzymes: BG+CELLO+XYLO) and BG (β-glucosidase).

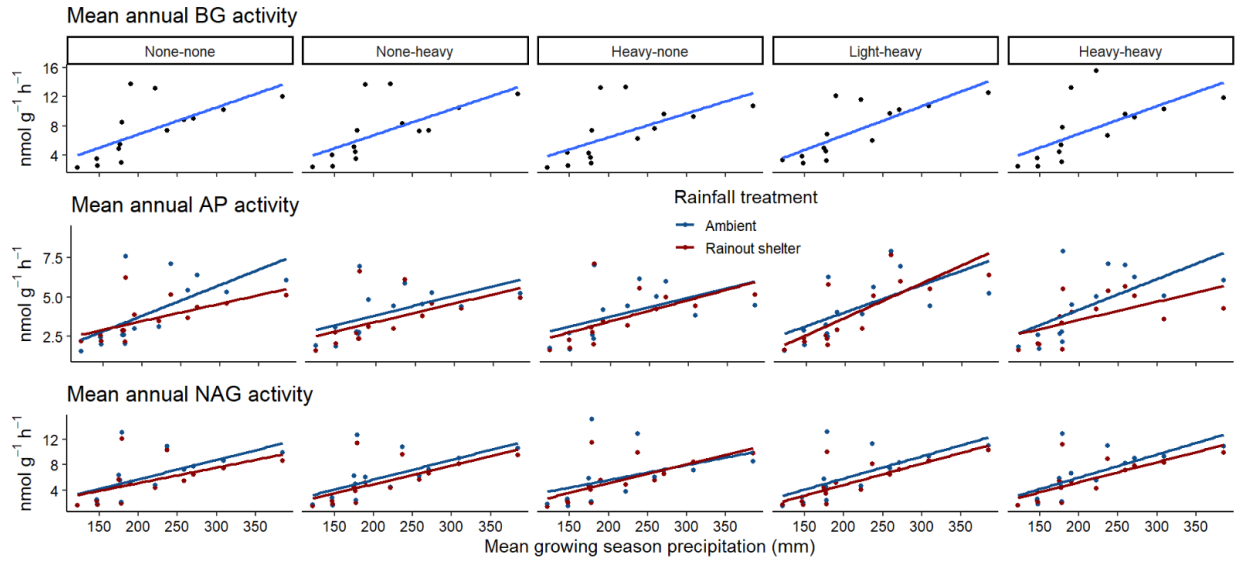


Figure 2.4 Two-way interaction of defoliation and mean growing season precipitation on average annual extracellular enzyme activity of BG (β-glucosidase); and three-way interaction of rainfall treatment, defoliation and mean growing season precipitation on average annual extracellular enzyme activity of AP (acid phosphatase) and NAG (N-acetyl-β-glucosaminidase).

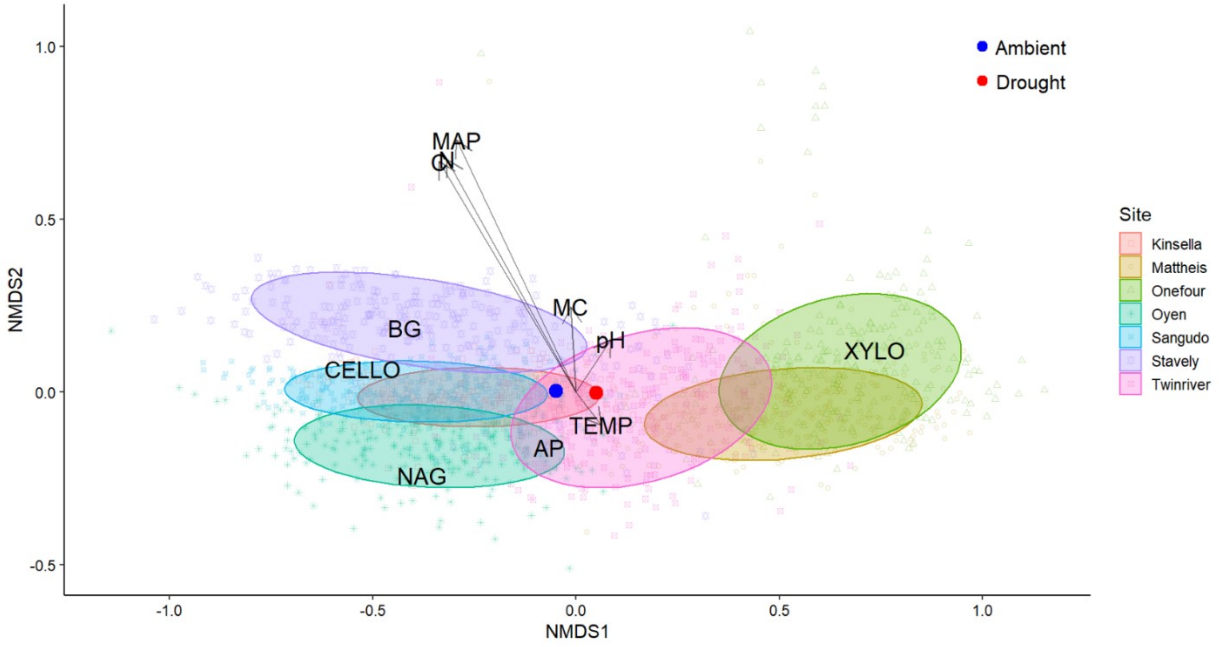


Figure 2.5 Non-metric multidimensional scaling (NMDS) ordination biplot (A) showing the relationship of extracellular enzyme activity (BG: β-glucosidase, CELLO: β-Cellobiosidase, AP: acid phosphatase, NAG: N-acetyl- β-glucosaminidase and XYLO: β-xylosidase) plotted as centroids with environmental factors (MAP: long-term mean annual precipitation, MC: soil moisture content, TEMP: soil temperature, pH, C: soil carbon content and N: soil nitrogen content) and association with the seven sites (shown as ellipses covering 75% of data points) included in the study and relationship of enzyme activity with the rainfall treatment (Ambient and Drought) across seven sites.

3. Chapter 3 - Soil CO_2 emissions were altered by the frequency, but not timing, of defoliation under drought conditions in northern temperate grasslands

3.1 Abstract

Grassland ecosystems play a major role in climate change due to their potential to mitigate greenhouse gas emissions. With proper grazing management, grasslands can act as a sink of atmospheric CO_2 and other greenhouse gases such as N_2O and CH_4. This study tested the effects of five defoliation treatments and multi-year growing season drought (45% rainfall reduction) at seven grassland locations in the Northern Great Plains covering a wide climatic gradient. Perennial grasses and forbs with unique vegetation, climate and soil characteristics dominated all experimental sites. Gas samples were collected from static chambers from April to September 2017 and 2018 (biweekly in April and May, followed by monthly). Samples were analyzed for CO_2, N_2O and CH_4 emissions. Plots defoliated only once a year consistently had reduced CO_2 emissions compared to those defoliated twice a year. Furthermore, defoliation frequency was more important in driving CO_2 emissions, while defoliation timing did not impact greenhouse gas emissions. Emissions of N_2O and CH_4 were not affected by defoliation or drought treatment. Regression models showed that soil temperature and total extracellular enzyme activity were the leading abiotic and biotic predictors for CO_2, N_2O and CH_4 emission. Both soil temperature and enzyme activity had a positive association with CO_2 and N_2O emissions. These findings suggest that limiting defoliation to a single event annually has the potential to reduce CO_2 emissions within these temperate grasslands, particularly under drought conditions and within drier agroclimatic locations.

3.2 Introduction

Grassland ecosystems can act as a source or sink of atmospheric CO_2 and play a major role in affecting climate change (Oates and Jackson, 2014). Proper management of grassland ecosystems, which are extensively grazed by livestock globally, has the potential to reduce emissions of CO_2 and other greenhouse gasses (GHG) such as N_2O and CH_4 by creating a carbon (C) sink (Allard et al., 2007; Chang et al., 2021; Reid et al., 2004). It has been estimated that grasslands have the potential to reduce global GHG emissions by 7.3 $GtCO_2$-equivalent $year^{-1}$ (IPCC 2022). Currently, the majority of arid and semiarid grasslands are affected by multiple anthropogenic activities (such as livestock grazing) and climate change, which can alter biogeochemical cycles and make grasslands a source rather than a sink of atmospheric CO_2 (Wilson et al., 2018; Zhu et al., 2016). Thus, understanding the response of soil CO_2, and other greenhouse gases (N_2O and CH_4), fluxes to grazing and global climate change is of vital importance to better predict future global C dynamics and the sustainability of grassland ecosystems (Chapin et al., 2002; McSherry and Ritchie, 2013). As grazing has been found to cause grassland soil GHG flux to increase (Gao et al., 2018), decrease (Owensby et al., 2006) or have no effect (Liebig et al., 2013), a more specific understanding of the processes regulating GHG fluxes in response to drought and defoliation is needed.

Grazing may affect soil GHG fluxes through a number of pathways as it affects plants and microbes, largely reflecting changes to plant root respiration and microbial soil respiration, respectively (Parkin et al., 2015). For example, grazing decreases aboveground biomass by defoliation, which in turn, may reduce root biomass, leading directly to reduced autotrophic respiration (Bai et al., 2015; Liu et al., 2015). Moreover, grazing affects soil aeration, and coupled with increases in soil temperature, may reduce litter (Tian et al., 2016), collectively

reducing soil moisture content, especially in arid and semiarid grasslands where increased evaporation and erosion are likely due to higher temperatures (Bai et al., 2012). Soil C fluxes in grasslands are also heavily influenced by biotic factors such as the soil microbial community, plant community, forage production, the intensity of grazing management and the type of grazing animal (Conant et al., 2001; Jones and Donnelly, 2004). As a result, grazed grassland soils can be either a source or sink of CO_2, N_2O and CH_4, and soil organic C may increase or decrease depending on how grazing management alters the balance of soil C inputs and decomposition processes (Allard et al., 2007; Plestenjak et al., 2012; Wang et al., 2010; Zhong et al., 2014). For example, heavy grazing can increase soil respiration by increasing soil temperature (Gao et al., 2018; Yates et al., 2000) or have no effect (Liebig et al., 2013). In contrast, cattle grazing may decrease soil respiration by reducing plant biomass and the availability of substrates for soil microorganisms (Owensby et al., 2006). In relation to grazing intensity, soil C typically increases under light to moderate grazing (Abdalla et al., 2018; Hewins et al., 2018) but decreases under heavy and very heavy grazing (Ganjegunte et al., 2005); however, in some systems, greater grazing intensity may promote soil C (Bork et al., 2020).

The effects of drought and precipitation on soil GHG flux can also be positive, neutral or negative. For example, drought can enhance tillering and photosynthetic efficiency in some plant species, thereby increasing C uptake in soils (Jentsch et al., 2011). By comparison, a net reduction in the gross primary productivity of grasslands due to extreme drought reduced net C uptake (Schwalm et al., 2012). A recent meta-analysis revealed that elevated precipitation increased CO_2 emissions and decreased CH_4 uptake, with no effect on N_2O emissions, thereby increasing GHG emissions (Yan et al., 2018); in contrast, decreased precipitation reduced CO_2 and N_2O emissions, while also increasing CH_4 uptake, leading to lower a lower GHG footprint.

Alteration of soil GHG flux under drought is likely the result of reduced microbial activity as reduced precipitation lowers soil microbial biomass C, the fungi:bacteria ratio in soil, litter biomass and root biomass, all of which could further reduce CO_2 fluxes (Yan et al., 2018).

Soil extracellular enzyme activity (EEA), an indicator of microbial activity, plays a key role in soil organic matter decomposition and nutrient cycling, and may therefore influence soil GHG fluxes (Cheng et al., 2017; Rao et al., 2017). Removal of vegetation due to grazing may affect EEA by altering soil microbial communities (Hewins et al., 2016). Furthermore, grazing and defoliation can alter soil properties via foliage and litter removal (Deutsch et al., 2010), which could also alter EEA (see Chapter 2). Observed decreases in EEA under drought conditions may be due to the direct dependence of microbial activity and EEA on soil moisture content (Sardands and Peñuelas, 2005), and could lead to reduced GHG emissions under drought. Soil EEA can be affected by biotic and abiotic factors within soil, and has been extensively studied in relation to soil C (Todd-Brown et al., 2012; Chuan et al., 2020; Bell et al., 2013; Luo et al., 2017). However, limited studies have observed the relationship of EEAs with soil GHG fluxes directly (Shrestha et al., 2020). Thus, exploring the relationship of soil EEA with GHG fluxes in grassland ecosystems may help to understand the role of EEA (and, indirectly, soil microbes) in regulating C and nutrient cycling.

Prior studies have investigated the effects of grazing and drought on GHGs (Aronson et al., 2019; Cardoso et al., 2017; Munjonji et al., 2020), but few have tested the interactive effects of grazing and drought due to the cost involved, methodological challenges and the individual complexity of grassland ecosystems (Zhou et al., 2014). Moreover, the majority of studies have focused on the interactive effects of different climate change factors, such as drought and temperature, rather than their combination with anthropogenic disturbances, such as grazing

(Yue et al., 2017). Results from interactive studies that have been done are often inconsistent or contradict each other, where warming and/or precipitation increased (Zhu et al., 2015), decreased (Lin et al., 2011) or had no effect on GHG emissions (Sharkhuu et al., 2016). Some studies suggest a stronger effect of grazing exists on soil C dynamics than agroclimatic conditions due to the direct effect of defoliation on plant biomass and litter inputs, community structure, soil microbial diversity and associated activity (Zhou et al., 2019). Despite the inconsistent results and complexity of conducting such experiments, knowledge of these interactive stressor effects is crucial to understanding and predicting soil C dynamics in grassland ecosystems. Furthermore, conducting these experiments across a wide range of agroclimatic conditions will provide a more robust test of C dynamics across temperate grasslands, including in relation to the predominant land use.

This study tested the interactive effects of simulated growing season drought and grazing, through the use of rainout-shelters and different defoliation regimes, on soil GHG fluxes at seven northern temperate grassland sites that differed in soils, vegetation and climate. The primary objectives of this study were to 1) test if combinations of defoliation intensity and frequency interact with drought to alter GHG emissions, and 2) identify which treatment and environmental factors were most important in regulating grassland soil GHG fluxes.

3.3 Materials and Methods

3.3.1 Study sites and experimental design

The experiment was conducted at seven grassland locations distributed across a distinct agroclimatic gradient of the northern Great Plains in Alberta, Canada. All study sites were

dominated by perennial grasses and forbs, and varied in climatic conditions, with mean annual rainfall ranging from 315 mm to 530 mm, and mean annual temperature from 2.3 to 5.2 °C (Table 3.1). Soils were representative of the region and mostly (Brown to Black) Chernozems, with one high rainfall site classified as a Luvisol (Table 3.1). The Agricultural Regions of Alberta Soil Inventory Database (AGRASID) was used to obtain soil classification information (Soil Classification Working Group, 1998).

The experiment utilized a full factorial design with five defoliation treatments to simulate grazing in combination with two levels of rainfall treatment, each of which was replicated three times at each of the seven sites, resulting in a total of 210 plots. Rainfall was reduced by 45% using rainout-shelters (Gherardi and Sala, 2013) within the drought plots between April and October each year, while the remaining plots experienced ambient precipitation. Defoliation treatments were applied manually by clipping to achieve the following five intensity-frequency combinations: heavy-heavy, light-heavy, heavy-none, none-heavy and none-none. Heavy-heavy and light-heavy plots were clipped two times a year in mid-June and mid-September to stubble heights of 7 cm - 7 cm and 3 cm - 7 cm, respectively. Heavy-none plots were clipped once a year (in June only) to a 3 cm stubble height, and none-heavy plots were clipped once a year (in September only) to a 3 cm height. The none-none treatment was not defoliated throughout the experiment to represent a non-grazed treatment. Treatments were applied to a 2.5 x 2.5 m area, the size of the rainout shelters, while all soil samples and GHG measures were collected from within the central 1 x 1 m area to avoid edge effects.

3.3.2 Gas sampling and processing

Soil GHG emissions (CO_2, N_2O and CH_4) were measured from April to September (twice a month in each of April and May, and once a month thereafter) during 2017 and 2018 using static round chambers (PVC pipes) (20 cm diameter). Gas chambers were inserted at least 6 cm into the soil at the start of the study and completed across all sites by July 2016. During sampling, the chamber was closed with a lid made of Plexiglas. The lid was lined with aluminum foil to prevent the air temperature inside the chamber from going up during gas sample collection. Gas samples were collected at 0, 10, 20 and 30 minutes after closing the lid using a 20 mL syringe and stored in 12 mL pre-evacuated glass exetainers (Labco Ltd., Lampeter, UK). During gas sampling, the air temperature inside the chamber was recorded with a temperature meter (HH806AU, Omega Engineering, CT, USA). The concentration of each GHG gas (CO_2, CH_4, and N_2O) was later determined on a gas chromatograph (Varian CP-3800, Varian Canada, Mississauga, Canada) equipped with a thermal conductivity detector, a flame ionization detector, and an electron capture detector. Cumulative emissions or consumption of each gas were calculated for each 6 month period of sampling throughout the year using the area under the curve by summing gas fluxes for each interval multiplied by the number of days within each interval.

Soil volumetric moisture content and temperature (°C) were measured using a FieldScout TD 350 Soil Moisture Meter and HH11B Digital Thermometer (Omega Engineering), respectively. Extracellular enzyme activity (EEA) of β-glucosidase (BG), β-D-cellobiosidase (CELLO), β-xylosidase (XYLO), acid phosphatase (AP) and N-acetyl-β-glucosaminidase (NAG) were measured; these EEA were selected based on their significance and function in the soil (Appendix 1), targeting the cycling of C, nitrogen (N) and phosphorus (P). Briefly, 1 g of soil was buffered in 50 mM sodium acetate buffer solution (Bell et al., 2013). The pH of the buffer

solution was adjusted to the field pH of the sample using acetic acid to stabilize and control fluorescence intensity, which is highly pH dependent (Marx et al., 2001). Enzyme activity was measured in 96-well Costar black polystyrene microplates (Corning Inc., NY, USA). After the addition of substrate, microplates were incubated in the dark at room temperature for 4 hr. Microplates were read using a SpectraMax M3 microplate reader (Molecular Devices LLC., Sunnyvale, CA, USA) at 365 nm excitation and 450 nm emission. Final enzyme activities were calculated in nmol per gram of dry soil per hour using the equation in Saiya-Cork et al. (2002).

3.3.3 Statistical analysis

The general effects of rainfall and defoliation treatments on cumulative gas fluxes across all sites were tested with a mixed-effects model using the lme4 package in R (Bates et al., 2015). Residuals were confirmed for normal distribution. Rainfall and defoliation treatments were treated as fixed factors (with interactions), while site and plots within the site were treated as random factors. Data were analyzed for each year separately (2017 and 2018). All statistical analyses were conducted in R (R Core Team, 2019); all graphs were produced using ggplot2 (Wickham, 2016) and means were calculated using emmeans (Lenth, 2019). Significance was assessed using an alpha value of 0.1 due to the limited number of replications at each site.

During preliminary analysis, we observed a clear pattern in the data related to defoliation frequency whereby different responses of CO_2 emissions occurred between the single vs twice defoliated treatments. Therefore, we tested the effect of grazing frequency directly, with comparisons made between the no-defoliation, 1x (single annual) defoliation and 2x (two times annual) defoliation treatments by combining plots based on the number of times defoliation was

applied each year. A linear mixed model was applied where rainfall and defoliation treatments were included as fixed factors and site as a random factor, similar to the previous model.

To test whether defoliation treatments affected GHG flux under drought differentially across an environmental gradient, the individual GHG response ratio of the drought to ambient treatment was calculated for each defoliation treatment within the replicate; this procedure was repeated for each GHG. The log response ratio (lnRR) is most frequently used in ecology to test the effect size (Koricheva and Gurevitch, 2014). A linear model was used to analyze lnRR where defoliation and study site, represented by their mean annual precipitation, were used as fixed factors (with interactions).

Model reduction was used to identify the best predictors (environmental variables and soil enzyme activities) for monthly GHG flux. Predictor variables included as fixed factors within the linear mixed models were soil temperature, soil moisture content, soil pH, and the aggregate activity of all EEAs (BG, CELLO, XYLO, AP and NAG), while site and plots were used as random factors. Aggregate EEA was included to avoid multicollinearity due to the high correlation between EEAs, with a geometric mean (Paz-Ferreiro et al., 2012) computed for all five EEAs; geometric means were used because the enzymes involved had a large variation in their activity levels (i.e., XYLO's mean was 3.36 and BG's mean was 7.53). The MuMIn package (Barton, 2020) in R was used to select among linear mixed models by the Akaike Information Criterion (AIC) estimator. The best-predicted model was selected based on the delta AICc (corrected version of AIC for a small sample size), which measures the relative difference between a particular model and the best-ranked model based on the smallest AICc value (Burnham and Anderson, 2002). The ΔAIC was used to identify the most parsimonious model by

selecting those models within 2 ΔAIC units, since these models are considered to be as good as the best model (Symonds and Moussalli, 2011).

3.4 Results

3.4.1 Drought and defoliation effects on GHG fluxes

In 2017, the interaction of rainfall treatment and defoliation had a significant effect at 0.10 alpha level ($P = 0.057$) on CO_2 emissions (Table 3.2). Under ambient moisture conditions, the CO_2 emissions did not significantly differ between defoliation treatments (Figure 3.1). However, there were differences between defoliation treatments under drought and ambient conditions; the none-none, none-heavy and heavy-none were reduced by drought, while the light-heavy and heavy-heavy were not. There were also differences between defoliation treatments under drought or ambient conditions. Emissions of CO_2 within the heavy-none treatment under drought were lower ($P < 0.05$) than under all the ambient moisture treatments, regardless of the defoliation regime (Figure 3.1A). Relative to the ambient none-none, defoliation did not have any effect on CO_2 emissions, but none-none, none-heavy and heavy-none reduced CO_2 emissions when drought was imposed. On the other hand, drought coinciding with defoliation light-heavy and heavy-heavy increased CO_2 emissions to the point of ambient none-none.

In 2018, the results suggested an interaction effect of rainfall treatment and defoliation ($P = 0.074$) on CO_2 emissions. While CO_2 fluxes once again did not differ among defoliation treatments within a rainfall treatment, there were two significant differences overall; relative to the ambient none-none treatment, CO_2 fluxes were lower within the heavy-none ($P = 0.013$) and none-heavy ($P = 0.045$; Figure 3.1B) when coinciding with drought. The other GHGs (N_2O and CH_4) were not affected ($P \geq 0.10$) by either rainfall treatment or defoliation in either year of sampling (Table 3.2).

When CO_2 emissions were examined in relation to specific defoliation frequency (no defoliation, 1x annual defoliation or 2x annual defoliation), effects of rainfall × defoliation frequency were evident (Table 3.3). Non-defoliated plots under ambient moisture had greater CO_2 emissions than all the defoliation treatments subject to drought conditions, including the non-defoliated control (non-grazed, 1x and 2x defoliation; $P = 0.001$, <0.001 and 0.021 respectively; see Figure 3.2A). Additionally, the ambient 1x and 2x defoliation treatments had greater CO_2 emissions than the drought treatment subject to 1x annual defoliation, but not the twice defoliated drought treatment (Figure 3.2A). Similar results were observed in 2018, with an interaction between rainfall treatment and defoliation frequency. Ambient non-defoliated plots had greater cumulative CO_2 emissions than the non-grazed drought treatment ($P = 0.035$), as well as the 1x defoliated drought ($P = 0.001$) regime (Figure 3.2B). The ambient 1x annual defoliation treatment was also greater in CO_2 flux than the 1x defoliation treatment under drought ($P = 0.004$) (Figure 3.2B).

3.4.2 Drought and defoliation effects on CO_2 fluxes across a precipitation gradient

Cumulative grassland CO_2 emissions varied across the gradient of mean annual precipitation, and were further impacted by defoliation, either alone (2018) or in combination with MAP (2017; see Table 3.4). In 2017 experimental drought decreased CO_2 emissions, though the reduction was larger at lower rainfall sites under all defoliation regimes with the exception of the light-heavy treatment (Figure 3.3A). Under light-heavy defoliation, drought showed a reverse trend of increasing CO_2 emissions, especially at drier sites. Notably, the effect of drought on CO_2 flux reductions tended to weaken and/or disappear at mesic sites, and this pattern was evident under all defoliation regimes (Figure 3.3A). In 2018, CO_2 flux response patterns were similar to

the year before, with drought reducing CO_2 emissions in single annual defoliation treatments and sites; while twice annual defoliated (light-heavy and heavy-heavy) treatments increased CO_2 emissions increased but only on the most mesic grasslands (Figure 3.3B).

3.4.3 Greenhouse gas flux and environmental variables

The best predictors of monthly CO_2 flux were soil temperature and the geometric mean of all five soil enzymes evaluated (Table 3.5; Figure 3.4). The proportion of variance in CO_2 flux explained by the entire model was 83% (conditional coefficient of determination $r^2 = 0.83$), whereas the variance explained by only the fixed effects (rainfall and defoliation treatments) was 12% (marginal coefficient of determination $r^2 = 0.12$). Due to the difference in units of predictors, standardized beta coefficients were calculated for EEA and soil temperature. Standardized coefficients revealed that EEA and temperature both had a positive effect (standardized coefficients of 0.332 and 0.340, respectively) on CO_2 emissions (Figure 3.4 - top). For N_2O, the most parsimonious model was the null model; however, additional models that had a delta AIC of less than 2 included EEA and pH. Standardized coefficients revealed that both EEA (-0.052) and pH (-0.082) had a negative effect on monthly N_2O flux (Figure 3.4 - bottom). For CH_4, the most parsimonious model included only EEA, and no abiotic variables (Table 3.5; Figure 3.4 - bottom). While soil moisture content was included in the model selection, it was not included in any of the most parsimonious models.

3.5 Discussion

This study provides insight into how soil CO_2, N_2O and CH_4 emissions respond to drought, and defoliation imposed at different intensities and frequency. Overall, CO_2 emissions declined under drought, though the exact nature of drought effects also depended on defoliation patterns and environmental characteristics (rainfall) of the site. Furthermore, the effects of defoliation depended primarily on frequency, and to a lesser extent, intensity, indicating that livestock grazing management has the potential to affect overall GHG fluxes within these northern temperate grasslands. Additionally, while soil moisture content is known to be an important control of soil GHG flux (Schaufler et al., 2010), we found that temperature and measures of EEA were ultimately superior predictors of GHG flux rather than moisture, highlighting the need to better understand the role of plants and microorganisms for producing enzymes responsible for regulating C and N cycling.

3.5.1 Defoliation and drought effects on greenhouse gas emissions

Grassland that was defoliated only once, as opposed to twice, had reduced CO_2 soil flux, while twice defoliated treatments consistently maintained elevated CO_2 flux, even under drought conditions. This pattern held through both years of the experiment, including when analyzed either across all defoliation treatments or when treatments were grouped based on a common defoliation frequency. The 2x annual defoliation treatments had more aggregate plant biomass removed and can be thought of as experiencing a higher overall season-long intensity of defoliation. Most previous studies have found contradictory results to ours, with greater defoliation or higher grazing intensity leading to reduced soil CO_2 flux (Cardoso et al., 2017; Wang et al., 2009; Wachiye et al., 2022), or at least a temporary reduction in CO_2 flux during

summer following defoliation (Bork et al., 2019; Shi et al., 2017). However, our study differs by emphasizing the soil GHG response to defoliation in combination with drought treatments.

There are a variety of biological explanations for the pattern observed, although, without further investigation, we cannot identify which may be most plausible. Twice defoliated treatments may have maintained CO_2 flux because of an increase of root exudates from dead roots, as repeated heavy defoliation is known to cause root death (Mikola et al., 2001). Root deaths may also occur under drought and increased temperatures that enhance microbial respiration (Arndal et al., 2018; Mcsherry and Ritchie, 2013). Furthermore, the reduction of CO_2 emissions in 1x defoliation may have been due to the transitory reduction in leaf area and aboveground biomass leading to reduced autotrophic respiration (Owensby et al., 2006; Shi et al., 2022). Increased CO_2 emissions in 2x defoliation are in agreement with previous studies (Frank et al., 2002; Wang et al., 2002; Zhang et al., 2016). Soil respiration, specifically heterotrophic respiration, largely depends on soil temperature, climate, moisture content, and their interactions (Raich et al., 2002). Keith et al. (1997) proposed that about 97% of variability in soil respiration can be explained by soil temperature and moisture content. Increased CO_2 emissions in 2x defoliation under drought conditions may have reduced autotrophic respiration due to the reduced aboveground biomass, but coincidently may boost heterotrophic respiration due to higher soil temperature and lower soil moisture content (Zhang et al., 2016). Similarly, we also identified temperature as an important factor driving CO_2 flux, which could increase in treatments with high-frequency defoliation due to reduced aboveground vegetation cover that allows more solar radiation to reach the soil surface (Deutsch and Bork, 2008), in turn causing greater soil respiration (Zhang et al., 2021).

Importantly, while the frequency of defoliation was important, we found no difference in GHG emissions due to the timing of defoliation (e.g., a single heavy event in spring vs fall), despite well known effects of the seasonality of defoliation and associated grazing management on plant communities (Ash and McIvor, 1998) and associated soil properties (Evans et al., 2012). Other studies that specifically examined different grazing systems have found either no difference in soil GHG flux due to intensive grazing practices (e.g., Ma et al., 2021) or that a higher grazing intensity led to lower GHG emissions (Frank et al., 2002; Gao et al., 2018). While seasonal responses of GHG in relation to grazing intensity have been extensively measured (Cardoso et al., 2017; Liu et al., 2017; Zhu et al., 2015), we find no other studies that specifically tested GHG responses to the seasonality of grazing (i.e., defoliation) frequency, suggesting a need for further research on this topic, including across different ecosystems.

Other GHGs contribute to the net effect of grasslands on climate change, but we found little to no overall effect of the drought and defoliation treatments on CH_4 or N_2O soil flux. Native grasslands in the region similar to these have been identified as CH_4 sinks (Bork et al., 2019; Ma et al., 2022; Thomas et al., 2017), but this was not the case in our study as mean CH_4 flux was very close to zero. This pattern happened despite relatively dry conditions throughout the observation period, which would be expected to encourage CH_4 consumption (Wu et al., 2010). The inconsistency of climate and defoliation effects on CH_4 flux across studies highlights the need for further investigation to understand changes in the total C balance of these grassland systems. Nitrous oxide similarly displayed no response to defoliation or drought, with this result being less surprising given that N_2O flux tends to be near zero and highly variable among grassland ecosystems (Flechard et al., 2007; Jones et al., 2011), although intensive grazing can reduce N_2O flux (Wolf et al., 2010), these grassland ecosystems seem to be resistant to such

effect. Other studies in the region have suggested that defoliation can cause grasslands to become a small source of N_2O under specific environmental conditions, although the amount emitted was very small (Bork et al., 2019; Ma et al., 2022).

As expected, drought reduced CO_2 flux, but had no effect on CH_4 or N_2O. Drought reduces soil moisture content (Batbaatar et al., 2021), which in turn, reduces plant growth, microbial activity, and, ultimately, microbial respiration (Deng et al., 2021). These results are consistent with numerous other studies (Aronson et al., 2019; Jentsch et al., 2011; Li et al., 2016; Munjonji et al., 2020). Methane typically decreases as conditions become less anaerobic; however, most of these grasslands are moisture-limited systems that typically have been observed to consume CH_4 (Galbally et al., 2008; Thomas et al., 2017). Nitrous oxide emissions tend to decline with drought, but given that these are arid systems and emission rates are low, the potential for further reductions may be limited because major sources of N_2O emissions in these grasslands are livestock excreta deposition and N application, both of which were not included as treatments, and did not occur, in our study (Jones et al., 2005; Oenema et al., 1997).

3.5.2 Drought and defoliation effects on cumulative CO_2 emissions across a precipitation gradient

The effect size of drought on CO_2 emissions varied with defoliation treatments, and was also larger at drier locations. Single defoliation regimes, when coupled with drought, tended to reduce CO_2 emissions, although the light-heavy defoliation treatment, in which plants were defoliated twice, had a smaller reduction in CO_2 flux, and in one year, increased CO_2 flux under drought conditions compared to ambient conditions. Other studies in arid grasslands of the Great Plains have found that mowing or grazing can increase CO_2 emissions, and attributed it to

increased soil temperatures enhancing plant growth rates (Braun et al., 2013; Lecain et al., 2000). In addition to that, plant defoliation can delay plant senescence under drought conditions because defoliated plants use less water and can grow for a longer period (Zheng et al., 2021). Compared to heavy-heavy defoliation, which may have slowed plant growth significantly, the light-heavy defoliation possibly allowed more plant growth in the summer prior to the second defoliation. Furthermore, significant removal of aboveground biomass could reduce photosynthetic activity, allowing plants to allocate and transport more C to roots, leading to increased soil microbial respiration (Gao et al., 2018; Lecain et al., 2000).

The reduction in CO_2 flux from drought at drier sites was markedly larger than at wetter grasslands. This could be due to extreme water limitations at drier sites inhibiting soil microbial processes, as precipitation at these sites was below their long-term average during the study (Batbaatar et al., 2021). Severe summertime drought can limit soil respiration rates by reducing autotrophic respiration and mitigating GHG increases that might arise due to warmer soils (Schindlbacher et al., 2012). Furthermore, microbial respiration is dependent on extracellular enzyme activity, and we measured enzymes associated with C cycling to be particularly reduced under drought conditions (see Chapter 2; Schindlbacher et al., 2012; Wang et al., 2014).

3.5.3 Relationship of greenhouse gas emissions with soil and environmental (biotic and abiotic) factors

The best abiotic predictor of CO_2 flux was soil temperature, not soil moisture content. Soil temperature is an important determinant of soil CO_2 flux (Rong et al., 2015; Silverthorn and Richardson, 2021), and can be more important than soil moisture in grasslands (Brito et al., 2015; Gao et al., 2018; Liebig et al., 2013). Temperature increases CO_2 flux because of enhanced

enzyme activity, as explained earlier, and general increases in soil microbial metabolic activity and root growth, provided there is enough soil moisture content (Dijkstra et al., 2011). The second-best predictor for CO_2 and the best predictor for N_2O and CH_4, was the observed level of EEA. A strong relationship between enzymes and CO_2 flux has been observed in the past, since extracellular enzymes catalyze rate-limiting procedures during soil organic matter decomposition (Chen et al., 2018), and are known to provide a direct expression for soil biogeochemical cycles (such as C and N turnover; Burns et al., 2013). Major components of the plant cell wall, such as cellulose and hemicellulose, are catalyzed by hydrolytic enzymes, thus supporting the release of CO_2, in part due to ongoing extracellular enzyme activity (Hildén and Mäkelä, 2018; Knob et al., 2010; Yan et al., 2021). Previously, we found that defoliation along with drought reduced enzyme activity, specifically those involved in C cycling, and in response to defoliation, which could thereby influence CO_2 emissions (see Chapter 2). These results highlight the need to better understand the biological responses of both plants and soil microbes to grazing/defoliation in relation to respiration (autotrophic vs heterotrophic respiration), as well as inherent agroclimatic factors, to understand overall soil GHG fluxes from grasslands.

3.6 Conclusion

Our data suggest that defoliation, particularly defoliation frequency, plays a major role in controlling CO_2 emissions from the grasslands studied. A single defoliation event during the growing season, especially in mid-June, may reduce CO_2 emissions under drought conditions. However, 2x annual defoliation may have the opposite impact (increase or maintain CO2 emissions), thereby increasing CO_2 emissions under drought. Furthermore, soil temperature was found to be the more important predictor of CO_2 flux than soil moisture content, along with

biotic factors (such as extracellular enzyme activity) that were equally (if not more) important in predicting grassland GHG (CO_2, N_2O, CH_4) fluxes. This finding highlights the importance of belowground biological responses to grazing, and calls for future long-term studies examining how nuanced grazing regimes (timing and/or intensity of defoliation) alter belowground biological controls on biogeochemical cycles. Results from this study suggest that single annual defoliation events have the potential to reduce CO_2 emissions from these grasslands under most conditions, including future drought, particularly at drier locations.

Tables

Table 3.1 Climatic, soil, and dominant vegetation attributes of each study site. Long-term mean annual precipitation and air temperature data were obtained from ClimateNA (Wang et al., 2016).

Site	MAP (mm)	MAT (°C)	Soil texture	Soil classification	pH (1:2)	Dominant plant species
Stavely	531.9	3.7	Silty clay loam	Orthic Black Chernozem	5.96	*Festuca campestris, Danthonia parryi, Poa pratensis*
Sangudo	501.4	2.6	Loam	Dark Grey Luvisol	6.71	*Elymus repens, Trifolium repens*
Kinsella	405.9	2.3	Clay	Orthic Black Chernozem	5.73	*Poa pratensis, Agropyron dasystachyum, Pascopyrum smithii*
Twin river	361.4	5.1	Clay loam	Orthic Dark Brown Chernozem	6.51	*Agropyron dasystachyum, Artemisia frigida, Festuca idahoensis*
Oyen	328.6	3	Clay loam	Orthic Dark Brown Chernozem	5.57	*Agropyron dasystachyum, Avenula hookeri, Hesperostipa curtiseta*
Onefour	323.9	5.2	Clay loam	Orthic Brown Chernozem	6.47	*Agropyron dasystachyum, Hesperostipa comata, Artemisia frigida*
Mattheis	315.8	3.9	Sandy loam	Orthic Brown Chernozem	6.21	*Hesperostipa comata, Artemisia frigida, Koeleria macrantha*

Table 3.2 Analysis of variance table of the linear mixed model effects of rainfall and defoliation treatment on cumulative greenhouse gas emissions. Df = numerator degrees of freedom, Df residual = denominator degrees of freedom. Values in bold are significant at $\alpha = 0.10$.

Treatment	Df	Df residual	F-stat	p-value	F-stat	p-value
			2017		2018	
					$\underline{CO_2}$	
Rainfall	1	194	2.25	0.136	1.93	0.166
Defoliation	4	194	1.50	0.204	2.37	**0.054**
Rainfall × Defoliation	4	194	2.33	**0.057**	2.17	**0.074**
					$\underline{N_2O}$	
Rainfall	1	194	1.50	0.222	0.01	0.911
Defoliation	4	194	0.45	0.775	0.90	0.422
Rainfall × Defoliation	4	194	0.40	0.805	0.91	0.461
					$\underline{CH_4}$	
Rainfall	1	194	0.23	0.631	0.10	0.753
Defoliation	4	194	0.25	0.909	0.27	0.900
Rainfall × Defoliation	4	194	0.21	0.934	0.79	0.537

Table 3.3 Analysis of variance table of the linear mixed model effects of rainfall treatment and defoliation frequency (non-grazed vs 1x grazed vs 2x grazed) on cumulative CO_2 emissions. Values in bold are significant at $\alpha = 0.10$. Df = numerator degrees of freedom, Df residual = denominator degrees of freedom.

Treatment	Df	Df residual	F-stat	p-value	F-stat	p-value
			2017		2018	
Rainfall treatment	1	198	24.30	**<0.001**	13.78	**<0.001**
Defoliation frequency	2	198	1.53	0.219	1.94	0.146
Rainfall treatment × Defoliation frequency	2	198	4.47	**0.013**	3.25	**0.041**

Table 3.4 ANOVA table of linear model testing the effects of mean annual precipitation (MAP) and defoliation treatments on the natural log-response ratio of drought treatment on CO_2 emissions. Values in bold are significant at $\alpha = 0.10$.

Treatment	Sum of squares	F-stat	p-value	Sum of squares	F-stat	p-value
	2017			2018		
MAP	0.052	3.56	**0.062**	0.142	5.66	**0.019**
Defoliation	0.246	4.24	**0.003**	0.359	3.59	**0.009**
MAP × Defoliation	0.164	2.83	**0.029**	0.182	1.81	0.132

Table 3.5 AIC, beta-coefficients and delta AICc scores for the selection of the top five best-ranked models explaining each of CO_2, N_2O and CH_4 emissions. Highlighted delta values are the most parsimonious models when delta < 2. Values for EEA, MC, pH and TEMP are model beta coefficients. MC: soil moisture content, EEA: geometric mean of soil extracellular enzyme activity of five enzymes (β-glucosidase, β-Cellobiosidase, β-xylosidase, acid phosphatase and N-acetyl-β-glucosaminidase), TEMP: soil temperature, df: degree of freedom, logLik: log-likelihood value, AICc: Second-order Akaike Information Criterion corrected for small sample size, delta: delta-AICc, weight: Akaike weights

(Intercept)	EEA	MC	pH	TEMP	df	logLik	AICc	delta	weight
				CO_2					
6.754	-0.112			0.014	5	-740.2	1490.5	**0**	0.926
7.023	-0.112		-0.045	0.014	6	-741.8	1495.6	5.123	0.071
6.744	-0.112	0.0003		0.014	6	-745.6	1503.3	12.851	0.001
7.030	-0.116				4	-747.8	1503.6	13.164	0.001
7.015	-0.113	0.0003	-0.044	0.014	7	-747.2	1508.5	17.986	0.0001
				N_2O					
0.383					3	-2062.9	4131.7	**0**	0.501
0.650	-0.052				4	-2062.8	4133.6	**1.806**	0.203
1.341			-0.158		4	-2062.8	4133.7	**1.972**	0.187
1.580	-0.051		-0.154		5	-2062.8	4135.7	3.925	0.070
0.222		0.009			4	-2065.6	4139.2	7.486	0.012
				CH_4					
0.362	-0.065				4	-584.8	1177.7	**0**	0.963
0.455	-0.065		-0.015		5	-587.3	1184.7	7.018	0.029
0.287	-0.064			0.004	5	-588.7	1187.5	9.872	0.007
0.368	-0.064	0.0004			5	-590.4	1190.8	13.131	0.001
0.385	-0.064		-0.016	0.004	6	-591.2	1194.5	16.858	0.0002

Figures

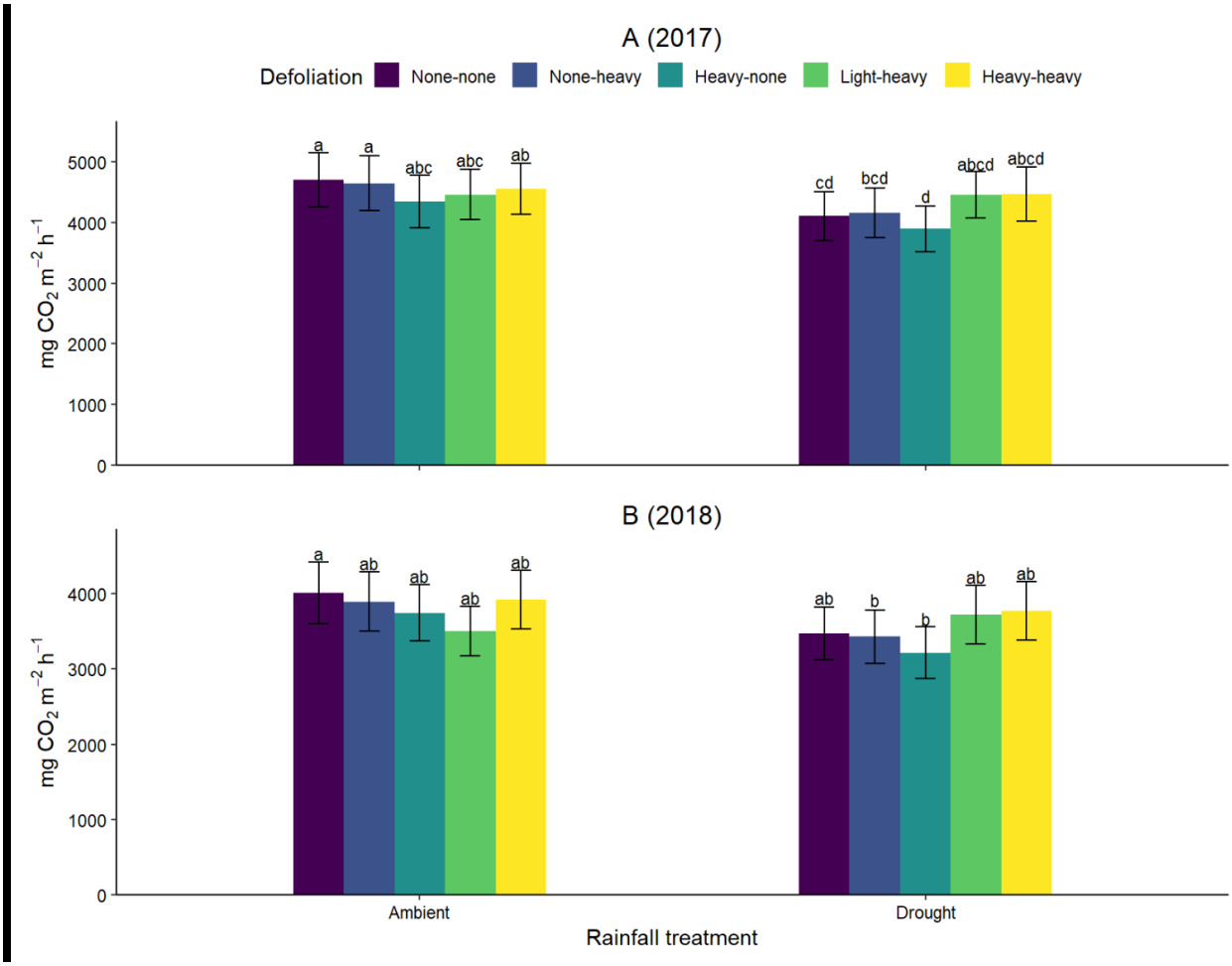


Figure 3.1 Rainfall treatment and defoliation effects on cumulative CO_2 emissions over the growing season from April to September (twice a month in each of April and May, and once a month thereafter) in (A) 2017 and (B) 2018. Different letters denote significant differences between groups at $\alpha = 0.05$.

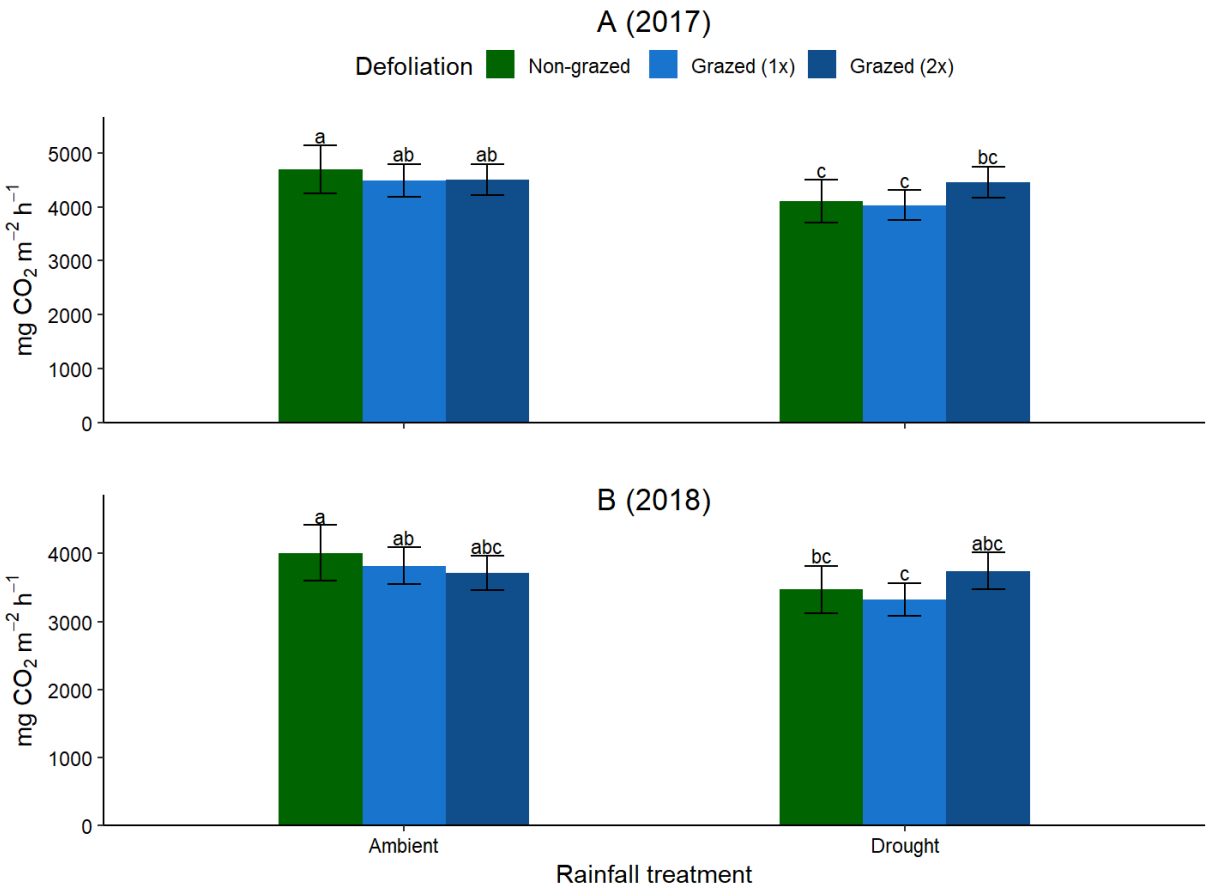


Figure 3.2 Rainfall treatment and defoliation effects on cumulative CO_2 emissions in (A) 2017 and (B) 2018. Grazed (1x) represents 1 time annual defoliated plots and Grazed (2x) represents 2 time annual defoliated plots regardless of timing and intensity. Different letters denote significant differences between groups at $\alpha = 0.10$.

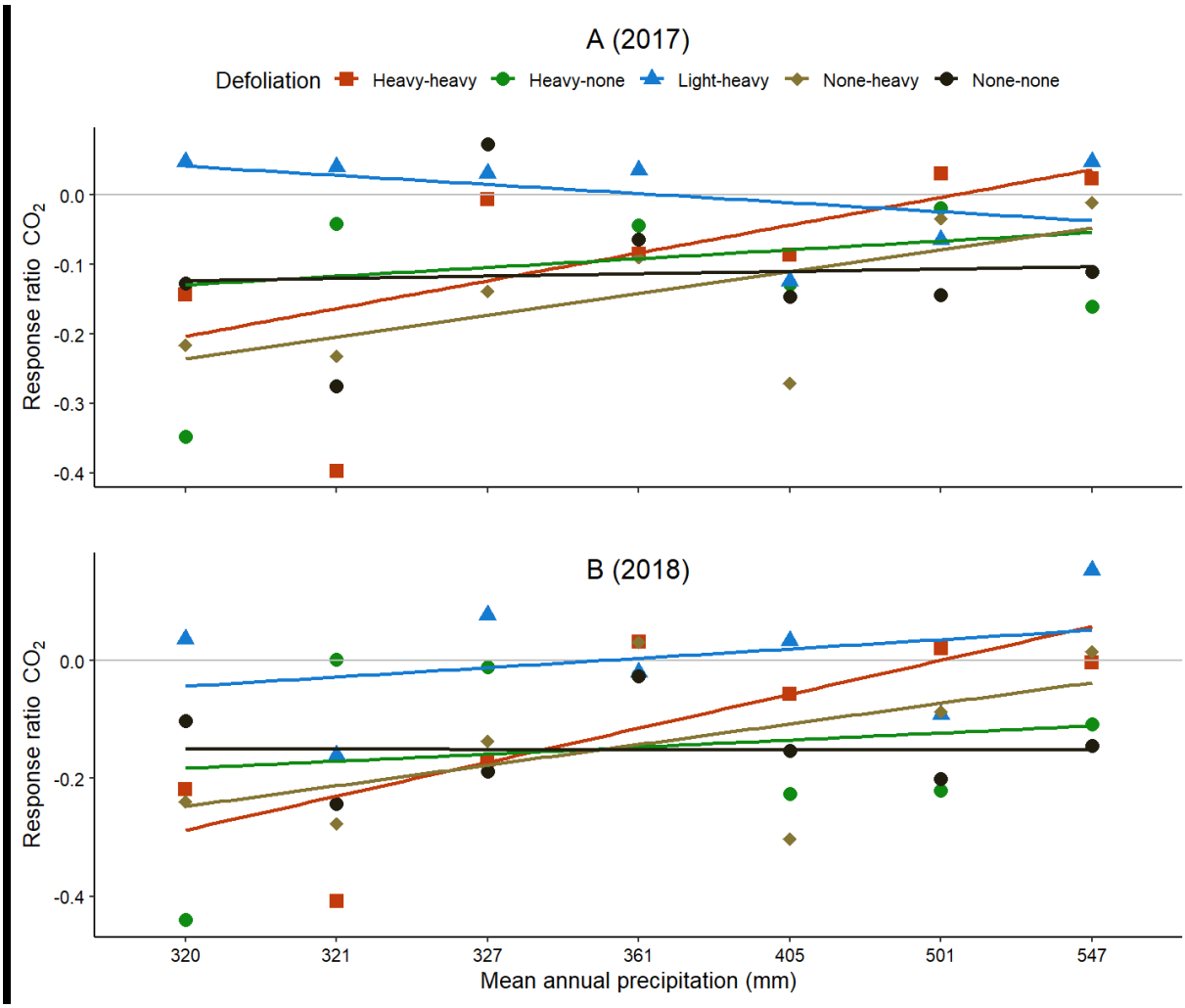


Figure 3.3 Simple linear regression between the log-Response ratio of rainfall treatment CO_2 emissions and mean annual precipitation (MAP) in (A) 2017 and (B) 2018.

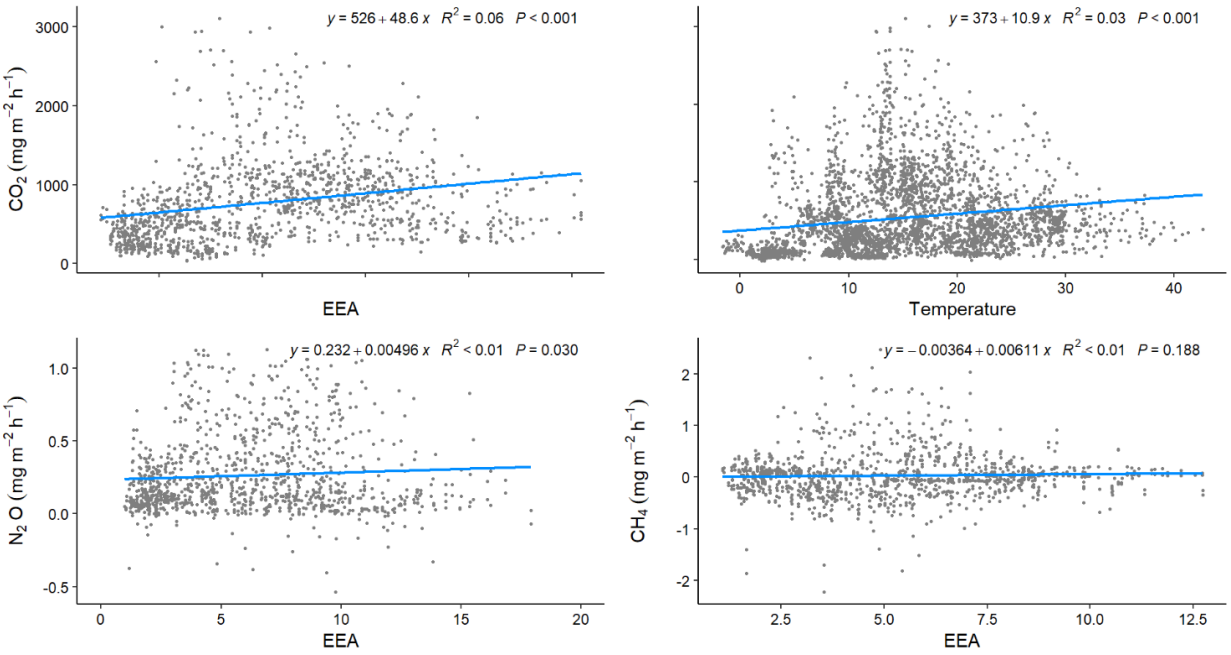


Figure 3.4 Simple linear regression between monthly greenhouse gas flux (CO_2, N_2O and CH_4) and TEMP: soil temperature and EEA: geometric mean of extracellular enzyme activity (β-glucosidase, β-Cellobiosidase, β-xylosidase, acid phosphatase and N-acetyl- β-glucosaminidase).

4. Chapter 4 Soil microbial communities' response to drought and defoliation in northern temperate grasslands

4.1 Abstract

Extreme drought events can negatively affect soil microbial communities in northern temperate grasslands, including their ecosystem functions such as nutrient cycling and plant productivity. Furthermore, grazing is the dominant land use in these ecosystems, which may interact with drought to affect soil microorganisms by altering the availability of microbial resources. This study tested how grazing at different intensities and frequencies (simulated using defoliation treatments) interacted with drought (45% rainfall reduction using rainout shelters) to affect soil microbial community composition, diversity and functions in two contrasting (mesic vs dry) grasslands in the Northern Great Plains of Alberta. Soil samples were analyzed for soil microbial community composition (bacteria and fungi), diversity and functional abundance using next-generation sequencing. Beta diversity of soil fungi increased under drought conditions at the mesic grassland site, where there was greater dispersion within drought treatment. Overall, soil fungal communities showed greater drought tolerance than bacterial communities at both sites. Soil moisture content and temperature reflected the changes in microbial composition at the mesic and dry site, respectively. At the functional group level, the abundance of chemoheterotrophs increased under drought at the dry site. In contrast, drought favored xylanolytic bacteria and saprophytic microorganisms at the mesic site and suggested that drought could increase the breakdown of xylan and xylooligosaccharides. Defoliation showed less consistent responses with alpha and beta diversity of fungal communities affected by defoliation at each of the dry and mesic sites, respectively, suggesting that soil microbial communities were relatively resistant to defoliation in these grasslands.

4.1 Introduction

Climate change caused by anthropogenic activities affects the soil microbial communities responsible for soil organic matter decomposition and influences soil nutrient cycling (Bellard et al., 2012; Zhang et al., 2016). In the northern great plains, extreme weather conditions in grassland ecosystems are expected to increase in the future, with more extreme drought and variable precipitation patterns causing moisture deficit conditions in these soils (Zeglin et al., 2013). Most of these grasslands are managed for livestock grazing; therefore, understanding the combined effects of grazing and drought on microbial communities is vital for developing management strategies to maintain ecosystem function.

Soil moisture content, driven principally by rainfall, is key in defining soil microbial communities (Hartmann et al., 2017; Zeglin et al., 2013). Generally, soil microbial community composition, diversity and biomass are negatively affected by drought (Hartmann et al., 2017; Ochoa-Hueso et al., 2012; Preece et al., 2019). Drought can lead to the death of viable microbial cells (Nocker et al., 2012) and shift microbial community composition (e.g., an increase in fungi:bacteria ratio) (Fukami, 2015; Placella et al., 2012). In particular, bacterial and archaeal communities are typically more affected than fungal communities (de Vries et al., 2018), although some bacterial communities can be tolerant of long-term drought (Evans and Wallenstein, 2014).

Defoliation is the removal of standing plant tissues by clipping, trampling or browsing (Heady and Child, 1994). While the effects of defoliation timing and intensity on plants are well studied (Bork et al., 2017; Broadbent et al., 2016), few investigations have examined their effects on soil microbial communities. Modification of timing and intensity of defoliation can provide insight into how different grazing management systems affect soil microbial communities and

associated nutrient cycling. Past studies have reported variable effects of defoliation on primary production and soil microbial community composition and diversity, ranging from no effect on soil microbial community composition and diversity (Macdonald et al., 2006; Attaeian et al., 2010) to shifts in composition (Carey et al., 2015) and reductions in alpha and beta diversities (Ma et al., 2018).

Many of these studies that provide important insights into soil microbial community composition and diversity have focused on the effects of single factors, such as reduced precipitation or grazing alone, due to the complexity of methodologies, costs involved and difficulty in controlling multiple factors (such as drought and grazing) under experimental designs (de Vries et al., 2018; Rui et al., 2015; Xiong et al., 2014; Zhang et al., 2016). While there have been numerous studies testing the effects of extreme weather events on soil microbial communities, grazing may have a stronger effect on soil microbial community composition and diversity in grassland ecosystems, and these can occur simultaneously with drought (Yang et al., 2013). Therefore, a study covering the effects of grazing regimes under drought would provide a broad investigation of grassland ecosystems, specifically in northern temperate grasslands which are grazed mainly by livestock and prone to future drought.

Soil microorganisms play a crucial role in ecosystem functioning, particularly in the cycling of C and other nutrients such as nitrogen (N) and phosphorus (P), by releasing extracellular enzymes that break down complex organic matter and plant residues to release nutrients back to the soil (Liang et al., 2017). Extracellular enzyme activity (EEA) is widely used as an indicator of soil microbial activity since most extracellular enzymes are released extensively by soil microorganisms (Cheng et al., 2017; Das and Varma, 2010). EEA of five different enzymes involved in C (β-glucosidase, Cellobiosidase, β-xylosidase), N (N-acetyl-β-

glucosaminidase: NAG) and P (acid-phosphatase: AP) was included to test the correlation of these enzymes with soil microbial functional abundance. Under drought conditions, EEA was reduced for most of the enzyme studies, except β-xylosidase, which tends to increase under drought conditions (Chapter 2; Gao et al., 2021). Furthermore, we found that defoliation altered EEA. For instance, β-glucosidase (an enzyme involved in C cycling) showed increased activity under heavy defoliation, with defoliation effects being dependent on precipitation and local climatic conditions of grasslands (Chapter 2). There have been various findings about the effects of stress on EEA and how it relates to soil microbial communities. For example, soil microbial communities could respond to environmental/anthropogenic stress by preferentially utilizing energy for survival rather than enzyme production (therefore leading to less enzyme activity), or drought may create favorable conditions for more enzyme production by soil microorganisms (Ylla et al., 2012). Studying the effect of stress (drought and/or defoliation) on soil microbial functional abundance in relation to EEA would provide a better understanding of the relationship between soil microbial communities and activity (here EEA). FAPROTAX is a database constructed manually to link prokaryotic taxa to their relevant ecological functions (such as denitrification, nitrogen fixation). The database includes information about available cultured taxa and the relative role/function they are involved in. FAPROTAX database is widely used in ecology to relate ecological or metabolic functions of specific microorganisms, however with its own limitations since the database contains information about only culturable microorganisms (Louca et el., 2016; Rivet et al., 2018; Yang et al., 2022). However, the database contains about 80 different detectable functions coving less than 5000 taxa so far, which may underestimate the analysis performed in grassland soils where microbial communities are diverse (Louca et al., 2016; Sansupa et al., 2021).

This study was conducted at two Northern grassland sites (Kinsella and Mattheis) in the northern Great Plains in Alberta, Canada, to provide insight into how soil microbial communities' different components (bacteria and fungi) respond to the effects of rainfall and defoliation treatments (and their interactions). Specifically, the objectives of this study were to test: i) community-level responses of soil bacteria and fungi to drought and defoliation imposed over several years, ii) taxa-level responses of soil bacterial and fungal communities to drought and defoliation, iii) potential ecological functional responses related to soil microbial community changes following drought and defoliation treatments (and their interactions), and iv) the relationship of environmental variables (such as pH, soil moisture content, soil temperature) and biotic factors (EEA) with soil microbial functional abundance.

4.3 Materials and Methods:

4.3.1 Study sites and experimental design:

The experiment was conducted at two grassland sites (Kinsella in the Aspen Parkland and Mattheis in the Mixedgrass Prairie) located in the northern great plains of Alberta, Canada. Both sites were dominated by perennial grasses and forbs, with unique plant composition and soil characteristics (Appendix 4). Mean annual precipitation (MAP) was 406 mm at Kinsella and 315 mm at Mattheis, with mean annual temperatures of 2.3 °C at Kinsella and 3.9 °C at Mattheis. At each site, we established a full factorial experiment in the summer of 2016 with two rainfall treatments, five defoliation treatments, and 3 replicates (30 plots per site). Defoliation treatments were applied with a lawnmower to create combinations of treatments with varying frequencies and intensities of defoliation. Defoliation The clipped plant biomass was captured and removed from the experimental plots. The defoliation frequency included a single clipping done in either

June or September, or in both months for plots clipped twice. Defoliation intensity included a "heavy" clipping that removed vegetation to a 3 cm stubble height, while a "light" clipping reduced vegetation to a 7 cm stubble height. We established five defoliation treatments: heavy-heavy, light-heavy, heavy-none, none-heavy and none-none (i.e., non-defoliated control), where the first intensity represents the June defoliation and the second a September defoliation. Heavy-heavy and light-heavy plots were clipped twice a year, in June and September. Heavy-none plots were clipped once a year in June, and none-heavy plots were clipped once a year in September. Rainfall was reduced to 45% of the ambient using rainout shelters (Gherardi and Sala, 2013) in drought plots, while the other half of the plots experienced ambient rainfall conditions (thereof called ambient). Each plot was 2.5 by 2.5 m, the size of the rainout shelter. Treatments were initiated in 2016 and continued through 2018.

4.3.2 Soil sampling and processing:

At each site, soil samples were collected from 3 replicates in early May, mid-June and mid-August of 2018 (three years after treatments commenced), creating a total of 180 samples (10 treatments x 3 replicates x 3 sampling times x 2 sites). Soil cores were collected using a 3.25 cm diameter soil corer to a depth of 15 cm. Five cores were collected from random points in each plot at each sampling time and mixed to form a composite sample. The soil corer was cleaned with 70% ethanol between plots. Samples were immediately placed in a cooler with dry ice, frozen during transport to the lab, and then stored at -20 °C until further analysis. All samples were sieved (2 mm) to remove coarse fragments and visible roots. Subsamples were taken from each sample to measure gravimetric soil water content and soil pH. Gravimetric soil water content was determined by drying 40 g of soil at 105 °C for 48 hr. Soil pH was determined using

a 1:5 soil:deionized water suspension based on volume (Rayment and Higginson, 1992) using a Fisherbrand Accumet AB150 benchtop pH meter (Fisher Scientific, MA, USA).

4.3.3 DNA extraction and sequencing:

Total DNA was extracted from 250 mg of each sample using the DNeasy PowerSoil Kit (QIAGEN Inc.) using manufacturer's protocol with the addition of bead beating step performed on FastPrep-24 Instrument (MP Biomedicals, Solon, OH). Amplicon libraries were constructed according to the Illumina Protocol (#15044224 Rev. B) with some modifications to explore soil bacterial, archaeal and fungal communities. The primers for bacteria and archaea were 515F and 806R (forward barcoded), similar to those used in Earth Microbiome (earthmicrobiome.org). Barcodes were on the forward primer 515F (Parada et al., 2016), which helps to use reverse primer constructs to obtain longer amplicons. For fungi, ITS1F and ITS2 primer sets were used, which were selected based on amplicon coverage, length and selective amplification (Gardes and Bruns, 1993; Toju et al., 2012; White et al., 1990). Full sequences of primers are listed in Appendix 5. During the 1st stage of the PCR, the number of cycles, annealing temperature and PCR mix were optimized. For bacteria and archaea, the 1st PCR mix included 13.2 µL of Platinum Hot Start PCR 2x Master Mix (Invitrogen, MA, USA), 0.4 µL of each 10 µM forward and reverse primers, 1 µL of diluted DNA (5 ng µL-1 in 10mM Tris pH 8.5) and 10 µL of nuclease-free water, creating a 24 µL of reaction volume (www.earthmicrobiome.org). The PCR (Thermocycler, ProFlex PCR system, Applied Biosystems, Foster City, CA, USA) conditions were: (i) bacteria and archaea: 2 min at 94 °C followed by 34 cycles of 30 s at 94 °C, 30 s at 50 °C, 30 s at 72 °C, and final elongation at 72 °C for 5 min; (ii) fungi: 2 min at 94 °C followed by 40 cycles of 30 s at 94 °C, 30 s at 50 °C, 30 s at 72 °C, and final elongation at 72 °C for 5 min.

Thereafter, all finalized 1st PCR products were cleaned and purified with 45 µL of AMPure XP Beads (#A63881, Beckman Coulter, CA, USA) as per Illumina (#15044224 Rev. B) and AMPure XP Beads (#001298v001) protocol. The 2nd PCR (Index PCR) was performed with 5 µL of DNA obtained from 1st PCR and cleanup, 5 µL of each Nextera XT Index Primer 1 and 2 (Nextera XT DNA Library Preparation Kit, Illumina Inc.), 25 µL of Platinum Hot Start PCR 2x Master Mix (Invitrogen, MA, USA) and 10 µL of nuclease-free water, creating a 50 µL of PCR reaction volume. The PCR (Thermocycler, ProFlex PCR system, Applied Biosystems, Foster City, CA, USA) conditions were: 2 min at 94 °C followed by 8 cycles of 30 s at 94 °C, 30 s at 55 °C, 30 s at 72 °C followed by final elongation at 72 °C for 5 min. After index PCR, a second cleanup was performed using AMPure XP Beads, followed by library quantification and pooling as per Illumina protocol. Finally, 5% PhiX was used as an internal control and paired-end sequencing (2x300) was performed using MiSeq Reagent Kit v3 (600 cycles) on Illumina MiSeq System (Illumina, Inc., San Diego, CA, USA) at the University of Alberta (Edmonton, AB, Canada).

4.3.4 Bioinformatics analysis:

Obtained FASTQ files were split into individual files for each sample and demultiplexed and processed in the DADA2 pipeline (Callahan et al., 2016). Primers were removed from amplicon sequencing data using the Cutadapt tool (Martin, 2011). The quality of reads was inspected using the plotQualityProfile function in the DADA2 pipeline, and low-quality reads were filtered and trimmed. Error rates were calculated, and samples were dereplicated to combine all identical sequencing reads. Next, inference algorithms were applied to dereplicated data to obtain true sequence variants from unique sequences in each sample. Then, forward and

reverse reads (paired) were merged by aligning denoised forward reads with the reverse complements of the corresponding reverse reads to construct contig sequences. Chimeric sequences were removed from all the sequences. Amplicon sequence variant (ASV) table was constructed using makeSequenceTable function of DADA2 package in R (Callahan et al., 2016). Taxonomy was assigned using the database Silva v.138.1 for bacteria and archaea, and UNITE for fungi. Assigned taxa with representative sequences and metadata were merged into phyloseq objects for further analysis (McMurdie and Holmes, 2013).

4.3.5 Statistical analysis:

All statistical analyses were performed in R (R Core Team, 2022). Initially, site was used as a random factor or as a block (in permutation); however, site had a significant effect on the results (alpha and beta diversity); therefore, all subsequent analyses were performed separately for both sites (see Appendix 15-18). Shannon diversity (H), Simpson index and Chao1 (species richness estimator) were calculated using the estimate_richness function from phyloseq package (McMurdie and Holmes, 2013), and were analyzed with a linear mixed model ANOVA using the lme4 package (Bates et al., 2015), where rainfall and defoliation treatments were treated as fixed factors (with interactions), and sampling month and plot were treated as random factors. For the mixed model, a significance level of 0.10 was used due to the low number of samples: however, all other models (such as those used to analyze functional groups) were tested at a significance level of 0.05. Permutational Multivariate Analysis of Variance (PerMANOVA) was performed with 999 permutations, where rainfall treatment and defoliation were treated as fixed factors, and month was used as a random factor on the distance matrix of microbial communities using the

adonis2 function in the vegan package in R (Oksanen et al., 2019). Furthermore, betadisper function of the vegan package was used to test the homogeneity of group dispersion (variance).

To detect the significant microbial biomarkers, linear discriminant analysis (LDA) Effect size (LEfSe) was used to detect which microbial taxa were significantly associated with each treatment. The LEfSe analysis was performed with an alpha value of 0.05, and the threshold for the LDA score for bacterial and fungal communities was set at >3.5 and >4, respectively (Segata et al., 2011). A 2.0 threshold LDA score is commonly used; however, at 2.0 score, we observed hundreds of significant phylogenetic level differences between ambient and drought. For that reason, we increased the threshold score according to the needs of the data to only highlight those taxa most associated with the treatments. The LEfSe analysis was used because it produces very similar results to other analysis, such as Kruskal-Wallis sum-rank test but with lower false positive rates, which are generally more common in biology than false negatives (Boulesteix, 2010; Segata et al., 2011). Furthermore, a cladogram was constructed to visualize the significant differences on a phylogeny level to observe the associations of clades with specific treatments. The microeco package in R was used to run the LDA analysis and construct a cladogram (Liu et al., 2021a). Redundancy analysis (RDA) was used to detect the effects of treatments and environmental factors on beta-diversity of microbial communities, where a community matrix of beta diversity is observed through a two-step process (initially multiple regression followed by principal component analysis; Legendre and Legendre, 2012).

Functional annotation of the prokaryotic taxa (FAPROTAX) database was used to link soil bacterial communities to their respective ecological functions. The FAPROTAX database includes over 80 functions collected from more than 4600 cultured bacterial taxa (Louca et al., 2016). For functional annotation of soil fungal communities, the FUNGuild v1.0 database was

used to determine functional groups (Nguyen et al., 2016). Furthermore, linear mixed modelling was performed to test the effects of rainfall and defoliation treatments on different ecological functions related to soil bacterial and fungal communities, where rainfall treatment and defoliation were treated as fixed factors (with interactions), sampling months, and plots were treated as random factors. Pearson correlation coefficients were calculated to test the correlation between ecological functions and environmental variables, including soil temperature, pH, soil moisture content, enzyme activity (collectively referred to as EEA) of BG, CELLO, XYLO, AP, NAG, CO_2, N_2O and CH_4 emissions.

4.4 Results

For bacteria, 2,774,328 high-quality sequences were obtained, averaging 15,412 reads per sample. For fungi, 5,631,985 high-quality sequences were obtained, averaging 31,288 reads per sample.

4.4.1 Alpha and beta diversity responses to drought

Shannon diversity of the fungi was affected by defoliation treatment at Mattheis ($F_{4,23.74}$ = 2.55; P = 0.065), while no other diversity indices were significantly affected (Appendix 6). Post-hoc pairwise comparisons showed that under ambient conditions, none-none defoliation had significantly greater alpha diversity than none-heavy treatment (Appendix 14). PERMANOVA results showed a significant effect of drought and defoliation on fungal beta diversity at Kinsella (Figure 4.1). Additionally, fungal communities had greater heterogeneity of dispersion under drought, indicating greater beta diversity (Figure 4.1).

The RDA biplot at Kinsella showed that the bacterial communities could be best explained by enzyme activity of XYLO, CELLO, BG, pH and soil moisture content (R^2 = 0.353. 0.166, 0.118, 0.185 and 0.219, respectively; Figure 4.2 – top left). At Mattheis, bacterial communities were best explained by CELLO, BG, NAG, AP, pH and temperature with R^2 values of 0.495, 0.378, 0.485, 0.345, 0.275 and 0.616, respectively (Figure 4.2 – bottom left).

The RDA biplot of fungal communities at Kinsella showed that the beta diversity can be best explained by the activity of BG, temperature, soil moisture content and activity of CELLO with R^2 values of 0.459, 0.433, 0.310 and 0.309, respectively (Figure 4.2 – top right). At Mattheis, the distribution of centroids for soil fungi can be best explained by temperature, the activity of NAG, CELLO and soil moisture content with R^2 values of 0.694, 0.436, 0.398 and 0.359, respectively (Figure 4.2 – bottom right)

4.4.2 Microbial community composition responses to drought

4.4.2.1 Bacteria

At Kinsella, the LEfSe analysis revealed that ambient treatment was significantly associated with eight bacterial ASVs. The drought treatment was significantly associated with 12 bacterial ASVs (Figure 4.3 – top). The phylogenetic cladogram showed that ASVs related to phylum Acidobacteriota (from phylum to genus) and Actinobacteriota were dominant under ambient conditions (Figure 4.3 – bottom). On the other hand, ASVs that could be related to phylum Verrcuomicrobiota and Firmicutes (from phylum to genus) were dominant under drought conditions. A particular ASV that could onlye be identified down to Phylum

Proteobacteria was dominant under drought conditions, except for one ASV which could be identified down to family Xanthobacteraceae, was dominant under ambient conditions.

In total, six bacterial ASVs were significantly associated with ambient treatment at Mattheis (Figure 4.4 – top). The drought treatment was significantly associated with 13 bacterial ASVs. At Mattheis, the cladogram of phylogeny revealed that ASV that could only be identified down to phylum Actinobacteriota (similar to Kinsella) was dominant under ambient conditions (Figure 4.4 – bottom). Furthermore, similar to Kinsella, ASVs belonging to phylum Verrucomicrobiota and Firmicutes (from phylum to genus) were dominant under drought conditions, while one ASV belonging to phylum Proteobacteria was largely dominant under drought, with 2 ASVs belonging families Xanthobacteraceae and Pseudomonodaceae being dominant under ambient conditions. Unlike Kinsella, ASV that could be identified down to genus Bradyrhizobium was not significantly associated with either treatment; however, the order to which they belong, Rhizobiales, that particular ASV was significantly associated with drought (opposite of Kinsella).

4.4.2.2 Fungi

For fungal communities at Kinsella, LEfSe analysis showed that ambient treatment was significantly associated with 11 fungal ASVs (Figure 4.5 – top). The drought treatment was significantly associated with nine fungal ASVs.

The cladogram of fungi communities at Kinsella revealed that ASVs that could be identified down to phylum Moriterellomycota (from phylum to genus) was dominant under ambient conditions (Figure 4.5 – bottom). One ASV particular to phylum Basidiomycota was

dominant under drought conditions, except for one ASV, which was significantly associated with ambient treatment. ASV that could be identified to class Sordariomycetes (of Phylum Ascomycota) from class to genus (genus Agaricus) was dominant under drought conditions, while ASVs belonging to families Didymellaceae and Pseudeurotiaceae were dominant under ambient.

For fungal communities at Mattheis, ambient treatment was significantly associated with one ASV (Figure 4.6 – top). Drought treatment was significantly associated with 18 fungal ASVs (Figure 4.6 – bottom). The cladogram at Mattheis showed that only one ASVs belonging to class Leotiomycetes (of phylum Ascomycota) was significantly dominant under ambient, while the rest of the ASVs related to phylum Ascomycota and phylum Mortierellomycota (from phylum to genus) were dominant under drought conditions.

4.4.3 Microbial functional group responses to drought

Analysis conducted on the FAPROTAX f functions was linked to soil bacterial communities. The most dominant bacterial functional groups were chemoheterotrophs, aerobic chemoheterotrophs, dark hydrogen oxidizers and nitrogen fixers. For soil fungal communities, the FUNguild database analysis showed a total of 24 functional groups were linked to soil fungal communities. The most dominant functional groups included saprotrophs, pathotrophs, symbiotrophs, plant pathogens and soil saprotrophs.

4.4.3.1 Bacteria

At Kinsella, the functional group xylanolytic bacteria had greater relative abundance under drought than under ambient ($F_{1,20.20} = 6.36$, $P = 0.02$) conditions (Appendix 7). At the other site, Mattheis, chemoheterotrophs had greater relative abundance under drought than under ambient conditions ($F_{1,14.96} = 5.12$, $P = 0.04$)

A correlation matrix was established to understand the relationship between microbial functional groups and EEA. At Kinsella, the correlation matrix showed a negative correlation between photosynthetic cyanobacteria and soil moisture content (Appendix 8). Observed CO_2 emissions were positively correlated with dark hydrogen oxidizers, nitrogen fixers, cellulolytic bacteria, and chemoheterotrophs. Furthermore, CH_4 consumption was negatively correlated with chitinolytic bacteria. The activity of XYLO had a significant negative correlation with dark hydrogen oxidizers and nitrogen fixers.

At Mattheis, soil moisture content and soil temperature had contrasting correlations with many of the bacterial functional groups (Appendix 8). For instance, cellulolytic bacteria and denitrifiers had positive correlations with soil moisture content, but negative correlations with soil temperature. On the other hand, nitrate reducers had positive correlations with soil temperature, but negative with soil moisture content. Additionally, photosynthetic cyanobacteria had a positive correlation with soil temperature, but a negative one with pH. CO_2 emissions positively correlated with nitrate reducers, while CH_4 consumption correlated negatively with denitrifiers. The activity of BG, CELLO, AP and NAG all positively correlated with cellulolytic bacteria and denitrifiers and negatively correlated with photoautotrophs and photosynthetic cyanobacteria.

4.4.3.2 Fungi

At Kinsella, the ecological functional group plant saprotrophs were significantly greater in relative abundance under ambient than under drought ($F_{1,17.13} = 4.79$, $P = 0.04$) conditions (Appendix 9). At Mattheis, fungal parasites were greater under drought than ambient conditions ($F_{1,17.29} = 5.78$, $P = 0.03$).

Similar to bacterial responses, soil moisture content and soil temperature had contrasting associations with fungal functional group abundance at Kinsella (Appendix 10). Dung and wood saprotrophs had negative correlations with soil moisture content, but were positively associated with temperature. The activity of BG, CELLO, AP, NAG had positive correlations with litter saprotrophs. When treatments were compared, litter saprotrophs were the only functional group which was significantly greater under ambient than drought ($P = 0.040$).

At Mattheis, saprotrophs, litter saprotrophs, symbiotrophs endomycorrhizal and endophyte abundance had positive correlations with soil moisture content, but were negatively associated with soil temperature (Appendix 10). On the other hand, arbuscular mycorrhizal and lichenized fungi had negative correlations with soil moisture content, but remained positively associated with soil temperature. Abundance of saprotrophs and symbiotrophs had negative correlations with both CO_2 and N_2O emissions. Activity of BG, CELLO, AP and NAG had significant positive correlations with endophyte, saprotrophs, and symbiotrophs.

4.5 Discussion

The effects of drought and defoliation (main and interactions) were tested in this study at two contrasting grassland sites, and we found that soil microbial communities were more responsive to drought treatment than defoliation at both sites.

4.5.1 Drought affected beta-diversity differentially at a dry vs a wet grassland

Beta diversity of bacteria and fungi was differentially affected at the two sites. Fungal beta diversity increased under drought only at the wetter grassland (Kinsella), where the dispersion was greater under drought conditions suggesting a heterogeneous community composition within group (drought). These findings suggest that, overall, drought negatively affected bacterial communities while fungal communities were more tolerant of drought conditions, which is consistent with other reports (de Oliveira et al., 2020). Furthermore, RDA showed that the soil moisture content and extracellular enzymes (specifically enzymes involved in carbon cycling; BG, CELLO and XYLO) reflected the changes in soil bacterial and fungal community composition at the wetter site, since a greater proportion of variance was explained by enzymes and soil moisture content. Similar positive correlations of soil moisture content and enzymatic activity with soil microbial community composition were observed in grasslands of with varying levels of degradation (Chao et al., 2022). These results support our findings that soil extracellular enzyme activity and soil moisture content are equally important for shifts in soil microbial communities.

Soil moisture content is widely known to regulate soil microbial activity and diversity in grassland ecosystems (Chen et al., 2015; Waldrop and Firestone, 2006). Furthermore, soil enzyme activity is regulated mainly by soil moisture content and temperature and has shown a

positive association with soil moisture content and hence responded to microbial diversity (Gao et al., 2021; Burns and Dick, 2002).

In contrast, RDA showed that the beta diversity of bacteria and fungi at the dry site was best explained by soil temperature and pH (in addition to enzyme activity). These results agree with previous studies that found pH to be a strong predictor of soil bacterial diversity (Kaiser et al., 2016; Nacke et al., 2011; Will et al., 2010). Differential relationships with predictor variables could be due to the other environmental and soil characteristics. For instance, a meta-analysis study observed that soil texture was a more important driver in altering soil microbial communities than soil moisture (Zhou et al., 2018). This suggest, sites may have responded to different environmental and/or biotic factors due to soil properties such as texture. Our RDA graph showed similar effects at the dry site (Mattheis), where the best explanatory variables were soil temperature and pH. In addition, we also found that biotic factors (i.e., extracellular enzymes) equally reflect changes in soil microbial communities at the wetter site (Zhang et al., 2017). This is important because changes/shifts in soil microbial communities will affect biogeochemical processes in the soils which will affect overall C cycling.

4.5.2 Microbial responses to drought at the phylogenetic level

The family Bacillaceae (phylum Firmicutes) showed a positive association with drought conditions at both sites. Family Bacillaceae are gram-positive bacteria and possess unique physiological characteristics due to their thicker cell wall, which makes them less susceptible to drought conditions (Schimel et al., 2007; Manzoni et al., 2012). These microorganisms produce a

large set of stress proteins which help them to survive even under severe drought conditions (Xu et al., 2018).

Family Chthoniobacteraceae was found to be positively associated with drought conditions at both sites in our experiment. Our results are in agreement with previous findings from a pot experiment of grassland soils where Chthoniobacteraceae increased under severe drought (Cordero et al., 2021). This bacterial taxon was also observed to be positively correlated with plant performance under water-limited conditions in agricultural land (Moore et al., 2023). Interestingly, Chthoniobacteraceae are gram-negative bacteria more susceptible to drought conditions than gram-positive (Maznoni et al., 2012). This warrants further investigation on this taxon in grassland ecosystems, because, to the best of our knowledge, there is a lack of in-depth research on this taxa in relation to drought and at our both sites Phylum Mortierellomycota was the third most dominant in terms or relative abundance.

At both sites, order Rhizobiales of phylum Proteobacteria was found to be enriched under drought conditions. Rhizobiales are rhizospheric symbiotic bacteria responsible for nitrogen fixation and are known to promote plant roots under drought conditions due to their drought-resistant characteristics (Liu et al., 2021b; Pieterse et al., 2014). Previously in agricultural soils, rhizobiales are widely known to increase under drought conditions in potatoes (Martins et al., 2023), sugarcane (Liu et al., 2021b) and rice fields (Santos-Medellín et al., 2017). Despite the fact that the relative abundance of nitrogen fixers in these grassland soils was very little, we further confirmed these findings by testing functional abundance where the mean relative abundance of nitrogen fixers was greater under drought conditions at both sites (see Appendix 11).

Both sites were dominant by phylum Ascomycota, Mortierellomycota and Basidiomycota, however, the composition of significant taxa associated with the treatments at a finer level was different. Genus Fusarium includes plant pathogens that cause rot, death, and wilting and reduce plant growth (Aoki et al., 2014). This genus has been observed to increase under drought conditions in tropical grasslands (de Oliveira et al., 2021). We found a similar response of Fusarium in arid/semi-arid grasslands.

Order Agaricales (phylum Basidiomycota) are involved in lignin degradation and found in oligotrophic environments (Entwistle et al., 2013). We found a significant association of Agaricales with drought which is in agreement with a previous study from northern mixed-grass prairie where they found increased Agaricales under drought conditions (She et al., 2018), presumably due to their oligotrophic nature, which makes them drought tolerant (Ho et al., 2017)

Almost all the significantly associated taxa were related to drought treatment at Mattheis. The genus Cladonia (class Lecanoromycetes) is worth mentioning, which was associated with drought conditions. In general, Cladonia is grown in cool to cold climates. However, they have the ability to absorb water directly from the atmosphere; therefore, they can colonize and survive even under drought conditions (Ahti and Hepburn, 1967). Our results are in agreement with previous studies in arid regions where lichenized biocrusts dominated under drought and warm conditions (Li et al., 2021). Surprisingly, the genus Mortierella (phylum Mortierellomycota) was associated with ambient at Kinsella (wetter) and drought at Mattheis (dry site). Mortierella is often found in the rhizosphere; however, there were very limited studies on the functioning of this genus. Some studies have suggested it to be a phosphate-solubilizing fungus and observed its important role in phosphorus cycling (Osorio and Habte, 2001). It has also been known to degrade pollutants and help in soil remediation (Wang et al., 2022). Conversely, it has been

reported as a plant pathogen as well (Guo et al., 2021). These mixed findings suggest that the role of this genus in soil could be species-specific, and therefore, we possibly had different compositions (at the species level) at both sites, leading to differential responses. Overall, soil fungal communities were more resistant to drought as compared to bacteria, where fungal communities mostly dominated under drought conditions (Hawkes et al., 2001). Soil fungal communities produce networks of hyphae in soils to facilitate nutrient transfer in soils and are generally known to be more drought tolerant than bacterial communities, and therefore thrive under dry conditions (Joergensen and Wichern, 2008). However, some contrasting responses of specific taxa at two sites raise the need for further investigation at the phylogenetic level of fungal communities.

4.5.3 Drought affected microbial functional groups prominently at the drier site

Bacterial functional groups responded to a greater extent to treatments at Mattheis than at Kinsella. At Kinsella, the only bacterial functional group that increased or decreased was xylanolytic bacteria, which break down xylan, a major component of hemicellulose (Chávez et al., 2006). Our results showed that drought conditions likely favored the breakdown of xylan and xylooligosaccharides (polymers of xylose) by increasing the abundance of xylanolytic bacteria, saprophytic microorganisms (Actinomycetes, fungi and other Actinobacteria) and activity of XYLO (Mganga et al., 2019). Furthermore, this functional response may be linked explicitly to the XYLO enzyme activity, which we found to thrive under drought conditions (Chapter 2).

At Mattheis, heterotrophic bacteria generally increased under drought conditions, while photoautotrophs and photosynthetic cyanobacteria decreased under drought, which suggests a

possible shift within the bacterial communities. Soil microorganisms either die or become dormant under environmental stresses. Dead microbial cells provide nutrients to the tolerant microbial communities, such as those that form spores (Bacillus, filamentous Actinobacteria, fungi), causing an increase in stress-tolerant communities (Bogati and Walczak, 2022). In our study, photoautotrophs (e.g. cyanobacteria) showed a negative correlation with soil moisture content and a positive correlation with soil temperature. An increase in cyanobacteria is strongly attributed to an increase in temperature, nutrient availability and drought (Hui et al., 2021). Abiotic stresses, such as drought caused by high temperatures and lower precipitation, could put cyanobacteria in hydric stress causing an oxidative response and may increase cyanobacteria via their osmotic adjustment ability (Hui et al., 2021; Zhang et al., 2016). Compositional shifts in soil microbial communities can cause either by elevated CO_2 emissions or warming (Yu et al., 2018; Sheik et al., 2011). We observed a similar correlation of microbial functional groups to CO_2 emissions and soil temperature, as we found that the rise in CO_2 emissions was best explained by increasing temperatures in our study (see Chapter 2).

4.6 Conclusions

Drought increased beta diversity only at the wetter site (Kinsella), where there was greater dispersion (heterogeneity). Furthermore, microbial community composition was driven by soil moisture content and enzyme activity at the wetter site and by soil temperature, enzyme activity and pH at the drier site. At the phylogenetic level, drought similarly affected soil bacterial communities at both sites. Specifically, Rhizobiales (nitrogen-fixing bacteria) were associated with drought at both sites suggesting greater nitrogen fixation under drought.

At the phylogenetic level, fungal communities were affected differentially at both sites, which suggests more diversity between and within groups (ambient vs drought). Genus Mortierella was associated with ambient at Kinsella and drought at Mattheis; this finding suggests further investigation of phylum Mortierellomycota at finer (species) level to understand functional roles since the taxa contain both beneficial and pathogenic fungal species.

At the mesic site (Kinsella), only xylanolytic bacteria (bacteria involved in the breakdown of xylan and xylooligosachharides) were increased by drought; however, at the dry site, chemoheterotrophs (the most common microbial group) increased under drought conditions. Defoliation did not affect soil microbial communities at both sites (except on fungal beta diversity at the mesic grassland), suggesting that soil microbial communities are quite resistant to defoliation applied in these ecosystems. However, long-term studies with more intensities and frequencies of defoliation may provide further insights into the effect of defoliation on soil microorganisms.

Figures

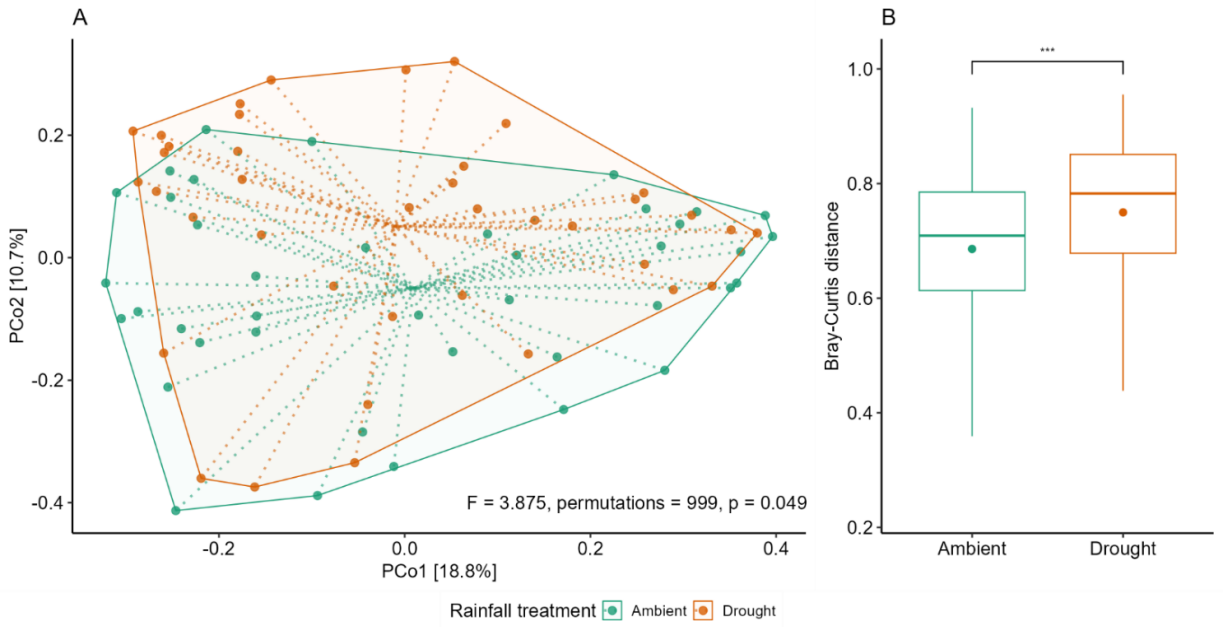


Figure 4.1 Biplot and boxplot showing the beta diversity of fungal communities at Kinsella plotted as Principal Coordinates Analysis (PCoA) based on Bray-Curtis distance matrix from PERMANOVA analysis (betadisper) to visualize dispersion within (A) and between groups (B). Asterisks denote significant different at $\alpha = 0.05$.

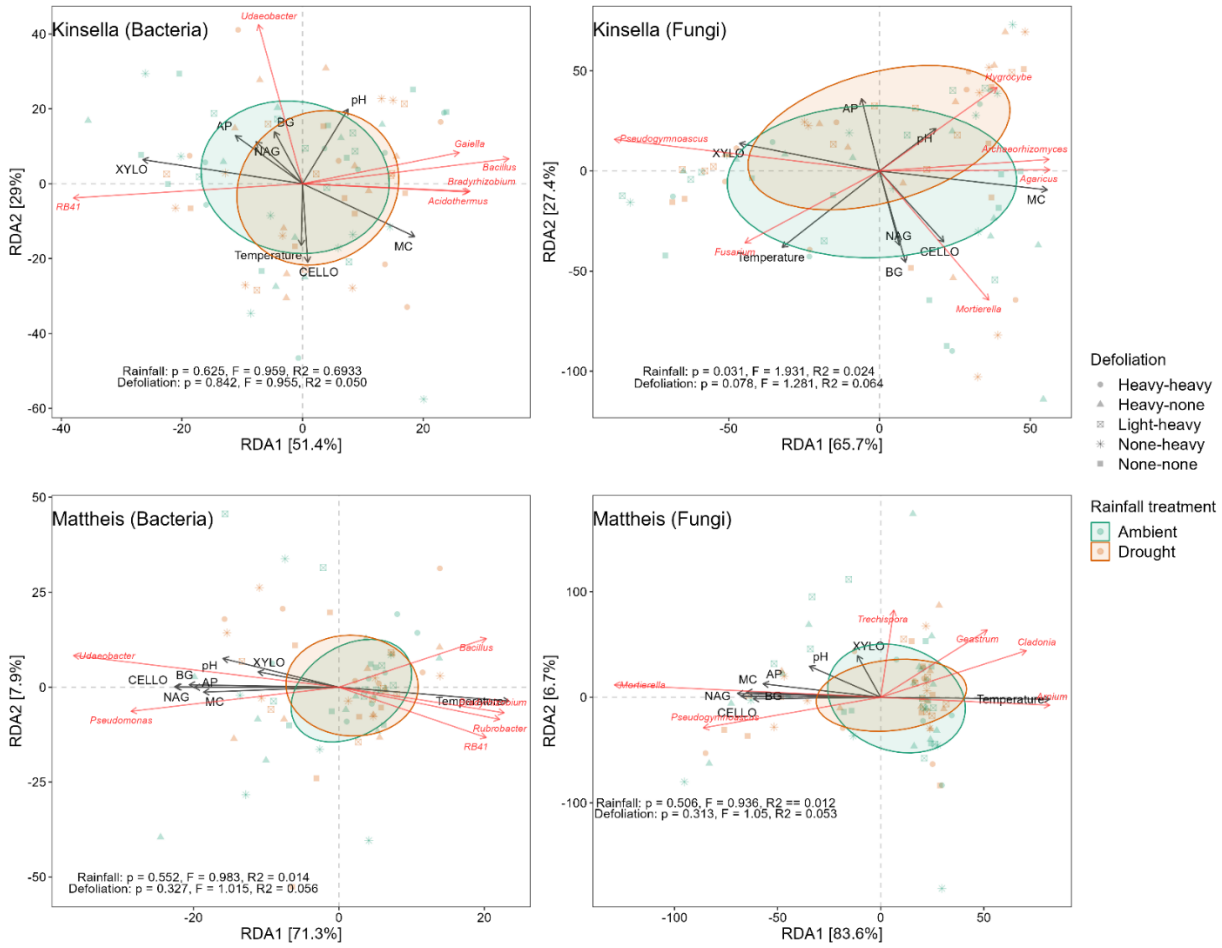


Figure 4.2 Redundancy analysis (RDA) of soil bacterial communities at genus level at Kinsella (Bacteria – top left, Fungi – top right) and Mattheis (Bactiera – bottom left, Fungi – bottom right). Environmental variables and the top 6 associated taxa are used as vectors to show the relationship of soil bacterial communities with environmental variables. BG: β-glucosidase, CELLO: β-Cellobiosidase, AP: acid phosphatase, NAG: N-acetyl- β-glucosaminidase, XYLO: β-xylosidase and MC: soil moisture content.

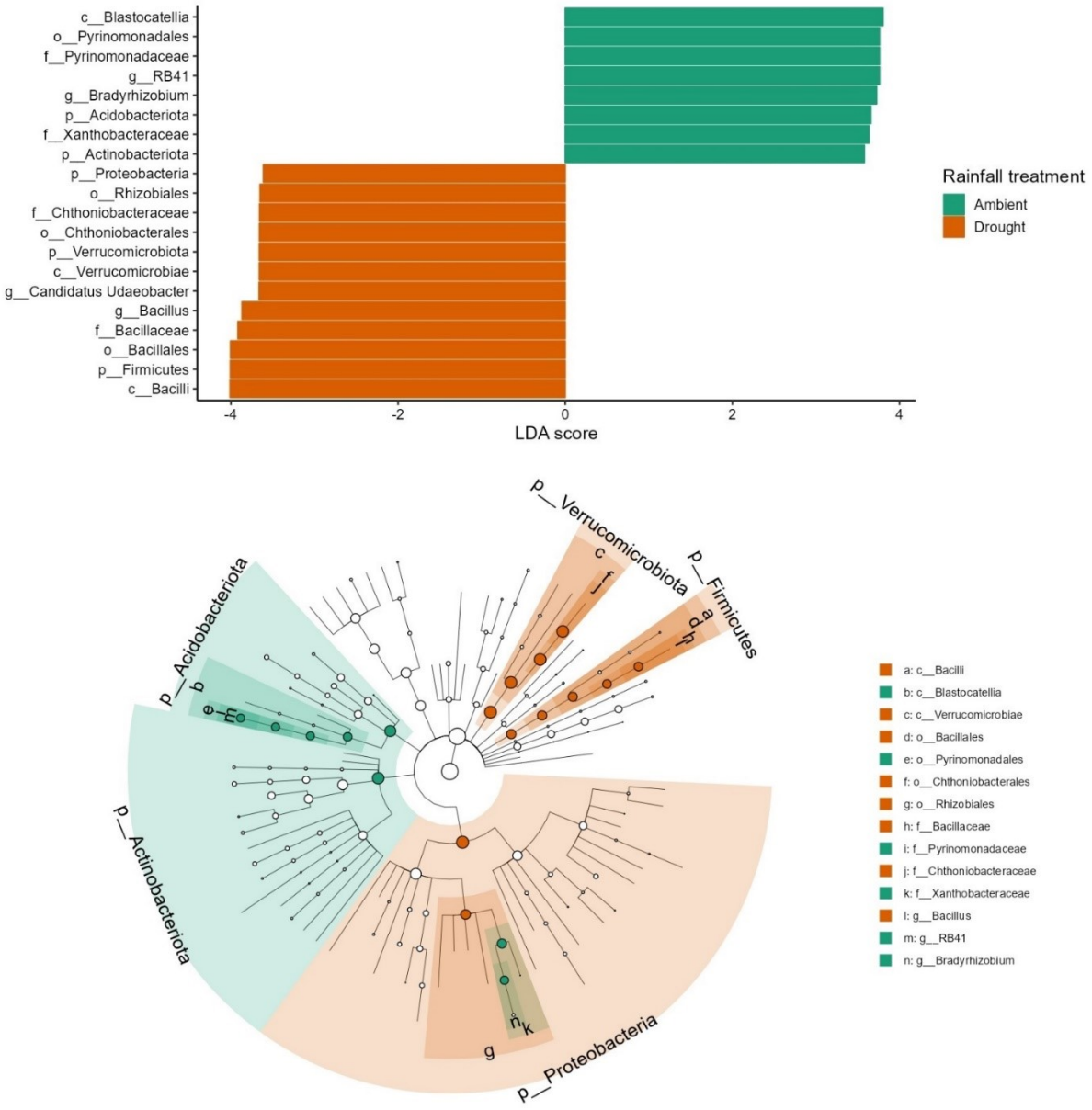


Figure 4.3 Linear Discriminant Analysis (LDA) Effect Size (LEfSe) with LDA scores of 3.5 and greater for soil bacterial communities at Kinsella to show statistically abundant taxa as biomarkers (top). A cladogram (bottom) of the phylogenetic tree of bacterial communities responding to rainfall treatments (ambient vs drought). The circles in the cladogram indicate phylogenetic levels from phylum to genus (from inner to outer circle).

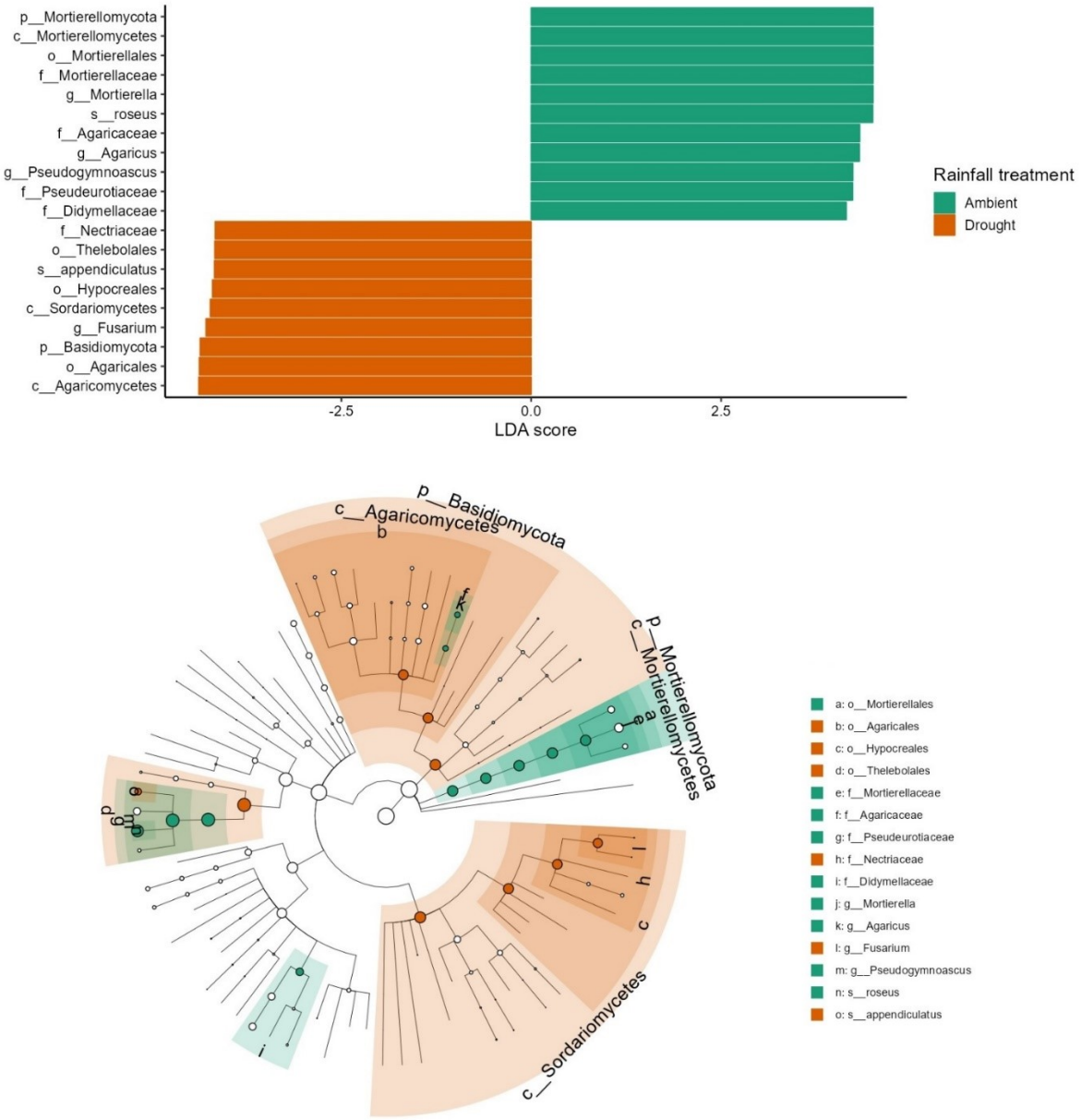


Figure 4.4 Linear Discriminant Analysis (LDA) Effect Size (LEfSe) with LDA scores of 3.5 and greater for soil fungal communities at Kinsella to show statistically abundant taxa as biomarkers (top). A cladogram (bottom) of the phylogenetic tree of bacterial communities responding to rainfall treatments (ambient vs drought). The circles in the cladogram indicate phylogenetic levels from phylum to genus (from inner to outer circle).

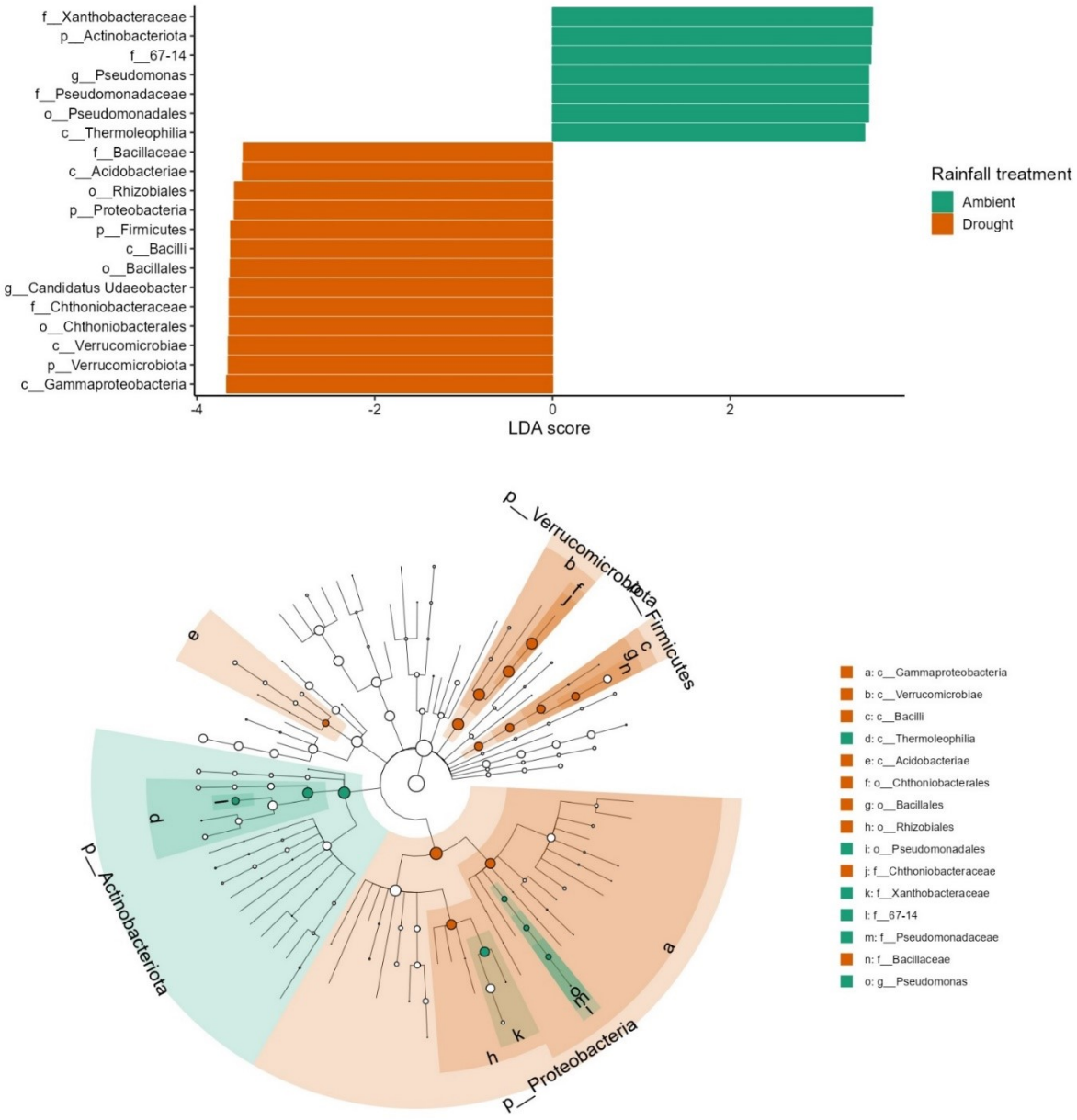


Figure 4.5 Linear Discriminant Analysis (LDA) Effect Size (LEfSe) with LDA scores of 3.5 and greater for soil bacterial communities at Mattheis to show statistically abundant taxa as biomarkers (top). A cladogram (bottom) of the phylogenetic tree of bacterial communities responding to rainfall treatments (ambient vs drought). The circles in the cladogram indicate phylogenetic levels from phylum to genus (from inner to outer circle).

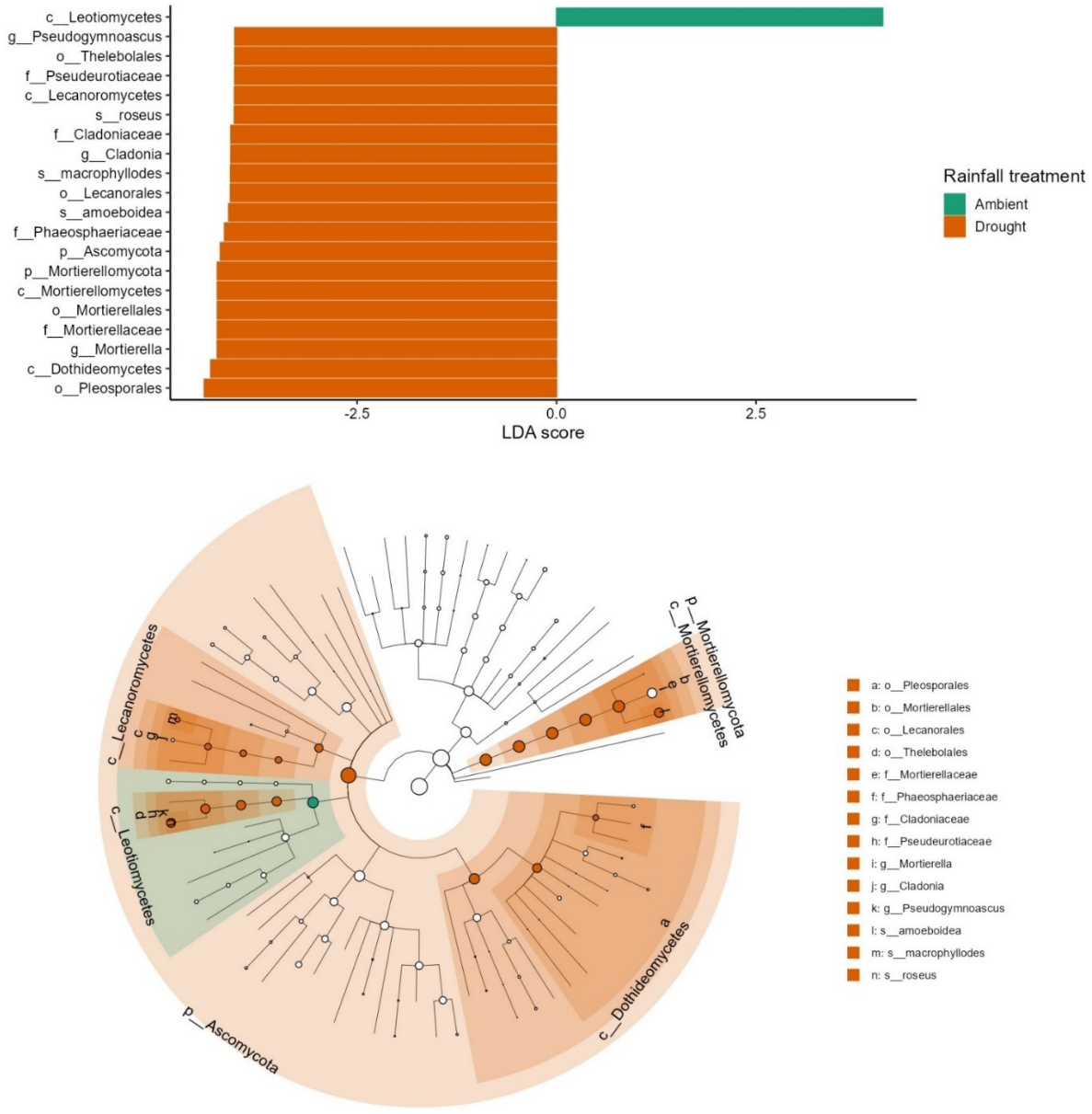


Figure 4.6 Linear Discriminant Analysis (LDA) Effect Size (LEfSe) with LDA scores of 3.5 and greater for soil fungal communities at Mattheis to show statistically abundant taxa as biomarkers (top). A cladogram (bottom) of the phylogenetic tree of bacterial communities responding to rainfall treatments (ambient vs drought). The circles in the cladogram indicate phylogenetic levels from phylum to genus (from inner to outer circle).

5. Chapter 5 Summary and conclusion

Grassland ecosystems are very complex and difficult to investigate because they are exposed to various management and environmental conditions (Soussana et al., 2004). In this study, I tested how grazing intensity and frequency can affect soil C and nutrient cycling under drought conditions. For the homogenous application of treatments and better control, I simulated drought conditions via rainout shelters to reduce the precipitation (45% by area) on experimental plots and mimicked grazing by manual removal of grass (defoliation) at certain stubble heights (to mimic intensity) at different times (to mimic frequency) throughout the growing season (April to early October). Furthermore, to generalize the results and conclusion for the northern temperate grasslands of Alberta, Canada, the experiment was done at seven different sites in Alberta covering a climate gradient. To understand the effect of drought and defoliation on soil C and nutrient cycling on a broader level, I divided this experiment into three individual studies (Chapters two, three and four).

Chapter two tested the effects of drought and defoliation on soil extracellular enzyme activity, which is a proximate indicator of soil microbial activity and plays a key role in organic matter decomposition, thus in carbon (C) and nutrient cycling (Das and Varma, 2010; Liang et al., 2017). Out of the five enzymes involved in C, nitrogen (N) and phosphorus (P) cycling in soil, activities of four enzymes were reduced under reduced rainfall (drought conditions). Activity of enzymes in the soil can reduce either due to the direct effect of limited substrate availability (Bardgett et al., 2008), which was confirmed as aboveground primary production was reduced due to drought (Batbaatar et al., 2022). Furthermore, enzyme activity can reduce indirectly when soil microorganisms (the main source of extracellular enzymes in the soil) produce fewer extracellular enzymes under drought conditions and allocate resources for

survival (Gao et al., 2021). This could be the cause of enzyme activity reduction in this study as rainout-shelter treatment led to a significant reduction in growing season precipitation, and all seven sites showed severe drought conditions (under drought treatment) measured by Standardized Precipitation-Evapotranspiration Index (Batbaatar et al., 2022). β-xylosidase (an extracellular enzyme involved in C cycling) activity was slightly increased under drought conditions and showed a positive relationship with drought, while the activity of two other enzymes (β-glucosidase and β-cellobiosidase) involved in C cycling was reduced. This finding suggests a shift in extracellular enzyme composition within these ecosystems, possibly due to the increased soil temperature because of drought treatment which favors β-xylosidase activity (Batbaatar et al., 2022; Ylla et al., 2012). Furthermore, fungi (the main producers of extracellular β-xylosidase) were more resilient to drought conditions in these ecosystems (Chapter 4; Naraian and Gautam). Defoliation effects were largely dependent on rainfall treatment and local climate and were only noticeable on one C-cycling enzyme activity. Findings suggested that rainfall and local climate are more important factors in regulating soil extracellular enzyme activity as compared to defoliation. Furthermore, observations on the defoliation effect suggest that repeated defoliation is not recommended in these ecosystems as it may lead to greater CO_2 emissions by accelerating the organic matter decomposition (Conant et al., 2011)

Chapter three tested the effects of drought and defoliation on greenhouse gas emissions (CO_2, N_2O, CH_4) over a growing season of two years (2017 and 2018). Grasslands are widely known as a sink of C and can help mitigate climate change; however, they can also become a source of C if not managed properly (Oates and Jackson, 2014; Derner and Schuman, 2007). In this chapter, I found that defoliation frequency is a more important driving factor which could affect CO_2 emissions particularly, as compared to defoliation timing. Single annual defoliation

(as compared to twice defoliated treatments) either reduced CO_2 emissions under drought conditions. This may have happened possibly due to two reasons, i) repeated defoliation causes more root deaths which could increase root exudates (Mikola et al., 2001), ii) temporary reduction of leaf area in single defoliated treatment can reduce autotrophic respiration (Shi et al., 2022). Furthermore, I found that soil temperature and extracellular enzyme activity were more important predictors of CO_2 emissions as compared to soil moisture content. The findings of this chapter suggested that single annual defoliation events are encouraged under future drought conditions to reduce CO_2 emissions from these grassland ecosystems.

In chapter four, I analyzed soil microbial communities (diversity, composition and functional ecology) to test how they are affected by drought and defoliation at two contrasting sites (mesic vs dry). Soil fungi were found to be more drought-resistant at both sites than soil bacterial communities. At the mesic site, I found that xylanolytic bacteria increased under drought, suggesting increased decomposition of xylan (Bretkreuz et al., 2021), and xylooligosaccharides (hemicellulose components). At the dry site, chemoheterotrophs were increased under drought conditions. Defoliation had limited effects on soil microbial diversity (fungi only), and overall microbial communities were found to be resistant to defoliation treatments in these ecosystems. However, the functioning of soil microbial communities was affected (such as those analyzed by enzymes). From a climate change perspective, these results suggest that single annual defoliation (more specifically, early season defoliation) is recommended in these temperate grasslands to reduce CO_2 emissions as compared to repeated defoliation.

In summary, this thesis concludes that multiple drivers affect the overall nutrient cycling in these temperate grassland ecosystems. For instance, my results showed that enzyme activity in

these soils was reduced under single defoliation events, specifically early season defoliation (e.g. heavy-none). Reduction in enzyme activity could lead to reduced/slowed organic matter decomposition, thereby reducing heterotrophic respiration. This conclusion was further supported by reduced CO_2 emissions (Chapter 3) under early single-event defoliation. Worth noting is that early season defoliation did not reduce CO_2 emissions under ambient rainfall conditions but only under drought conditions. This implies that in future drought, single event defoliation (or grazing), especially early season, is the best practice in these grasslands for the ranchers not only from a climate change perspective, but also will make these grasslands more sustainable in the long-term for primary productivity due to slowed organic matter decomposition. Furthermore, soil microbial communities themselves showed resilience to defoliation but were mainly affected by drought. Overall, drought-affected soil bacteria, to a greater extent, soil fungal communities were mostly resistant to drought conditions, or even some fungal taxa favoured drought conditions. This implies a microbial community shift under future drought conditions, which in turn will affect soil organic carbon storage in these ecosystems. I also observed that we lack detailed research/information about various fungal taxa (e.g. genus Mortierella) and their specific functioning due to the diversity within the taxa itself. This demands further investigation of microbial taxa in these ecosystems since microorganisms are the main, if not only, driving factors of organic matter decomposition and C cycling. Therefore, in-depth and long-term research on soil microbial communities in northern temperate grassland ecosystems is much needed to increase our understanding of the effects of multiple stressors (such as drought and defoliation). Finally, the findings of this thesis suggest that I found clear trends of reduction of CO_2 emissions and C-cycling enzymes in early season single

defoliation which suggest that early season single grazing/defoliation events would likely reduce greenhouse gas emissions (specifically CO_2) under future drought conditions.

References

Abdalla, M., Hastings, A., Chadwick, D. R., Jones, D. L., Evans, C. D., Jones, M. B., Rees, R. M., & Smith, P. (2018). Critical review of the impacts of grazing intensity on soil organic carbon storage and other soil quality indicators in extensively managed grasslands. *Agriculture, Ecosystems and Environment, 253*, 62–81. https://doi.org/10.1016/j.agee.2017.10.023

Ahti, T. T., & Hepburn, R. L. (1967). Preliminary Studies on Woodland Caribou Range: Especially on Lichen Stands in Ontario. *Research Branch,* Ontario Department of Lands and Forests

Alef, K., & Nannipieri, P. (1995). *Methods in applied soil microbiology and biochemistry (No. 631.46 M592ma)*. Academic Press

Allard, V., Soussana, J.-F., Falcimagne, R., Berbigier, P., Bonnefond, J. M., Ceschia, E., D'hour, P., Hénault, C., Laville, P., Martin, C., & Pinarès-Patino, C. (2007). The role of grazing management for the net biome productivity and greenhouse gas budget (CO2, N2O and CH4) of semi-natural grassland. *Agriculture, Ecosystems and Environment*, *121*(1–2), 47–58. https://doi.org/10.1016/j.agee.2006.12.004

Allison, S. D., & Treseder, K. K. (2008). Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Global Change Biology, 14*(12), 2898–2909. https://doi.org/10.1111/j.1365-2486.2008.01716.x

Allison, S. D., McGuire, K. L., & Treseder, K. K. (2010). Resistance of microbial and soil properties to warming treatment seven years after boreal fire. *Soil Biology and Biochemistry, 42*(10), 1872–1878. https://doi.org/10.1016/j.soilbio.2010.07.011

Alster, C. J., German, D. P., Lu, Y., & Allison, S. D. (2013). Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. *Soil Biology and Biochemistry, 64*, 68–79. https://doi.org/10.1016/j.soilbio.2013.03.034

Amelung, W., Brodowski, S., Sandhage-Hofmann, A., & Bol, R. (2008). Combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic

matter. *Advances in Agronomy*, *100*, 155–250. https://doi.org/10.1016/S0065-2113(08)00606-8

Amgaa, B. (2022). *Ecosystem sensitivity to variation in precipitation and defoliation across grasslands of Alberta*. [Doctoral Dissertation]. University of Alberta]. Education and Research Archive

Apprill, A., McNally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic Microbial Ecology, 75*(2), 129–137. http://doi.org/10.3354/ame01753

Arndal, M. F., Tolver, A., Larsen, K. S., Beier, C., & Schmidt, I. K. (2018). Fine root growth and vertical distribution in response to elevated CO2, warming and drought in a mixed heathland–grassland. *Ecosystems, 21*(1), 15–30. https://doi.org/10.1007/s10021-017-0131-2

Aronson, E. L., Goulden, M. L., & Allison, S. D. (2019). Greenhouse gas fluxes under drought and nitrogen addition in a Southern California grassland. *Soil Biology and Biochemistry, 131*, 19–27. https://doi.org/10.1016/j.soilbio.2018.12.010

Ash, A. J., & McIvor, J. G. (1998). How season of grazing and herbivore selectivity influence monsoon tall-grass communities of northern Australia. *Journal of Vegetation Science, 9*(1), 123–132. https://doi.org/10.2307/3237230

Attaeian, B. (2010). *Biogeochemical cycling and microbial Communities in native grasslands: Responses to climate change and defoliation*. [Doctoral Dissertation]. University of Alberta]. Education and Research Archive.

Bai, W., Fang, Y., Zhou, M., Xie, T., Li, L., & Zhang, W. H. (2015). Heavily intensified grazing reduces root production in an Inner Mongolia temperate steppe. *Agriculture, Ecosystems and Environment, 200*, 143–150. https://doi.org/10.1016/j.agee.2014.11.015

Bai, Y., Wu, J., Clark, C. M., Pan, Q., Zhang, L., Chen, S., Wang, Q., & Han, X. (2012). Grazing alters ecosystem functioning and C:N:P stoichiometry of grasslands along a regional precipitation gradient. *Journal of Applied Ecology, 49*(6), 1204–1215. https://doi.org/10.1111/j.1365-2664.2012.02205.x

Baldrian, P. (2014). Distribution of extracellular enzymes in soils: Spatial heterogeneity and determining factors at various scales. *Soil Science Society of America Journal, 78*(1), 11–18. https://doi.org/10.2136/sssaj2013.04.0155dgs

Banerjee, M. R., Burton, D. L., McCaughey, W. P., & Grant, C. A. (2000). Influence of pasture management on soil biological quality. *Journal of Range Management, 53*(1), 127–133. https://doi.org/10.2307/4003403

Bardgett, R. D., Freeman, C., & Ostle, N. J. (2008). Microbial contributions to climate change through carbon cycle feedbacks. *ISME Journal*, *2*(8), 805–814. https://doi.org/10.1038/ismej.2008.58

Barnard, R. L., Osborne, C. A., & Firestone, M. K. (2015). Changing precipitation pattern alters soil microbial community response to wet-up under a Mediterranean-type climate. *ISME Journal*, *9*(4), 946–957. https://doi.org/10.1038/ismej.2014.192

Barton, K. (2020). Mumin: Multi-model inference. R package version 1.43.17. https://cran.r-project.org/package=MuMIn

Batbaatar, A., Carlyle, C. N., Bork, E. W., Chang, S. X., & Cahill, J. F. (2022). Multi-year drought alters plant species composition more than productivity across northern temperate grasslands. *Journal of Ecology, 110*(1), 197–209. https://doi.org/10.1111/1365-2745.13796

Batbaatar, A., Carlyle, C. N., Bork, E. W., Chang, S. X., & Cahill Jr, J. F. (2023). Differential sensitivity of above-and belowground plant biomass to drought and defoliation in temperate grasslands. *Agriculture, Ecosystems & Environment*, *356*, 108660.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. Journal of Statistical Software, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bell, C. W., Fricks, B. E., Rocca, J. D., Steinweg, J. M., McMahon, S. K., & Wallenstein, M. D. (2013). High-throughput fluorometric measurement of potential soil extracellular enzyme activities. *Journal of Visualized Experiments: JoVE. Jove, 81*(81), e50961. https://doi.org/10.3791/50961

Bell, T. H., & Henry, H. A. L. (2011). Fine scale variability in soil extracellular enzyme activity is insensitive to rain events and temperature in a mesic system. *Pedobiologia, 54*(2), 141–146. https://doi.org/10.1016/j.pedobi.2010.12.003

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., & Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. *Ecology Letters*, *15*(4), 365–377. https://doi.org/10.1111/j.1461-0248.2011.01736.x

Bogati, K., & Walczak, M. (2022). The impact of drought stress on soil microbial community, enzyme activities and plants. *Agronomy, 12*(1), 189. https://doi.org/10.3390/agronomy12010189

Bork, E. W., Attaeian, B., Cahill, J. F., & Chang, S. X. (2019). Soil nitrogen and greenhouse gas dynamics in a temperate grassland under experimental warming and defoliation. *Soil Science Society of America Journal, 83*(3), 780–790. https://doi.org/10.2136/sssaj2018.04.0150

Bork, E. W., Broadbent, T. S., & Willms, W. D. (2017). Intermittent Growing Season Defoliation Variably Impacts Accumulated Herbage Productivity in Mixed Grass Prairie. *Rangeland Ecology and Management, 70*(3), 307–315. https://doi.org/10.1016/j.rama.2016.10.007

Bork, E. W., Raatz, L. L., Carlyle, C. N., Hewins, D. B., & Thompson, K. A. (2020). Soil carbon increases with long-term cattle stocking in northern temperate grasslands. *Soil Use and Management, 36*(3), 387–399. https://doi.org/10.1111/sum.12580

Boulesteix, A. L. (2010). Over-optimism in bioinformatics research. *Bioinformatics*, *26*(3), 437-439.

Bouwman, A. F. (1998). Nitrogen oxides and tropical agriculture. *Nature, 392*(6679), 866–867. https://doi.org/10.1038/31809

Braun, M., Bai, Y., McConkey, B., Farrell, R., Romo, J. T., & Pennock, D. (2013). Greenhouse gas flux in a temperate grassland as affected by landform and disturbance. *Landscape Ecology*, *28*(4), 709–723. https://doi.org/10.1007/s10980-013-9878-9

Breitkreuz, C., Herzig, L., Buscot, F., Reitz, T., & Tarkka, M. (2021). Interactions between soil properties, agricultural management and cultivar type drive structural and functional

adaptations of the wheat rhizosphere microbiome to drought. *Environmental Microbiology, 23*(10), 5866–5882. https://doi.org/10.1111/1462-2920.15607

Brito, L. F., Azenha, M. V., Janusckiewicz, E. R., Cardoso, A. S., Morgado, E. S., Malheiros, E. B., La Scala, N., Reis, R. A., & Ruggieri, A. C. (2015). Seasonal fluctuation of soil carbon dioxide emission in differently managed pastures. *Agronomy Journal, 107*(3), 957–962. https://doi.org/10.2134/agronj14.0480

Broadbent, T. S., Bork, E. W., and Willms, W. D. (2016). Contingency in defoliation and moisture effects on northern mixedgrass prairie composition and diversity. *Rangeland Ecology and Management, 69*(4), 292–299. https://doi.org/10.1016/j.rama.2016.02.003

Burnham, K. P., & Anderson, D. R. (2002). Model selection and multimodel inference: A practical information-theoretic approach (2nd ed.). *Springer*

Burns, R. G., & Dick, R. P. (2002). Enzymes in the environment: Activity, ecology, and applications. CRC Press

Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallenstein, M. D., Weintraub, M. N., & Zoppini, A. (2013). Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biology and Biochemistry, 58*, 216–234. https://doi.org/10.1016/j.soilbio.2012.11.009

Byrd, K. B., Flint, L. E., Alvarez, P., Casey, C. F., Sleeter, B. M., Soulard, C. E., Flint, A. L., & Sohl, T. L. (2015). Integrated climate and land use change scenarios for California rangeland ecosystem services: Wildlife habitat, soil carbon, and water supply. *Landscape Ecology, 30*(4), 729–750. https://doi.org/10.1007/s10980-015-0159-7

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods, 13*(7), 581–583.

Carbone, M. S., Still, C. J., Ambrose, A. R., Dawson, T. E., Williams, A. P., Boot, C. M., Schaeffer, S. M., & Schimel, J. P. (2011). Seasonal and episodic moisture controls on plant and microbial contributions to soil respiration. *Oecologia, 167*(1), 265–278. https://doi.org/10.1007/s00442-011-1975-3

Cardoso, A. D. S., Brito, L. D. F., Janusckiewicz, E. R., Morgado, E. D. S., Barbero, R. P., Koscheck, J. F. W., Reis, R. A., & Ruggieri, A. C. (2017). Impact of grazing intensity and seasons on greenhouse gas emissions in tropical grassland. *Ecosystems, 20*(4), 845–859. https://doi.org/10.1007/s10021-016-0065-0

Carey, C. J., Beman, J. M., Eviner, V. T., Malmstrom, C. M., and Hart, S. C. (2015). Soil microbial community structure is unaltered by plant invasion, vegetation clipping, and nitrogen fertilization in experimental semi-arid grasslands. *Frontiers in Microbiology, 6*, 466. https://doi.org/10.3389/fmicb.2015.00466

Carlyle, C. N., Fraser, L. H., & Turkington, R. (2011). Tracking soil temperature and moisture in a multi-factor climate experiment in temperate grassland: Do climate manipulation methods produce their intended effects? *Ecosystems*, *14*(3), 489–502. https://doi.org/10.1007/s10021-011-9425-y

Chang, J., Ciais, P., Gasser, T., Smith, P., Herrero, M., Havlík, P., Obersteiner, M., Guenet, B., Goll, D. S., Li, W., Naipal, V., Peng, S., Qiu, C., Tian, H., Viovy, N., Yue, C., & Zhu, D. (2021). Climate warming from managed grasslands cancels the cooling effect of carbon sinks in sparsely grazed and natural grasslands. *Nature Communications*, *12*(1), 118. https://doi.org/10.1038/s41467-020-20406-7

Chao, L., Ma, X., Tsetsegmaa, M., Zheng, Y., Qu, H., Dai, Y., Li, J., & Bao, Y. (2022). Response of soil microbial community composition and diversity at different gradients of grassland degradation in central Mongolia. *Agriculture, 12*(9), 1430. https://doi.org/10.3390/agriculture12091430

Chapin, F. S., Matson, P., & Mooney, H. A. (2002). Principles of terrestrial ecosystem ecology. *Springer*

Chávez, R., Bull, P., & Eyzaguirre, J. (2006). The xylanolytic enzyme system from the genus Penicillium. *Journal of Biotechnology*, *123*(4), 413–433. https://doi.org/10.1016/j.jbiotec.2005.12.036

Chen, J., Luo, Y., García-Palacios, P., Cao, J., Dacal, M., Zhou, X., Li, J., Xia, J., Niu, S., Yang, H., Shelton, S., Guo, W., & van Groenigen, K. J. (2018). Differential responses of

carbon-degrading enzyme activities to warming: Implications for soil respiration. *Global Change Biology, 24*(10), 4816–4826. https://doi.org/10.1111/gcb.14394

Chen, J., Shi, W., & Cao, J. (2015). Effects of grazing on ecosystem CO_2 exchange in a meadow grassland on the Tibetan Plateau during the growing season. *Environmental Management*, *55*(2), 347–359. https://doi.org/10.1007/s00267-014-0390-z

Cheng, L., Zhang, N., Yuan, M., Xiao, J., Qin, Y., Deng, Y., Tu, Q., Xue, K., van Nostrand, J. D., Wu, L., He, Z., Zhou, X., Leigh, M. B., Konstantinidis, K. T., Schuur, E. A., Luo, Y., Tiedje, J. M., & Zhou, J. (2017). Warming enhances old organic carbon decomposition through altering functional microbial communities. *ISME Journal, 11*(8), 1825–1835. https://doi.org/10.1038/ismej.2017.48

Chuan, X., Carlyle, C. N., Bork, E. W., Chang, S. X., & Hewins, D. B. (2020). Extracellular enzyme activity in grass litter varies with grazing history, environment and plant species in temperate grasslands. *Science of the Total Environment, 702*, 134562. https://doi.org/10.1016/j.scitotenv.2019.134562

Chuan, X., Carlyle, C. N., Bork, E. W., Chang, S. X., & Hewins, D. B. (2018). Long-term grazing accelerated litter decomposition in northern temperate grasslands. *Ecosystems, 21*(7), 1321–1334. https://doi.org/10.1007/s10021-018-0221-9

Clark, J. S., Campbell, J. H., Grizzle, H., Acosta-Martìnez, V., & Zak, J. C. (2009). Soil microbial community response to drought and precipitation variability in the Chihuahuan Desert. *Microbial Ecology, 57*(2), 248–260. https://doi.org/10.1007/s00248-008-9475-7

Conant, R. T., Paustian, K., & Elliott, E. T. (2001). Grassland management and conversion into grassland: Effects on soil carbon. *Ecological Applications*, *11*(2), 343–355. https://doi.org/10.1890/1051-0761(2001)011[0343:GMACIG]2.0.CO;2

Cordero, I., Leizeaga, A., Hicks, L. C., Rousk, J., and Bardgett, R. D. (2021). Extreme drought triggers transition to an alternative soil microbial state. *bioRxiv*. 12.

Crain, C. M., Kroeker, K., & Halpern, B. S. (2008). Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters, 11*(12), 1304–1315. https://doi.org/10.1111/j.1461-0248.2008.01253.x

Cui, X., Wang, Y., Niu, H., Wu, J., Wang, S., Schnug, E., ... & Tang, Y. (2005). Effect of long-term grazing on soil organic carbon content in semiarid steppes in Inner Mongolia. *Ecological Research*, *20*, 519-527.

Das, S. K., & Varma, A. (2010). Role of enzymes in maintaining soil health. In *Soil enzymology* (pp. 25–42). Springer. https://doi.org/10.1007/978-3-642-14225-3_2

de Oliveira, T. B., de Lucas, R. C., Scarcella, A. S. D. A., Contato, A. G., Pasin, T. M., Martinez, C. A., and Polizeli, M. D. L. T. D. M. (2020). Fungal communities differentially respond to warming and drought in tropical grassland soil. *Molecular Ecology, 29*(8), 1550-1559.

de Vries, F. T., Griffiths, R. I., Bailey, M., Craig, H., Girlanda, M., Gweon, H. S., Hallin, S., Kaisermann, A., Keith, A. M., Kretzschmar, M., Lemanceau, P., Lumini, E., Mason, K. E., Oliver, A., Ostle, N., Prosser, J. I., Thion, C., Thomson, B., & Bardgett, R. D. (2018). Soil bacterial networks are less stable under drought than fungal networks. *Nature Communications, 9*(1), 3033. https://doi.org/10.1038/s41467-018-05516-7

Deng, L., Peng, C., Kim, D. G., Li, J., Liu, Y., Hai, X., Liu, Q., Huang, C., Shangguan, Z., & Kuzyakov, Y. (2021). Drought effects on soil carbon and nitrogen dynamics in global natural ecosystems. *Earth-Science Reviews,* 214, 103501. https://doi.org/10.1016/j.earscirev.2020.103501

Derner, J. D., & Schuman, G. E. (2007). Carbon sequestration and rangelands: A synthesis of land management and precipitation effects. *Journal of Soil and Water Conservation, 62*(2), 77–85.

Derner, J. D., Boutton, T. W., & Briske, D. D. (2006). Grazing and ecosystem carbon storage in the North American Great Plains. *Plant and Soil, 280*(1–2), 77–90. https://doi.org/10.1007/s11104-005-2554-3

Derrien, D., Barré, P., Basile-Doelsch, I., Cécillon, L., Chabbi, A., Crème, A., Fontaine, S., Henneron, L., Janot, N., Lashermes, G., Quénéa, K., Rees, F., & Dignac, M. F. (2023). Current controversies on mechanisms controlling soil carbon storage: Implications for interactions with practitioners and policy-makers. A review. *Agronomy for Sustainable Development, 43*(1), 21. https://doi.org/10.1007/s13593-023-00876-x

Deutsch, E. S., & Bork, E. W. (2008). Combined effects of litter and defoliation on soil moisture and associated forage production in aspen parkland grasslands. In The 2008 Joint Meeting of the Society for Range Management and the America Forage and Grassland Council.

Deutsch, E. S., Bork, E. W., & Willms, W. D. (2010). Soil moisture and plant growth responses to litter and defoliation impacts in Parkland grasslands. *Agriculture, Ecosystems and Environment, 135*(1–2), 1–9. https://doi.org/10.1016/j.agee.2009.08.002

Dick, W. A., & Tabatabai, M. A. (1992). Significance and potential uses of soil enzymes. *Soil microbial ecology: applications in agricultural and environmental management*, 95–127

Dijkstra, P., Thomas, S. C., Heinrich, P. L., Koch, G. W., Schwartz, E., & Hungate, B. A. (2011). Effect of temperature on metabolic activity of intact microbial communities: Evidence for altered metabolic pathway activity but not for increased maintenance respiration and reduced carbon use efficiency. *Soil Biology and Biochemistry*, *43*(10), 2023–2031. https://doi.org/10.1016/j.soilbio.2011.05.018

Dixon, A. P., Faber-Langendoen, D., Josse, C., Morrison, J., & Loucks, C. J. (2014). Distribution mapping of world grassland types. *Journal of Biogeography, 41*(11), 2003–2019. https://doi.org/10.1111/jbi.12381

Ekenler, M., & Tabatabai, M. A. (2004). β-glucosaminidase activity as an index of nitrogen mineralization in soils. *Communications in Soil Science and Plant Analysis*, *35*(7–8), 1081–1094. https://doi.org/10.1081/CSS-120030588

Eldridge, D. J., Poore, A. G., Ruiz-Colmenero, M., Letnic, M., & Soliveres, S. (2016). Ecosystem structure, function, and composition in rangelands are negatively affected by livestock grazing. *Ecological Applications, 26*(4), 1273–1283. https://doi.org/10.1890/15-1234

Entwistle, E. M., Zak, D. R., and Edwards, I. P. (2013). Long-term experimental nitrogen deposition alters the composition of the active fungal community in the forest floor. *Soil Science Society of America Journal, 77*(5), 1648-1658.

Esch, E. H., Hernández, D. L., Pasari, J. R., Kantor, R. S. G., & Selmants, P. C. (2013). Response of soil microbial activity to grazing, nitrogen deposition, and exotic cover in a

serpentine grassland. *Plant and Soil, 366*(1–2), 671–682. https://doi.org/10.1007/s11104-012-1463-5

Evans, C. R. W., Krzic, M., Broersma, K., & Thompson, D. J. (2012). Long-term grazing effects on grassland soil properties in southern British Columbia. *Canadian Journal of Soil Science*, *92*(4), 685–693. https://doi.org/10.4141/cjss2011-070

Evans, S. E., and Wallenstein, M. D. (2014). Climate change alters ecological strategies of soil bacteria. *Ecology letters, 17*(2), 155-164.

Fansler, S. J., Smith, J. L., Bolton, H., & Bailey, V. L. (2005). Distribution of two C cycle enzymes in soil aggregates of a prairie chronosequence. *Biology and Fertility of Soils*, *42*(1), 17–23. https://doi.org/10.1007/s00374-005-0867-2

Filazzola, A., and Cahill, J.F. (2021). Replication in field ecology: Identifying challenges and proposing solutions. *Methods in Ecology and Evolution, 12:*1780-1792

Flechard, C. R., Ambus, P., Skiba, U., Rees, R. M., Hensen, A., Van Amstel, A., Dasselaar, AvdP., Soussana, J.-F., Jones, M., Clifton-Brown, J., Raschi, A., Horvath, L., Neftel, A., Jocher, M., Ammann, C., Leifeld, J., Fuhrer, J., Calanca, P., Thalman, E., . . . and Grosz, B. (2007). Effects of climate and management intensity on nitrous oxide emissions in grassland systems across Europe. *Agriculture, Ecosystems and Environment*, *121*(1–2), 135–152. https://doi.org/10.1016/j.agee.2006.12.024

Follett, R. F., Kimble, J. M., & Lal, R. (2001). The potential of US grazing lands to sequester soil carbon. In *The potential of US grazing lands to sequester carbon and mitigate the greenhouse effect* (pp. 401–430). CRC Press

Ford, H., Rousk, J., Garbutt, A., Jones, L., & Jones, D. L. (2013). Grazing effects on microbial community composition, growth and nutrient cycling in salt marsh and sand dune grasslands. *Biology and Fertility of Soils, 49*(1), 89–98. https://doi.org/10.1007/s00374-012-0721-2

Frank, A. B., Liebig, M. A., & Hanson, J. D. (2002). Soil carbon dioxide fluxes in northern semiarid grasslands. *Soil Biology and Biochemistry, 34*(9), 1235–1241. https://doi.org/10.1016/S0038-0717(02)00062-7

Fterich, A., Mahdhi, M., Lafuente, A., Pajuelo, E., Caviedes, M. A., Rodriguez-Llorente, I. D., & Mars, M. (2012). Taxonomic and symbiotic diversity of bacteria isolated from nodules of Acacia tortilis subsp. raddiana in arid soils of Tunisia. *Canadian Journal of Microbiology*, *58*(6), 738–751. https://doi.org/10.1139/w2012-048

Fukami, T. (2015). Historical Contingency in Community Assembly: Integrating Niches, Species Pools, and Priority Effects. *Annual Review of Ecology, Evolution, and Systematics*, *46*(1), 1–23. https://doi.org/10.1146/annurev-ecolsys-110411-160340

Galbally, I. E., Kirstine, W. V., Meyer, C. P., & Wang, Y. P. (2008). Soil–atmosphere trace gas exchange in semiarid and arid zones. *Journal of Environmental Quality*, *37*(2), 599–607. https://doi.org/10.2134/jeq2006.0445

Ganjegunte, G. K., Vance, G. F., Preston, C. M., Schuman, G. E., Ingram, L. J., Stahl, P. D., & Welker, J. M. (2005). Soil organic carbon composition in a northern mixed-grass prairie: Effects of grazing. *Soil Science Society of America Journal*, *69*(6), 1746–1756. https://doi.org/10.2136/sssaj2005.0020

Gao, W., Reed, S. C., Munson, S. M., Rui, Y., Fan, W., Zheng, Z., Li, L., Che, R., Xue, K., Du, J., Cui, X., Wang, Y., & Hao, Y. (2021). Responses of soil extracellular enzyme activities and bacterial community composition to seasonal stages of drought in a semiarid grassland. *Geoderma*, *401*(115327), 115327. https://doi.org/10.1016/j.geoderma.2021.115327

Gao, X., Thomas, B. W., Beck, R., Thompson, D. J., Zhao, M., Willms, W. D., & Hao, X. (2018). Long-term grazing alters soil trace gas fluxes from grasslands in the foothills of the Rocky Mountains, Canada. *Land Degradation and Development*, *29*(2), 292–302. https://doi.org/10.1002/ldr.2664

Garcia, M. R. L., Sampaio, A. A. M., & Nahas, E. (2011). Impact of different grazing systems for bovine cattle on the soil microbiological and chemical characteristics. *Revista Brasileira de Zootecnia, 40*(7), 1568–1575. https://doi.org/10.1590/S1516-35982011000700024

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. *Molecular Ecology*, *2*(2), 113–118. https://doi.org/10.1111/j.1365-294x.1993.tb00005.x

Gherardi, L. A., & Sala, O. E. (2013). Automated rainfall manipulation system: a reliable and inexpensive tool for ecologists. *Ecosphere (Washington, D.C), 4*(2), art18. https://doi.org/10.1890/es12-00371.1

Giel, P., & Bojarczuk, K. (2011). Effects of high concentrations of calcium salts in the substrate and its pH on the growth of selected rhododendron cultivars. *Acta Societatis Botanicorum Poloniae*, *80*(2), 105–114. https://doi.org/10.5586/asbp.2011.021

Gomez, E. J., Delgado, J. A., & Gonzalez, J. M. (2020). Environmental factors affect the response of microbial extracellular enzyme activity in soils when determined as a function of water availability and temperature. *Ecology and Evolution*, *10*(18), 10105-10115

Gougoulias, C., Clark, J. M., & Shaw, L. J. (2014). The role of soil microbes in the global carbon cycle: Tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. *Journal of the Science of Food and Agriculture*, *94*(12), 2362–2371. https://doi.org/10.1002/jsfa.6577

Guitian, R., & Bardgett, R. D. (2000). Plant and soil microbial responses to defoliation in temperate seminatural grassland. *Plant and Soil, 220*(1–2), 271.

Guo, N., Li, L., Cui, J., & Cai, B. (2021). Effects of Funneliformis mosseae on the fungal community in and soil properties of a continuously cropped soybean system. *Applied Soil Ecology: A Section of Agriculture, Ecosystems & Environment*, *164*(103930), 103930. https://doi.org/10.1016/j.apsoil.2021.103930

Gutknecht, J. L. M., Henry, H. A. L., & Balser, T. C. (2010). Inter-annual variation in soil extra-cellular enzyme activity in response to simulated global change and fire disturbance. *Pedobiologia*, *53*(5), 283–293. https://doi.org/10.1016/j.pedobi.2010.02.001

Hartmann, M., Brunner, I., Hagedorn, F., Bardgett, R. D., Stierli, B., Herzog, C., Chen, X., Zingg, A., Graf-Pannatier, E., Rigling, A., & Frey, B. (2017). A decade of irrigation

transforms the soil microbiome of a semi-arid pine forest. *Molecular Ecology, 26*(4), 1190–1206. https://doi.org/10.1111/mec.13995

Havstad, K. M., Peters, D. P. C., Skaggs, R., Brown, J., Bestelmeyer, B., Fredrickson, E., Herrick, J., & Wright, J. (2007). Ecological services to and from rangelands of the United States. *Ecological Economics, 64*(2), 261–268. https://doi.org/10.1016/j.ecolecon.2007.08.005

Hawkes, C. V., Kivlin, S. N., Rocca, J. D., Huguet, V., Thomsen, M. A., and Suttle, K. B. (2011). Fungal community responses to precipitation. *Global Change Biology, 17*(4), 1637-1645.

Heady, H. F., and Child, R. D. (1994). Rangeland ecology and management. Westview Press, Boulder, CO. *Rangeland ecology and management*. 2nd ed. Westview Press, Boulder, CO.

Henry, H. A. L. (2012). Soil extracellular enzyme dynamics in a changing climate. *Soil Biology and Biochemistry, 47,* 53–59. https://doi.org/10.1016/j.soilbio.2011.12.026

Hewins, D. B., Broadbent, T., Carlyle, C. N., & Bork, E. W. (2016). Extracellular enzyme activity response to defoliation and water addition in two ecosites of the mixed grass prairie. *Agriculture, Ecosystems and Environment, 230*, 79–86. https://doi.org/10.1016/j.agee.2016.05.033

Hewins, D. B., Fatemi, F., Adams, B., Carlyle, C. N., Chang, S. X., & Bork, E. W. (2015). Grazing, regional climate and soil biophysical impacts on microbial enzyme activity in grassland soil of western Canada. *Pedobiologia, 58*(5–6), 201–209. https://doi.org/10.1016/j.pedobi.2015.10.003

Hewins, D. B., Lyseng, M. P., Schoderbek, D. F., Alexander, M., Willms, W. D., Carlyle, C. N., Chang, S. X., & Bork, E. W. (2018). Grazing and climate effects on soil organic carbon concentration and particle-size association in northern grasslands. *Scientific Reports, 8*(1), 1336. https://doi.org/10.1038/s41598-018-19785-1

Hildén, K., & Mäkelä, M. R. (2018). Role of fungi in wood decay. In *Reference module in life sciences*. Elsevier.

Ho, A., Di Lonardo, D. P., & Bodelier, P. L. (2017). Revisiting life strategy concepts in environmental microbial ecology. *FEMS Microbiology Ecology*, 93(3). https://doi.org/10.1093/femsec/fix006

Hueso, S., García, C., & Hernández, T. (2012). Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. *Soil Biology and Biochemistry, 50*, 167–173. https://doi.org/10.1016/j.soilbio.2012.03.026

Hui, R., Li, X., Zhao, R., Tan, H., & Jia, R. (2021). Physiological response of moss/cyanobacteria crusts along a precipitation gradient from semi-arid to arid desert in China. *Plant and Soil*, 468(1–2), 97–113. https://doi.org/10.1007/s11104-021-05117-2

IPCC. (2021). Climate Change 2021: The physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change. Climate Change. Cambridge University Press.

IPCC. (2022). Climate Change 2022: Impacts, adaptation, and vulnerability. Contribution of working Group II to the sixth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press.

Jentsch, A., Kreyling, J., Elmer, M., Gellesch, E., Glaser, B., Grant, K., Hein, R., Lara, M., Mirzae, H., Nadler, S. E., Nagy, L., Otieno, D., Pritsch, K., Rascher, U., Schädler, M., Schloter, M., Singh, B. K., Stadler, J., Walter, J., … Beierkuhnlein, C. (2011). Climate extremes initiate ecosystem-regulating functions while maintaining productivity: Drought effect on multiple ecosystem services. *The Journal of Ecology*, *99*(3), 689–702. https://doi.org/10.1111/j.1365-2745.2011.01817.x

Jiang, R., Gan, T. Y., Xie, J., Wang, N., & Kuo, C. C. (2017). Historical and potential changes of precipitation and temperature of Alberta subjected to climate change impact: 1900–2100. *Theoretical and Applied Climatology, 127*(3–4), 725–739. https://doi.org/10.1007/s00704-015-1664-y

Joergensen, R., & Wichern, F. (2008). Quantitative assessment of the fungal contribution to microbial tissue in soil. *Soil Biology and Biochemistry*, *40*(12), 2977–2991. https://doi.org/10.1016/j.soilbio.2008.08.017

Jonas, J. L., Buhl, D. A., & Symstad, A. J. (2015). Impacts of weather on long-term patterns of plant richness and diversity vary with location and management. *Ecology, 96*(9), 2417–2432. https://doi.org/10.1890/14-1989.1

Jones, M. B., & Donnelly, A. (2004). Carbon sequestration in temperate grassland ecosystems and the influence of management, climate and elevated CO2. *New Phytologist*, *164*(3), 423–439. https://doi.org/10.1111/j.1469-8137.2004.01201.x

Jones, S. K., Famulari, D., Di Marco, C. F., Nemitz, E., Skiba, U. M., Rees, R. M., & Sutton, M. A. (2011). Nitrous oxide emissions from managed grassland: A comparison of eddy covariance and static chamber measurements. *Atmospheric Measurement Techniques*, *4*(10), 2179–2194. https://doi.org/10.5194/amt-4-2179-2011

Jones, S. K., Rees, R. M., Skiba, U. M., & Ball, B. C. (2005). Greenhouse gas emissions from a managed grassland. *Global and Planetary Change*, *47*(2–4), 201–211. https://doi.org/10.1016/j.gloplacha.2004.10.011

Kaiser, K., Wemheuer, B., Korolkow, V., Wemheuer, F., Nacke, H., Schöning, I., Schrumpf, M., & Daniel, R. (2016). Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests. *Scientific Reports*, 6(1). https://doi.org/10.1038/srep33696

Kakumanu, M. L., Cantrell, C. L., & Williams, M. A. (2013). Microbial community response to varying magnitudes of desiccation in soil: A test of the osmolyte accumulation hypothesis. *Soil Biology and Biochemistry, 57*, 644–653. https://doi.org/10.1016/j.soilbio.2012.08.014

Kardol, P., & Wardle, D. A. (2010). How understanding aboveground–belowground linkages can assist restoration ecology. *Trends in Ecology and Evolution, 25*(11), 670–679. https://doi.org/10.1016/j.tree.2010.09.001

Kardol, P., Cregger, M. A., Campany, C. E., & Classen, A. T. (2010). Soil ecosystem functioning under climate change: Plant species and community effects. *Ecology, 91*(3), 767–781. https://doi.org/10.1890/09-0135.1

Kassambara, A. (2020). ggpubr: "ggplot2" Based Publication Ready Plots. R package version 0.4.0. https://cran.r-project.org/package=ggpubr

Keiluweit, M., Nico, P. S., Kleber, M., & Fendorf, S. (2016). Are oxygen limitations under recognized regulators of organic carbon turnover in upland soils? *Biogeochemistry, 127*(2–3), 157–171. https://doi.org/10.1007/s10533-015-0180-6

Keith, H., Jacobsen, K. L., & Raison, R. J. (1997). Effects of soil phosphorus availability, temperature and moisture on soil respiration in Eucalyptus pauciflora forest. *Plant and Soil*, *190*(1), 127–141. https://doi.org/10.1023/A:1004279300622

Kelley, A. M., Fay, P. A., Polley, H. W., Gill, R. A., & Jackson, R. B. (2011). Atmospheric CO2 and soil extracellular enzyme activity: A meta-analysis and CO2 gradient experiment. *Ecosphere, 2*(8), 1–20.

Knob, A., Terrasan, C. R. F., & Carmona, E. C. (2010). β-xylosidases from filamentous fungi: An overview. *World Journal of Microbiology and Biotechnology*, *26*(3), 389–407. https://doi.org/10.1007/s11274-009-0190-4

Koricheva, J., & Gurevitch, J. (2014). Uses and misuses of meta-analysis in plant ecology. *Journal of Ecology*, *102*(4), 828-844.

Kreyling, J., Beierkuhnlein, C., Elmer, M., Pritsch, K., Radovski, M., Schloter, M., Wöllecke, J., & Jentsch, A. (2008). Soil biotic processes remain remarkably stable after 100-year extreme weather events in experimental grassland and heath. *Plant and Soil*, *308*(1–2), 175–188. https://doi.org/10.1007/s11104-008-9617-1

Kwon, M. J., Haraguchi, A., & Kang, H. (2013). Long-term water regime differentiates changes in decomposition and microbial properties in tropical peat soils exposed to the short-term drought. *Soil Biology and Biochemistry*, *60*, 33–44. https://doi.org/10.1016/j.soilbio.2013.01.023

Lal, R. (2009). Sequestering carbon in soils of arid ecosystems. *Land Degradation & Development*, *20*(4), 441-454.

Landesman, W. J., & Dighton, J. (2010). Response of soil microbial communities and the production of plant-available nitrogen to a two-year rainfall manipulation in the New Jersey Pinelands. *Soil Biology and Biochemistry*, *42*(10), 1751–1758. https://doi.org/10.1016/j.soilbio.2010.06.012

Lavallee, J. M., Soong, J. L., & Cotrufo, M. F. (2020). Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. *Global Change Biology, 26*(1), 261–273. https://doi.org/10.1111/gcb.14859

Lecain, D. R., Morgan, J. A., & Schuman, G. E. (2000). Carbon exchange rates in grazed and ungrazed pastures of Wyoming. *Journal of Range Management, 53*(2). https://doi.org/10.2458/azu_jrm_v53i2_lecain

Legendre, P., & Legendre, L. (2012). *Numerical ecology*. Elsevier.

Lenth, R. V. (2021). Estimated marginal means, aka least-squares means [R Package Emmeans Version 1.6. 0]. *Comprehensive R Archive Network (CRAN)*.

Li, J., Benti, G., Wang, D., Yang, Z., & Xiao, R. (2022). Effect of alteration in precipitation amount on soil microbial community in a semi-arid Grassland. *Frontiers in Microbiology, 13*, 842446. https://doi.org/10.3389/fmicb.2022.842446

Li, L., Fan, W., Kang, X., Wang, Y., Cui, X., Xu, C., Griffin, K. L., & Hao, Y. (2016). Responses of greenhouse gas fluxes to climate extremes in a semiarid grassland. *Atmospheric Environment, 142*, 32–42. https://doi.org/10.1016/j.atmosenv.2016.07.039

Li, X., Hui, R., Zhang, P., & Song, N. (2021). Divergent responses of moss-and lichen-dominated biocrusts to warming and increased drought in arid desert regions. *Agricultural and Forest Meteorology, 303*, 108387.

Liang, C., Schimel, J. P., & Jastrow, J. D. (2017). The importance of anabolism in microbial control over soil carbon storage. *Nature Microbiology*, *2*(8), 17105. https://doi.org/10.1038/nmicrobiol.2017.105

Liebig, M. A., Kronberg, S. L., Hendrickson, J. R., Dong, X., & Gross, J. R. (2013). Carbon dioxide efflux from long-term grazing management systems in a semiarid region. *Agriculture, Ecosystems and Environment*, *164*, 137–144. https://doi.org/10.1016/j.agee.2012.09.015

Lin, X., Zhang, Z., Wang, S., Hu, Y., Xu, G., Luo, C., Chang, X., Duan, J., Lin, Q., Xu, B., Wang, Y., Zhao, X., & Xie, Z. (2011). Response of ecosystem respiration to warming and grazing during the growing seasons in the alpine meadow on the Tibetan Plateau.

Agricultural and Forest Meteorology, 151(7), 792–802. https://doi.org/10.1016/j.agrformet.2011.01.009

Liu, C., Cui, Y., Li, X., & Yao, M. (2021a). microeco: An R package for data mining in microbial community ecology. *FEMS Microbiology Ecology*, 97(2). https://doi.org/10.1093/femsec/fiaa255

Liu, H., Zang, R., & Chen, H. Y. (2016). Effects of grazing on photosynthetic features and soil respiration of rangelands in the Tianshan Mountains of Northwest China. *Scientific Reports*, *6*(1), 30087. https://doi.org/10.1038/srep30087

Liu, N., Kan, H. M., Yang, G. W., & Zhang, Y. J. (2015). Changes in plant, soil, and microbes in a typical steppe from simulated grazing: Explaining potential change in soil C. *Ecological Monographs, 85*(2), 269–286. https://doi.org/10.1890/14-1368.1

Liu, Q., Zhao, X., Liu, Y., Xie, S., Xing, Y., Dao, J., Wei, B., Peng, Y., Duan, W., & Wang, Z. (2021b). Response of Sugarcane Rhizosphere Bacterial Community to Drought Stress. *Frontiers in Microbiology*, 12. https://doi.org/10.3389/fmicb.2021.716196

Liu, Y., Yan, C., Matthew, C., Wood, B., & Hou, F. (2017). Key sources and seasonal dynamics of greenhouse gas fluxes from yak grazing systems on the Qinghai-Tibetan Plateau. *Scientific Reports*, *7*(1), 40857. https://doi.org/10.1038/srep40857

Logsdon, S., Clay, D., & Moore, D. (Eds.). (2008). *Soil science: Step-by-step field analysis*. American Society of Agronomy.

Louca, S., Parfrey, L. W., & Doebeli, M. (2016). Decoupling function and taxonomy in the global ocean microbiome. *Science (New York, N.Y.)*, *353*(6305), 1272–1277. https://doi.org/10.1126/science.aaf4507

Lu, X., Kelsey, K. C., Yan, Y., Sun, J., Wang, X., Cheng, G., & Neff, J. C. (2017). Effects of grazing on ecosystem structure and function of alpine grasslands in Qinghai–Tibetan Plateau: A synthesis. *Ecosphere*, *8*(1), e01656.

Luo, L., Meng, H., & Gu, J. D. (2017). Microbial extracellular enzymes in biogeochemical cycling of ecosystems. *Journal of Environmental Management, 197*, 539–549. https://doi.org/10.1016/j.jenvman.2017.04.023

Lyseng, M. P., Bork, E. W., Hewins, D. B., Alexander, M. J., Carlyle, C. N., Chang, S. X., & Willms, W. D. (2018). Long-term grazing impacts on vegetation diversity, composition, and exotic species presence across an aridity gradient in northern temperate grasslands. *Plant Ecology*, *219*(6), 649–663. https://doi.org/10.1007/s11258-018-0824-4

Ma, B., Cai, Y., Bork, E. W., & Chang, S. X. (2018). Defoliation intensity and elevated precipitation effects on microbiome and interactome depend on site type in northern mixed-grass prairie. *Soil Biology and Biochemistry, 122*, 163–172. https://doi.org/10.1016/j.soilbio.2018.04.015

Ma, Z., Bork, E. W., Attaeian, B., Cahill, Jr., J. F., & Chang, S. X. (2022). Altered precipitation rather than warming and defoliation regulate short-term soil carbon and nitrogen fluxes in a northern temperate grassland. *Agricultural and Forest Meteorology*, *327*, 109217. https://doi.org/10.1016/j.agrformet.2022.109217

Ma, Z., Bork, E. W., Li, J., Chen, G., & Chang, S. X. (2021). Photosynthetic carbon allocation to live roots increases the year following high intensity defoliation across two ecosites in a temperate mixed grassland. *Agriculture, Ecosystems and Environment*, *316*, 107450. https://doi.org/10.1016/j.agee.2021.107450

Ma, Z., Chen, H. Y. H., Li, Y., & Chang, S. X. (2020). Interactive effects of global change factors on terrestrial net primary productivity are treatment length and intensity dependent. *Journal of Ecology, 108*(5), 2083–2094. https://doi.org/10.1111/1365-2745.13379

Ma, Z., Shrestha, B. M., Bork, E. W., Chang, S. X., Carlyle, C. N., Döbert, T. F., Sobrinho, L. S., & Boyce, M. S. (2021). Soil greenhouse gas emissions and grazing management in northern temperate grasslands. *Science of the Total Environment, 796*, 148975. https://doi.org/10.1016/j.scitotenv.2021.148975

Macdonald, L. M., Paterson, E., Dawson, L. A., & McDonald, A. J. S. (2004). Short-term effects of defoliation on the soil microbial community associated with two contrasting Lolium perenne cultivars. *Soil Biology and Biochemistry, 36*(3), 489–498. https://doi.org/10.1016/j.soilbio.2003.11.001

Macdonald, L. M., Paterson, E., Dawson, L. A., & McDonald, A. J. S. (2006). Defoliation and fertiliser influences on the soil microbial community associated with two contrasting Lolium perenne cultivars. *Soil Biology and Biochemistry*, *38*(4), 674–682. https://doi.org/10.1016/j.soilbio.2005.06.017

Manucharova, N. A., Yaroslavtsev, A. M., Kornyushenko, E. G., Stepanov, A. L., Smagin, A. V., Zvyagintsev, D. G., and Sudnitsyn, I. I. (2007). Methane production and growth of microorganisms under different moisture conditions in soils with added chitin and without it. *Eurasian Soil Science, 40*, 860-865

Manzoni, S., Schimel, J. P., & Porporato, A. (2012). Responses of soil microbial communities to water stress: results from a meta-analysis. *Ecology*, *93*(4), 930–938. https://doi.org/10.1890/11-0026.1

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10. https://doi.org/10.14806/ej.17.1.200

Martins, B. R., Siani, R., Treder, K., Michalowska, D., Radl, V., Pritsch, K., & Schloter, M. (2023). Low soil moisture induces recruitment of Actinobacteria in the rhizosphere of a drought-sensitive and Rhizobiales in a drought-tolerant potato cultivar. In *bioRxiv*. https://doi.org/10.1101/2023.05.26.542284

Marx, M.-C., Wood, M., & Jarvis, S. C. (2001). A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biology and Biochemistry*, *33*(12–13), 1633–1640. https://doi.org/10.1016/S0038-0717(01)00079-7

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS One*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

McSherry, M. E., & Ritchie, M. E. (2013). Effects of grazing on grassland soil carbon: A global review. *Global Change Biology, 19*(5), 1347–1357. https://doi.org/10.1111/gcb.12144

Meehl, G. A., Stocker, T. F., Collins, W. D., Friedlingstein, P., Gaye, A. T., Gregory, J. M., Kitoh, A., Knutti, R., Murphy, J. M., Noda, A., Raper, S. C. B., Watterson, I. G., Weaver, A. J., & Zhao, Z.-C. (2007): The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.

Global climate projections. http://www.ipcc.ch. In *Climate change*. Cambridge University Press.

Mganga, K. Z., Razavi, B. S., Sanaullah, M., & Kuzyakov, Y. (2019). Phenological stage, plant biomass, and drought stress affect microbial biomass and enzyme activities in the rhizosphere of Enteropogon macrostachyus. *Pedosphere*, *29*(2), 259–265. https://doi.org/10.1016/s1002-0160(18)60799-x

Mikola, J., Yeates, G. W., Barker, G. M., Wardle, D. A., & Bonner, K. I. (2001). Effects of defoliation intensity on soil food-web properties in an experimental grassland community. *Oikos*, *92*(2), 333–343. https://doi.org/10.1034/j.1600-0706.2001.920216.x

Mitchell, J. E. (2010). *Criteria and indicators of sustainable rangeland management* (p. 242). J. E. Mitchell (Ed.). Laramie, WY, USA: University of Wyoming, Cooperative Extension Service.

Moore, E. R., Carter, K. R., Heneghan, J. P., Steadman, C. R., Nachtsheim, A. C., Anderson-Cook, C., ... and Albright, M. B. (2023). Microbial Drivers of Plant Performance during Drought Depend upon Community Composition and the Greater Soil Environment. *Microbiology Spectrum, 11*(2), e01476-22.

Morgan, J. A., LeCain, D. R., Pendall, E., Blumenthal, D. M., Kimball, B. A., Carrillo, Y., Williams, D. G., Heisler-White, J., Dijkstra, F. A., & West, M. (2011). C4 grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. *Nature*, *476*(7359), 202–205. https://doi.org/10.1038/nature10274

Munjonji, L., Ayisi, K. K., Mudongo, E. I., Mafeo, T. P., Behn, K., Mokoka, M. V., & Linstädter, A. (2020). Disentangling drought and grazing effects on soil carbon stocks and CO2 fluxes in a semi-arid African savanna. *Frontiers in Environmental Science*, 8. https://doi.org/10.3389/fenvs.2020.590665

Nacke, H., Thürmer, A., Wollherr, A., Will, C., Hodac, L., Herold, N., Schöning, I., Schrumpf, M., & Daniel, R. (2011). Pyrosequencing-based assessment of bacterial community structure along different management types in German forest and grassland soils. *PLOS ONE*, 6(2), e17000. https://doi.org/10.1371/journal.pone.0017000

Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., Masciandaro, G., Fornasier, F., Moscatelli, M. C., & Marinari, S. (2012). Soil enzymology: Classical and molecular approaches. *Biology and Fertility of Soils, 48*(7), 743–762. https://doi.org/10.1007/s00374-012-0723-0

Naraian, R., & Gautam, R. L. (2018). Penicillium enzymes for the saccharification of lignocellulosic feedstocks. In *New and future developments in microbial biotechnology and bioengineering* (pp. 121–136). Elsevier.

Narayanan, A., Ismert, K. J., Smith, M. D., & Jumpponen, A. (2021). Soil fungal communities are compositionally resistant to drought manipulations–Evidence from culture-dependent and culture-independent analyses. *Fungal Ecology*, *51*, 101062.

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., Schilling, J. S., and Kennedy, P. G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology, 20*, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Nocker, A., Fernández, P. S., Montijn, R., & Schuren, F. (2012). Effect of air drying on bacterial viability: A multiparameter viability assessment. *Journal of Microbiological Methods*, *90*(2), 86–95. https://doi.org/10.1016/j.mimet.2012.04.015

Oates, L. G., & Jackson, R. D. (2014). Livestock management strategy affects net ecosystem carbon balance of subhumid pasture. *Rangeland Ecology and Management*, *67*(1), 19–29. https://doi.org/10.2111/REM-D-12-00151.1

Ochoa-Hueso, R., Collins, S. L., Delgado-Baquerizo, M., Hamonts, K., Pockman, W. T., Sinsabaugh, R. L., Smith, M. D., Knapp, A. K., & Power, S. A. (2018). Drought consistently alters the composition of soil fungal and bacterial communities in grasslands from two continents. *Global Change Biology, 24*(7), 2818–2827. https://doi.org/10.1111/gcb.14113

Oenema, O., Velthof, G. L., Yamulki, S., & Jarvis, S. C. (1997). Nitrous oxide emissions from grazed grassland. *Soil Use and Management*, *13*(s4), 288–295. https://doi.org/10.1111/j.1475-2743.1997.tb00600.x

Oertel, C., Matschullat, J., Zurba, K., Zimmermann, F., & Erasmi, S. (2016). Greenhouse gas emissions from soils – A review. *Geochemistry, 76*(3), 327–352. https://doi.org/10.1016/j.chemer.2016.04.002

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., ... & Imports, M. A. S. S. (2019). Package 'vegan'. *Community ecology package, version*, *2*(9).

Osorio, N. W., and Habte, M. (2001). Synergistic influence of an arbuscular mycorrhizal fungus and a P solubilizing fungus on growth and P uptake of Leucaena leucocephala in an Oxisol. *Arid Land Research and Management, 15*(3), 263-274.

Owensby, C. E., Ham, J. M., & Auen, L. M. (2006). Fluxes of CO2 from grazed and ungrazed tallgrass prairie. *Rangeland Ecology and Management*, *59*(2), 111–127. https://doi.org/10.2111/05-116R2.1

Panagos, P., Van Liedekerke, M., Borrelli, P., Köninger, J., Ballabio, C., Orgiazzi, A., Lugato, E., Liakos, L., Hervas, J., Jones, A., & Montanarella, L. (2022). European Soil Data Centre 2.0: Soil data and knowledge in support of the EU policies. *European Journal of Soil Science, 73*(6), e13315. https://doi.org/10.1111/ejss.13315

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, *18*(5), 1403–1414. http://doi.org/10.1111/1462-2920.13023

Parham, J. A., & Deng, S. P. (2000). Detection, quantification and characterization of β-glucosaminidase activity in soil. *Soil Biology and Biochemistry*, *32*(8–9), 1183–1190. https://doi.org/10.1016/S0038-0717(00)00034-1

Parker, S. S., & Schimel, J. P. (2011). Soil nitrogen availability and transformations differ between the summer and the growing season in a California grassland. *Applied Soil Ecology*, *48*(2), 185–192. https://doi.org/10.1016/j.apsoil.2011.03.007

Parkin, T. B., Doran, J. W., & Franco-Vizcaíno, E. (2015). Field and laboratory tests of soil respiration. In *SSSA Special Publications* (pp. 231–245). Soil Science Society of America.

Parry, M. L., Canziani, O. F., Palutikof, J. P., Van Der Linden, P. J., & Hanson, C. E. (Eds.). (2007). *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press.

Parton, W. J., Ojima, D. S., & Schimel, D. S. (1994). Environmental change in grasslands: Assessment using models. *Climatic Change*, *28*(1–2), 111–141. https://doi.org/10.1007/BF01094103

Paz-Ferreiro, J., Gascó, G., Gutiérrez, B., & Méndez, A. (2012). Soil biochemical activities and the geometric mean of enzyme activities after application of sewage sludge and sewage sludge biochar to soil. *Biology and Fertility of Soils*, *48*(5), 511–517. https://doi.org/10.1007/s00374-011-0644-3

Pieterse, C. M., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C., and Bakker, P. A. (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology, 52*, 347–375. https://doi.org/10.1146/annurev-phyto-082712-102340

Piñeiro, G., Paruelo, J. M., Oesterheld, M., & Jobbágy, E. G. (2010). Pathways of grazing effects on soil organic carbon and nitrogen. *Rangeland Ecology & Management*, *63*(1), 109-119.

Piton, G., Foulquier, A., Martínez-García, L. B., Legay, N., Hedlund, K., Martins da Silva, P., Nascimento, E., Reis, F., Sousa, J. P., De Deyn, G. B., & Clement, J. C. (2020). Disentangling drivers of soil microbial potential enzyme activity across rain regimes: An approach based on the functional trait framework. *Soil Biology & Biochemistry*, *148*(107881), 107881. https://doi.org/10.1016/j.soilbio.2020.107881

Placella, S. A., Brodie, E. L., and Firestone, M. K. (2012). Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. Proceedings of the National Academy of Sciences of the United States of America, *109(27),* 10931–10936.

Plestenjak, G., Eler, K., Vodnik, D., Ferlan, M., Čater, M., Kanduč, T., Simončič, P., & Ogrinc, N. (2012) Sources of soil CO2 in calcareous grassland with woody plant encroachment. *Journal of Soils and Sediments, 12*(9), 1327–1338. https://doi.org/10.1007/s11368-012-0564-3

Polizeli, M. L. T. M., Rizzatti, A. C. S., Monti, R., Terenzi, H. F., Jorge, J. A., & Amorim, D. S. (2005). Xylanases from fungi: Properties and industrial applications. *Applied Microbiology and Biotechnology*, *67*(5), 577–591. https://doi.org/10.1007/s00253-005-1904-7

Preece, C., Verbruggen, E., Liu, L., Weedon, J. T., & Peñuelas, J. (2019). Effects of past and current drought on the composition and diversity of soil microbial communities. *Soil Biology and Biochemistry, 131*, 28–39. https://doi.org/10.1016/j.soilbio.2018.12.022

Qi, S., Zheng, H., Lin, Q., Li, G., Xi, Z., & Zhao, X. (2011). Effects of livestock grazing intensity on soil biota in a semiarid steppe of Inner Mongolia. *Plant and Soil, 340*(1–2), 117–126. https://doi.org/10.1007/s11104-010-0463-6

R Core Team. (2019). *R: A Language and Environment for Statistical Computing*. http://www.r-project.org/. R Foundation for Statistical Computing.

R Core Team. (2022). *R: A Language and Environment for Statistical Computing*. https://www.r-project.org/. R Foundation for Statistical Computing.

Raich, J. W., Potter, C. S., & Bhagawati, D. (2002). Interannual variability in global soil respiration, 1980–94. *Global Change Biology*, *8*(8), 800–812. https://doi.org/10.1046/j.1365-2486.2002.00511.x

Rao, C. S. (2017). Soil enzymes. In R. Lal (Ed.), *Encyclopedia of soil science* (pp. 2100–2107). CRC Press.

Rayment, G. E., & Higginson, F. R. (1992). *Australian laboratory handbook of soil and water chemical methods*. Inkata Press Pty. Ltd.

Reid, R. S., Thornton, P. K., McCrabb, G. J., Kruska, R. L., Atieno, F., & Jones, P. G. (2004). Is it possible to mitigate greenhouse gas emissions in pastoral ecosystems of the tropics? In. *Environment, Development and Sustainability*. Springer, 6(1/2), (91–109). https://doi.org/10.1023/B:ENVI.0000003631.43271.6b

Rivett, D. W., & Bell, T. (2018). Abundance determines the functional role of bacterial phylotypes in complex communities. *Nature Microbiology*, *3*(7), 767–772. https://doi.org/10.1038/s41564-018-0180-0

Rong, Y., Ma, L., Johnson, D. A., & Yuan, F. (2015). Soil respiration patterns for four major land–use types of the agro–pastoral region of northern China. *Agriculture, Ecosystems and Environment*, *213*, 142–150. https://doi.org/10.1016/j.agee.2015.08.002, Google Scholar.

Rounsevell, M. D. A., Evans, S. P., & Bullock, P. (1999). Climate change and agricultural soils: impacts and adaptation. *Climatic Change*, *43*(4), 683-709.

Rui, J., Li, J., Wang, S., An, J., Liu, W.-T., Lin, Q., Yang, Y., He, Z., & Li, X. (2015). Responses of bacterial communities to simulated climate changes in alpine meadow soil of the Qinghai-Tibet plateau. *Applied and Environmental Microbiology*, *81*(17), 6070–6077. https://doi.org/10.1128/AEM.00557-15

Ruppert, J. C., Harmoney, K., Henkin, Z., Snyman, H. A., Sternberg, M., Willms, W., & Linstädter, A. (2015). Quantifying drylands' drought resistance and recovery: The importance of drought intensity, dominant life history and grazing regime. *Global Change Biology*, *21*(3), 1258–1270. https://doi.org/10.1111/gcb.12777

Saiya-Cork, K. R., Sinsabaugh, R. L., & Zak, D. R. (2002). The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. *Soil Biology and Biochemistry*, *34*(9), 1309–1315. https://doi.org/10.1016/S0038-0717(02)00074-3

Sala, O. E., Yahdjian, L., Havstad, K., & Aguiar, M. R. (2017). Rangeland ecosystem services: Nature's supply and humans' demand. In *Rangeland Systems* (pp. 467–489). Springer International Publishing.

Salvati, L., & Carlucci, M. (2015). Towards sustainability in agro-forest systems? Grazing intensity, soil degradation and the socioeconomic profile of rural communities in Italy. *Ecological Economics, 112*, 1–13. https://doi.org/10.1016/j.ecolecon.2015.02.001

Sansupa, C., Wahdan, S. F. M., Hossen, S., Disayathanoowat, T., Wubet, T., & Purahong, W. (2021). Can we use functional annotation of prokaryotic taxa (FAPROTAX) to assign the ecological functions of soil bacteria? *Applied Sciences (Basel, Switzerland)*, *11*(2), 688. https://doi.org/10.3390/app11020688

Santos-Medellín, C., Edwards, J., Liechty, Z., Nguyen, B., & Sundaresan, V. (2017). Drought Stress Results in a Compartment-Specific Restructuring of the Rice Root-Associated Microbiomes. *mBio*, *8*(4), e00764-17. https://doi.org/10.1128/mBio.00764-17

Sardans, J., & Peñuelas, J. (2005). Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. forest. *Soil Biology and Biochemistry, 37*(3), 455–461. https://doi.org/10.1016/j.soilbio.2004.08.004

Sardans, J., & Peñuelas, J. (2010). Soil enzyme activity in a Mediterranean forest after six years of drought. *Soil Science Society of America Journal, 74*(3), 838–851. https://doi.org/10.2136/sssaj2009.0225

Schaeffer, S. M., Homyak, P. M., Boot, C. M., Roux-Michollet, D., & Schimel, J. P. (2017). Soil carbon and nitrogen dynamics throughout the summer drought in a California annual grassland. *Soil Biology and Biochemistry*, *115*, 54–62. https://doi.org/10.1016/j.soilbio.2017.08.009

Schaufler, G., Kitzler, B., Schindlbacher, A., Skiba, U., Sutton, M. A., & Zechmeister-Boltenstern, S. (2010). Greenhouse gas emissions from European soils under different land use: effects of soil moisture and temperature. *European Journal of Soil Science*, *61*(5), 683–696. https://doi.org/10.1111/j.1365-2389.2010.01277.x

Schimel, J. P. (2018). Life in dry soils: Effects of drought on soil microbial communities and processes. Annual Review of Ecology, Evolution, and Systematics, 49(1), 409–432. https://doi.org/10.1146/annurev-ecolsys-110617-062614

Schimel, J. P., & Weintraub, M. N. (2003). The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biology and Biochemistry*, *35*(4), 549-563. https://doi.org/10.1016/S0038-0717(03)00015-4

Schimel, J., Balser, T. C., & Wallenstein, M. (2007). Microbial stress-response physiology and its implications for ecosystem function. *Ecology, 88*(6), 1386–1394. https://doi.org/10.1890/06-0219

Schindlbacher, A., Wunderlich, S., Borken, W., Kitzler, B., Zechmeister-Boltenstern, S., & Jandl, R. (2012). Soil respiration under climate change: Prolonged summer drought

offsets soil warming effects. *Global Change Biology, 18*(7), 2270–2279. https://doi.org/10.1111/j.1365-2486.2012.02696.x

Schwalm, C. R., Williams, C. A., Schaefer, K., Baldocchi, D., Black, T. A., Goldstein, A. H., Law, B. E., Oechel, W. C., Paw U, K. T., & Scott, R. L. (2012). Reduction in carbon uptake during turn of the century drought in western North America. *Nature Geoscience, 5*(8), 551–556. https://doi.org/10.1038/ngeo1529

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome biology*, *12*, 1-18

Sharkhuu, A., Plante, A. F., Enkhmandal, O., Gonneau, C., Casper, B. B., Boldgiv, B., & Petraitis, P. S. (2016). Soil and ecosystem respiration responses to grazing, watering and experimental warming chamber treatments across topographical gradients in northern Mongolia. *Geoderma*, *269*, 91–98. https://doi.org/10.1016/j.geoderma.2016.01.041

She, W., Bai, Y., Zhang, Y., Qin, S., Feng, W., Sun, Y., Zheng, J., & Wu, B. (2018). Resource availability drives responses of soil microbial communities to short-term precipitation and nitrogen addition in a desert shrubland. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.00186

Sheik, C. S., Beasley, W. H., Elshahed, M. S., Zhou, X. H., Luo, Y. Q., and Krumholz, L. R. (2011). Effect of warming and drought on grassland microbial communities. *ISME Journal, 5*(10), 1692–1700. https://doi.org/10.1038/ismej.2011.32

Sherwood, S., & Fu, Q. (2014). Climate change. A drier future? *Science, 343*(6172), 737–739. https://doi.org/10.1126/science.1247620

Shi, B., Fu, X., Smith, M. D., Chen, A., Knapp, A. K., Wang, C., Xu, W., Zhang, R., Gao, W., & Sun, W. (2022). Autotrophic respiration is more sensitive to nitrogen addition and grazing than heterotrophic respiration in a meadow steppe. *CATENA*, *213*, 106207. https://doi.org/10.1016/j.catena.2022.106207

Shi, H., Hou, L., Yang, L., Wu, D., Zhang, L., & Li, L. (2017). Effects of grazing on CO2, CH4, and N2O fluxes in three temperate steppe ecosystems. *Ecosphere*, *8*(4), e01760. https://doi.org/10.1002/ecs2.1760

Shrestha, B. M., Bork, E. W., Chang, S. X., Carlyle, C. N., Ma, Z., Döbert, T. F., Kaliaskar, D., & Boyce, M. S. (2020). Adaptive multi-paddock grazing lowers soil greenhouse gas emission potential by altering extracellular enzyme activity. *Agronomy, 10*(11), 1781. https://doi.org/10.3390/agronomy10111781

Silverthorn, T. K., & Richardson, J. S. (2021). Temporal and microtopographical variations in greenhouse gas fluxes from riparian forest soils along headwater streams. *Biogeochemistry*, *155*(3), 401–412. https://doi.org/10.1007/s10533-021-00832-5

Sinsabaugh, R. L. (2010). Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biology and Biochemistry*, *42*(3), 391-404

Smith, P., Bustamante, M., Ahammad, H., Clark, H., Dong, H., Elsiddig, E. A., ... & Bolwig, S. (2014). Agriculture, forestry and other land use (AFOLU). In *Climate change 2014: mitigation of climate change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 811-922). Cambridge University Press.

Soil Classification Working Group. (1998). *The Canadian system of soil classification* (3rd ed) p. 1646. Agriculture and Agri-Food Canada Publication.

Soussana, J. F., Allard, V., Pilegaard, K., Ambus, P., Amman, C., Campbell, C., Ceschia, E., Clifton-Brown, J., Czobel, S., Domingues, R., Flechard, C., Fuhrer, J., Hensen, A., Horvath, L., Jones, M., Kasper, G., Martin, C., Nagy, Z., Neftel, A., . . . and Valentini, R. (2007). Full accounting of the greenhouse gas (CO2, N2O, CH4) budget of nine European grassland sites. *Agriculture, Ecosystems and Environment, 121*(1–2), 121–134. https://doi.org/10.1016/j.agee.2006.12.022

Soussana, J.-F., Soussana, J.-F., Loiseau, P., Vuichard, N., Ceschia, E., Balesdent, J., Chevallier, T., & Arrouays, D. (2004). Carbon cycling and sequestration opportunities in temperate grasslands. *Soil Use and Management, 20*(2), 219–230. https://doi.org/10.1079/SUM2003234

Steinweg, J. M., Dukes, J. S., & Wallenstein, M. D. (2012). Modeling the effects of temperature and moisture on soil enzyme activity: Linking laboratory assays to continuous field

data. *Soil Biology and Biochemistry*, *55*, 85–92. https://doi.org/10.1016/j.soilbio.2012.06.015

Steinweg, J. M., Dukes, J. S., Paul, E. A., & Wallenstein, M. D. (2013). Microbial responses to multi-factor climate change: Effects on soil enzymes. *Frontiers in Microbiology, 4*, 146. https://doi.org/10.3389/fmicb.2013.00146

Stockmann, U., Adams, M. A., Crawford, J. W., Field, D. J., Henakaarchchi, N., Jenkins, M., Minasny, B., McBratney, A. B., Courcelles, R., Singh, K., Wheeler, I., Abbott, L., Angers, D. A., Baldock, J., Bird, M., Brookes, P. C., Chenu, C., Jastrow, J. D., Lal, R., . . . Zimmermann, M. (2013). The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agriculture, Ecosystems and Environment*, *164*, 80–99. https://doi.org/10.1016/j.agee.2012.10.001

Sun, Y., Chen, H. Y. H., Jin, L., Wang, C., Zhang, R., Ruan, H., & Yang, J. (2020). Drought stress induced increase of fungi:bacteria ratio in a poplar plantation. *CATENA*, *193*, 104607. https://doi.org/10.1016/j.catena.2020.104607

Symonds, M. R. E., & Moussalli, A. (2011). A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioral Ecology and Sociobiology*, *65*(1), 13–21. https://doi.org/10.1007/s00265-010-1037-6

Tan, Z., Liu, S., Johnston, C. A., Loveland, T. R., Tieszen, L. L., Liu, J., & Kurtz, R. (2005). Soil organic carbon dynamics as related to land use history in the northwestern Great Plains. *Global Biogeochemical Cycles, 19*(3). https://doi.org/10.1029/2005GB002536

Tarafdar, J. C., & Claassen, N. (1988). Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. *Biology and Fertility of Soils*, *5*(4), 308–312. https://doi.org/10.1007/BF00262137

Thomas, B. W., Gao, X., Zhao, M., Bork, E. W., & Hao, X. (2017). Grazing altered carbon exchange in a dry mixed-grass prairie as a function of soil texture. *Canadian Journal of Soil Science*, *98*(1), 136–147.

Tian, D., Niu, S., Pan, Q., Ren, T., Chen, S., Bai, Y., & Han, X. (2016). Nonlinear responses of ecosystem carbon fluxes and water-use efficiency to nitrogen addition in Inner Mongolia grassland. *Functional Ecology, 30*(3), 490–499. https://doi.org/10.1111/1365-2435.12513

Tibbett, M., Gil-Martínez, M., Fraser, T., Green, I. D., Duddigan, S., De Oliveira, V. H., Raulund-Rasmussen, K., Sizmur, T., & Diaz, A. (2019). Long-term acidification of pH neutral grasslands affects soil biodiversity, fertility and function in a heathland restoration. *CATENA, 180*, 401–415. https://doi.org/10.1016/j.catena.2019.03.013

Tisdale, S. L., Nelson, W. L., & Beaton, J. D. (1975). *Soil fertility and fertilisers*. Macmillan.

Todd-Brown, K. E. O., Hopkins, F. M., Kivlin, S. N., Talbot, J. M., & Allison, S. D. (2012). A framework for representing microbial decomposition in coupled climate models. *Biogeochemistry, 109*(1–3), 19–33. https://doi.org/10.1007/s10533-011-9635-6

Toju, H., Tanabe, A. S., Yamamoto, S., & Sato, H. (2012). High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PloS one*, *7*(7), e40863.

Török, P., Valkó, O., Deák, B., Kelemen, A., Tóth, E., & Tóthmérész, B. (2016). Managing for species composition or diversity? Pastoral and free grazing systems in alkali steppes. *Agriculture, Ecosystems and Environment, 234*, 23–30. https://doi.org/10.1016/j.agee.2016.01.010

Wachiye, S., Pellikka, P., Rinne, J., Heiskanen, J., Abwanda, S., & Merbold, L. (2022). Effects of livestock and wildlife grazing intensity on soil carbon dioxide flux in the savanna grassland of Kenya. *Agriculture, Ecosystems and Environment, 325*, 107713. https://doi.org/10.1016/j.agee.2021.107713

Wakelin, S. A., Gregg, A. L., Simpson, R. J., Li, G. D., Riley, I. T., & McKay, A. C. (2009). Pasture management clearly affects soil microbial community structure and N-cycling bacteria. *Pedobiologia, 52*(4), 237–251. https://doi.org/10.1016/j.pedobi.2008.10.001

Waldrop, M. P., Firestone, M. K. (2006). Response of Microbial Community Composition and Function to Soil Climate Change. *Microbial Ecology*, 52(4), 716–724. https://doi.org/10.1007/s00248-006-9103-3

Wang, C., Lu, X., Mori, T., Mao, Q., Zhou, K., Zhou, G., Nie, Y., & Mo, J. (2018). Responses of soil microbial community to continuous experimental nitrogen additions for 13 years in a nitrogen-rich tropical forest. *Soil Biology and Biochemistry, 121*, 103–112. https://doi.org/10.1016/j.soilbio.2018.03.009

Wang, J., Wang, G., Hu, H., & Wu, Q. (2010). The influence of degradation of the swamp and alpine meadows on CH4 and CO2 fluxes on the Qinghai-Tibetan Plateau. *Environmental Earth Sciences*, *60*(3), 537–548. https://doi.org/10.1007/s12665-009-0193-3

Wang, T., Hamann, A., Spittlehouse, D., & Carroll, C. (2016). Locally Downscaled and Spatially Customizable Climate Data for Historical and Future Periods for North America. *PLOS ONE*, 11(6), e0156720. https://doi.org/10.1371/journal.pone.0156720

Wang, X., Liu, L., Piao, S., Janssens, I. A., Tang, J., Liu, W., Chi, Y., Wang, J., & Xu, S. (2014). Soil respiration under climate warming: Differential response of heterotrophic and autotrophic respiration. *Global Change Biology, 20*(10), 3229–3237. https://doi.org/10.1111/gcb.12620

Wang, Y. S., Hu, Y. Q., Ji, B. M., Liu, G. R., & Xue, M. (2002). Research of grazing effects on greenhouse gas emission in Inner Mongolian grasslands. *China Environmental Science*, *22*(6), 490–494.

Wang, Y., Wang, L., Suo, M., Qiu, Z., Wu, H., Zhao, M., & Yang, H. (2022). Regulating Root Fungal Community Using Mortierella alpina for Fusarium oxysporum Resistance in Panax ginseng. *Frontiers in Microbiology*, *13*, 850917. https://doi.org/10.3389/fmicb.2022.850917

Wang, Z. W., Jiao, S. Y., Han, G. D., Zhao, M. L., & Willms, W. D. (2009). Soil respiration response to different stocking rates on Stipa breviflora Griseb. Desert Steppe. *Journal of Inner Mongolia University*, *40*(2), 186–193.

Waterman, J. M., Cazzonelli, C. I., Hartley, S. E., & Johnson, S. N. (2019). Simulated herbivory: the key to disentangling plant defence responses. *Trends in ecology & evolution*, *34*(5), 447-458. https://doi.org/10.1016/j.tree.2019.01.008

White, R. P., Murray, S., Rohweder, M., Prince, S. D., & Thompson, K. M. (2000). *Grassland ecosystems* (p. 81). Washington, DC, USA: World Resources Institute.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols* (pp. 315–322). Elsevier.

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag New York.

Will, C., Thürmer, A., Wollherr, A., Nacke, H., Herold, N., Schrumpf, M., Gutknecht, J., Wubet, T., Buscot, F., & Daniel, R. (2010). Horizon-specific bacterial community composition of German grassland soils, as revealed by pyrosequencing-based analysis of 16S rRNA genes. *Applied and Environmental Microbiology*, 76(20), 6751–6759. https://doi.org/10.1128/AEM.01063-10

Wilson, C. H., Strickland, M. S., Hutchings, J. A., Bianchi, T. S., & Flory, S. L. (2018). Grazing enhances belowground carbon allocation, microbial biomass, and soil carbon in a subtropical grassland. *Global Change Biology*, *24*(7), 2997–3009. https://doi.org/10.1111/gcb.14070

Wolf, B., Zheng, X., Brüggemann, N., Chen, W., Dannenmann, M., Han, X., Sutton, M. A., Wu, H., Yao, Z., & Butterbach-Bahl, K. (2010). Grazing-induced reduction of natural nitrous oxide release from continental steppe. *Nature, 464*(7290), 881–884. https://doi.org/10.1038/nature08931

Wrage, N., Velthof, G. L., van Beusichem, M. L., & Oenema, O. (2001). Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biology and Biochemistry, 33*(12–13), 1723–1732. https://doi.org/10.1016/S0038-0717(01)00096-7

Wu, J., & Brookes, P. C. (2005). The proportional mineralisation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. *Soil Biology and Biochemistry, 37*(3), 507–515. https://doi.org/10.1016/j.soilbio.2004.07.043

Wu, X., Yao, Z., Brüggemann, N., Shen, Z. Y., Wolf, B., Dannenmann, M., Zheng, X., & Butterbach-Bahl, K. (2010). Effects of soil moisture and temperature on CO2 and CH4 soil–atmosphere exchange of various land use/cover types in a semi-arid grassland in Inner Mongolia, China. *Soil Biology and Biochemistry, 42*(5), 773–787. https://doi.org/10.1016/j.soilbio.2010.01.013

Wu, Z., Dijkstra, P., Koch, G. W., Peñuelas, J., & Hungate, B. A. (2011). Responses of terrestrial ecosystems to temperature and precipitation change: A meta-analysis of experimental manipulation. *Global Change Biology, 17*(2), 927–942. https://doi.org/10.1111/j.1365-2486.2010.02302.x

Wuebbles, D.J., D.W. Fahey, D., Hibbard, K., Dokken, D., Stewart, B., and Maycock, T. (2017). Climate Science Special Report: Fourth National Climate Assessment, Volume I. U.S. Global Change Research Program, Washington, DC, USA, 470 pp., doi: 10.7930/J0J964J6.

Xiao, W., Chen, X., Jing, X., & Zhu, B. (2018). A meta-analysis of soil extracellular enzyme activities in response to global change. *Soil Biology and Biochemistry, 123*, 21–32. https://doi.org/10.1016/j.soilbio.2018.05.001

Xiong, J., Sun, H., Peng, F., Zhang, H., Xue, X., Gibbons, S. M., Gilbert, J. A., & Chu, H. (2014). Characterizing changes in soil bacterial community structure in response to short-term warming. *FEMS Microbiology Ecology*, *89*(2), 281–292. https://doi.org/10.1111/1574-6941.12289

Xu, L., Naylor, D., Dong, Z., Simmons, T., Pierroz, G., Hixson, K. K., Kim, Y.-M., Zink, E. M., Engbrecht, K. M., Wang, Y., Gao, C., DeGraaf, S., Madera, M. A., Sievert, J. A., Hollingsworth, J., Birdseye, D., Scheller, H. V., Hutmacher, R., Dahlberg, J., … Coleman-Derr, D. (2018). Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(18), E4284–E4293. https://doi.org/10.1073/pnas.1717308115

Xu, S., Silveira, M. L., Inglett, K. S., Sollenberger, L. E., & Gerber, S. (2017). Soil microbial community responses to long-term land use intensification in subtropical grazing lands. *Geoderma*, *293*, 73–81. https://doi.org/10.1016/j.geoderma.2017.01.019

Xun, W., Yan, R., Ren, Y., Jin, D., Xiong, W., Zhang, G., Cui, Z., Xin, X., & Zhang, R. (2018). Grazing-induced microbiome alterations drive soil organic carbon turnover and productivity in meadow steppe. *Microbiome, 6*(1), 170. https://doi.org/10.1186/s40168-018-0544-y

Yan, G., Mu, C., Xing, Y., & Wang, Q. (2018). Responses and mechanisms of soil greenhouse gas fluxes to changes in precipitation intensity and duration: A meta-analysis for a global perspective. *Canadian Journal of Soil Science, 98*(4), 591–603. https://doi.org/10.1139/cjss-2018-0002

Yan, Z., Kang, E., Zhang, K., Li, Y., Hao, Y., Wu, H., Li, M., Zhang, X., Wang, J., Yan, L., & Kang, X. (2021). Plant and soil enzyme activities regulate CO2 efflux in alpine peatlands after 5 years of simulated extreme drought. *Frontiers in Plant Science, 12*, 756956. https://doi.org/10.3389/fpls.2021.756956

Yang, Y., Wu, L., Lin, Q., Yuan, M., Xu, D., Yu, H., Hu, Y., Duan, J., Li, X., He, Z., Xue, K., van Nostrand, J., Wang, S., & Zhou, J. (2013). Responses of the functional structure of soil microbial community to livestock grazing in the Tibetan alpine grassland. *Global Change Biology*, 19(2), 637–648. https://doi.org/10.1111/gcb.12065

Yang, Z., Peng, C., Cao, H., Song, J., Gong, B., Li, L., Wang, L., He, Y., Liang, M., Lin, J., & Lu, L. (2022). Microbial functional assemblages predicted by the FAPROTAX analysis are impacted by physicochemical properties, but C, N and S cycling genes are not in mangrove soil in the Beibu Gulf, China. *Ecological Indicators*, *139*(108887), 108887. https://doi.org/10.1016/j.ecolind.2022.108887

Yates, C. J., Norton, D. A., & Hobbs, R. J. (2000). Grazing effects on plant cover, soil and microclimate in fragmented woodlands in south-Western Australia: Implications for restoration. *Austral Ecology*, *25*(1), 36–47. https://doi.org/10.1046/j.1442-9993.2000.01030.x

Ylla, I., Romaní, A. M., & Sabater, S. (2012). Labile and recalcitrant organic matter utilization by river biofilm under increasing water temperature. *Microbial Ecology*, 64(3), 593–604. https://doi.org/10.1007/s00248-012-0062-6

Yu, H., He, Z., Wang, A., Xie, J., Wu, L., Van Nostrand, J. D., Jin, D., Shao, Z., Schadt, C. W., Zhou, J., & Deng, Y. (2018). Divergent responses of forest soil microbial communities under elevated CO2 in different depths of upper soil layers. *Applied and Environmental Microbiology*, *84*(1). https://doi.org/10.1128/AEM.01694-17

Yue, K., Fornara, D. A., Yang, W., Peng, Y., Peng, C., Liu, Z., & Wu, F. (2017). Influence of multiple global change drivers on terrestrial carbon storage: Additive effects are common. *Ecology Letters, 20*(5), 663–672. https://doi.org/10.1111/ele.12767

Zeglin, L. H., Bottomley, P. J., Jumpponen, A., Rice, C. W., Arango, M., Lindsley, A., McGowan, A., Mfombep, P., and Myrold, D. D. (2013). Altered precipitation regime affects the function and composition of soil microbial communities on multiple time scales. *Ecology*, *94*(10), 2334–2345. https://doi.org/10.1890/12-2018.1

Zhang, K., Shi, Y., Jing, X., He, J.-S., Sun, R., Yang, Y., Shade, A., & Chu, H. (2016). Effects of short-term warming and altered precipitation on soil microbial communities in alpine grassland of the Tibetan plateau. *Frontiers in Microbiology*, *7*, 1032. https://doi.org/10.3389/fmicb.2016.01032

Zhang, X., Tan, Y., Zhang, B., Li, A., Daryanto, S., Wang, L., & Huang, J. (2017). The impacts of precipitation increase and nitrogen addition on soil respiration in a semiarid temperate steppe. *Ecosphere*, 8(1), e01655. https://doi.org/10.1002/ecs2.1655

Zhang, Y., Xie, Y. Z., Ma, H. B., Zhang, J., Jing, L., Wang, Y. T., & Li, J. P. (2021). The responses of soil respiration to changed precipitation and increased temperature in desert grassland in northern China. *Journal of Arid Environments*, *193*, 104579. https://doi.org/10.1016/j.jaridenv.2021.104579

Zheng, Z., Powell, J. J., Ye, X., Liu, X., Yuan, Z., & Liu, C. (2021). Overcompensation can be an ideal breeding target. *Agronomy*, *11*(7), 1376. https://doi.org/10.3390/agronomy11071376

Zhong, L., Du, R., Ding, K., Kang, X., Li, F. Y., Bowatte, S., Hoogendoorn, C. J., Wang, Y., Rui, Y., Jiang, L., & Wang, S. (2014). Effects of grazing on N2O production potential and abundance of nitrifying and denitrifying microbial communities in meadow-steppe grassland in northern China. *Soil Biology and Biochemistry, 69*, 1–10. https://doi.org/10.1016/j.soilbio.2013.10.028

Zhou, G., Luo, Q., Chen, Y., Hu, J., He, M., Gao, J., Zhou, L., Liu, H., & Zhou, X. (2019). Interactive effects of grazing and global change factors on soil and ecosystem respiration

in grassland ecosystems: A global synthesis. *Journal of Applied Ecology, 56*(8), 2007-2019. https://doi.org/10.1111/1365-2664.13443

Zhou, G., Zhou, X., He, Y., Shao, J., Hu, Z., Liu, R., ... & Hosseinibai, S. (2017). Grazing intensity significantly affects belowground carbon and nitrogen cycling in grassland ecosystems: A meta-analysis. *Global change biology*, *23*(3), 1167-1179.

Zhou, L., Zhou, X., Zhang, B., Lu, M., Luo, Y., Liu, L., & Li, B. O. (2014). Different responses of soil respiration and its components to nitrogen addition among biomes: A meta-analysis. *Global Change Biology, 20*(7), 2332–2343. https://doi.org/10.1111/gcb.12490

Zhou, Z., Wang, C., & Luo, Y. (2018). Response of soil microbial communities to altered precipitation: A global synthesis. *Global Ecology and Biogeography*, 27(9), 1121–1136. https://doi.org/10.1111/geb.12761

Zhu, K., Chiariello, N. R., Tobeck, T., Fukami, T., & Field, C. B. (2016). Nonlinear, interacting responses to climate limit grassland production under global change. Proceedings of the National Academy of Sciences of the United States of America, 113(38), 10589–10594. https://doi.org/10.1073/pnas.1606734113

Zhu, X., Luo, C., Wang, S., Zhang, Z., Cui, S., Bao, X., Jiang, L., Li, Y., Li, X., Wang, Q., & Zhou, Y. (2015). Effects of warming, grazing/cutting and nitrogen fertilization on greenhouse gas fluxes during growing seasons in an alpine meadow on the Tibetan Plateau. *Agricultural and Forest Meteorology, 214–*215, 506–514. https://doi.org/10.1016/j.agrformet.2015.09.008

Appendices

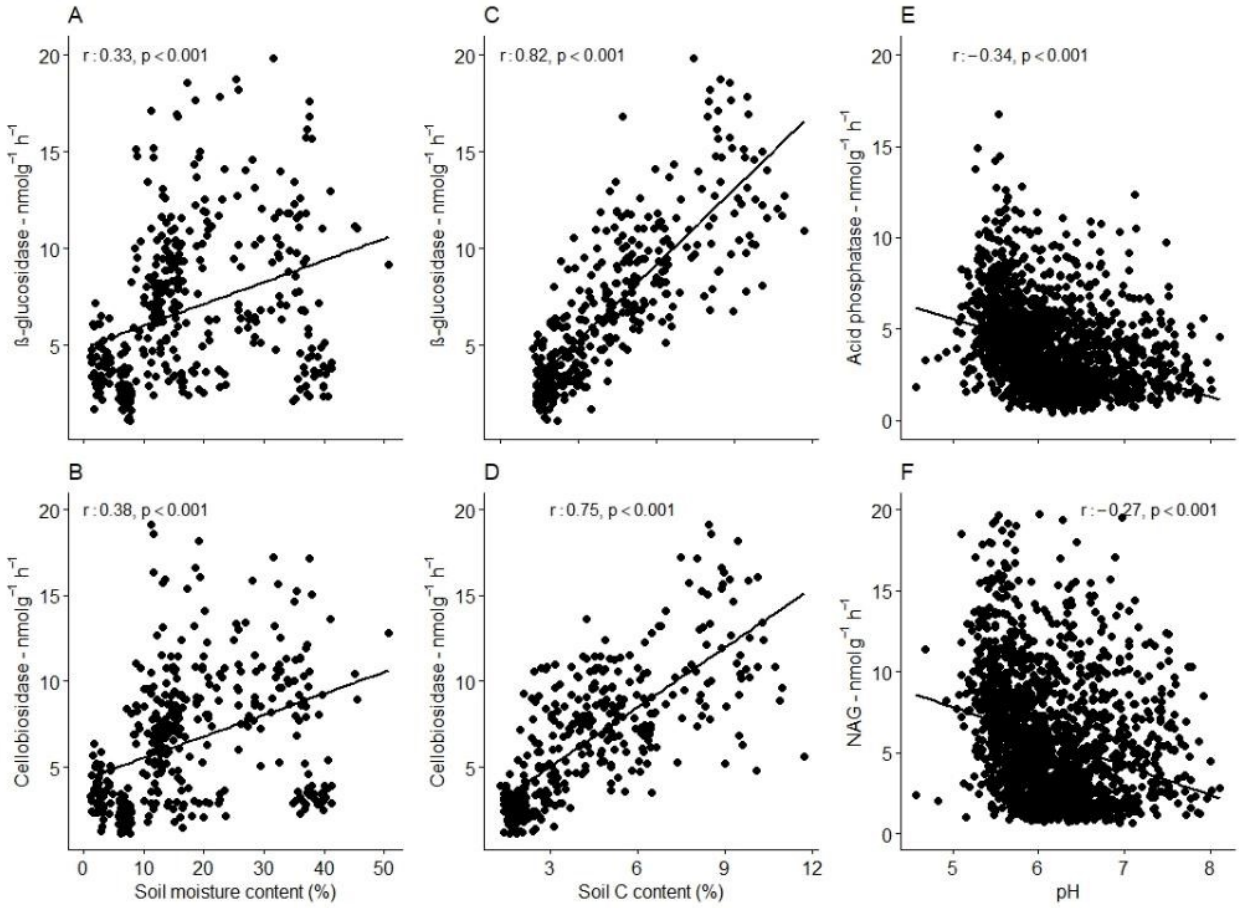
Appendix 1. Extracellular enzymes assayed in this study, their class and functions in soil.

Enzyme	EC number	Enzyme class	Function
β-glucosidase (BG)	3.2.1.21	Cellulase	Converts cellobiose to glucose by performing the final step of hydrolysis (Alef and Nannipieri, 1995)
β-D—cellobiosidase (CELLO)	3.2.1.91	Cellulase	Releases oligosaccharides and cellobiose from polysaccharides (Hildén and Mäkelä, 2018)
β-xylosidase (XYLO)	3.2.1.37	Hemicellulase	Responsible for the hydrolysis of the major component (xylan) of hemicellulose and releases xylose (Knob et al., 2010)
Acid phosphatase (AP)	3.1.3.2	Phosphatase	Hydrolysis of phosphate esters, converts organic phosphate to inorganic P (Tarafdar and Claassen, 1988)
N-acetyl-β-glucosaminidase (NAG)	3.2.1.52	Glucosaminidase	Involved in chitin degradation, hydrolyses N-acetyl-b-glucosamine (Ekenler and Tabatabai, 2004)

Appendix 2. Results of the NMDS analysis showing the relationship between EEAs and environmental factors (MAP: mean annual precipitation, MC: soil moisture content, TEMP: soil temperature, pH, C: soil carbon content and N: soil nitrogen content), analyzed using the envfit function (R-package: vegan). Significant values ($p < 0.05$) are in bold.

Variable	NMDS1	NMDS2	r^2	*p*
MAP	-0.50	0.86	0.62	**<0.001**
TEMP	0.51	0.86	0.05	**< 0.001**
MC	-0.93	0.38	0.11	**< 0.001**
pH	0.70	0.72	0.05	**< 0.001**
N	-0.60	0.80	0.61	**< 0.001**
C	-0.63	0.78	0.63	**< 0.001**

Appendix 3. Pearson correlation coefficient bivariate plots of β-glucosidase and cellobiosidase with soil moisture content (A and B), β-glucosidase and cellobiosidase with soil C content (%) (C and D) and acid phosphatase and N-acetyl-β-glucosaminidase (NAG) with soil pH (E and F).



Appendix 4. Soil and environmental characteristics and dominant vegetation at the study sites. Long-term mean annual precipitation and air temperature data were obtained from ClimateNA (Wang et al., 2016). MAP: mean annual precipitation, MAT: mean annual temperature.

Site	MAP (mm)	MAT (°C)	Soil texture	pH (1:2)	Dominant species
Kinsella	405.9	2.3	Clay	5.73	*Poa pratensis, Agropyron dasystachyum, Pascopyrum smithii*
Mattheis	315.8	3.9	Sandy loam	6.21	*Hesperostipa comata, Artemisia frigida, Koeleria macrantha*

Appendix 5. Details of primers used for next-generation sequencing (NGS) of bacteria, archaea and fungi. Illumina adapters added to the forward and reverse primers were TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG and GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G, respectively.

Primer Name	Primer Sequence 5'-3'	Target	Reference
515F	GTGYCAGCMGCCGCGGTAA	Bacteria and archaea	Parada et al., 2016
806R	GGACTACNVGGGTWTCTAAT	Bacteria and archaea	Apprill et al., 2015
ITS1F	CTTGGTCATTTAGAGGAAGTAA	Fungi	Gardes and Burns, 1993
ITS2	GCTGCGTTCTTCATCGATGC	Fungi	White et al., 1990

Appendix 6. ANOVA results (p-values) of a linear mixed model of rainfall and defoliation treatments on Shannon and Simpson diversity indices, Chao1 (species richness estimator), and Faith's PD (phylogenetic diversity) of soil bacterial and fungal communities. The P-value in bold is significant at alpha = 0.10.

Microbial group	Treatment	Kinsella				Mattheis			
		Shannon	Simpson	Chao1	Faith's PD	Shannon	Simpson	Chao1	Faith's PD
Bacteria	Rainfall (R)	0.435	0.846	0.291	0.278	0.802	0.787	0.920	0.916
	Defoliation (D)	0.844	0.990	0.809	0.848	0.704	0.682	0.664	0.776
	R x D	0.645	0.570	0.610	0.633	0.653	0.488	0.722	0.739
Fungi	Rainfall (R)	0.934	0.845	0.969	0.991	0.671	0.802	0.540	0.774
	Defoliation (D)	0.978	0.962	0.999	0.989	**0.065**	0.139	0.255	0.147
	R x D	0.732	0.693	0.915	0.747	0.242	0.353	0.383	0.303

Appendix 7. ANOVA results (p-values) from mixed models testing the effects of rainfall and defoliation treatments on ecological functions related to bacterial communities were obtained from the FAPROTAX database. Rainfall and defoliation treatments were treated as the main factors, and plot ID and sampling month were treated as random factors. Bold values are significant at alpha = 0.05.

	----------Kinsella----------		----------Mattheis----------	
Ecological functions	Rainfall (R)	Defoliation (D)	Rainfall (R)	Defoliation (D)
Aerobic chemoheterotrophs	0.108	0.176	**0.047**	0.442
Anaerobic chemoheterotrophs	0.196	0.443	0.846	0.946
Anoxygenic photoautotrophs	0.453	0.892	**0.041**	0.135
Cellulolytic bacteria	0.281	0.235	0.983	0.382
Chemoheterotrophs	0.080	0.158	**0.048**	0.437
Chitinolytic bacteria	0.251	0.247	0.935	0.839
Denitrifiers	0.965	0.742	0.142	0.886
Methanol oxidizers	1	0.542	0.697	0.215
Methanotrophs	1	0.534	0.729	0.265
Methylotrophs	1	0.588	0.725	0.269
Nitrate reducers	0.363	0.451	0.450	0.629
Nitrate respiration bacteria	0.216	0.977	0.094	0.165
Nitrite respiration bacteria	0.613	0.934	0.069	0.193

Nitrogen fixers	0.302	0.329	0.581	1
Nitrogen respiration bacteria	0.230	0.803	0.142	0.829
Nonphotosynthetic cyanobacteria	0.269	0.171	0.645	0.257
Oxygenic photoautotrophs	0.716	0.434	0.147	0.745
Photoautotrophs	0.812	0.587	0.314	0.782
Photoheterotrophs	0.186	0.812	**0.013**	0.118
Photosynthetic cyanobacteria	0.711	0.432	0.147	0.745
Phototrophs	0.466	0.646	0.406	0.817
Xylanolytic bacteria	**0.026**	0.843	0.060	0.577

Appendix 8. Pearson correlation coefficients of soil bacterial ecological functional groups (relative abundance) with soil and environmental biotic and abiotic factors at Kinsella and Mattheis. BG: β-glucosidase (nmol g^{-1} h^{-1}), AP: Acid phosphatase (nmol g^{-1} h^{-1}), CELLO: β-cellobiosidase (nmol g^{-1} h^{-1}), NAG: N-acetyl-β-glucosaminidase (nmol g^{-1} h^{-1}), XYLO: β-xylosidase (nmol g^{-1} h^{-1}), MC: soil moisture content (%, g/g), TEMP: soil temperature (°C). The bold values denote significant correlation.

	BG	AP	CELLO	NAG	XYLO	MC	pH	TEMP	CO_2*	N_2O*	CH_4*
Ecological functions					Kinsella						
Aerobic chemoheterotrophs	0.155	0.246	-0.102	0.241	0.230	0.179	0.185	0.035	**0.352**	0.128	0.093
Anaerobic chemoheterotrophs	0.004	0.031	0.057	0.115	0.175	0.178	0.036	0.050	0.049	0.103	0.141
Anoxygenic photoautotrophs	0.136	0.088	-0.148	0.121	0.023	0.203	0.055	0.173	0.169	0.031	0.008
Cellulolytic bacteria	0.124	0.142	-0.041	0.103	0.185	0.313	0.024	0.043	**0.394**	0.032	0.079
Chemoheterotrophs	0.152	0.244	-0.097	0.230	0.236	0.186	0.183	0.031	**0.349**	0.132	0.100
Chitinolytic bacteria	0.003	0.027	-0.112	0.112	0.061	0.017	0.158	0.032	0.155	0.074	**0.323**
Dark hydrogen oxidizers	0.209	0.315	-0.138	0.243	**0.518**	0.257	0.340	0.073	**0.461**	0.142	0.157
Denitrifiers	0.188	0.136	-0.192	0.188	0.036	0.171	0.125	0.271	0.142	0.125	0.048
Methanotrophs	0.013	0.011	-0.102	0.068	0.013	0.101	0.048	0.090	0.060	0.009	0.026

Nitrate reducers	0.215	-0.134	-0.175	0.142	0.044	-0.253	0.141	0.267	-0.189	0.063	-0.257
Nitrogen fixers	0.119	-0.227	-0.080	0.149	**0.462**	-0.292	0.305	0.015	**-0.386**	0.157	-0.186
Oxygenic photoautotrophs	0.174	-0.014	-0.268	0.108	0.274	**-0.368**	0.077	0.277	-0.209	0.071	-0.131
Photoautotrophs	0.214	-0.064	-0.292	0.156	0.220	**-0.402**	0.092	0.315	-0.261	0.034	-0.104
Photoheterotrophs	0.128	-0.107	-0.152	0.116	0.094	-0.019	0.052	0.100	-0.067	0.076	-0.016
Photosynthetic cyanobacteria	0.174	-0.014	-0.268	0.108	0.274	**-0.368**	0.077	0.277	-0.209	0.071	-0.131
Phototrophs	0.223	-0.086	-0.311	0.164	0.142	-0.293	0.095	0.281	-0.206	0.000	-0.088
Ureolytic bacteria	0.075	-0.048	-0.129	0.002	0.254	-0.170	0.087	0.097	-0.051	0.007	-0.058
Xylanolytic bacteria	0.032	-0.109	-0.022	0.209	0.218	-0.118	0.126	-0.044	0.270	-0.101	0.115
					Mattheis						
Aerobic chemoheterotrophs	0.136	-0.056	0.023	0.045	0.117	0.216	0.043	-0.132	-0.251	-0.076	**-0.362**
Anaerobic chemoheterotrophs	0.042	-0.043	0.086	0.081	-0.172	0.092	0.029	-0.039	-0.074	-0.052	-0.163
Anoxygenic photoautotrophs	0.078	-0.080	0.087	0.093	-0.053	0.028	0.120	-0.067	-0.022	-0.059	-0.134

Cellulolytic bacteria	**0.550**	**0.571**	**0.655**	**0.610**	0.048	**0.334**	**0.460**	**-0.604**	0.293	**-0.301**	0.276
Chemoheterotrophs	0.133	0.050	0.037	0.051	0.101	0.232	0.038	-0.131	0.254	0.079	**-0.370**
Chitinolytic bacteria	0.060	0.062	0.154	0.068	-0.040	-0.086	0.047	-0.084	0.026	-0.121	0.024
Dark hydrogen oxidizers	-0.115	-0.213	**-0.366**	**-0.390**	0.063	0.125	0.212	-0.254	0.032	0.195	0.103
Denitrifiers	**-0.392**	**-0.325**	**-0.417**	**-0.429**	0.030	0.220	0.194	**-0.410**	0.295	0.211	**-0.564**
Methanotrophs	-0.234	-0.247	0.000	-0.128	0.005	0.002	-0.024	0.082	0.078	-0.094	0.068
Nitrate reducers	-0.266	-0.226	**-0.453**	**-0.333**	0.112	**-0.379**	0.183	**-0.557**	**-0.446**	0.113	0.231
Nitrogen fixers	-0.151	-0.295	**-0.428**	**-0.417**	0.011	0.088	0.254	0.260	0.010	-0.140	0.109
Oxygenic photoautotrophs	**-0.511**	**-0.483**	**-0.686**	**-0.558**	0.092	**-0.462**	**-0.480**	**-0.573**	0.093	0.036	0.188
Photoautotrophs	**-0.433**	**-0.415**	**-0.591**	**-0.456**	0.120	**-0.375**	**-0.429**	**-0.478**	0.089	0.024	0.161
Photoheterotrophs	0.241	0.169	0.285	0.183	-0.080	0.123	0.277	0.205	0.102	0.099	0.083
Photosynthetic cyanobacteria	**-0.511**	**-0.483**	**-0.686**	**-0.558**	0.092	**-0.462**	**-0.480**	**-0.573**	0.093	0.036	0.188
Phototrophs	**-0.391**	**-0.406**	**-0.536**	**-0.427**	0.150	**-0.324**	**-0.380**	**-0.444**	0.070	0.012	0.173

	-	-		-			-		-	-	
Ureolytic bacteria	0.164	0.206	0.048	0.110	0.260	0.092	0.077	0.006	0.102	0.026	0.051
					-			-		-	-
Xylanolytic bacteria	0.230	0.255	0.152	0.249	0.129	0.136	0.139	0.124	0.014	0.002	0.317

*Measurement units for CO_2, N_2O and CH_4 are mg m^{-2} h^{-1}

Appendix 9. ANOVA results (p-values) from mixed models testing the effects of rainfall and defoliation treatments on ecological functional groups related to fungal communities were obtained from the FunGUILD database. Rainfall and defoliation treatments were treated as the main factors, and plot ID and sampling month were treated as random factors. Bold values are significant at alpha = 0.05.

	----------Kinsella----------			----------Mattheis----------		
Ecological functions	Rainfall (R)	Defoliation (D)	R x D	Rainfall (R)	Defoliation (D)	R x D
Arbuscular Mycorrhizal	0.27	0.24	0.61	0.36	0.25	0.71
Bryophyte Parasite	0.18	0.99	0.57	0.37	0.54	0.18
Clavicipitaceous Endophyte	0.35	0.36	0.48	0.08	0.26	0.65
Dung Saprotroph	0.32	0.23	0.71	0.44	0.32	0.60
Ectomycorrhizal	0.77	0.39	0.71	0.09	0.22	0.31
Endomycorrhizal	0.85	0.98	0.70	0.44	0.49	0.68
Endophyte	0.12	0.46	0.20	0.88	0.31	0.25
Epiphyte	0.94	0.38	0.64	0.23	0.09	0.24
Ericoid Mycorrhizal	0.79	0.12	0.58	1	0.13	0.14
Fungal Parasite	0.09	0.14	0.25	**0.03**	0.92	0.15
Leaf Saprotroph	0.34	0.51	0.18	0.66	0.39	0.50
Lichenized	0.19	0.18	0.34	0.82	0.68	0.47

Lichen Parasite	0.44	0.93	0.58	0.83	0.54	0.62
Litter Saprotroph	0.18	0.55	0.13	0.62	0.50	0.06
Orchid Mycorrhizal	0.25	0.54	0.40	0.09	0.15	0.21
Pathotroph	0.12	0.24	0.16	0.18	0.90	0.77
Plant Parasite	0.44	0.89	0.31	0.36	0.56	0.36
Plant Pathogen	0.27	0.55	0.41	0.56	0.73	0.96
Plant Saprotroph	**0.04**	0.24	0.35	0.31	0.41	0.57
Saprotroph	0.90	0.65	0.57	0.11	0.92	0.37
Soil Saprotroph	0.23	0.24	0.29	0.88	0.77	0.43
Symbiotroph	0.92	0.36	0.34	0.07	0.19	0.05
Wood Saprotroph	0.08	0.07	0.06	0.57	0.46	0.49

Appendix 10. Pearson correlation coefficients of soil fungal ecological functional groups (relative abundance) with soil and environmental biotic and abiotic factors at Kinsella and Mattheis. BG: β-glucosidase (nmol g^{-1} h^{-1}), AP: Acid phosphatase (nmol g^{-1} h^{-1}), CELLO: β-cellobiosidase (nmol g^{-1} h^{-1}), NAG: N-acetyl-β-glucosaminidase (nmol g^{-1} h^{-1}), XYLO: β-xylosidase (nmol g^{-1} h^{-1}), MC: soil moisture content (%, g/g), TEMP: soil temperature (°C). The bold values denote significant correlation.

	BG	AP	CELLO	NAG	XYLO	MC	pH	TEMP	CO_2	N_2O	CH_4
Ecological functional groups				Kinsella							
Animal pathogen	0.162	0.280	0.024	-0.003	0.130	**-0.330**	-0.206	-0.071	-0.232	-0.012	-0.100
Arbuscular mycorrhizal	-0.034	-0.001	-0.035	-0.123	-0.212	0.032	-0.118	-0.132	-0.215	-0.135	-0.020
Bryophyte parasite	-0.097	-0.125	0.042	-0.009	0.101	-0.136	-0.056	-0.121	-0.028	-0.075	-0.168
Clavicipitaceous endophyte	0.034	-0.101	-0.086	-0.084	-0.140	0.011	-0.006	-0.014	-0.188	-0.082	-0.102
Dung saprotroph	-0.253	0.024	0.294	-0.294	-0.105	**-0.513**	-0.180	**-0.436**	-0.219	-0.209	-0.159

	-0.189	-0.176		-0.073		-0.063	-0.114	-0.141	-0.110	-0.235	-0.117
Ectomycorrhizal			-0.128		-0.046						
	-0.050	-0.009		-0.155		-0.172	-0.074	-0.036	-0.157	-0.075	-0.039
Endomycorrhizal			-0.086		0.269						
	-0.242	-0.295				-0.178	-0.110	-0.080	-0.143	-0.053	-0.066
Endophyte			0.156								
	-0.011	-0.108		-0.018		-0.062	-0.030	-0.022	-0.208	-0.057	-0.180
Epiphyte			-0.087		0.029						
	-0.274	-0.260		-0.301		-0.055	-0.056	-0.195	-0.155	-0.004	-0.161
Ericoid mycorrhizal			**-0.318**		-0.128						
	-0.237	-0.188				-0.167	-0.259	-0.094	-0.103	-0.136	-0.039
Fungal parasite			**0.362**		0.187						
	-0.126	-0.180				-0.027	-0.067	-0.087	-0.001	-0.097	-0.138
Leaf saprotroph			-0.022		-0.163						
	-0.137	-0.270				-0.122	-0.094	-0.004	-0.144	-0.010	-0.028
Lichen parasite			0.100		0.082						

								-		-	-
	0.04	0.0				0.21	0.14	0.02	0.14	0.11	0.14
Lichenized	3	22	0.080	0.239	0.028	6	4	5	5	7	9
							-	**-**	-		
	0.48	**0.4**				0.13	0.18	**0.35**	0.04	0.05	0.01
Litter saprotroph	**4**	**47**	**0.420**	**0.384**	0.131	5	2	**9**	2	4	8
	-										-
	0.12	0.0		-		0.18	0.13	0.09	0.19	0.12	0.09
Orchid mycorrhizal	4	00	0.013	0.014	-0.041	4	3	4	0	8	7
						-	-		-		-
	0.16	0.2				0.16	0.26	0.10	0.25	0.12	0.07
Pathotroph	6	56	0.092	0.016	0.162	8	3	6	1	9	0
	-	-				-					-
	0.09	0.1		-		0.08	0.01	0.09	0.00	0.07	0.09
Plant parasite	2	38	-0.052	0.027	-0.110	5	5	4	9	8	2
						-	-		-		
	0.00	0.2		-		0.19	0.21	0.20	0.24	0.16	0.00
Plant pathogen	4	16	-0.042	0.092	0.117	3	5	3	7	7	0
	-								-		-
	0.04	0.1		-		0.00	0.03	0.18	0.08	0.08	0.00
Plant saprotroph	1	06	-0.055	0.120	0.195	0	8	6	3	8	9
						-	-		-		-
	0.20	0.2		-		0.21	0.16	0.03	0.19	0.09	0.13
Saprotroph	0	61	0.059	0.010	0.159	4	9	9	7	4	7

						-	-	-	-	-	
	0.43	**0.3**				0.07	0.26	0.26	0.24	0.10	0.01
Soil saprotroph	**7**	**82**	0.275	0.283	0.250	9	3	9	3	5	5
						-	-	-	-		-
	0.34	**0.3**				0.06	0.27	0.15	0.27	0.01	0.03
Symbiotroph	**5**	**39**	0.300	0.207	0.210	8	3	6	4	6	6
	-	-				**-**	-		-		-
	0.28	0.1		**-**		**0.48**	0.00	**0.32**	0.28	0.01	0.09
Wood saprotroph	0	46	**-0.332**	**0.391**	-0.058	**2**	4	**7**	0	1	4

Mattheis

								-	**-**	-	-
	0.27	0.2				**0.32**	0.17	**0.55**	**0.38**	0.18	0.17
Animal pathogen	8	58	**0.432**	**0.468**	0.067	**2**	7	**9**	**0**	7	3
	-					**-**					
Arbuscular	0.08	0.0		-		**0.44**	0.17	**0.32**	**0.41**	0.07	0.13
mycorrhizal	5	64	-0.097	0.117	0.169	**9**	7	**9**	**5**	7	5
	-	-						-	-		
	0.02	0.0				0.06	0.05	0.00	0.05	0.06	0.05
Bryophyte parasite	3	70	-0.035	0.043	0.021	4	8	2	7	7	1
	-	-				-	-	-	-		-
Clavicipitaceous	0.00	0.0				0.13	0.03	0.08	0.06	0.19	0.20
endophyte	9	14	0.002	0.030	-0.158	0	2	6	9	4	1
						-		-	-		
	0.03	0.0				0.05	0.06	0.01	0.02	0.17	0.12
Dung saprotroph	9	49	0.026	0.058	-0.010	8	6	2	1	5	2

	-	-				-				-	
	0.04	0.1		-		0.03	0.06	0.05	0.08	0.11	0.13
Ectomycorrhizal	8	63	-0.204	0.154	-0.048	5	0	2	4	5	4
							-	-	-	-	-
	0.20	0.0				**0.34**	0.01	0.27	0.25	0.03	0.29
Endomycorrhizal	1	71	0.169	0.156	-0.069	**3**	6	3	0	0	0
								-	**-**	-	-
	0.46	**0.4**				**0.45**	0.20	**0.61**	**0.34**	0.30	0.23
Endophyte	**0**	**19**	**0.643**	**0.589**	0.101	**6**	1	**6**	**3**	0	0
								-	-	-	-
	0.13	0.0				0.27	0.05	0.20	0.19	0.06	0.20
Epiphyte	5	52	**0.338**	0.274	0.022	5	4	8	0	0	8
		-						-		-	
	0.04	0.0		-		0.13	0.06	0.01	0.02	0.08	0.06
Ericoid mycorrhizal	1	98	-0.132	0.047	0.273	6	6	9	6	0	5
								-	-	-	-
	0.01	0.0				**0.37**	0.00	**0.37**	0.31	0.05	0.00
Fungal parasite	5	17	0.163	0.146	0.130	**4**	5	**0**	6	6	8
						-		-	-	-	-
	0.04	0.0				0.03	0.06	0.20	0.23	0.17	0.15
Leaf saprotroph	2	92	0.251	0.148	0.189	3	4	2	9	3	8
								-	-		-
	0.25	0.2				0.11	0.15	0.25	0.22	0.00	0.06
Lichen parasite	6	93	0.234	0.192	0.025	9	9	6	7	1	1

	-	-				-	-				
	0.14	0.1		-		0.23	0.13	**0.35**	0.11	0.06	0.05
Lichenized	4	25	-0.286	0.234	-0.110	3	6	**4**	4	5	1
								-	**-**	**-**	-
	0.37	**0.3**				**0.53**	0.19	**0.66**	**0.36**	**0.41**	0.14
Litter saprotroph	**2**	**46**	**0.675**	**0.547**	0.142	**7**	7	**0**	**2**	**3**	8
		-					-			-	
	0.03	0.0		-		0.15	0.06	0.10	0.04	0.10	0.07
Orchid mycorrhizal	1	63	-0.111	0.073	0.080	3	0	6	9	4	0
								-	**-**	-	-
	0.29	0.2				**0.41**	0.12	**0.54**	**0.35**	0.12	0.18
Pathotroph	9	03	**0.368**	**0.371**	0.190	**4**	3	**7**	**4**	9	4
	-	-							-		
	0.13	0.2		-		0.01	0.01	0.02	0.11	0.08	0.06
Plant parasite	9	30	-0.081	0.045	-0.065	9	3	2	4	7	0
								-	-	-	-
	0.27	0.1				0.28	0.08	**0.37**	0.28	0.07	0.22
Plant pathogen	6	71	0.254	0.255	0.112	5	0	**1**	7	1	9
								-	-		-
	0.20	0.1				0.23	0.17	0.25	0.18	0.04	0.13
Plant saprotroph	7	64	0.151	0.163	0.129	3	8	1	0	7	2
								-	**-**	-	-
	0.34	0.2				**0.47**	0.17	**0.64**	**0.42**	0.20	0.21
Saprotroph	**7**	26	**0.505**	**0.464**	-0.014	**5**	4	**3**	**8**	0	2

								-	**-**	**-**	-
	0.38	**0.3**				**0.45**	0.26	**0.63**	**0.32**	**0.43**	0.20
Soil saprotroph	**0**	**20**	**0.586**	**0.564**	0.103	**0**	4	**4**	**5**	**5**	5
								-	**-**	**-**	-
	0.40	**0.3**				**0.44**	0.27	**0.54**	**0.35**	**0.38**	0.17
Symbiotroph	**6**	**10**	**0.541**	**0.473**	0.042	**3**	1	**7**	**3**	**7**	5
								-	-	-	-
	0.19	0.2				0.19	0.13	0.28	0.06	0.16	0.05
Wood saprotroph	0	31	**0.328**	**0.399**	0.022	7	6	2	9	4	5

Appendix 11. Mean of relative abundance of bacteria for each ecological functional group for rainfall treatments (ambient and drought).

	Rainfall treatment			
	----------Kinsella----------		----------Mattheis----------	
Ecological functions	Ambient	Drought	Ambient	Drought
Aerobic chemoheterotrophs	21.94	22.79	**23.99**	**26.74**
Anaerobic chemoheterotrophs	0.477	0.585	0.445	0.352
Anoxygenic photoautotrophs	0.107	0.218	0.176	0.169
Cellulolytic bacteria	0.833	0.795	0.328	0.387
Chemoheterotrophs	22.42	23.38	**24.44**	**27.09**
Chitinolytic bacteria	0.106	0.102	0.075	0.090
Denitrifiers	0.252	0.560	0.698	0.781
Methanol oxidizers	0.014	0.037	0.103	0.037
Methanotrophs	0.024	0.029	0.103	0.054
Methylotrophs	0.024	0.037	0.103	0.054
Nitrate reducers	0.963	0.997	1.609	1.376
Nitrate respiration bacteria	0.210	0.408	0.287	0.268
Nitrite respiration bacteria	0.192	0.324	0.248	0.225
Nitrogen fixers	7.887	8.108	8.114	9.535
Nitrogen respiration bacteria	0.270	0.645	0.737	0.824
Nonphotosynthetic cyanobacteria	0.023	0.023	0.028	0.021
Oxygenic photoautotrophs	0.268	0.191	1.920	1.501
Photoautotrophs	0.375	0.409	2.096	1.671
Photoheterotrophs	0.246	0.296	**0.261**	**0.274**
Photosynthetic cyanobacteria	0.268	0.191	1.920	1.501
Phototrophs	0.514	0.486	2.181	1.775
Xylanolytic bacteria	**0.324**	**0.401**	0.238	0.113

Appendix 12. The number of amplicon sequence variants (ASVs) assigned to specific ecological functional groups of bacteria for mesic (Kinsella) and dry (Mattheis) site.

Ecological functions	Kinsella	Mattheis
Aerobic chemoheterotrophs	2686	2557
Anaerobic chemoheterotrophs	84	64
Anoxygenic photoautotrophs	36	43
Cellulolytic bacteria	58	41
Chemoheterotrophs	2770	2621
Chitinolytic bacteria	17	10
Denitrifiers	51	55
Methanol oxidizers	7	12
Methanotrophs	7	13
Methylotrophs	8	13
Nitrate reducers	155	182
Nitrate respiration bacteria	49	53
Nitrite respiration bacteria	42	47
Nitrogen fixers	246	369
Nitrogen respiration bacteria	58	61
Nonphotosynthetic cyanobacteria	7	6
Oxygenic photoautotrophs	44	186
Photoautotrophs	80	229
Photoheterotrophs	58	60
Photosynthetic cyanobacteria	44	186
Phototrophs	102	246
Xylanolytic bacteria	47	27

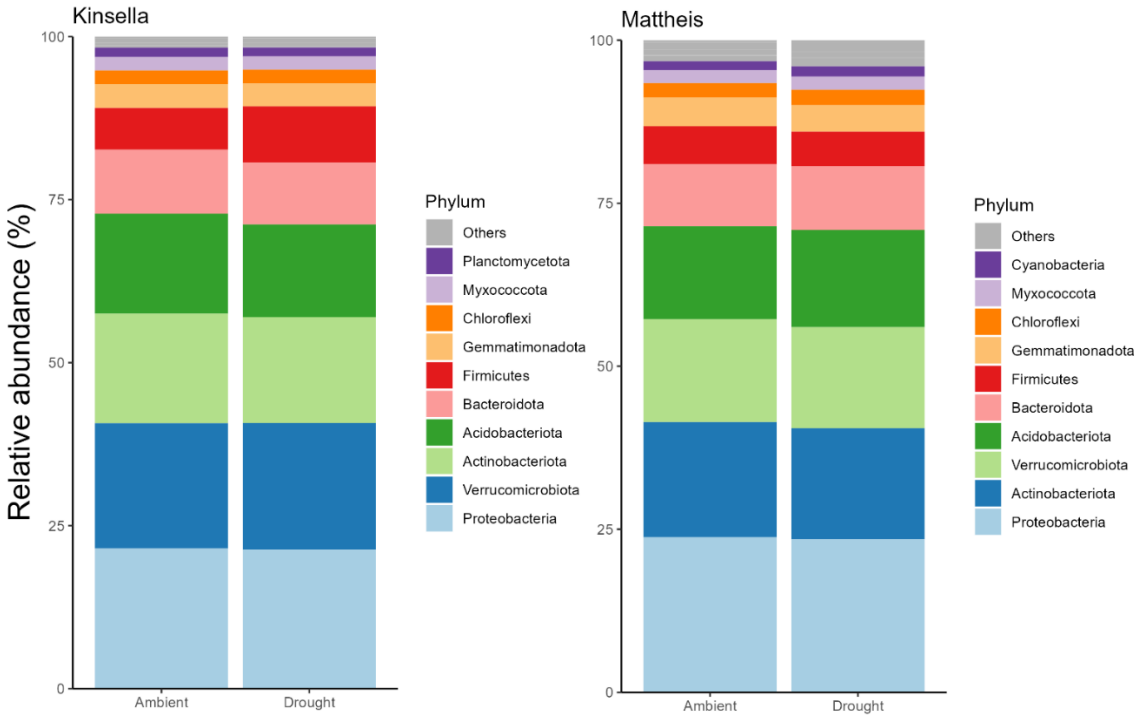
Appendix 13. Number of amplicon sequence variants (ASVs) assigned to specific ecological functional groups of fungi for mesic (Kinsella) and dry (Mattheis) site.

Ecological functions	Kinsella	Mattheis
Arbuscular Mycorrhizal	56	110
Bryophyte Parasite	11	9
Clavicipitaceous Endophyte	4	3
Dung Saprotroph	192	136
Ectomycorrhizal	77	75
Endomycorrhizal	44	76
Endophyte	303	299
Epiphyte	24	27
Ericoid Mycorrhizal	10	4
Fungal Parasite	279	263
Leaf Saprotroph	46	28
Lichenized	64	137
Lichen Parasite	95	128
Litter Saprotroph	121	83
Orchid Mycorrhizal	14	20
Pathotroph	563	878
Plant Parasite	8	7
Plant Pathogen	572	636
Plant Saprotroph	139	169
Saprotroph	1680	1598
Soil Saprotroph	205	187
Symbiotroph	656	799
Wood Saprotroph	282	263

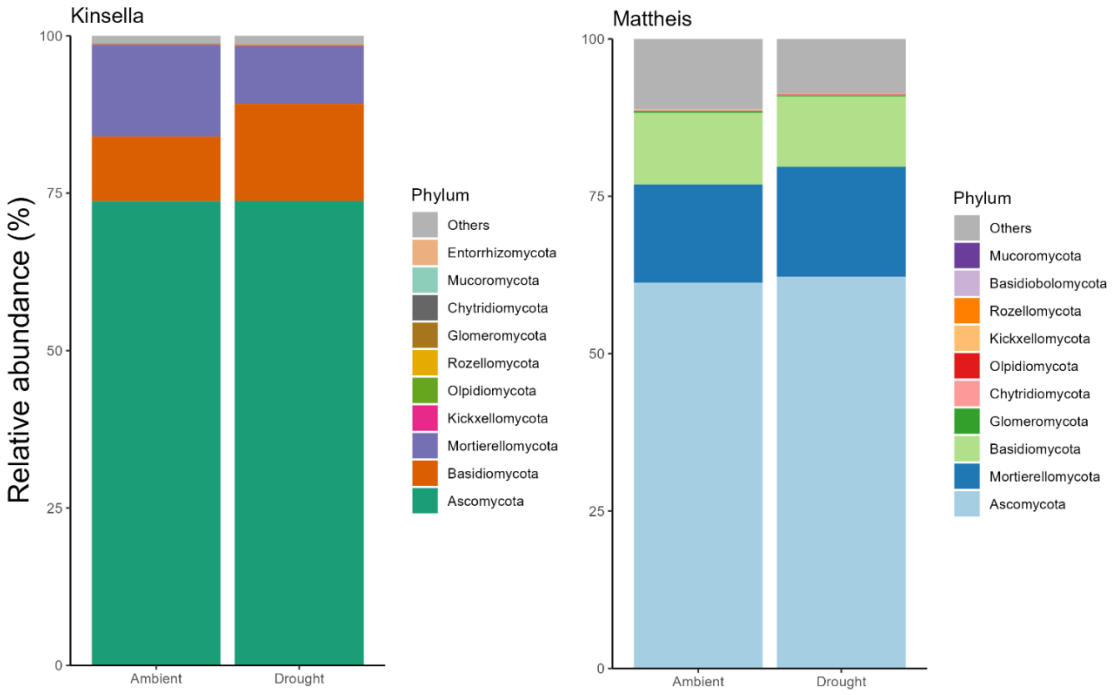
Appendix 14. Post-hoc pairwise comparisons of alpha diversity of soil fungi at Mattheis, showing the differences between defoliation treatments. Number of amplicon sequence variants (ASVs) assigned to specific ecological functional groups of fungi for mesic (Kinsella) and dry (Mattheis) site. Bold values are significant at alpha = 0.10.

Ambient	estimate	SE	df	t.ratio	p.value
(Heavy-heavy) - (Heavy-none)	0.570	0.355	22.10	1.605	0.510
(Heavy-heavy) - (Light-heavy)	0.266	0.387	21.10	0.687	0.957
(Heavy-heavy) - (None-heavy)	0.917	0.428	23.10	2.142	0.237
(Heavy-heavy) - (None-none)	-0.295	0.425	27.00	-0.693	0.956
(Heavy-none) - (Light-heavy)	-0.304	0.329	20.80	-0.924	0.884
(Heavy-none) - (None-heavy)	0.348	0.376	23.40	0.924	0.885
(Heavy-none) - (None-none)	-0.865	0.373	28.60	-2.320	0.168
(Light-heavy) - (None-heavy)	0.651	0.407	22.30	1.601	0.512
(Light-heavy) - (None-none)	-0.561	0.404	26.40	-1.390	0.639
(None-heavy) - (None-none)	-1.212	0.443	27.70	-2.736	**0.074**
Drought					
(Heavy-heavy) - (Heavy-none)	0.038	0.414	18.10	0.093	1.000
(Heavy-heavy) - (Light-heavy)	0.011	0.413	15.40	0.026	1.000
(Heavy-heavy) - (None-heavy)	-0.029	0.415	16.70	-0.070	1.000
(Heavy-heavy) - (None-none)	0.069	0.413	15.40	0.167	1.000
(Heavy-none) - (Light-heavy)	-0.028	0.384	20.90	-0.072	1.000
(Heavy-none) - (None-heavy)	-0.067	0.386	23.20	-0.174	1.000
(Heavy-none) - (None-none)	0.031	0.384	20.90	0.080	1.000
(Light-heavy) - (None-heavy)	-0.040	0.385	18.90	-0.103	1.000
(Light-heavy) - (None-none)	0.058	0.383	17.10	0.152	1.000
(None-heavy) - (None-none)	0.098	0.385	18.90	0.254	0.999

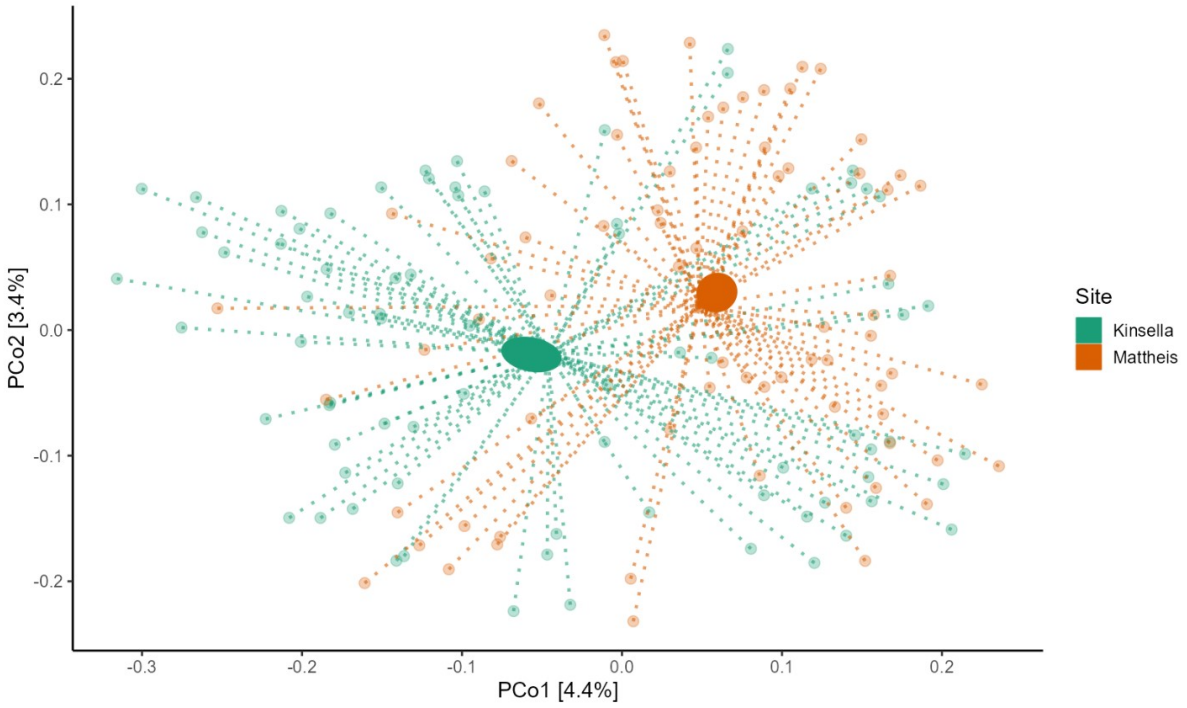
Appendix 15. Relative abundance (%) of soil bacterial phyla at Kinsella (left) and Mattheis (right)



Appendix 16. Relative abundance (%) of soil fungi phyla at Kinsella (left) and Mattheis (right)



Appendix 17. Principal coordinate analysis (PCoA) plot with Bray-Curtis dissimilarity of soil bacterial communities at both sites. Circles in the center of the data points represent the mean of the centroids with both sites clustered apart.



Appendix 18. Principal coordinate analysis (PCoA) plot with Bray-Curtis dissimilarity of soil fungal communities at both sites. Circles in the center of the data points represent the mean of the centroids with both sites clustered apart.