Soil Microbial, Physical, and Chemical Response to Cattle Grazing Management in the Northern Great Plains

by

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Abstract

Globally, 25% of the terrestrial surface is covered by grasslands, 40% of which is used for grazing livestock and is estimated to hold 30% of global soil carbon. Native grasslands in the Canadian prairie cover 12 million hectares and are used extensively for grazing cattle. Livestock grazing behaviors such as feeding, trampling, and fouling lead to changes in soil characteristics, which will directly affect the innate soil microbial communities. Soil microbes play a major role in carbon and nitrogen cycling. Grazing may also alter the stability of carbon pools through changes in soil fractions and the carbon held within them, as soil microbes play a critical role in carbon pools and stability in soil. Thus, small changes in grazing management that lead to reductions in greenhouse gases through carbon sequestration in soil could lead to large offsets due to the spatial extent of the grassland ecosystem.

Among different grazing management systems, a specialized form of rotational grazing known as adaptive multi-paddock grazing (AMP) is considered a regenerative grazing management practice that can improve soil carbon sequestration, productivity, and sustainability of grasslands by altering soil microbial community. However, it is not known how AMP grazing affects microbial communities or subsequent effects on SOC pools in the Northern Great Plains. The overall objective of this thesis research is to understand the effects of grazing systems and specific management metrics on soil microbial community and soil carbon pools.

In this study, soil samples were collected from 19 ranch pairs (38 ranches in total) located across the Canadian prairie, where, in each pair, one ranch practiced AMP grazing while the other practiced conventional grazing (varying from continuous to slow to fast rotational grazing). Grazing management metrics such as stocking rate, stocking density, and rest periods were calculated based on management information data obtained from each landowner. We used soil

phospholipid fatty acid profiles and chloroform fumigation extraction to quantify microbial functional groups and measured microbial biomass carbon. Gene abundance of total bacteria and total fungi were enumerated by targeting the 16S rRNA, and ITS through quantitative real-time PCR; alpha, beta diversity and co-occurrence pattern were assessed using 16S/ITS amplicon sequencing. Further, different SOC pools (i.e., labile to recalcitrant carbon) was quantified through different soil particle size [fine (<53 μm), medium (53-250 μm), and coarse (>250 μm)] and density fractions [light (>1.6 g cm^{-3}) and heavy (<1.6 g cm^{-3})]. To understand AMP grazing effects, we applied two step statistical approaches 1) direct comparison of AMP vs. conventional grazing and 2) analysis of management metrics effect.

I found AMP grazing promotes bacterial to fungal ratios by enhancing labile nutrients (water-soluble organic carbon). Additionally, we found that AMP grazing increased fungal diversity and evenness and led to more complex microbial associations by reducing soil pH. In general, I found the largest SOC stock was in fine fraction, which was 1.1 times and 1.7 times higher than in the coarse and medium size fractions, respectively in AMP grazing. In contrast, under conventional grazing, the coarse fraction held the largest SOC stock, which was more than 2.1 and 1.6 times higher than that in the medium and fine fractions on average. Furthermore, SOC (concentration and stock) were significantly higher in the fine soil fractions from AMP grassland than conventionally grazed grasslands in association with clay particles and corresponding fungi: bacterial (F:B) ratios.

My findings also highlighted stocking density and rest periods are equally important management metrics besides stocking rate in structuring resilient microbial communities and carbon storage in soil. I found that stocking rates play a key role in bacterial and fungal richness while stocking density plays a major role in F: B ratio and soil aggregate distribution. Rest

periods were vital for fungal richness and diversity; where long rest periods boost fungal richness and diversity. Thus, proper implementation of management metrics is equally important to enhance benefits. Moreover, soil properties like pH, texture, and aridity were important factors in structuring soil microbial communities. Maintaining AMP grazing is crucial for grassland stability, sustainability, and SOC in the prairie regions with long rest periods and low to medium stocking rate and stocking density. Thus, my results support the idea that AMP grazing is generally beneficial for increased soil carbon storage.

Preface

This thesis is an original work by Upama Khatri-Chhetri.

Chapter 2 of this thesis has been published in Journal of Applied Soil Ecology in 2022 as

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I was responsible for soil sample collection, all data collation, lab analysis, data analysis and manuscript writing. Dr. Cameron N. Carlyle provided significant input on the conception of the ideas, funding, feedback on the manuscript and supervision. Dr. Karen A. Thompson provided significant input on the conception of the ideas and editing manuscript. Drs. Sylvie S. A. Quideau and Scott X. Chang provided lab facilities, technical expertise and contributed to editing manuscript. Dr. Edward W. Bork contributed significantly to the editing manuscript. Drs. Dr. Mark S. Boyce contributed to funding and editing manuscript. Mr. Dauren Kaliaskar contributed to some of the lab analysis and editing manuscript. Data is deposited in the University of Alberta Dataverse

https://borealisdata.ca/dataverse/carlyle;jsessionid=8d55d0353a406aea9414756bc570

Chapter 3 of this thesis is under review in Journal Science of the Total Environment in 2023 as

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I was responsible for the conception of ideas, sample collection, all data collation, lab analysis, data analysis and manuscript writing. Dr. Cameron N. Carlyle provided significant input on the conception of the ideas, funding, feedback on the manuscript and supervision. Dr. Karen A. Thompson provided input on the conception of the ideas, experimental design and feedback on the manuscript. Drs. Edward W. Bork and Mark S. Boyce contributed to funding and editing the manuscript. Dr. Sylvie A. Quideau provided validation and feedback on the manuscript. Dr. Samiran Banerjee advised on data analysis and data visualization. The gene sequences data has been deposited at DDBJ/ENA/GenBank under the accession numbers KHUT00000000 and KHUX00000000. Chapter 4 of this thesis is in preparation for submission to a peer-reviewed journal as

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I was responsible for the sample collection, all data collation, lab analysis, data analysis and manuscript writing. Dr. Cameron N. Carlyle provided significant input on the conception of the ideas, funding, feedback on the manuscript and supervision. Dr. Scott X. Chang provided lab facilities, technical expertise and contributed to editing the manuscript. Dr. Edward contributed to funding and provided significant input in editing the manuscript. Drs Karen A. Thompson, Sylvie A. Quideau, and Mark S. Boyce provided validation and feedback on the manuscript. Data deposited in the University of Alberta Dataverse:

https://borealisdata.ca/dataverse/carlyle;jsessionid=8d55d0353a406aea9414756bc570

Dedication

This degree is dedicated to my parents Ramesh Khatri Chhetri and Indira Devi Sharma K C

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Arya who is lovely and understanding, for all her mischievous smile and giggles which motivated me at times.

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List of Abbreviations

AHM: annual heat: moisture

AMF: Arbuscular mycorrhizal fungi

AMP: Adaptive multi-paddock grazing

ANOVA: analysis of variance

BD: bulk density

C: carbon

C: N: carbon to nitrogen ratio

CO_2: carbon dioxide

F: B: fungal to bacterial ratio

GHG: greenhouse gas

GP: gram-positive

GN: gram-negative

GP: GN: gram-positive to gram-negative

MBC: microbial biomass carbon

MBN: microbial biomass nitrogen

MBC: MBN: microbial biomass carbon to nitrogen

N: nitrogen

OTU: operational taxonomic unit

PLFA: phospholipid fatty acid analysis

qPCR: quantitative polymerase chain reaction

SOC: soil organic carbon

SR: stocking rate

SD: stocking density

R: G: rest-to-grazing ratio

Chapter 1. Introduction

1.1 Research Background

Soil is a major global carbon (C) sink (Whitman et al., 1998), which holds 3.3 times more carbon than the atmosphere and 4.5 times that of terrestrial plants (Lal, 2004). Grasslands hold about 30% of terrestrial soil organic carbon (SOC), as they comprise more than 40% of Earth's terrestrial surface (Follett et al., 2001; Ingram et al., 2008; Lal, 2002; Schuman et al., 2002). Canadian grasslands and rangelands cover 11.4 million hectares of area in the Canadian prairie provinces (Bailey et al., 2010). These grasslands and pastures are used extensively for grazing cattle (Follett et al., 2001; Ingram et al., 2008), which affects soil C through complex pathways (Pinheiro et al., 2004). In particular, soil microbial communities play a major role in C and N cycling and are considered the main driver of C storage in soil (Klotz and Stein, 2008). Thus, small changes in grazing management that affect microbial communities could lead to reductions in atmospheric GHG through enhanced sequestration in soil. Therefore, knowledge of the effects of cattle management on the soil microbial community could help to increase C storage in the soil to mitigate climate change.

Livestock contribute significantly to GHG emissions and cattle, in particular, have been vilified for the methane they create (Johnson and Johnson, 1995; Lassey et al., 1997). However, Allan Savory claimed in a highly viewed TED talk, which received tremendous media attention that we should "increase the number of cows to fight against climate change" (Savory Institute, 2015). His claim was based on the premise that specialized management systems of cattle grazing can dramatically increase the amount of carbon stored in grassland soil thereby offsetting greenhouse gas emissions. These management systems, called adaptive multi-paddock grazing

(AMP), place cattle in small pastures for short periods of time (hours – days) at very high densities. In contrast, traditional grazing management in the Canadian prairies has cattle in pastures for long periods of time (weeks-months) at low densities. Recent studies have shown AMP grazing can improve soil moisture and water infiltration (Döbert et al., 2021; Teague et al., 2011) thereby stimulating the activity of soil microorganisms and increasing carbon storage (Stanley et al., 2018a; Teague et al., 2011). Whether AMP grazing stimulates the microbial community to increase soil C storage has not been thoroughly assessed across the broad range of climatic conditions present in the Canadian Prairie, nor have the underlying mechanisms been thoroughly explored.

Soil microbes play a major role in soil ecosystem processes and services including organic matter decomposition, nutrient cycling, and soil aggregate stabilization, and can structure ecosystem composition like plant diversity (Bender et al., 2016; Van Der Heijden et al., 2008). Soil microbes are the unseen living component of soil and comprise a major percentage of life's genetic diversity (Van Der Heijden et al., 2008; Whitman et al., 1998), and soil contains >1,000 kg/ha of microbial biomass C (Fierer, 2017). The soil microbial community includes bacteria, fungi, archaea, and other organisms. Soil microbes are well-known as key players in C sequestration (Bhattacharyya et al., 2022; Malik et al., 2016; Schimel and Schaeffer, 2012; Six et al., 2006). Furthermore, microbes play a major role in the decomposition of organic matter in the soil, however, microbes also protect organic matter from decomposition either by entangling soil particles through fungal and bacterial hyphae, or cementing soil aggregates by producing gluey substances like polysaccharides (Chaney and Swift, 1986; Oades, 1984; Six et al., 2002; Wiesmeier et al., 2012).

Soil organic C is held in different fractions (pools) with different stability (Sollins et al., 1996; von Lützow et al., 2007), where soil microbial communities play a key role in organic carbon protection, decomposition, turnover rate, and storage in the different pools (Bhattacharyya et al., 2022). Most of the microbial organic C in the fine fraction is mainly due to bacterial colonization whereas, a higher proportion of fungal biomass is found in the coarse fractions (Kandeler et al., 2007). Soil organic matter is divided into mineral-associated organic matter (MAOM) and particulate organic carbon matter (POM) pool to better understand soil C sequestration and stability (Castellano et al., 2015; Cotrufo et al., 2015). The MAOM is the organic C that is predominately microbial products associated with minerals, such as iron and aluminum oxides, and present in <50 μm soil fraction, whereas, POM is composed of organic compounds mainly of plant origin, such as lipid, protein, and carbohydrates available in >50 μm fraction (Cotrufo et al., 2015; Emde et al., 2022). POM is more sensitive to soil disturbance resulting from land management practices like agriculture, cattle grazing, etc. and is susceptible to microbial breakdown leading to relatively fast turnover times, in contrast, MAOM is more stable and can remain in the soil for decade to centuries as it is physically protected against microbial decomposition (Emde et al., 2022; Emilia Hannula and Morriën, 2022; Lavallee et al., 2020; Sequeira and Alley, 2011). Particle size and density fractionation have been commonly used to separate these pools of different soil organic matter quality and turnover time (Kandeler et al., 2007).

Soil microbial communities are sensitive to grazing activities (Huhe et al., 2017; Patra et al., 2015). Livestock behaviors such as feeding, trampling, and fouling lead to changes in the plant community, specifically on plant biomass, root biomass, exudation (Clegg et al. 2003;

Pineiro et al. 2010), and the physical, chemical, and biological properties of soil, which will directly affect the soil microbial communities (Bardgett et al., 2001). A previous study examined soil fungal and bacterial abundance response to AMP grazing, using direct microscopy (Van Veen and Paul, 1979) to estimate bacterial and fungal counts, which is a highly qualitative (not quantitative) method. It focused on soil microbial communities described by total bacteria and total fungi, rather than a more comprehensive analysis of microbial community as an indicator of soil health (Teague et al., 2011). Given the large extent of the Canadian prairie, the quantification of soil microbial response to grazing along with C stocks in the different fractions of the prairie is essential to determine how the grazing management system could provide a practical approach for C sequestration on a larger scale. Previous studies have focused on cattle grazing effects on C stocks (Bork et al., 2015, 2020; Hewins et al., 2018) in some of the prairie regions; however, no studies have been reported on the relationship between soil microbial communities and soil carbon stocks in different fractions in the Canadian prairie. Further, information about the microbial response to AMP grazing and its subsequent effect on soil C stocks across large geographic reactions in the Northern great plain is limited. This thesis work will explore the detailed soil microbial community characterization and its relationship with soil carbon fraction stocks, allowing a better understanding of the mechanism through which grazing management alters soil C, including the specific role of stocking rates, stocking density, and rest periods. The work will provide data that can support recommendations for grazing systems that will improve ecological and environmental values in the Canadian prairie.

1.2 Research Objective

The aim of this thesis research is to enhance our understanding of soil microbial community (abundance, biomass, diversity) response to different grazing treatments, and the resulting effects

on soil carbon storage. Further, this thesis work has addressed more specific questions like whether, how, and why grazing practices may alter the soil microbial community to improve long-term C storage. We addressed three primary research questions:

1) Does AMP vs. non-AMP grazing affect soil microbial communities and C stocks?
2) How do specific management metrics influence soil microbial communities and C stocks?
3) How do associated soil environment and climate effects help in shaping soil microbial communities and C pools?

1.3 Thesis overview

The thesis has five chapters. The first chapter introduces the background and objectives of this thesis work. The second to fourth chapters present original research completed as a part of this thesis. The final chapter summarizes and synthesizes the contribution of this thesis to rangeland and soil science.

The general experimental design involved collection of soil samples from paired AMP and conventional ranches distributed over an environmental gradient to address whether the effects of AMP have generality across a diversity of grassland types. This research was completed in collaboration with 38 private and independent ranch owners responsible for the management of cattle in their operations. The paired designed comparison provided grazing effects at the local level and sampling in overall Canadian prairie in larger ranch numbers provided a broad knowledge of the AMP grazing system at a national level.

I use three different microbial analytical techniques- -soil microbial biomass-chloroform fumigation/extraction, phospholipid fatty acid analysis (PLFA) (Ford et al., 2013), quantitative

polymerase chain reaction (qPCR), and sequencing of DNA (Stone et al., 2015; Zheng et al., 2012) derived from the soil. Each technique has its strength. For example, soil microbial biomass provides information about soil microbial changes to perturbation at the community level. PLFA will provide overall microbial community structure, whereas DNA quantification and sequencing of total bacteria (16S rRNA) and total fungi (ITS) can provide detailed microbial community information at the species level. Therefore, applying all three techniques will provide complementary information and results can be comprehensively applicable for larger spatial scales like the Canadian prairie. Further, SOC quantification in bulk soil and indifferent fractions provides soil C storage and potential stability information in the prairie region. Moreover, soil physical and chemical properties including soil moisture, texture, pH, bulk density, organic C, total nitrogen, dissolved organic carbon (DOC), and dissolved organic nitrogen (DON) were analyzed and compared with the microbial community.

In Chapter 2, I examined the response of soil microbial abundance and biomass to different grazing treatments and how they varied by specific management metrics across the Canadian Prairie. Soil microbial abundance and biomass were quantified with PLFA, components of microbial cell membranes, and microbial biomass- chloroform fumigation/extraction. The PLFA provided fungal and bacterial abundance in terms of arbuscular mycorrhizal fungi (AMF), fungi, gram-positive, gram-negative bacteria, while microbial biomass C (MBC), and microbial biomass nitrogen (MBN) were obtained from the chloroform fumigation method. PLFAs are the most commonly used method to identify microbial response to perturbation and it is comparatively rapid and less expensive than molecular methods (Frostegård et al., 2011; Kaur et al., 2010). Our data show that microbial PLFA abundances and MBC were not different between the two grazing systems. However, AMP grazing resulted in

lower ratios of microbial biomass C: N (MBC: MBN), fungi: bacteria (F: B), and Gram-positive: Gram-negative bacteria in grassland soils compared to conventional grazing. Thus, there is a potential to affect soil function by altering the composition of soil microbial communities through AMP grazing.

In Chapter 3, I examined the response of soil microbial diversity and co-occurrence patterns to different grazing treatments and by specific management metrics. Soil microbial diversity quantification is not possible with PLFA, while some studies have quantified diversity from PLFA, the approach is heavily criticized because fungi have very few different PLFAs in their membrane, thus a large number of different fungal species can be represented by the same PLFA (Frostegård et al., 2011). To quantify microbial diversity and to obtain detailed microbial abundance at the species level gene sequencing was performed. For that, DNA was extracted from soil and gene abundance of total bacteria was enumerated by targeting the 16S rRNA (180bp), and for total fungi by targeting ITS (380bp) by quantitative real-time PCR Further, to assess corresponding changes in metagenome and diversity (alpha and beta), high-throughput sequencing was performed. Multiplexed sequencing was conducted using Illumina MiSeq reagent kit V3 and resulting sequences were clustered into operational taxonomic units (OTUS). qPCR and rRNA gene sequencing has become more popular and advanced molecular techniques to quantify soil microbial diversity (Escobar-Zepeda et al., 2015). Soil microbial diversity dominates soil biodiversity and is extremely complex because one gram of soil may have >10, 000 different microbial species and are strongly interconnected forming dense network structures (Allison and Martiny, 2008). Soil microbial diversity promotes valuable ecosystem services and plays a key role in C and N and nutrient cycling (Maron et al., 2018; Waring et al., 2013; Yeates

et al., 1997). Specifically, fungal diversity is of high significance in ecosystem functioning. Fungi play multiple functions in grassland soil such as involvement in nutrient cycling, especially in organic matter decomposition (Orellana, 2013), influence sources of CO_2 emission (Faghihinia et al., 2020), and enhance plant grazing tolerance and vegetation productivity (Walling and Zabinski, 2006). Grassland soil conserves a wide and diverse microbial community (Van Der Heijden et al., 2008; Yeates et al., 1997); however, they are poorly characterized. Additionally, some of the studies have established that microbial community response to cattle grazing, where the response of microbial diversity and co-occurrence pattern was inconsistent across various microbial groups, types of livestock, and different grazing intensities (Bardgett et al., 2001; Gou et al., 2015; Yang et al., 2013; Zhou et al., 2010). Increases in soil microbial diversity are associated with enhanced soil C stock and C stability (Bastida et al., 2021; Mau et al., 2015), while complex microbial network and diversity improved ecosystem multifunctionality and stability (Delgado-Baquerizo et al., 2016). A detailed understanding of cattle grazing effects on soil microbial diversity and co-occurrence pattern is crucial to understand the effects on grassland ecosystem functioning and sustainability (Eldridge et al., 2017; Maron et al., 2018). Thus, the effect of AMP grazing on soil microbial diversity is unknown, and this is the first time in Canadian prairie that the soil microbial community is being assessed under different grazing systems. Our data show that stocking rate and rest-to-grazing ratio play major roles in shaping bacterial and fungal richness and diversity. Furthermore, AMP grazing enhances fungal diversity and microbial network complexity, indicating that the grazing system could lead to improved ecosystem functioning and stability.

In Chapter 4, I quantified soil C pools under different grazing systems in 12 ranch pairs (n=24) in Alberta rangeland to address whether AMP grazing increases stable soil C stocks. The

labile and stable soil carbon pools were quantified using size and density fractions (Crow et al., 2007; Six et al., 2002; J. Q. Yang et al., 2021). Some studies have suggested that AMP grazing can store more SOC (Rasmussen et al., 2016; Stanley et al., 2018a). I sought to understand the mechanism for enhanced SOC under AMP grazing and assessed the association between SOC in different fractions and soil microbial communities. The identification of a grazing system that promotes soil carbon in different pools and its corresponding stability would help to facilitate C storage for a long duration in soil and eventually can slow down C cycling (Malik et al., 2016; Waring et al., 2013). Soil microbial communities influence C in different pools (Hemkemeyer et al., 2018). Unlike plants, we have limited knowledge of the relationship between microbial communities and soil C pool, particularly in grassland ecosystems. To examine these relationships, the soil microbial community and soil C pools were assessed. Our data show that AMP grazing influenced macroaggregates and enhanced SOC in fine soil fractions than conventional grazing. Additionally, soil microbial community associations existed with the amount of soil C sequestration across different soil fractions. Given that SOC in the fine fractions is more recalcitrant, our result indicates that AMP grazing increased the size of the stable SOC pool.

In Chapter 5, the primary findings of the thesis research were summarized and synthesized into general conclusions. Additionally, this chapter includes research limitations and future research directions, and recommendations for ranchers and policymakers.

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Chapter 2. Adaptive multi-paddock grazing increases soil nutrient availability and bacteria to fungi ratio in grassland soils

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Summary

Grasslands are used extensively for grazing livestock, and variation in grazing management may affect the soil microbial community and ecosystem functions, such as nutrient cycling, in grasslands. In particular, adaptive multi-paddock (hereafter 'multi-paddock') grazing is considered a regenerative grazing management practice that can improve the sustainability of grasslands. However, little is known about how multi-paddock grazing affects the soil microbial community, which plays an important role in global biogeochemical cycling. In this study, grassland soils were collected from 15 ranch pairs located across the Canadian prairie, where, in each pair, one ranch practiced multi-paddock grazing while the other practiced conventional grazing (varying from continuous to slow to fast rotational grazing). We used soil phospholipid fatty acid (PLFA) profiles to quantify microbial functional groups, and measured microbial biomass C and soil properties. Our data show that microbial PLFA abundances and microbial biomass C were not different between the two grazing systems. However, multi-paddock grazing resulted in lower ratios of microbial biomass C: N (MBC:MBN), fungi: bacteria (F:B) and Gram-positive: Gram-negative bacteria in grassland soils compared to conventional grazing. Further investigation of specific management metrics revealed that MBC:MBN and F:B ratios were most affected by cattle stocking rate and stocking density, respectively. Thus, there is potential to affect soil function by altering the composition of soil microbial communities through multi-paddock grazing.

Keywords

Stocking density; Stocking rate; Fungi: Bacteria ratio; Grazing system;

Microbial biomass carbon; Phospholipid fatty acids

Highlights

- Adaptive multi-paddock grazing (AMP) altered microbial composition
- AMP reduced microbial biomass C:N
- AMP reduced gram positive to gram negative and fungi to bacteria ratios
- Increased stocking rates reduced microbial biomass C:N
- Increased stock density decreased fungi: bacteria

2.1 Introduction

Agriculture is one of the key global economic drivers and provides food for human welfare, but is also responsible for 14% of global greenhouse gas (GHG) emissions (IPCC, 2014). Soils are an important GHG sink (Rumpel et al., 2018), and grassland, which covers about 25% of Earth's terrestrial surface, store around 30% of the global carbon stocks in soil (Adams et al., 1990; Bailey et al., 2010; Lal, 2004; Ojima et al., 1993). A primary use of grasslands is livestock grazing, which can affect soil microbial communities, and thereby alter carbon (C) and nitrogen (N) cycling (Klotz and Stein, 2008) along with the production and consumption of GHGs (Nielsen et al., 2011). Grazing can change both above- and belowground plant biomass, root exudation patterns (Clegg et al., 2003; Pineiro et al., 2010), and soil chemical and physical characteristics (Ingram et al., 2008), which further affect soil microbial communities (Bardgett et al., 2001) and biogeochemical processes and ecosystem properties such as C storage (Young and Crawford, 2004). Despite the wealth of studies on the effect of grazing on aboveground processes, considerably fewer have looked at belowground responses and particularly the effect of specific grazing management practices on soil microbial communities.

Grazing systems can take many forms, which may have different effects on ecosystem properties and processes. The simplest grazing systems use continuous (or season-long) grazing, which places livestock in a single pasture for the duration of the grazing season (many months). Complex grazing systems involve moving livestock through multiple pastures frequently (weeks to months), commonly known as rotational grazing. Further, highly complex grazing systems, such as Adaptive Multi-Paddock (hereafter known as "multi-paddock") management, places cattle in small pastures at high stocking densities for short durations (hours to days) with long rest periods between grazing events (Savory, 1983; Savory and Parsons, 1980; Bork et al., 2021).

Decisions regarding movements of livestock between paddocks are typically based on vegetation type and phenology, as well as requirements to facilitate vegetation recovery after grazing (weeks-months) (Milton, 1999; Valentine, 1967) with the aim to provide long resting periods for plants to recover from grazing.

Multi-paddock grazing is thought to improve conditions for soil microorganisms through altered soil moisture, nutrient availability and plant growth (Döbert et al., 2021; Stanley et al., 2018b; Teague et al., 2013, 2011; Teague, 2018). The purported underlying reason for this is that high animal densities allow for longer rest periods for plants and create a phenomenon called the "Herd effect" or "Hoof effect" in which animals trample vegetation and incorporate it into the soil surface (Burleson and Leininger, 1988; Savory and Butterfield, 2016; Warren et al., 1986). However, most previous research examining livestock management on soil has accounted only for stocking rate and not stocking density (Briske et al., 2008; Wang et al., 2015; Warren et al., 1986). Stocking rate (number of cattle/area/time) is a standard measure of grazing (forage use) intensity and is considered one of the most important livestock management metrics affecting soil microbial communities (Wakelin et al., 2009), plant productivity (Briske et al., 2008) and animal production (Venter et al., 2019). However, the response of microbial communities to stocking rates is not consistent; for example, microbial biomass can decrease or increase, with increasing stocking rate (Banerjee et al., 2000; Bardgett and Wardle, 2003; Harrison and Bardgett, 2004; Wang et al., 2006). Variation in microbial community responses to grazing and stocking rates opens the possibility that other management metrics, such as stocking density and length of the rest period may have important effects on soil microbial communities. Understanding the effects of specific grazing management metrics, in addition to the overall

grazing system, on soil microbial communities will aid in identifying grazing management practices that improve soil quality and functionality through soil microbial communities.

Several measures of soil microbial communities can be used to examine their responses to grazing, which may additionally indicate responses of soil C stores. Microbial Phospholipid fatty acids (PLFA) are the components of cell membranes, by identifying PLFA derived from the living microbial community they can be used as a biological index of overall soil quality (Kaur et al., 2005; Quideau et al., 2016). A number of functional groups and their relative abundances, derived from PFLA markers, can also indicate soil C change. Arbuscular mycorrhizal fungi (AMF), fungi, the ratio of Gram-positive: Gram-negative bacteria (GP: GN) and ratio of fungi:bacteria (F:B) are associated with soil C, moisture (Pan et al., 2016), pH (Rousk et al., 2010) and nutrient content (Clegg, 2006). Arbuscular mycorrhizal fungi, which form symbiotic relationships with plants (Heyde et al., 2017), can promote nutrient availability (Francisco et al., 2016), crop productivity (Naher et al. 2013) and enhance soil carbon sequestration (Wang et al., 2016). Typically, AMF abundance decreases with livestock grazing because of reduced aboveground plant biomass (Dudinszky et al., 2019) and increased nutrient input from livestock manure (Conant et al., 2003, 2001; Schuman et al., 1999). Grazing can either increase (Bagchi et al., 2017; Bardgett and Leemans, 1997), or decrease F:B ratios (Ghani et al., 2003). Soils with higher F:B ratios can store more soil C (Malik et al., 2016; Waring et al., 2013), and increase the persistence of stored C (Bailey et al. 2002). Similarly, higher GP:GN ratios are related to C availability in soil (Fanin et al., 2019) and more complex C forms (carbonyls), whereas a lower ratio of GP:GN bacteria is associated with more labile C derived from plants (Fanin et al., 2019; Herman et al., 2012). Microbial biomass C can serve as an indicator of soil stress and disturbance, an early indicator of changes in total organic C (Liu et al., 2012; Ramesh et al.,

2019) and can either increase or decrease with grazing (Banerjee et al., 2000; Zhang et al., 2005). Further, grazing increases microbial biomass C to N (MBC:MBN) compared to ungrazed plot and is not affected by N addition (Bagchi et al., 2017). Thus, AMF, GP:GN, F:B, and MBC: MBN ratios can be used to examine grazing effects on soil quality, C, and N storage (Bagchi et al., 2017; Heyde et al., 2017; Patra et al., 2015; Teague et al., 2011). However, the majority of studies have compared grazed to non-grazed areas, but few studies have examined the response of these microbial communities to AMP grazing or specific grazing management metrics.

Studies of the effects of cattle grazing systems and management metrics on these microbial groups could aid understanding of how grazing ultimately affects soil C and nutrient cycling. Using data collected from 30 ranches (15 multi-paddock and 15 conventional grazing) in a paired design, we sought to: (1) characterize microbial communities and soil properties in grasslands subject to either multi-paddock or conventional grazing; and (2) to identify key grazing management practices and environmental variables affecting the soil microbiome using specific management metrics (i.e. stocking rate, stocking density, and rest to grazing ratio) collected from each ranch.

2.2 Materials and Methods

2.2.1 Study sites, sampling design and soil sampling

We sampled paired ranches at 15 locations across a large area of the northern Great Plains in the Canadian prairie provinces of Alberta, Saskatchewan, and Manitoba. Sites were distributed across a broad environmental gradient that included the Mixedgrass Prairie, Fescue Grassland, Aspen Parkland and Boreal Transition ecoregions, and four major soil types, including Black Chernozemic, brown Chernozemic, gray Luvisolic and black Regosolic soils. Mean annual

temperature for the sites ranged from 0.6 °C to 4.8 °C, with annual precipitation from 299 mm to 590 mm based on the 30 year normal (1989-2018) data (Table S1). Most grasslands were dominated by agronomic grasses such as smooth brome (*Bromus inermis* Leyss.), meadow brome (*Bromus biebersteinii* Roem and Schult.), crested wheatgrass (*Agropyron cristatum* (L.) Gaertn) and Russian wild rye (Psathyrostachys /*Elymus junceus Fisch.*), along with legumes such as alfalfa (*Medicago sativa* L.), sweet clover (*Melilotus officinalis* (L.) Pall.), and alsike clover (*Trifolium hybridum* L.). A few sites were dominated by a native grass, rough fescue (*Festuca campestris* Rydb.).

All sites were privately owned ranches, except for one owned by the University of Alberta. We first identified multi-paddock ranches to locate study sites through a two-step process. First, multi-paddock ranches were identified through online questionnaires completed by willing participants. Questionnaires helped to identify whether a ranch met the criteria of multi-paddock grazing, in which livestock are grazed at high densities for short periods, and the pasture is given a long period of rest (Teague et al., 2013). Second, follow-up phone calls were conducted to screen potential multi-paddock ranches to ensure they met the following conditions: a) the only livestock were beef cattle, b) a suitable area of grazed grassland at least 10 ha in size was available for sampling, and c) there were areas free of supplementary bale feeding on the sample area (to isolate grazing-system effects). Initial screening reduced the number of potential multi-paddock ranches from 93 to 60. Next, we identified a suitable adjacent or nearby ranch (hereafter collectively known as "conventional grazing") within 5 km distance for sampling. To isolate the role of grazing effects and minimize the effects of environmental variability, the sampling locations with both ranches in a pair were located on similar ecosites (topography, soils, and vegetation type). There was

a minimumdistance of 25 km between ranch pairs, and pairs were distributed ~1000 km from east to west and ~550 km from north to south in western Canada.

The cattle management history of each ranch was obtained from each landowner through a detailed survey in which we documented mean pasture stocking rate, stocking density, and rest to grazing ratios. Stocking rates (animal-units-months (AUM) ha^{-1}) and stocking densities (animal-units (AU) ha^{-1}) were calculated based on the mean paddock size, the number of animals, which was adjusted based on the class of animal using typical animal unit size equivalents for the region (Bao et al., 2019), and for stocking rate the length of the grazing period. The rest to grazing ratio was derived from the minimum number of days of rest reported by ranchers relativized to the mean length of the early season grazing period (prior to August 1). The ranch pairs in this study are a sub-set of sites reported on in Bork et al. (2021), which thoroughly describes management differences between multi-paddock and conventional grazing. The two grazing systems could be distinguished from each other based on stocking density (multi-paddock: 39.9± 40.1 AU ha^{-1}; conventional grazing: 2.49± 2.05 AU ha^{-1}) and rest to grazing ratio (multi-paddock: 46.1± 32.8; conventional grazing: 0.93± 1.21), but not stocking rate (Tables S1 & S2).

Study sites were distributed across a broad range of climatic conditions; thus, we used an index of aridity, annual heat: moisture (AHM) (derived from ClimateNA) to describe their climate in a single variable. AHM is the ratio of mean annual temperature (MAT) to mean annual precipitation (MAP), and is known to be an important predictor of soil and vegetation properties in the study region (Bork et al., 2019; Hewins et al., 2018).

$$AHM = (MAT+10)/(MAP/1000)$$

From each ranch, 10 soil cores (2.54 cm diameter) were collected from a depth of 0-15 cm from June through August 2017, where the paired ranches were sampled at the same time. Core locations were randomly selected within the 10-ha area of grassland within each ranch, from within areas that were on a similar ecosite to its neighbouring ranch. Cores were combined, immediately frozen in the field with dry ice, and transported to the lab and stored at -20 ^{o}C until further analysis. Four separate soil cores were randomly collected (3.81 cm diameter ×15 cm deep) to determine soil bulk density.

2.2.2 PLFA extraction

A sub-sample of frozen soil was used for PLFA analysis to characterize the microbial community. Individual PLFAs can be differentiated based on chemical structure and have been used as biomarkers for different microbial functional groups, including GP bacteria (represented by saturated, terminally branched, iso and anteiso-branched fatty acids), GN bacteria (represented by monounsaturated fatty acids), actinomycetes (represented by saturated PLFAs with methyl chain branching on the 10th C), fungi (usually represented by polyunsaturated PLFAs, with some monounsaturated PLFAs) and Eukaryotes (represented by polyunsaturated fatty acids) (Quideau et al., 2016). However, some of these PLFAs can be found in two different microbial functional groups, so caution must be applied while associating individual PLFAs with microbial functional groupings (Frostegård et al., 2011; Quideau et al., 2016). PLFAs were extracted from soils using an extended extraction method with a chloroform: methanol: citrate buffer mixture (Quideau et al., 2016), which is a user-friendly modified extraction protocol of prior methods (Chowdhury and Dick 2012) that improves extraction efficiency compared to the original method (Bligh, and Dyer, 1959). Flame ionization detector gas chromatography (GC-FID), Agilent 7890A, FID, SSI, 7683 (Agilent Technologies, Inc., USA) was used to identify

fatty acids. Out of 120 PLFAs (Chain lengths 10-24 C atoms) detected, only 84 PLFAs (chain lengths 14 to 20 C atoms) were used for analysis because PLFAs chain lengths between 14 to 20 C atoms are largely bacterial and fungal origin (Quideau et al., 2016). Individual PLFAs were automatically assigned to specific microbial functional groups through the Microbial Identification Inc (MIDI) system, with the details on the identification provided in Table S3. Altogether, 6 functional groups: AMF, Fungi, GP, GN, Actinomycetes and Anaerobes, were identified based on MIDI (Table S3). Abundances of actinomycetes and GP bacteria were summed to obtain total GP for estimation of the GP: GN ratios. The number of bacterial PLFAs (GP, GN, Actinomycetes and Anaerobes) were summed to obtain total bacterial abundance, and AMF (16:1 ω 5c) and Fungi (18:2 ω 6c) were summed to obtain estimates of total fungal abundance. Finally, the fungi: bacteria (F:B) ratios were calculated based on total fungal (pmol g^{-1}) and total bacterial PLFA abundance (pmol g^{-1}). Thus, five microbial PLFA markers, namely Fungi, AMF, total fungi, GP:GN and F:B are presented.

2.2.3 Microbial Biomass C and N

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined with a chloroform fumigation-extraction (CFE) method (Brookes et al., 1985). From each ranch, two sets of duplicate 10 g samples (oven-dried equivalent) were analyzed. One set of soil samples was incubated under chloroform fumigation for 24 hr to lyse microbial cells. Then, both sets (with and without fumigation) of soil samples were mixed with 50 ml of 0.5 M K_2SO_4 (potassium sulfate) (1:5 soil: solution ratio). To prepare 0.5 M K_2SO_4, 87 g of potassium sulfate was dissolved in 1000 ml of deionized water. Then the soil samples were shaken for 1 hr at 180 rpm and filtered. Fumigated and unfumigated soil extracts were run on a Shimadzu TOC-V CSH/CSN Total Organic Carbon Analyzer (Shimadzu Corporation, Kyoto, Japan). The

difference between fumigated and non-fumigated samples was used as estimates of MBC and MBN (Vance et al., 1987).

2.2.4 Soil physicochemical properties

Soil ancillary properties were measured on air-dried sub-samples from the same composite freezer stored soil to describe the soil environment at each ranch. Soil pH was measured with an Accumet AB150 pH/mV Meter (Fisher Scientific, America) in a soil and deionized water (1:5) solution after being shaken for 30 minutes (Kalra, 1995). For total soil C and N, sub-samples were oven-dried at 60 °C for 48 hr and passed through a 2 mm sieve, then ground to a fine powder in a ball mill grinder (Hewins et al. 2018) and quantified with a Flash 2000 CHNS elemental analyzer (Thermo Scientific, America). Bulk density samples were sieved (2 mm). The fine fraction was dried and weighed, and the volume of coarse material was measured by displacement, and the latter was subtracted from the core volume (Blake and Hartge, 1986). Soil texture was characterized using the hydrometer method (Kroetsch and Wang, 2008); all samples were pretreated for organic matter using 30% hydrogen peroxide (H_2O_2) (Jensen et al., 2017) and samples with pH > 6.5 were treated with hydrochloric acid (HCl) to remove carbonates and obtain a measure of SOC without soil inorganic C (Francis and Aguilar, 1995). Water-extractable carbon (C) and nitrogen (N) were extracted from 10 g of oven-dry equivalent samples in distilled water (in 1:10 ratio), then centrifuged at 12,000 g for 10 min; the slurry was removed with a 0.4 μm polycarbonate filter, and C and N measured using a Shimadzu TOC-V CSH/CSN Total Organic Carbon Analyzer (Shimadzu Corporation, Kyoto, Japan) (Jones and Willett, 2006; Kalbitz et al., 2003; Zsolnay, 1996).

2.2.5 Statistical analyses

We first examined the effects of grazing system (multi-paddock vs. conventional grazing) and

climate (AHM) on the soil microbial community and ancillary soil properties with linear mixed-effects models in which ranch pair was included as a random effect. Response variables included soil MBC and MBN, the relative abundance of fungal and bacterial functional groups derived from PLFAs, and soil pH, bulk density, total C, total N, C: N ratio, and texture (Sand: Clay ratios). Models were estimated using the "lmer" function in "lme4" (Bates et al., 2015) and "car" package in R 4.0.0 (R Development Core Team, 2020). Before estimating the model, data were assessed for outliers, normality, homogeneity of variance and collinearity. Homogeneity of variance was checked with residual plots and collinearity among covariates was assessed by computing variance inflation factors (VIF values less than 10 indicate the absence of collinearity) (Belsley et al., 1980). Microbial biomass C, MBN, GP:GN were log-transformed, and abundances of fungi, AMF, MBC: MBN, GP, total fungi and F: B ratios were square-root transformed. Model assumptions were validated by inspection of diagnostic plots (residuals vs. fitted, Q-Q and Cooks distance).

Grazing system is a coarse category of management, which in reality represents a wide range of variations in specific management metrics (Bork et al., 2021) that could regulate soil microbial community composition. Thus, we conducted a more detailed assessment of the effects of specific grazing metrics on soil microbial communities using a multi-model inference to identify the most parsimonious predictor variables from a broader set that included grazing metrics and environmental variables. We used a two-stage approach in analysis using the 'dredge' function in the 'MuMIn' package for R (Barton, 2020). First, we identified the most parsimonious grazing metrics from a model that included only stocking rate, stocking density and rest-to-grazing ratio. The best grazing metrics were chosen based on the lowest Akaike information criterion (AICc) score. However, if the null model had the lowest score, the next

best scoring model with a grazing metric was used so that at least one management metric was included in subsequent modelling. Once the best set of grazing metrics was identified for each response, these were combined in a "global" model that included a soil variable based on principle component analysis (PCA) and climatic aridity (AHM). To minimize redundancy and collinearity among soil properties, which was apparent through examination of correlation coefficients (Figure S1 and S2), PCA (Pearson 1901; Hoteling 1933) was used to create an integrated soil variable. Soil properties included pH, bulk density, C, N, water-extractable C, water-extractable N, and the ratio of sand: clay (i.e., soil texture). We used the first principal component to represent soils, named "Soil PC 1". Soil PC 1 explained 41.3% of the variation in soil attributes (Figure S3) and was represented primarily by soil C, N and water-extractable C and N (Table S4), with higher PC1 axes scores negatively associated with these properties (Table S5). Thus, the global model included AHM, soil PC 1, and the most parsimonious set of grazing metrics identified from the first step, with "ranch pair" included as a random effect. To avoid model overfitting, no model contained more than four covariates. Model assumptions were validated by inspection of diagnostic plots (residuals vs. fitted, Q-Q and Cooks distance). We selected the best predictors based on the model with the lowest AICc (Beier et al., 2001), with model coefficients used to report effect sizes and confidence intervals calculated with the "standardize_parameters" function in the "effectsize" package for R (Ben-Shachar et al., 2020).

2.3 Results

2.3.1 Grazing system effects

2.3.1.1 Soil properties and Microbial biomass carbon to nitrogen

Some soil properties were affected by grazing system, and AHM (full model results for all variables are in Table S6). All means are reported with standard error (mean ± standard error).

Water-extractable C (F= 6.51, df= 13.99, p=0.023; multi-paddock: 26.3 ± 2.40 mg kg $^{-1}$; conventional: 21.5 ± 1.66 mg kg $^{-1}$), water-extractable N (F= 6.58, df= 13.99, p=0.022; multi-paddock: 3.79 ± 0.91 mg kg $^{-1}$; conventional: 2.11 ± 0.17mg kg $^{-1}$) and silt content (F= 6.49, df= 13.99, p=0.023; AMP: 35.1 ± 2.73 %; N-AMP: 29.9±1.82 %) were all greater under multi-paddock than conventional grazing. Soil C:N ratios decreased with increasing AHM (Figure S4). Soil pH, bulk density, and sand:clay did not vary with grazing system or AHM. Microbial biomass carbon and nitrogen were not affected by grazing system or climate, though their ratio (MBC:MBN) (Table 2.1) was lower in grassland soils exposed to multi-paddock rather than conventional grazing (Figure 2.1A); MBC:MBN also increased with AHM (p=0.008) (Figure 2.1B).

2.3.1.2 Relative abundance of PLFA markers

Arbuscular mycorrhizal fungi (16:1 ω 5c), fungal PLFA (18:2 ω 6c), and total fungi PLFA abundance were not affected by grazing system or AHM. However, both the F:B ratio and GP:GN ratio were lower in soils of multi-paddock ranches compared to conventional ranches (Table 2.1, Figure 2.2A and 2.2B). The underlying cause of the change in ratios was examined, in AMP grazing, bacterial abundance increased while fungal abundance decreased, whereas, in conventional grazing, fungal abundance increased while bacterial abundance remained the same as AMP grazing (Figure S6C & D). Similarly, in conventional grazing, Gram-positive bacterial abundance increases while Gram-negative bacterial abundance remains the same (Figure S6E &F). Additionally, fungal PLFA abundance increased with AHM (Table 2.1, Figure 2.2C). Other individual and total PLFA functional groups like GP, actinomycetes, GN, anaerobic and total bacteria were not affected by the grazing system or AHM (Table S7).

2.3.2 Grazing metrics and soil property effects on soil microbial biomass and PLFA abundance

Through model selection we identified stocking rate as the most consistently parsimonious grazing metric affecting soil PLFAs marker (Table S8). However, there was some variation from this pattern, for e.g., fungi (18:2 ω 6c) and total fungi (sum of AMF-16:1 ω 5c and fungi-18:2 ω 6c) had a model with stocking rate and stocking density within an AICc of 2. The null model had the lowest AICc score for a number of response variables, including MBC, MBN, MBC:MBN, GP:GN and F:B ratios (Table S8); however, to maintain at least one grazing metric in the subsequent global model, we used the second best model after the null model for each of these responses. For each of these responses, the second best model was stocking rate except for F:B ratio, for which stocking density alone was the best model (Table S8).

In the global model we ran the leading grazing metric (derived from Table S8), as well as AHM, and soil PC1 to identify the most parsimonious model for all eight microbial markers (Table S9). For further validation, we ran a linear mixed effect model of the most parsimonious global model to estimate parameters and weights (Table 2.2) to account for model selection uncertainty. Soil MBC:MBN decreased with increasing stocking rate, while F:B decreased with increasing stock density (Figure 2.3C and D, Table 2.2). Both MBC and MBN were negatively related to soil PC1 (Figure 2.3A and 2.3B, Table 2.2), indicating a positive association with soil C, water-extractable C, and water-extractable N (Figure S5A-D). However, there were no significant predictors identified for GP:GN, AMF or total Fungi.

2.3 Discussion

The effects of the cattle grazing system and specific grazing metrics on grassland soil microbial community abundance and biomass were tested on 15 pairs of ranches in the Canadian

Prairies. We found that multi-paddock grazing decreased F:B, GP:GN and MBC:MBN ratios. Among specific grazing practices, stocking density was an important metric regulating soil F:B ratios where the F:B ratio decreased with increasing cattle densities. In contrast, MBC:MBN ratios declined with increasing cattle stocking rates along with some soil attributes including soil C, nitrogen and water-extractable carbon. The combined patterns highlight the potential for grazing management to increase soil C storage and turnover through changes in the microbial community.

2.4.1 Multi-paddock grazing and stocking rate reduced MBC:MBN ratios

Microbial biomass carbon and nitrogen were not affected by the grazing system, but their ratio was greater in soils of conventional compared to multi-paddock ranches, indicating that biogeochemical cycling of C and N is affected by the grazing system. Soil MBC represents the living soil organic C fraction (Ramesh et al., 2019) comprises 1-3% of total soil C, and MBN is the most labile organic N fraction (Smith and Paul, 1990), which accounts for 3-5% of total soil N pool (Murphy et al., 2000). Microbial biomass C and N were positively associated with water-extractable C and water-extractable N; the pattern is consistent with previous studies where microbial biomass C and N were strongly correlated to soil organic C and soil total N (Liu et al., 2012; Sankaran and Augustine, 2004). Lower MBC:MBN in multi-paddock grassland soils might be due to more vigorous vegetation out-competing microbes for available nitrogen during the growing season (Michael and Billings, 1999).

Microbial biomass carbon to nitrogen ratios increases with aridity and decline with cattle stocking rates, which may result through three possible pathways. First, higher stocking rates often reduce plant aboveground biomass, which reduces photosynthetic capacity and subsequent C input to roots, and ultimately reduces available C for microbial communities (Banerjee et al.,

2000; Kowalchuk et al., 2002; Smith and Paul, 1990). Second, higher stocking rates can reduce litter inputs and increase litter breakdown (Chuan et al., 2018; Manley et al., 1995), which alters the microbial environment (Rousk et al., 2010) by maintaining soil temperatures and retaining soil moisture (Van Der Heijden et al. 2008; Nielsen et al. 2011; de Vries et al. 2012) which directly influence soil microbial biomass (Nielsen et al., 2011; Rousk et al., 2010). Third, higher stocking rates may increase labile mineral or organic N to soil through feces and urine deposition in soil (Tierling and Kuhlmann, 2018), which are readily assimilated by plants, thereby lowering the C:N ratio (L. Wang et al., 2022) and limiting microbial activity and biomass (Leff et al., 2015; Männistö et al., 2016; Ramirez et al., 2012; Treseder, 2008). Higher MBC:MBN ratios at low stocking rates may suggest a greater potential for C sequestration (Liu et al., 2012) in lower stocked grasslands than in heavily stocked grasslands (Schuman et al., 2002). However, the transition of MBC into stable soil organic C is regulated by processes controlling microbial necromass (Kästner et al., 2021), the quantity and chemical composition of which may vary under various grazing scenarios, ultimately affecting C storage (Buckeridge et al., 2022; Wang et al., 2021). Furthermore, higher stocking rates have been associated with more soil C within the study region (Bork et al., 2020), so additional understanding of the mechanisms stabilizing MBC as SOC is needed.

2.4.2 Grazing system and cattle stock density alter F:B ratios

The F:B ratio was greater in conventionally grazed grasslands than multi-paddock grasslands and declined with stocking density. This result is contrary to a previous study that suggests the F:B ratio was higher under multi-paddock grazing than light continuous, heavy continuous, and non-grazed tallgrass prairie (Teague et al., 2011). Discrepancies between studies might be due to differences in a grazing system, vegetation type, climate or methodology. Fungal

to bacterial ratios are associated with increased soil C, and fungal abundance is associated with slower C cycling (Malik et al., 2016; Waring et al., 2013), thus this pattern suggests that the sensitivity of fungi to grazing with higher stocking densities could affect soil C (Xun et al., 2018; Y. Yang et al., 2021). While we found no effect of stocking rate on F:B, other studies have shown that less intense grazing leads to increased F:B and enhances C-storage (Klumpp et al., 2009). Fungal communities are more sensitive than bacterial communities (Hartmann et al., 2014) to physical disruption in the soil (Ford et al., 2013), particularly nutrient inputs to soil (Bardgett and McAlister, 1999; Seaton et al., 2022; Yeates et al., 1997). The added nutrients from cattle urination and defecation can reduce fungal abundance due to a lower C:N ratio, which favours bacteria (Bailey et al., 2002; Sinsabaugh et al., 2013; Yeates et al., 1997). Thus, conventionally grazed ranches, which use lower stocking densities, might accumulate relatively more organic C in soil and have slower turnover rates of soil C (Malik et al., 2016; Waring et al., 2013) compared to multi-paddocks grasslands.

2.4.3 Grazing system influences GP: GN ratios

The ratio of Gram-positive to Gram-negative bacteria was greater under conventional grazing than multi-paddock grazing, which can be a consequence of reduced labile C availability in these soils (Fanin et al., 2014). Indeed, we observed lower water-extractable C and water-extractable N values in conventionally grazed soils than in multi-paddock grazed soils. Gram-negative bacteria are more efficient in using water-extractable C than GP (Wang et al., 2021) because GN bacteria prefer plant-derived C sources and have higher growth rates than GP (Ford et al., 2013; Garcia-Pausas and Paterson, 2011). Additionally, multi-paddock grazing improved soil hydrological functioning (Döbert et al., 2021), which can benefit plant productivity (Franzluebbers, 2002) that enhances plant-derived C sources such as root exudates, which benefit

GN bacteria (Ford et al., 2013). Generally, GN bacteria are more tolerant of soil physical and chemical disturbances (Cui et al., 2016; Kaur et al., 2005) and more abundant in heavily grazed areas (Ford et al., 2013). Both Gram-positive and GN bacteria were reduced by increasing stocking rate, while the difference was not significant, our multi-paddock grazing ranched did tend to have a slightly greater stocking rate which may be contributing to these patterns.

2.4 Conclusions

Microbial biomass C and N and individual PLFA abundances were not affected by grazing system, but the composition, specifically the ratios of microbial biomass C and N, and PLFA functional groups often associated with labile nutrients, were consistently increased under multi-paddock grazing. Notably, multi-paddock grazing management increased soil nutrient availability (water-extractable C and N) and shifted the soil microbiome toward a bacterial, rather than fungal dominated community which could ultimately lead to loss of soil carbon. (Bagchi et al., 2017; Six et al., 2006). The broad variation in management practices within each grazing system (Bork et al., 2021) may underly why the binary comparison of "grazing system" revealed few effects on response variables. However, climate and specific grazing management metrics, particularly AHM, stocking density, and stocking rate, did affect the absolute abundance of individual PLFA functional groups and microbial biomass. While stocking rate is commonly examined in grazing studies, few have examined stocking density and given the potential importance suggested here, it should be examined further. So, grazing management metrics could potentially influence net soil C-storage in grassland by altering microbial abundance and biomass mediated by aridity. Consequently, these results provide novel insight into microbial responses to grazing metrics, and suggest potential mechanisms as to how grazing can alter soil C and nutrient cycling.

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Tables

Table 2. 1: Results of the ANOVA test (mixed effect model) of grazing system and AHM on each soil microbial PLFA (F:B = Fungi: bacteria, GP:GN= Gram-positive to Gram-negative bacteria, AMF = arbuscular mycorrhizal fungi) and microbial biomass carbon (MBC) and nitrogen (MBN). Bold text indicates significant effects ($p \leq 0.05$).

		Intercept	Grazing	AHM
MBC (mg kg $^{-1}$)	F	24.58	2.58	0.24
	Df.res	13.27	13.99	13.13
	Pr(>F)	0.00	0.13	0.63
MBN (mg kg $^{-1}$)	F	10.72	0.05	1.68
	Df.res	13.38	13.98	13.33
	Pr(>F)	0.01	0.83	0.22
MBC:MBN	F	2.76	5.91	4.62
	Df.res	13.28	13.99	13.10
	Pr(>F)	0.12	**0.03**	**0.05**
GP:GN	F	98.77	4.54	0.13
	Df.res	13.27	13.99	13.11
	Pr(>F)	0.00	**0.05**	0.72
AMF (pmol g^{-1})	F	32.70	0.96	0.10
	Df.res	13.50	14.00	13.04
	Pr(>F)	0.00	0.34	0.75
Fungi (pmol g^{-1})	F	0.01	1.42	12.81
	Df.res	13.35	14.00	13.06
	Pr(>F)	0.91	0.25	**0.001**
Total Fungi (pmol g^{-1})	F	0.35	0.82	3.77
	Df.res	13.43	14.00	13.05
	Pr(>F)	0.56	0.38	0.07
F:B	F	57.97	4.25	0.03
	Df.res	13.30	13.99	13.08
	Pr(>F)	0.00	**0.05**	0.87

Table 2. 2**:** Summary of the most parsimonious linear mixed models for soil microbial markers (Microbial biomass carbon (MBC) and nitrogen (MBN), Fungi: Bacteria (F:B) ratio, Gram-positive: Gram-negative (GP:GN) bacteria, Arbuscular mycorrhizal fungi (AMF)) including soil PC1, AHM and the leading grazing metrics (stocking rare (SR) and stocking density (SD)) derived from supplementary Table 8. Ranch pair was specified as a random effect to address geographic variation in climate and soil type. Marginal R^2 (variance explained by fixed effects) and conditional R^2 (variance explained by both fixed and random effects) were calculated for the models. To account for model selection uncertainty, we report unconditional standard errors (SE). Standardized coefficients (ω_p^2) used to determine effect size with 95% confidence intervals as a measure of significance are provided; bold variables have p-value <0.05 and confidence intervals that do not overlap 0.

Microbial markers	**Model:**	**R^2_m**	**R^2_c**	**Explanatory variable**	**Estimate**	**Std. Error**	**p**	**ω_p^2**	**95%**
MBC (mg kg $^{-1}$)	Soil PC1 +AHM+SR	0.23	0.72	Intercept	3.83	0.65	0.00	0	[0.00, 0.00]
				Soil PC1	**-0.121**	**0.04**	**0.01**	**-0.44**	**[-0.76, -0.12]**
				AHM	-0.014	0.02	0.5	-0.15	[-0.58, 0.28]
				SR	-0.054	0.03	0.09	-0.23	[-0.49, 0.03]
MBN (mg kg $^{-1}$)	Soil PC1+AHM+SR	0.22	0.89	intercept	2.39	0.65	0.002	0	[0.00, 0.00]
				Soil PC1	**-0.09**	**0.03**	**0.006**	**-0.35**	**[-0.58, -0.12]**
				AHM	-0.03	0.02	0.17	-0.32	[-0.76, 0.12]
				SR	-0.01	0.01	0.41	-0.07	[-0.23, 0.09]
MBC:MBN	Soil PC1+AHM+SR	0.28	0.6	Intercept	1.86	0.71	0.02	0	[0.00, 0.00]
				Soil PC1	0.04	0.05	0.47	0.12	[-0.21, 0.45]
				AHM	0.04	0.02	0.07	0.38	[-0.01, 0.76]

				SR	**-0.08**	**0.04**	**0.05**	**-0.3**	**[-0.59, -0.02]**
GP:GN	Soil PC1+AHM+SR	0.05	0.64	Intercept	1.23	0.1	0.00	0	[0.00, 0.00]
				Soil PC1	0.008	0.007	0.24	0.23	[-0.15, 0.62]
				AHM	0.001	0.003	0.76	0.08	[-0.43, 0.59]
				SR	0.001	0.005	0.77	0.05	[-0.27, 0.36]
AMF (pmol g^{-1})	Soil PC1+SR+AHM	0.09	0.27	Intercept	447.56	74.91	0.00	0	[0.00, 0.00]
				Soil PC1	7.57	6.27	0.24	0.24	[-0.15 ,0.62]
				SR	-5.67	5.09	0.27	-0.21	[-0.58, 0.16]
				AHM	-1.43	2.39	0.55	-0.13	[-0.54, 0.28]
Fungi (pmol g^{-1})	Soil PC1+AHM+SR+SD	0.4	0.65	Intercept	18.41	89.51	0.84	0	[0.00, 0.00]
				Soil PC1	2.84	7.67	0.71	0.06	[-0.27, 0.40]
				AHM	**9.93**	**2.88**	**0.004**	**0.63**	**[0.27, 0.99]**
				SR	3.96	6.91	0.57	0.11	[-0.25, 0.47]
				SD	-0.55	0.4	0.17	-0.25	[0.60, 0.10]
Total Fungi (pmol g^{-1})	Soil PC1+AHM+SD+SR	0.205	0.43	Intercept	350.28	103.28	0.004	0	[0.00, 0.00]
				PC1	6.67	9.13	0.47	0.15	[-0.25, 0.54]
				AHM	5.53	3.29	0.11	0.34	[-0.06, 0.74]
				SD	-9.51	8.57	0.91	-0.02	[-0.46, 0.41]
				SR	-0.488	0.5	0.34	-0.21	[-0.65, 0.22]
F:B	Soil PC1+AHM+SD	0.31	0.75	Intercept	0.34	0.03	0.00	0	[0.00, 0.00]
				Soil PC1	0.005	0.002	0.06	0.32	[-0.01, 0.65]
				AHM	-0.0001	0.001	0.93	-0.02	[-0.42, 0.39]
				SD	**0.0003**	**0.0001**	**0.003**	**-0.42**	**[-0.66, -0.18]**

Figures

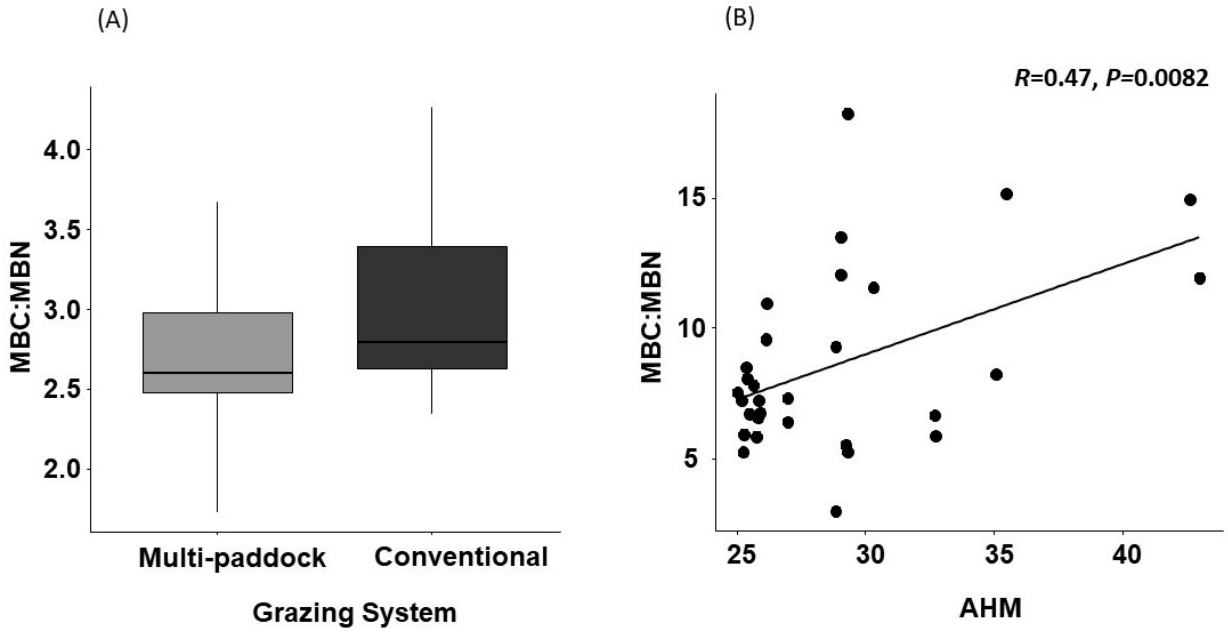

Figure 2. 1: Microbial biomass carbon to nitrogen (MBC:MBN) ratio under multi-paddock and conventional grazing. **B)** Relationship between MBC:MBN ratio to AHM.

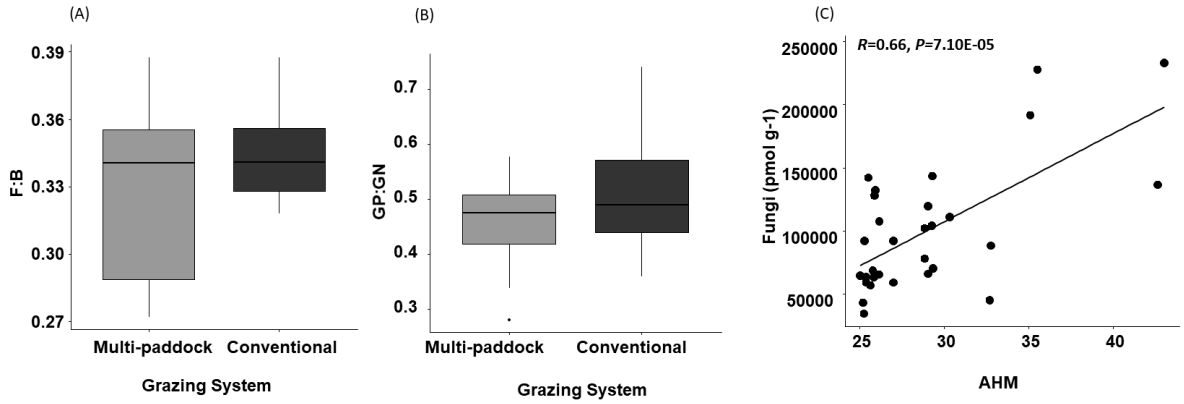

Figure 2. 2: **A)** Gram-positive: Gram-negative bacterial ratio and **B)** Fungi: Bacteria ratio under multi-paddock and conventional grazing system. **C)** Relationship between Fungi and AHM.

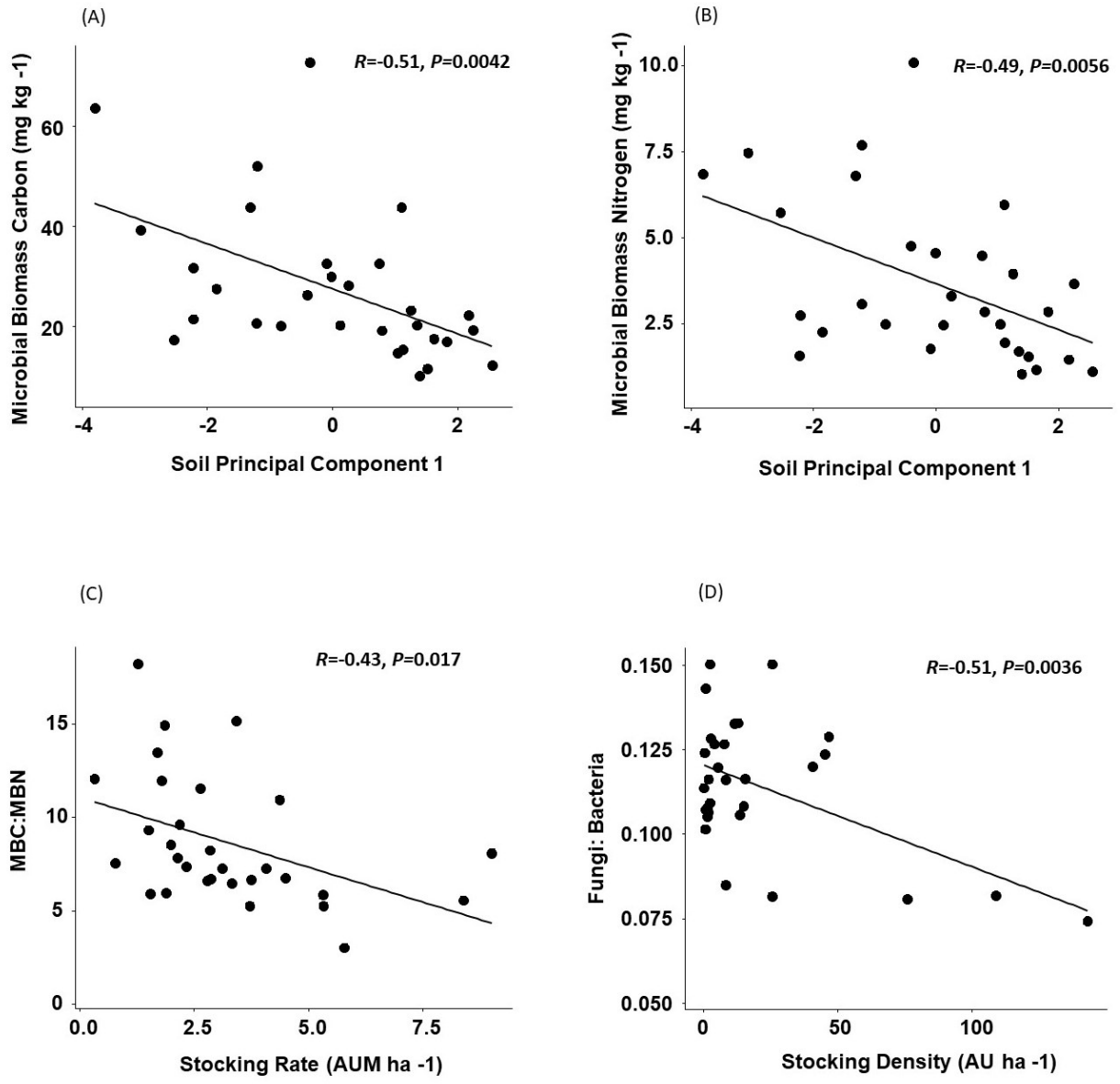

Figure 2. 3: Relationship between A. Microbial biomass carbon, and **B.** Microbial biomass nitrogen, and soil principal component 1. Relationships between **C.** Microbial biomass carbon to nitrogen ratio and stocking rate (AUM ha^{-1}), **D.** F:B ratio and Stocking density (AU ha^{-1}). Scatter plots represent the output from model selection and regression lines are linear fits of microbial markers to soil and climate factors where the gray area indicates 95% confidence interval.

Chapter 3. Cattle grazing management affects soil microbial diversity and community network complexity in the Northern Great Plains

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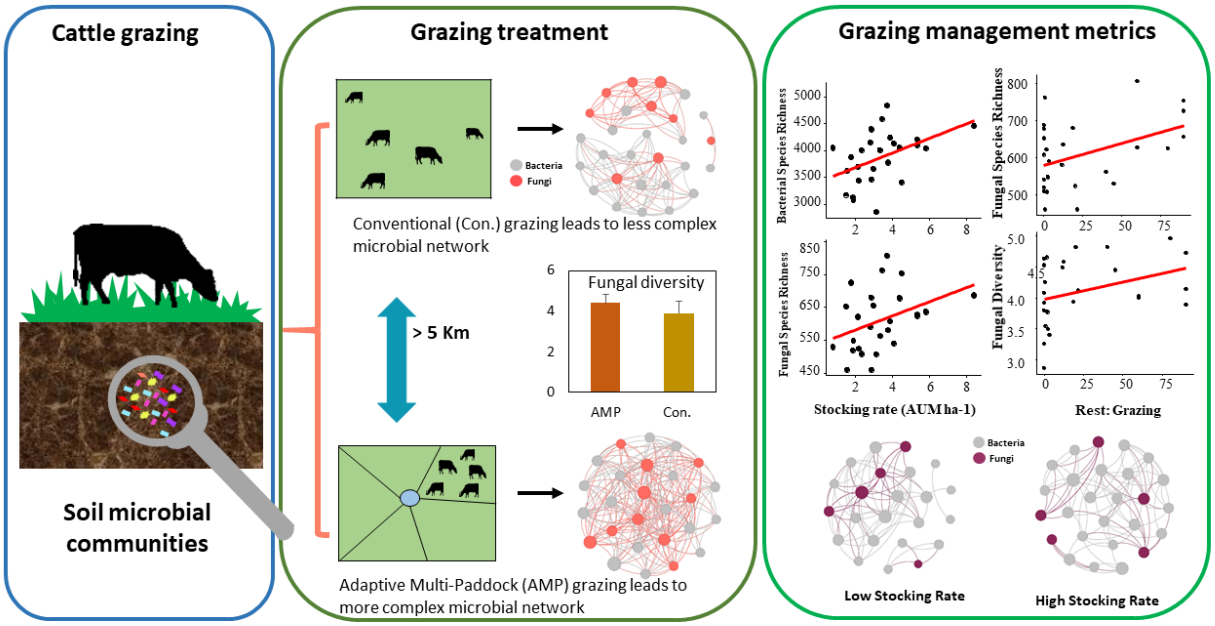
Summary

Soil microbial communities play a vital role in the biogeochemical cycling and ecological functioning of grassland, but may be affected by common land uses such as cattle grazing. Changes in microbial diversity and network complexity can affect key ecosystem functions such as nutrient cycling. However, it is not well known how microbial diversity and network complexity respond to grazing in the Northern Great Plains. Consequently, it is important to understand whether variation in grazing management alters the diversity and complexity of grassland microbial communities. We compared the effect of intensive adaptive multi-paddock (AMP) grazing and conventional grazing practices on soil microbial communities using 16S/ITS amplicon sequencing. Samples were collected from grasslands in 13 AMP ranches and 13 neighboring, conventional ranches located across the Canadian prairies. We found that AMP grazing increased fungal diversity and evenness, and led to more complex microbial associations. *Acidobacteria, Actinobacteria, Gemmatimonadetes*, and *Bacteroidetes* were keystone taxa associated with AMP grazing, while *Actinobacteria, Acidobacteria, Proteobacteria,* and *Armatimonadetes* were keystone taxa under conventional grazing. Besides overall grazing treatment effects, specific grazing metrics like cattle stocking rate and rest-to-grazing ratio affected microbial richness and diversity. Bacterial and fungal richness increased with elevated stocking rate, and fungal richness and diversity increased directly with the rest-to-grazing ratio. These results suggest that AMP grazing may improve ecosystem functioning and ecosystem stability by enhancing fungal diversity and increasing microbial network complexity and connectivity.

Graphical abstract



Keywords: Adaptive Multi-Paddock (AMP) grazing; Cattle grazing; Grassland; Keystone taxa; Network analysis; Soil microbial diversity

Highlights

- Adaptive multi-paddock (AMP) grazing enhances fungal diversity.
- AMP grazing enhances soil microbial network complexity.
- Higher cattle stocking rates increased bacterial and fungal species richness.
- Higher cattle stocking rates lead to a more complex microbial network.
- Grazing rest periods enhances fungal richness and diversity.

3.1 Introduction

Soil microbial communities play key roles in ecosystem functioning and associated services including litter decomposition, nutrient cycling, carbon (C) storage, and mineralizing nutrients for plant uptake (Jacoby et al., 2017; Van Der Heijden et al., 2008; Wagg et al., 2014). Grasslands cover about one-third of terrestrial ecosystems (Bengtsson et al., 2019; Suttie et al., 2005) and are one of the world's largest C sinks (Boval and Dixon, 2012). Livestock grazing can affect soil C storage and offset GHG emissions (Liu et al., 2012; Shrestha et al., 2020) through changes in the soil microbial community (Kohler et al., 2005; Liu et al., 2012). Microbial communities respond promptly to changes in the environment, for instance, moisture and nutrient content as well as physical disturbances (Maron et al., 2018) caused by livestock grazing (Khatri-Chhetri et al., 2022; Qu et al., 2016; Xun et al., 2018). Therefore, identifying the response of the soil microbial community to grazing systems can provide insight on how soil-based ecosystem functions might be altered by cattle management.

Increases in soil microbial diversity are associated with enhanced soil C stock and C stability (Bastida et al., 2021; Mau et al., 2015), together with improved ecosystem multifunctionality (Delgado-Baquerizo et al., 2016). Multifunctionality is the ability of an ecosystem to provide multiple ecological functions and services such as litter decomposition, nutrient cycling, primary production, and climate regulation (Delgado-Baquerizo et al., 2016; Wagg et al., 2014). Moreover, communities with higher microbial diversity can include functionally redundant taxa that help maintain ecosystem functioning through periods of altered soil environmental conditions or the loss of specific taxa (Wagg et al., 2019b). Specifically, fungal diversity is positively associated with ecosystem stability, resistance and resilience (Liu et al., 2022). Grazing alters the richness, diversity, and composition of soil microbial communities

in grassland ecosystems (Ingram et al., 2008; Qu et al., 2016; Wang et al., 2022, 2019). Microbial diversity can increase with grazing (Qu et al., 2016), but not if the system is overgrazed or the grazing intensity is too high (Xun et al., 2018). Grazing intensity, commonly known as stocking rate (number of cattle/area/time), is an important management factor affecting soil microbial communities as was demonstrated in other studies (Briske et al., 2008; Khatri-Chhetri et al., 2022). However, a few recent studies suggest that stocking density (instantaneous number of cattle/area), and the length of rest periods between grazing events are equally important management metrics that influence soil properties and soil microbial communities (Döbert et al., 2021; Khatri-Chhetri et al., 2022; Teague, 2017). Thus, it is important to identify grazing systems and specific management metrics that can enhance microbial diversity and richness.

Soil microbes are involved in a multitude of functions and depend on each other. Individual microbes maintain positive or negative, and direct or indirect, relationships through the processes of competition, facilitation, and inhibition with other microbes, thereby forming a complex interconnected network (Banerjee et al., 2016; Barberán et al., 2012; Ma et al., 2016). The complex interactions arising between microorganisms can be examined through network analysis that explores co-occurrence patterns among soil microorganisms (Zhou et al., 2010). Analysis of microbial networks has been widely used to determine if microbiome complexity has implications for ecosystem functioning (Barberán et al., 2012; Wagg et al., 2019a; J. Zhou et al., 2010). Complex networks with higher connectivity (associations) can be more resilient to environmental perturbations than networks with lower connectivity (Santolini and Barabási, 2018). Furthermore, network analysis also aids in identifying keystone taxa - microbes that have greater biotic connectivity in the community than other taxa (Banerjee et al., 2018; Berry and

Widder, 2014; Herren and McMahon, 2018; Yuan et al., 2021) and facilitate ecosystem processes such as organic matter decomposition (Banerjee et al., 2016). Few studies have examined microbial associations in response to grazing. Short-term grazing exclusion has been found to reduce network complexity and the connectivity of microorganisms in temperate grasslands (Wang et al., 2022). Examination of soil microbial networks under different grazing systems in the Northern Great Plains will provide new insights on grassland ecosystem function.

A variety of grazing systems are practiced globally and across the northern Great Plains. Conventional grazing often places cattle in pastures either at low densities for long periods (many months) or move cattle through a modest number of pastures at relatively infrequent intervals (typically weeks to months) depending on vegetation productivity (Milton, 1999; Valentine, 1967). In contrast, a more complex grazing system known as adaptive multi-paddock grazing (hereafter AMP grazing) places cattle in small pastures for short periods (hours to days) at high densities with long rest periods for vegetation between grazing events (Bork et al., 2021; Teague, 2018). However, a broad variation in management practices has been found within both AMP and conventional grazing management approaches (Bork et al., 2021).

Much of the previous research in pastures has focused on grazing vs. grazing exclusion effects on soil microbial alpha- and beta-diversity patterns (Wang et al., 2022; Xun et al., 2018). However, the impact of AMP grazing and specific grazing management metrics on soil microbial diversity and network complexity has not been assessed, particularly across a broad range of climatic conditions. Grazing can alter the structure of soil microbial networks, and either support or negatively impact keystone taxa under different grazing practices (Wang et al., 2022), which in turn, will have implications for C storage, organic matter decomposition, and vegetation productivity in grassland ecosystems (Banerjee et al., 2016). Thus, the objective of this research

was to document the effects of AMP grazing in relation to conventional grazing practices, as well as the influence of grazing management metrics (i.e. stocking rate, stocking density and rest-to-grazing ratio) on soil microbial diversity and network complexity. Using soil microbial composition data collected from 26 ranches in the northern Great Plains, we address the following questions: (a) Does soil microbial community diversity and complexity differ between AMP and conventional grazing? (b) Which grazing management metrics (stocking rate, stocking density and rest-to-grazing ratio) affect soil microbial diversity and complexity? and (c) Which taxa act as keystone groups under various grazing practices?

3.2 Material and Methods

3. 2.1 Site selection and sampling

Soil samples were collected from 26 grasslands located on privately owned ranches in the northern Great Plains within the prairie provinces of Alberta, Saskatchewan, and Manitoba, Canada. Most grasslands were dominated by agronomic grasses and legumes such as crested wheatgrass (*Agropyron cristatum* (L.) Gaertn), smooth brome (*Bromus inermis* Leyss.), meadow brome (*Bromus biebersteinii* Roem and Schult.), Russian wild rye (Psathyrostachys /*Elymus junceus Fisch.*), alfalfa (*Medicago sativa* L.), sweet clover (*Melilotus officinalis* (L.) Pall.), and alsike clover (*Trifolium hybridum* L.). A few sites were dominated by a native grass, foothills rough fescue (*Festuca campestris* Rydb.). Pastures in this study were sampled in a paired design where each pair contained a ranch known to practice AMP grazing (for at least five years) and a neighboring ranch practicing conventional grazing. The latter is best thought of as a representative random sample of beef cattle ranches in the region, wherein grazing comprised of a wide range of systems, including continuous (season-long) grazing or rotational grazing at low to moderate intensity (Bork et al., 2021). Paired design sampling was done to address the role of

grazing treatment and minimize the effects of environmental variability. Consequently, both ranches within a pair were located on a similar ecosite with similar topography, soils, and vegetation type, including cultivation history (Bork et al., 2021). Ranches within a pair were no more than 5 km apart and the distance between pairs was at least 25 km.

Sites in this study were spread across a broad range of climatic conditions and we used an index of aridity, the annual heat: moisture (AH: M) index (derived from http://tinyurl.com/ClimateNA) to describe site environmental conditions. The AHM is the ratio of mean annual temperature (MAT) to mean annual precipitation (MAP) and is an important predictor of soil and vegetation properties across the study region (Bork et al., 2019; Hewins et al., 2018).

$$AHM = (MAT+10)/ (MAP/1000) \quad (1)$$

Mean annual temperatures for the sites ranged from 1.4 ºC to 4.8 ºC, and annual precipitation ranged from 364.8 mm to 533.6 mm based on the 30-year normal (1989-2018) data.

Cattle management practices were obtained from each landowner through a survey, with detailed management differences between AMP and conventional grazing reported previously (Bork et al., 2021); ranch pairs in this study are a subset of these sites (Table S1). The two grazing treatments could be distinguished from each other based on stocking density (AMP: 60.9± 19.1 AU ha $^{-1}$; conventional grazing: 2.58± 0.41 AU ha $^{-1}$) and rest-to-grazing ratio (AMP: 49.2± 6.09; conventional grazing: 0.87± 0.23), but not stocking rate (AMP: 3.47± 0.29 AUM ha $^{-1}$; conventional grazing: 3.18± 0.36 AUM ha $^{-1}$) (Table S2). Stocking density is the number of animal units per unit area for a single pasture, expressed as AU/ha. Contrasting rest periods in this study are indicated by the "rest-to-grazing ratio." Rest-to-grazing ratio was derived from the minimum number of days of rest reported by ranchers relativized to the mean length of the early season grazing period (before August 1) (Bork et

al., 2021). Soil samples were collected in June-July 2017. From each ranch, five soil cores (2.54 cm diameter) were collected at a depth of 0-15 cm and were randomly selected within a 10-ha area of grassland within each ranch. The five soil cores were combined to create one composite sample per ranch, and frozen immediately in the field with dry ice, transported to the lab, and stored at -20 ^{0}C until further analysis. Sub-samples were taken to determine soil pH, soil texture, soil organic carbon (SOC), and total soil nitrogen (TSN); details of the analysis is described by Khatri-Chhetri et al. (2022).

3.2.2 DNA extraction and quantitative PCR

DNA was extracted from 0.25 g of composite soil using the Power Soil DNA Isolation kit (Qiagen, Germantown, MD, USA) according to the manufacturer's protocol. DNA concentration and quality were assessed with a NanoDrop2000C spectrophotometer (ThermoScientific, Wilmington, DE, USA). Extracted DNA was stored at -80 °C prior to molecular analysis. Gene abundance of total bacteria was enumerated by targeting the 16S rRNA (180bp), and for total fungi by targeting ITS (380bp) using primer pairs: 338f/518r (16S rRNA; Fierer et al., 2005), and nu-SSU-0817/nu-SSU-1196 (ITS; Borneman and Hartin, 2000) respectively, by quantitative real-time PCR (Table S3). The qPCR conditions consisted of an initial 2 min each at 50°C and 95°C. This was followed by 40 (bacteria and fungi) cycles of denaturing at 95°C for 15 sec, annealing at 55°C (bacteria), and 56°C (fungi) for 15 sec, and elongation at 72°C for 1 min. The total qPCR reaction volume was 10 µl, which contained 5 µl of PowerUP ^{TM} SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA), 0.8 (bacteria), or 0.6 (fungi) µl of 10µM each of forward and reverse primer, 1 µl of template DNA, and an adjusted volume of RNase/DNase-free water to a final volume of 10 µl. For the no template control, 1 µl of nuclease-free water was added instead of template DNA. All samples were analyzed in duplicate on the reaction plates (Applied Biosystems, Foster City, CA, USA) using the thermocycler

StepOne program (Applied Biosystems, Foster City, CA, USA). Standard curves for qPCR quantification were constructed using triplicate serial dilution of plasmid DNA containing the target gene (10^1 -10^8 copies per reaction). Standards were custom-made gBlocks® (Integrated DNA Technology, Coralville, USA), the design of which was based on partial sequences of *Clostridium thermocellum*, and *Aspergillus niger* strain P-19 for bacterial 16S rRNA and fungal 18S rRNA, respectively. Gene copy numbers were expressed as the copy number per gram of dry soil weight (oven-dried at 105ºC for 48 hrs). All qPCR assays were optimized to ensure reaction efficiencies of 98-104% (bacteria), and 90-98% (fungi) and standard curve slopes of -3.1 to -3.6 with R^2 values between 0.990 and 1.000.

3.2.3 Illumina sequencing

Sequencing (Paired-end) was performed on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) at the University of Alberta, AFNS department (Edmonton, AB, Canada). Illumina 16S rRNA and ITS amplicon sequencing were performed to quantify microbial diversity. For total bacteria, amplicon libraries were prepared according to the Illumina protocol (Part #15044223 Rev.B), whereas for total fungi, the protocol was slightly modified. For total bacteria, "16S Amplicon PCR forward and reverse primer =5'" (Table S3) were selected from the Illumina protocol (Illumina, 2013), whereas fungal primers were selected based on amplicon length, coverage, and selective amplification (Toju et al 2012) (Table S3). PCR reactions were prepared at 25 µl volume for all samples containing 10 µl of Platinum Hot start PCR 2x Master Mix (Invitrogen, Lithuania, Vilnus), and 0.5 µl of each 10 µM forward and reverse primers, 1 µl of the diluted DNA (5ng l^{-1} in 10mM Tris pH 8.5) and 13 µl of nuclease-free water. For fungi, the first stage PCR condition began at 94° C for 2 min, and was followed by 40 cycles of denaturation at 94° C for 30 s, annealing at 50° C for 30 s, elongation at 72° C for 30 s, and a final extension of 72° C for 5 min. As per the Illumina protocol, all PCR products were purified

with AMPure XP beads (Beckman Coulter, Pasadena, CA, USA), however, volume ratio (45 µl of beads to 25 µl reaction) was followed as suggested in AMPure XP beads manufacturer protocol (#001298v001). Thereafter, second stage PCR was performed at 50 µl volume containing 25µl of Platimun Hot Start PCR 2x Master Mix, 5 µl of the DNA obtained from first stage PCR, and 10 µl of nuclease-free water. Conditions for the second PCR were an initiation step at 94° C for 2 min, followed by 8 cycles of denaturation at 94° C for 30 s, annealing at 55° C for 30 s, followed by elongation at 72° C for 30 s, and a final extension of 72° C for 5 min. After the second PCR, further steps of library preparation were followed according to Illumina protocol. PhiX (10%) was used as an internal control. The 16S rRNA and ITS gene sequencing data of all samples were submitted to the NCBI SRA database under accession numbersnumbers KHUT00000000 and KHUX00000000.

3.2.4 Statistical analysis

All statistical analyses were performed using RStudio, version R 4.0.0 (R Development Core Team, 2020). An alpha value of <0.05 was used to assess significant differences. The effect of grazing treatment on the size of bacterial and fungal communities (gene copy abundance) was tested with a linear mixed effects model using the "lmer" function in "lme4" (Bates et al., 2015) and "car" package in R 4.0.0 (R Development Core Team, 2020). Grazing treatment and aridity (AHM) were fixed effects, while ranch pairs were used as random effects. Taxa and Operational Taxonomic Unit (OTU) tables were prepared using "Phyloseq" package in R 4.0.0 to assess microbial richness, alpha diversity metrics, and evenness. Singletons were removed before diversity analysis. The number of OTUs detected after removing singletons was 16307 for bacteria and 5156 for fungi. Alpha diversity indices such as richness, Shannon's index (H),

Pielou's evenness, and Simpson's index (D) of the rarefied OTUs were calculated for bacteria and fungi using the "vegan" package (Oksanen et al., 2019).

The effect of grazing treatment and aridity on alpha diversity and evenness of bacterial and fungal OTUs was assessed with a mixed-effects model with the same model structure as described above. Further, the effects of specific management metrics on soil microbial diversity indices were assessed through mixed-effect models where stocking rate, stocking density, and rest: grazing ratio were used as fixed factors and ranch pair as a random effect using the "lmer" function in "lme4" (Bates et al., 2015) and "car" package in R 4.0.0 (R Development Core Team, 2020).

Beta diversity was measured on total sum scaling (TSS) transformed data after removing singletons. The TSS transformation is a normalization method that uses total read count for each sample as the size factor (Chen et al., 2018). Grazing and aridity effects on beta diversity were assessed using the Bray Curtis distance matrix in R (Lozupone and Knight, 2005) through Permutational Multivariate Analysis of Variance (PERMAVOVA), and ordination plots (principal coordinate analysis, PCoA) were created using the vegan package (Oksanen et al., 2019).

3.2.5 Network analysis:

To explore co-occurrence patterns in fungal and bacterial communities under different grazing treatments, correlation-based network analysis was conducted. We analyzed microbial networks (bacterial and fungal communities combined) for two grazing treatments (AMP and conventional) and two stocking rates (low and high). Ranches were evenly divided into two groups practicing low (AMP= 6, Conventional= 7) and high (AMP= 7, Conventional= 6) stocking rates (low: 0.78-2.96 AUM ha $^{-1}$ and high: 3.11-8.38 AUM ha $^{-1}$) independent of

location, pair and grazing treatment. For the network analysis, we only used OTUs that were present in at least 50% of sites, which were identified using the "remote" and "vmikk/metaMisc" packages in R (Appelhans et al., 2015). This resulted in a total of 4065 OTUs for AMP grazing, 3935 OTUs for conventional grazing, 3784 OTUs for low stocking rate pastures, and 4284 OTUs for high stocking rate pastures. Then, a correlation matrix describing all pair-wise associations between OTUs was created based on Spearman correlation coefficients (Weiss et al. 2016) using "Hmisc" package in R 4.0.0 (R Core Team, 2020). To test the significance of correlation coefficient a *P*-value was calculated using the Fisher z-transformation (Fisher, 1915) and corrected for multiple testing using the false discovery rate (FDR) correction (Benjamini and Hochberg, 1995). To select highly significant associations, overall networks were constructed with significant ($p<0.00001$), strong positive ($r>0.9$) and strong negative correlation ($r< -0.9$) coefficients. This resulted in a total of 4977 associations among 1824 OTUs (nodes) for AMP grazing, 2884 associations among 1791 nodes for conventional grazing, 3923 associations among 1724 nodes for the low stocking rate, and 5448 associations among 2025 nodes for the high stocking rate. Additionally, to simplify networks for better visualization and analysis, we used OTUs with relative abundance higher than 0.01% to construct the networks for different grazing treatments and stocking rates (Gao et al., 2021; Guo et al., 2022; Wang et al., 2022; Yang et al., 2022). Relationships among microbial communities under different grazing treatments and stocking rates were visualized in Gephi version 9.5 (Bastian et al., 2009) using the undirected network (where edges have no direction) and the Fruchterman-Reingold layout (Fruchterman and Reingold, 1991). Each empirical network was compared with a corresponding random network generated with an equal number of nodes and edges in *random graph* plugin in Gephi to assess any non-random patterns. Topological parameters such as degree, clustering

coefficient, and average path length of random networks were different from empirical networks, thereby confirming that these networks were non-random (Table S12).

Various network structural attributes including node level topological properties such as degree, clustering coefficient, diameter, and average path length were calculated in Gephi. Nodes (fungal and bacterial OTUs) are the essential units of a network, while edges represent links/connections between nodes. Microbial complexity was assessed using the degree metric (Landi et al., 2018; Wagg et al., 2019b). Additionally, to support complex co-occurrence patterns, the connectivity between OTUs was assessed using other network topological properties including the number of edges, clustering coefficient, and average path length. The degree is the number of edges (correlations) of each node (OTUs) with other nodes in the network (Berry and Widder, 2014). Average path length is the average distance in the shortest paths between two nodes of the network (Faust and Raes, 2012; Wang et al., 2022). The clustering coefficient reflects the connectedness among nodes, also called transitivity (Ma et al., 2016). The diameter of a network refers to the largest distance between two nodes, while closeness centrality represents the closeness of a node to all other nodes in the network (Lupatini et al., 2014). Grazing effects on network topological properties such as degree, closeness centrality, correlation coefficient, and betweenness centrality were assessed through a Wilcoxon rank sum test in R (Haynes, 2013). Average degree and closeness centrality were used to identify keystone taxa (Banerjee et al., 2019; Berry and Widder, 2014; Lupatini et al., 2014). A Z-score of the sum of these two factors was formulated and the top 10 taxa with high z-scores were selected as keystone taxa for each grazing system (Zheng et al., 2021).

3.3 Results

3.3.1 Effect of grazing treatment on microbial gene copies and soil properties

AMP grazing marginally (F=3.5, Df.res=12, *P*= 0.08) reduced the abundance of bacterial gene copies; however, grazing did not affect fungal gene abundance (Table S4). Some soil properties also were affected by grazing treatment and specific management metrics. Soil pH was higher (F=5.4, df= 1, *P*= 0.03) in grasslands subject to conventional grazing than AMP grazing (Table 3.1 & S5). Further, soil pH and C: N ratio decreased with increasing aridity (Table S5 & Figure S1). Stocking rate effects were evident on the abundance of clay in soil (F=17.1, df= 1, *P*= 0.0006), where soil clay percentage decreased with increasing stocking rate (Table S6 & Figure S1). Soil pH and C: N ratios were affected by stocking density and its interaction with stocking rate and rest-to-grazing ratio (Table S6).

3.3.2 Effect of grazing treatment on microbial diversity, richness and abundance

Average bacterial OTU richness (AMP: 3944±409 and conventional: 3783±573) was marginally higher (F=3.6, df.res=12, *P* =0.08) in soils under AMP grazing, while fungal richness was not different in soils between grazing treatments (Figures 3.1A & 3.1E). Alpha diversity indices (Shannon and Simpson) of fungi were significantly higher in the AMP (Shannon: F=10.7, df.res=12, *P* = 0.006 and Simpson: F=7.7, df.res= 12, *P*= 0.01) grazed pastures, whereas alpha diversity indices of bacteria were significantly higher in conventionally (Shannon: F=7.5, df.res=12, *P* = 0.01 and Simpson: F=5.6, df.res= 12, *P*= 0.03) grazed pastures (Figure 3.1B, 3.1C, 3.1F & 3.1G;). Similarly, Pielou's index of evenness for fungi was significantly higher (F=11.4, df.res=12, *P* =0.006) in AMP than in conventional soils, while for bacteria Pielou's index was higher (F=8.7, df.res=12, *P* =0.01) under conventional grazing than in AMP grazed areas (Figure 3.1D & 3.1H). Mean summary average and full model results are in supplementary

Tables S7 and S8. Fungal and bacterial beta diversities, based on Bray Curtis distance, were not significantly different between AMP and conventional grazing (Table S9 & Figure S2). However, fungal and bacterial beta diversities (fungi: F= 2, df= 1, *P*= 0.001 and bacteria: F=1.13, df = 1, *P*= 0.007) were affected by aridity (Table S9).

Among grazing management metrics, stocking rate and rest-to-grazing ratio both affected microbial diversity while we found no evidence for a stocking density effect. Specifically, stocking rate directly increased both bacterial (F=3.9, df.res=22.8, *P* =0.05) and fungal (F=3.5, df.res=23.7, *P* =0.07) species richness (Figure 3.2A & 3.2B; Table S10). Similarly, increases in rest-to-grazing ratio increased fungal species richness and diversity (richness: *P*<0.05; Shannon: *P*=0.08; Simpson: *P*=0.07) (Figure 3.2C- 3.2E; Table S10). Grazing treatment affected the abundance of different phyla (Table S11). At the phylum level, the abundance of Proteobacteria (mean ±SE) (AMP: 29±0.99% and conventional: 29±0.88%), Actinobacteria (AMP: 20±1.3% and conventional: 23±1.1%), and Acidobacteria (AMP: 21±1.1% and conventional: 20±1.2%) were the dominant bacterial groups under both grazing treatments (Figure 3.3 & S3). In contrast, the relative abundance of Actinobacteria was significantly higher (F= 4.6, df.res=12, *P* =0.05) in conventionally grazed soils. Further, the relative abundance of Firmicutes (F= 6.2, df.res=12, *P* =0.02), Verrucomicrobia (F= 8.4, df.res=12, *P* =0.01), and other phyla (F= 4.4, df.res=12, *P* =0.05) were significantly higher in soils under AMP grazing than in conventional grazing (Figure 3.3; Table S11). Additionally, Cyanobacteria were marginally higher (F= 4, df.res=12, *P* =0.06) in grasslands subject to conventional grazing compared to AMP grazing (Figure 3.3; Table S11). Among fungi, Ascomycota (AMP: 66±3.1% and conventional: 69±4.3%) and Basidiomycota (AMP: 16±2.1% and conventional: 14±3.7%) were the dominant fungi under both grazing treatments (Figure 3.3 and Figure S3). The relative abundance of Zygomycota was

higher (F=5.6, df.re=12, *P*= 0.04) in soils under AMP grazing while the relative abundance of Chytridiomycota (F=3.2, df.re=12, *P*= 0.09) and Blastocladiomycota (F=3.4, df.re=12, *P*= 0.08) were marginally higher in soils under AMP grazing than conventional grazing (Figure 3.3; Table S11).

3.3.3 Effect of grazing management system on soil microbial networks

Network analysis revealed distinctly different correlation structures within microbial communities in soils under AMP vs conventional grazing (Figure 3.4). Under AMP grazing the microbial network comprised 390 nodes (372 bacterial and 18 fungal OTUs) forming 709 highly significant ($p<0.00001$) and robust ($r>0.9$) correlations. Conventional grazing led to a microbial network consisting of 325 nodes (310 bacterial and 15 fungal OTUs) with 355 significant ($p<0.00001$) and robust ($r>0.9$) correlations (Table S12 & Figure 3.4). The number of positive (AMP: 614 and conventional: 315) and negative (AMP: 95 and conventional: 40) correlations were higher in grasslands subject to AMP grazing than under conventional grazing (Table S12). Further, compared to conventional grazing, the AMP grazing network exhibited a significantly higher (*P*= 0.002) average degree (AMP grazing: 3.6 and conventional: 2.2), almost double the edges (connectivity), and a lower average path length (AMP grazing: 5.6 and conventional: 7.5), indicating that the AMP grazing treatment had higher complexity and connectivity (Table S12; Figure 3.4). However, the clustering coefficient was not significantly different (Figure 3.4). Keystone taxa under both grazing networks were bacterial, and no fungal taxa were identified to be keystone in these grassland soils. Under AMP grazing, identified keystone taxa belonged to Acidobacteria, Actinobacteria, Bacteroidetes, and Gemmatimonadetes phyla, while in conventional grazing the keystone taxa belonged to Proteobacteria, Acidobacteria, Actinobacteria, and Armatimonadetes (Figure 3.5A). Acidobacteria was the most abundant of the

keystone taxa in soil under AMP grazing, while Actinobacteria was most abundant in soils under conventional grazing (Figure 3.5B).

After identifying stocking rate as an important management factor affecting microbial richness and diversity, we further evaluated the resulting microbial networks based on stocking rate. We constructed two separate networks based on low and high stocking rates, which displayed a remarkable difference in their structure and topology (Figure 3.6). The low stocking rate network consisted of 332 nodes (311 bacterial and 21 fungal OTUs) with 486 (positive:451 and negative:35) significant ($p<0.00001$) and robust ($r>0.9$) edges (correlations), while the high stocking rate network had 444 nodes (417 bacterial and 27 fungal OTUs) with 810 (positive:695 and negative:115) significant ($p<0.00001$) and robust ($r>0.9$) edges (Table S12; Figures 3.6A and 3.6B). High stocking rate networks had a higher degree, greater number of edges, and larger clustering coefficients, but reduced average path lengths, relative to networks from low stocking rates (Table S12; Figure 3.6C). Thus, the microbial network in soils under a high stocking rate was more complex than the network under low stocking (Figure 3.6C). Keystone taxa under both stocking rates were bacterial and belonged to three main phyla; i.e., Acidobacteria, Actinobacteria, and Proteobacteria (Figure S4A). Acidobacteria was the most abundant of the keystone taxa in soils under low stocking, while all three phyla were equally abundant in soils under high stocking (Figure S4 B).

3.4 Discussion

We found that AMP grazing increased fungal diversity, bacterial richness and influenced soil pH, which led to a highly complex microbial network under AMP grazing. Both stocking rate and pasture rest-to-grazing ratio were important management metrics affecting bacterial and

fungal richness and diversity. Additionally, network connectivity was positively associated with cattle stocking rates.

3.4.1 AMP grazing enhances fungal diversity

Adaptive multi-paddock grazing increased fungal diversity and evenness, whereas conventional grazing increased bacterial diversity and evenness. Fungal diversity and evenness were increased by AMP grazing indicating that the latter may enhance soil functions responsible for vegetation productivity, C storage, and soil multifunctionality (Hannula and Morriën, 2022; Li et al., 2022). Generally, fungi can store and assimilate nutrients (e.g. C) more efficiently than bacteria (Bardgett and McAlister, 1999; de Vries et al., 2006) and fungal diversity and richness are more strongly related to ecosystem multifunctionality (Delgado-Baquerizo et al., 2016) than bacterial diversity and richness (Li et al., 2022). AMP grazing might enhance fungal diversity through increased soil moisture and litter availability. AMP grazing is known to improve soil moisture and litter availability on the soil surface (Döbert et al., 2021; Hamonts et al., 2017; Hillenbrand et al., 2019) which might increase fungal diversity because fungi are important litter decomposers as they produce a wide range of extracellular enzymes (Kjoller and Struwe, 1982). Fungi are generally more directly dependent on plant litter and rhizodeposits than bacteria (Millard and Singh, 2010). Moreover, AMP grazing includes longer rest periods between consecutive grazing activities compared to conventional grazing, and we observed that an extended rest-to-grazing ratio increased soil fungal richness and diversity. Fungal richness can enhance both functional redundancy and functional uniqueness within the community (Delgado-Baquerizo et al., 2016; Galand et al., 2018) which could improve ecosystem functioning.

In contrast, conventional grazing promoted bacterial diversity but reduced fungal diversity and bacterial richness. The result is consistent with previous studies where grazing (no

specific grazing system mentioned) increased bacterial diversity (Wu et al., 2022) and decreased fungal diversity in grassland soil (Yang et al., 2021). Reduced fungal diversity and bacterial richness in conventionally grazed soil may lead to a less stable ecosystem due to fewer taxa supporting the same function (redundancy) and a lower diversity of taxa that support different functions (reduced functional uniqueness) (Delgado-Baquerizo et al., 2016; Galand et al., 2018; Li et al., 2019). Thus, relative to conventional grazing, AMP grazing may have improved ecosystem functioning by enhancing fungal diversity, evenness, and richness (Liu et al., 2022).

3.4.2 AMP grazing enhances microbial complexity

Microbial network complexity in soils under AMP grazing was higher than that in soils of conventionally grazed ranches. Higher network complexity and connectivity were demonstrated by the higher value of network topological indices such as degree, edges (correlations), and clustering coefficient, and the lower average path length and diameter, within AMP grazing networks relative to the conventional grazing network (Banerjee et al., 2019; Wagg et al., 2019b; Yang et al., 2022). Higher network complexity reveals closer and more concentrated connections among microbial taxa (Berry and Widder, 2014; Chen and Wen, 2021; Guseva et al., 2022; Li et al., 2020; Ma et al., 2016) indicating better microbial cooperation and higher microbial linkage within the network (Guo et al., 2022). Enhanced microbial network complexity and connectivity may be the result of grazing treatment induced changes in soil properties like pH. Soil pH was comparatively low in AMP grazing compared to conventional grazing, which is consistent with previous work demonstrating a lower soil pH with a more complex microbial network (Yang et al., 2022). Additionally, higher stocking density and longer rest periods under AMP grazing could enhance network complexity through changes in C and N content availability (Wang et al., 2022), in turn influencing microbial diversity. This is supported by our data that show a

significant effect of stocking density, rest-to-grazing ratio, and stocking rate interaction effect on soil C: N ratios. Moreover, we also observed that a longer rest-to-grazing ratio increased soil fungal richness and diversity. Studies have shown that higher bacterial and fungal richness and higher fungal diversity both enhance microbial complexity (Chen and Wen, 2021; Li et al., 2020). Similarly, lower microbial complexity under conventional grazing may be due to reduced microbial richness and diversity (Chen and Wen, 2021; Li et al., 2020). Thus, AMP grazing can improve ecosystem functioning and stability by enhancing fungal diversity, richness, and evenness (Liu et al., 2022) leading to more complex and concentrated interconnected taxa within the network, as compared to conventional grazing.

There were more positive than negative associations (correlations) in both networks, but AMP had a higher proportion of negative correlations among microbes (OTUs) than conventional grazing. Positive and negative associations between taxa (nodes) in co-occurrence networks might indicate biological associations (Berry and Widder, 2014; Fuhrman, 2009), although the exact mechanisms underlying positive or negative associations within the network are unknown. Higher positive correlations under conventional grazing could indicate a shift in cooperative behaviors such as mutualistic associations, and cross-feeding behaviors, relative to that under AMP grazing. In contrast, higher negative correlations between nodes under AMP grazing might represent greater competition for resources and survival due to the presence of common predators (Berry and Widder, 2014; Blagodatskaya and Kuzyakov, 2008; Mau et al., 2015; Yuan et al., 2021). AMP grazing altered microbial co-occurrence patterns, specifically increasing and diversifying associations (enhancing negative correlations) within these grassland ecosystems.

3.4.3 Higher stocking rate increases microbial network complexity

Higher stocking rates increased soil microbial network complexity regardless of the grazing system. The connectivity (correlations), degree, and average clustering coefficients were higher, while the average path length and diameter were lower, within soil microbial networks under high stocking rates indicating more complexity than under low stocking. Complex microbial networks from high stocking could be due to higher bacterial and fungal richness, as well as to changes in soil pH. Our results indicate that bacterial and fungal richness increased with increasing stocking rate, and higher microbial richness potentially leads to heightened complexity in species associations. Previous studies on co-occurrence networks in pristine ecosystems also found that higher bacterial richness increased network complexity (Chen and Wen, 2021; Li et al., 2020). Further, higher complexity also could be due to soil pH which is considered the important factor driving microbial co-occurrence network complexity (Yang et al., 2022). Soil pH decreases with high grazing intensity (Zhang et al., 2018) and this trend was also apparent for our data, though not significant (high SR: 6.6; low SR: 7.0). Both positive and negative associations were higher under elevated stocking rates, indicating a more robust network to environmental perturbations (Santolini and Barabási, 2018) created through high grazing intensity. Generally, more complex networks suggest greater resource transfer and our study confirmed that microbial community networks under high stocking rates were more resilient to grazing regardless of the grazing treatment.

3.4.4 Distinct keystone taxa were identified under different grazing treatments and stocking rate

Different keystone taxa dominated each grazing treatment and stocking rate. Acidobacteria was the most abundant keystone taxa under AMP grazing while Actinobacteria and Acidobacteria were the most abundant keystone taxa under conventional grazing. Similarly, Acidobacteria was

the most abundant keystone taxa in soil under low stocking rate, while Proteobacteria, Actinobacteria and Acidobacteria were equally abundant in soil under a high stocking rate. Keystone taxa are the most connected taxa in networks playing an important role in the microbiome regardless of their abundance (Banerjee et al., 2019). Removal of such taxa can interrupt microbiome structure and functioning (Banerjee et al., 2019; Berry and Widder, 2014). Several studies have identified keystone taxa and revealed their vital functioning in ecosystems. For example, keystone taxa were involved in organic matter decomposition in agricultural soils (Banerjee et al., 2016) and litter decomposition in forests and farmland (Zheng et al., 2021). The presence of Acidobacteria as dominating the keystone taxa may be indicative of higher litter availability under AMP grazing and low stocking rates (Hillenbrand et al., 2019). Acidobacteria are linked to litter degradation (Schneider et al. 2012) and are an important decomposer and its abundance increased with litter addition (Kirby 2005; Baldrian 2012). In contrast, Actinobacteria as the most abundant of the keystone taxa may indicate higher labile carbon availability in soils under conventional grazing. Generally, Actinobacteria dominate in soils with high inputs of labile carbon (Aislabie and Deslippe 2013); however, the relative abundance of Actinobacteria was inversely correlated with SOC (Cheng et al., 2016). Thus, in AMP grazing and low stocking rate, Acidobacteria was dominant keystone taxa, while in conventional grazing and high stocking rate, Actinobacteria, Acidobacteria, and Proteobacteria were playing important role in the microbiome as keystone taxa.

3.5 Conclusions

This study provides insight into grazing effects on soil microbial diversity and co-occurrence patterns in grasslands of the Northern Great Plains. AMP grazing, characterized by short pulses of grazing and extended rest periods, may have improved ecosystem functioning and

stability as evidenced by enhanced fungal diversity and an increasing complexity of microbial co-occurrence networks. Proteobacteria, Acidobacteria, and Actinobacteria were the dominant bacterial groups, while Ascomycota and Basidomycota were the most abundant fungi under both AMP and conventional grazing. Soil fungal and bacterial diversity, richness, evenness, and co-occurrence patterns changed with grazing treatment, and these patterns were driven by specific grazing management metrics (i.e., elevated cattle stocking rates and rest-to-grazing ratios) and soil environmental condition (i.e., lower pH). Additionally, higher stocking rates led to more complex microbial co-occurrence patterns. Our study confirmed that both AMP grazing and stocking rate have marked effects on microbial diversity and connectivity, which consequently may impact ecosystem functioning and important processes such as nutrient cycling and soil C storage.

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Tables

Table 3. 1: Summary of physical and chemical properties (mean ± 1SE) at 13 paired locations. Ranches practiced either Adaptive Multi-Paddock (AMP) grazing or conventional grazing. A mixed effect model was used to test grazing effects on soil properties, where grazing system and annual heat: moisture AHM were fixed factors and ranch pair was a random effect. Bold text indicates significant effects ($P < 0.05$).

Management practices	AMP	Conventional
pH	**6.77 (0.2)**	**6.87 (0.2)**
Bulk Density (g cm^{-3})	0.89 (0.05)	0.91(0.4)
Carbon (%)	4.23 (0.4)	3.94 (0.6)
Nitrogen (%)	0.37 (0.04)	0.31 (0.05)
Carbon: Nitrogen ratio	12.36 (0.8)	12.85 (0.4)
Sand (%)	48.51 (3.3)	51.30 (2.4)
Silt (%)	32.78 (2.8)	28.95 (2.4)
Clay (%)	18.69 (0.9)	19.74 (1.6)

Figures

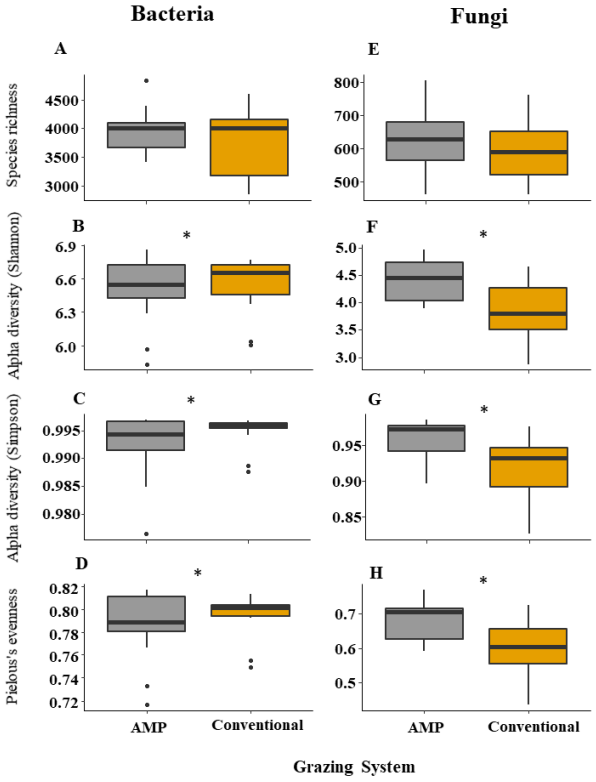

Figure 3. 1: Alpha diversity of the soil bacterial and fungal communities under Adaptive, Multi-Paddock (AMP) and conventional grazing. Shannon and Simpson's indices were calculated based on the phylogenetic distance at the Operational Taxonomic Unit (OTU) level, richness is the total count of OTUs and Pielous's evenness index is calculated from Shannon Weiner diversity and the total number of OTUs displayed in boxplots. The difference between diversity indices under the two grazing treatments was tested using a mixed-effect model. Pairwise means labeled with "*" differed significantly ($P<0.05$).

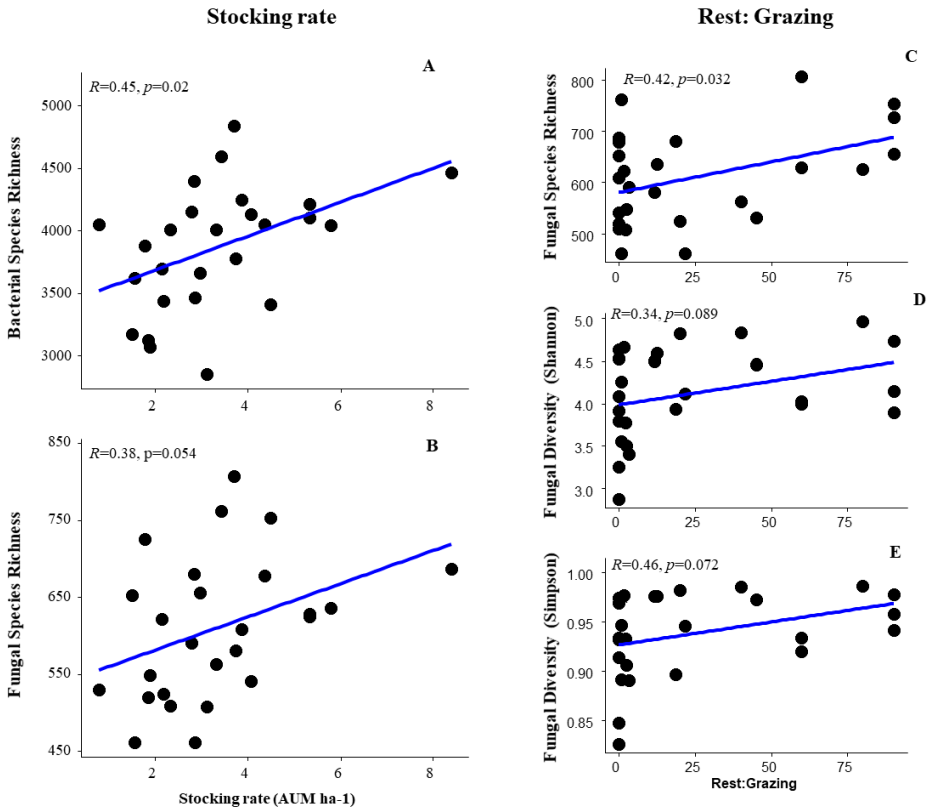

Figure 3. 2: Relationship between **A.** Bacterial species richness and **B.** Fungal species richness, with cattle season-long stocking rate (AUM ha^{-1}). Also shown is the relationship of **C.** Fungal species richness, **D.** Fungal Shannon diversity, and **E.** Fungal Simpson diversity index, with the rest-to-grazing (Rest: Grazing) ratio. Regression lines are linear fits of microbial richness and diversity to each grazing management metric.

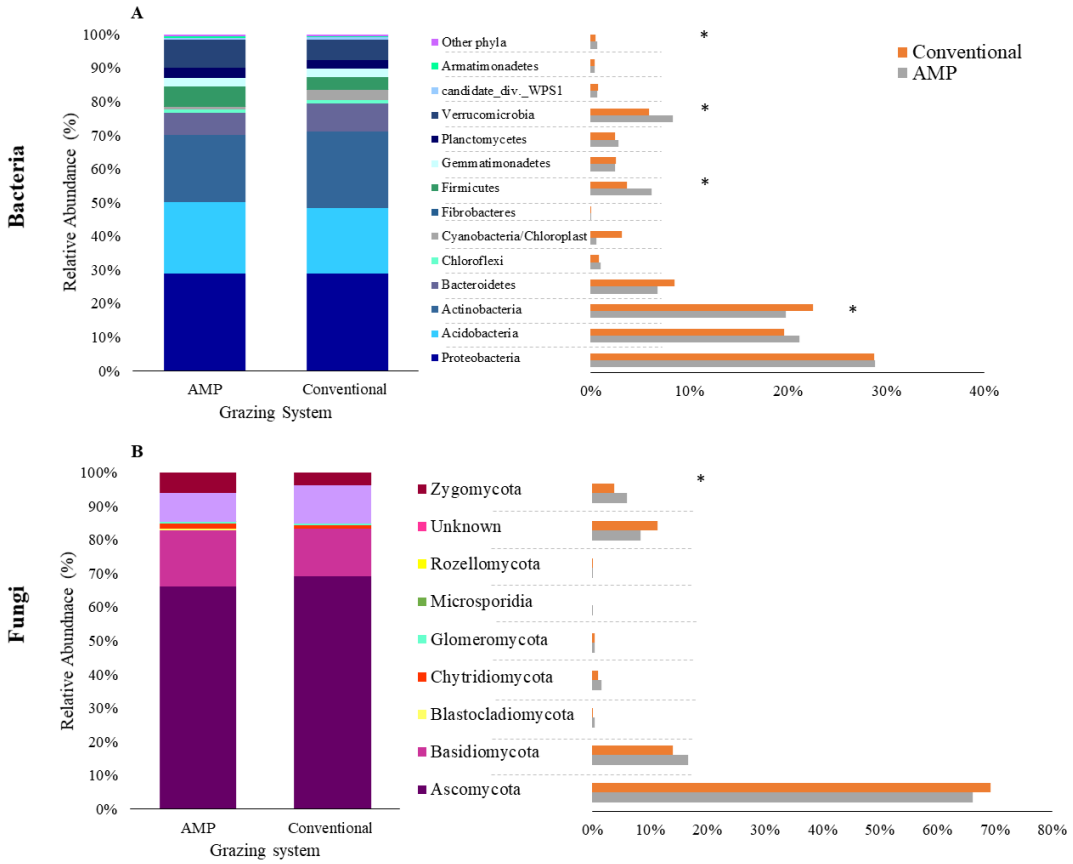

Figure 3. 3: Relative abundance of soil A. bacterial and B. fungal groups at the phylum level in relation to either Adaptive, Multi-Paddock (AMP) or conventional grazing. The left panel represents stack bar plots indicating the relative abundance (%) of microbial phylum composition, and the right panel includes the corresponding barplot of each phylum under the two grazing treatment. Bacteria phyla with a relative abundance of <1% are grouped as other phyla, whereas unidentified fungal Operational Taxonomic Unit (OTUs) are grouped as 'unknown'. The difference in relative abundance under the two grazing treatments was tested using a mixed-effect model, with paired means labeled with "*" showing significant differences ($P<0.05$).

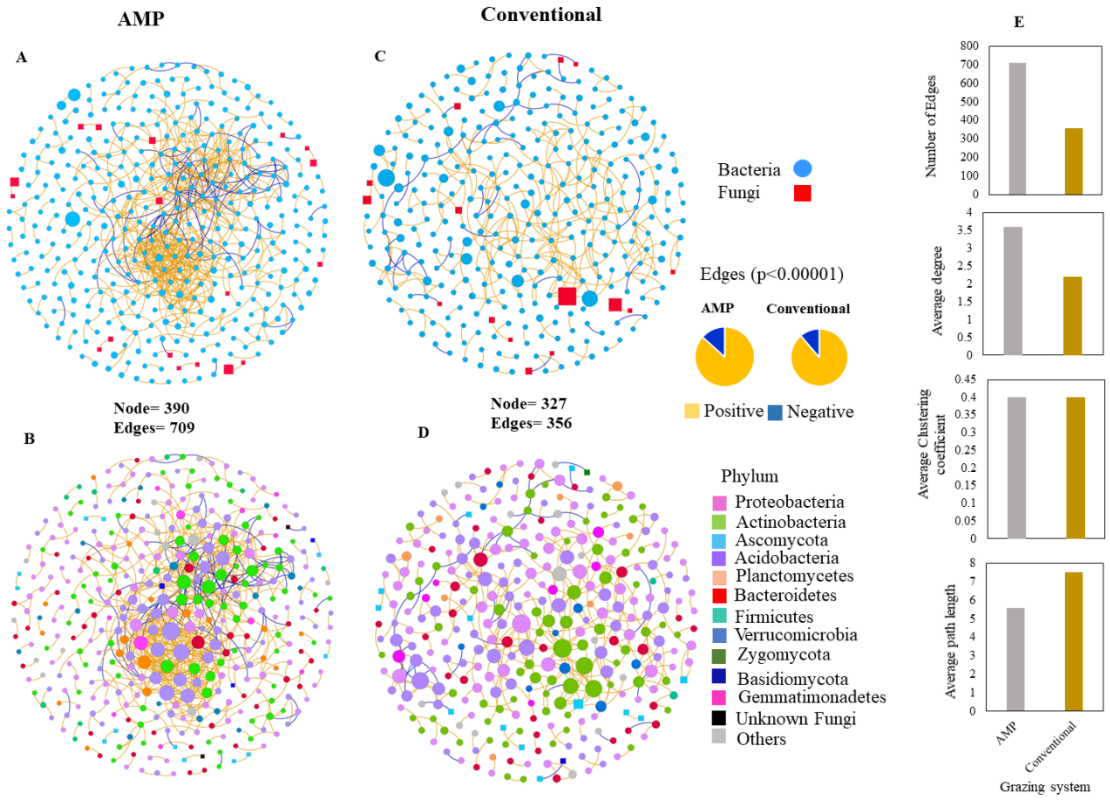

Figure 3. 4: Network analysis depicting the correlation (Spearman correlation, *P*<0.00001) pattern among microbial communities (bacterial and fungal) based on the taxonomic unit under AMP and conventional grazing. Orange lines indicate a significant strong (>0.9) positive correlation between nodes, while blue lines represent strong (<-0.9) negative correlation. **A.** Network showing the association between bacterial and fungal communities under AMP grazing, and **B.** the network arising from conventional grazing. Node colors reflect the domain while the size of the node is based on relative abundance. **C.** Bacterial and fungal communities found under AMP grazing and **D.** conventional grazing practices, where node color is based on the phylum class and the size of the node is based on the degree and **E.** Network topological indices such as the number of edges, average degree, average path length, and clustering coefficient. Pie charts depict the overall number of positive and negative correlations among the microbial community.

A

Keystone taxa: *Genus* (Phylum)	
AMP	Conventional
Terrimonas (Bacteroidetes)	*Anderseniella* (Proteobacteria)
Gaiella (Actinobacteria)	*Gaiella* (Actinobacteria)
Gp6 (Acidobacteria)	*Gp6* (Acidobacteria)
Gp3 (Acidobacteria)	*Gp4* (Acidobacteria)
Streptomyces (Actinobacteria)	*Jiangella* (Actinobacteria)
Gemmatimonas (Gemmatimonadetes)	*Massilia* (Proteobacteria)
	Brooklawnia (Actinobacteria)
	Chthonomonas Gp3 (Armatimonadetes)

B

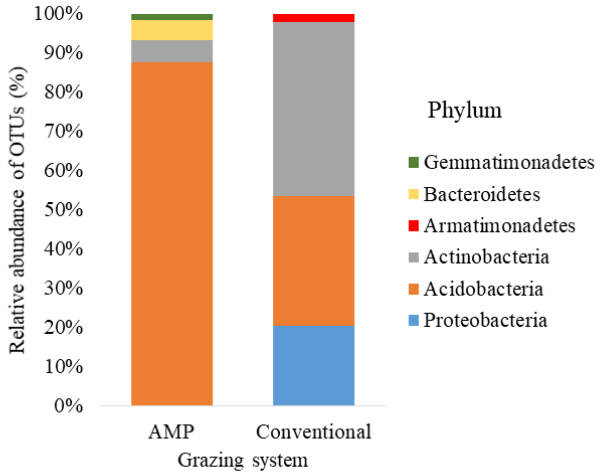

Figure 3. 5: Keystone taxa and their relative abundance under AMP and conventional grazing. **A.** List of keystone taxa; **B.** Stack diagram showing the relative abundance of keystone taxa by phyla. Values are the average relative abundance of 13 samples under either AMP grazing or conventional grazing.

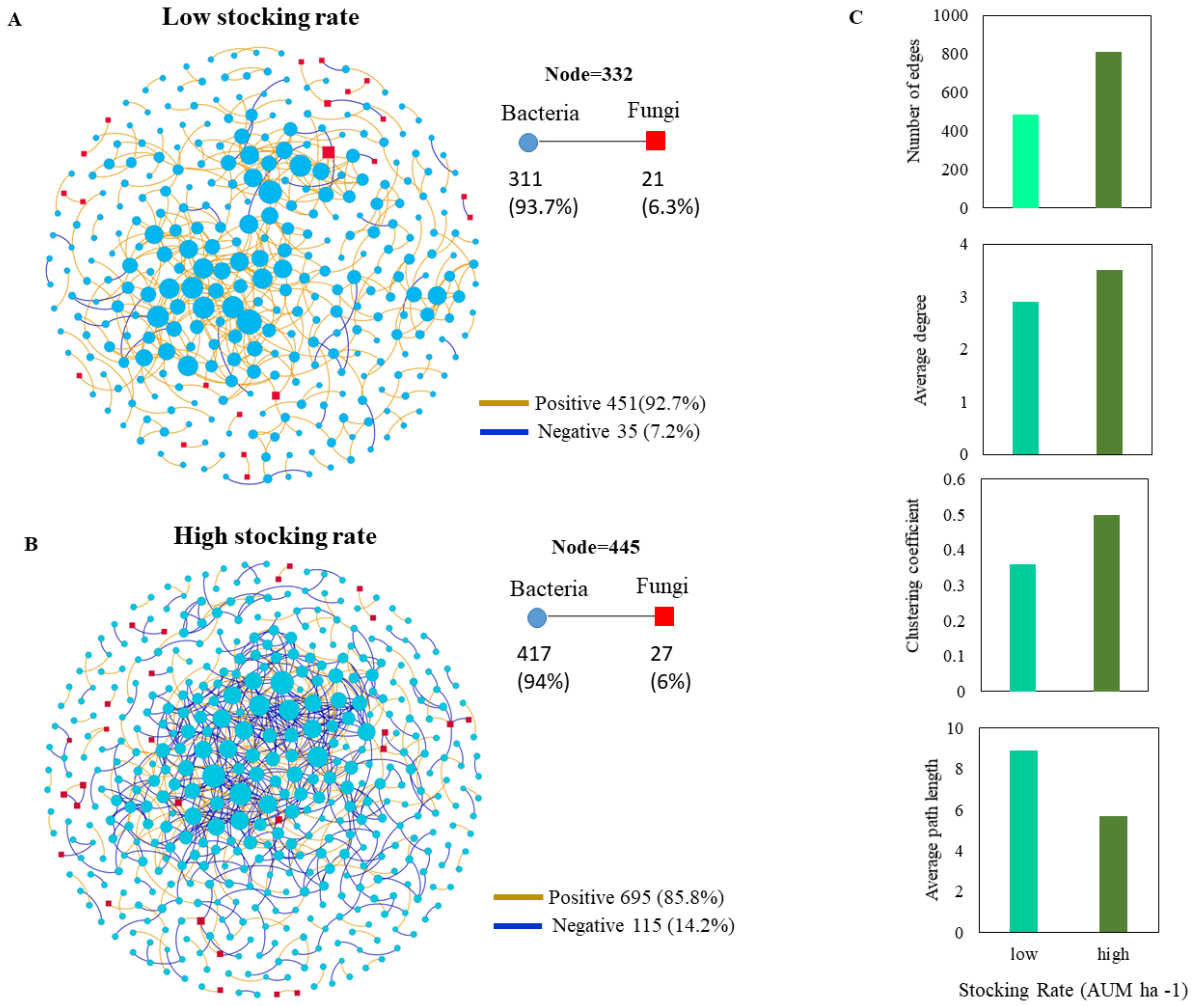

Figure 3. 6: Network analysis depicting the correlation (Spearman correlation, $P<0.0001$) pattern among microbial communities (bacterial and fungal) based on the taxonomic unit under either low or high stocking rates. Yellow lines indicate a significant strong (>0.8) positive correlation, and blue lines represent a strong (<-0.8) negative correlation. **A.** Network analysis showing an association between bacterial and fungal communities under low stocking rate and **B.** high stocking rate. Nodes colors reflect the domain: blue color represents bacteria and red color represents fungi. The size of the node is based on degree. **C.** Network topological indices such as the number of edges, average degree, average path length, and clustering coefficient. Line graphs depict the overall number of positive and negative correlations among microbial communities.

Chapter 4. Adaptive Multi-paddock grazing increases soil carbon in fine particle size fractions and its stability in Northern grasslands

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Summary

Grassland soils play an important role in sequestering carbon (C) on a global scale. Grasslands are primarily used for livestock grazing, and grazing management may increase the amount of C stored in soils and the distribution of C in different soil fractions by altering soil microbial community structure. Soil microbial communities affect the grassland C pool by decomposition and transformation of organic matter. Among different grazing management systems, a specialized form of rotational grazing known as adaptive multi-paddock grazing (AMP), which is considered to enhance grassland sustainability, productivity, and soil C sequestration. However, how different grazing systems affect the amount of C stored in different soil fractions has received little attention. In this study we evaluated 24 sites in a paired design where 12 ranches practiced AMP grazing and 12 neighboring ranches practiced conventional grazing. Soil organic C (SOC) and total nitrogen were measured in different soil particle size [fine (<53 μm), medium (53-250 μm) and coarse (>250 μm)] and density [light (>1.6 g cm^{-3}) and heavy (<1.6 g cm^{-3})] fractions. Soil bacterial and fungal biomass and abundance were quantified using phospholipid fatty acid analysis (obtained from previously published data). Soil microaggregates (fine and medium fractions) and heavy fractions were higher in soil exposed to AMP grazing, whereas macroaggregates (coarse) and light fractions were higher in conventionally grazed soils. Soil organic C (concentration and stock) were significantly higher in the fine soil fractions from AMP grasslands than conventionally grazed grasslands. Given that SOC in fine fractions is more recalcitrant, our result indicates that AMP grazing increased the size of the stable SOC pool found in association with clay particles, and corresponding fungi: bacterial (F:B) ratios. Additionally, F: B ratios were positively related to SOC stocks in the fine fraction, while the gram-positive to gram-negative bacterial ratio was negatively associated with SOC in the coarse

soil fraction, indicating soil microbial community associations existed with the amount of soil C sequestration across different soil fractions. In conclusion, larger amounts of stable C accumulation suggests that AMP grazing can reduce CO_2 levels in the atmosphere relative to conventional grazing

Keywords: AMP grazing; Cattle grazing; Density fractionation; Grassland; Size fractionation; Soil organic carbon

4.1 Introduction

Grasslands cover about 25% of Earth's terrestrial surface and store around 30% of global soil carbon (C) (Adams et al., 1990; Ojima et al., 1993). Grasslands store a larger proportion (about 90%) of C belowground than aboveground (Golubiewski, 2006). Grasslands are dominated by herbaceous vegetation with large below-ground biomass, that serves as an adaptation to grazing, harvest, fire and, seasonal senescence (Berhongarary et al., 2018; Ontl and Janowiak, 2017). Soil organic matter (SOM) is comprised of plant, animal, and microbial residues at different stages of decomposition, including fine roots and soil microbiota (Christensen, 1992). Soil organic C is the component of soil organic matter representing around 58% of organic matter. Based on the stages of degradation and turnover of organic matter, soil C is associated with different aggregate sizes (fractions) that are heterogeneous in structure (Christensen, 2001; Haile et al., 2008). The turnover of soil organic carbon (SOC) is associated with specific organic matter fractions, structure, and aggregation (Six et al., 2000). Soil C in grasslands is vulnerable and sensitive to management practices (Bork et al., 2020; Ontl and Janowiak, 2017) such as grazing (Hewins et al., 2018), prescribed fire (Page-Dumroese et al., 2002) and conversion to cultivation (Yang et al., 2019). Moreover, variation in grazing management practices among land managers is high, including in relation to grazing (Bork et al., 2021), indicating C may be lost from the ecosystem if grasslands are not managed properly.

Organic C in the soil can be stored in different size and density fractions (pools), which affects long-term stability of C (Sollins et al., 1996; von Lützow et al., 2007). Carbon associated with different size and density fractions differ in lability (Rita et al., 2011). Generally, C in coarse fractions (>250 μm, macroaggregates) is more labile (easily decomposable) than C associated with the fine (<53 μm, silt, and clay) and medium fractions (53-250 μm,

microaggregates). Specifically, C stored in fine fractions is more recalcitrant than C stored in larger fractions and represents the stable fractions (Bronick and Lal, 2005; Rita et al., 2011) with longer turnover times ranging from decades to centuries (Haile et al., 2008; Yamashita et al., 2006). Higher stability ensures C sequestration for long periods within the ecosystem and helps offset CO_2 emission (Haile et al., 2008). Furthermore, soil C in the heavy density (>1.85 g cm^{-3}) fraction is generally more stable because of the presence of less mineralizable C than in the light density (<1.85 g cm^{-3}) fraction (Christensen, 1992; Hassink, 1995). Light fraction material can easily decompose, whereas the heavy fraction material contains mineral associated organic material with a slower turnover rate (Crow et al., 2007; Rita et al., 2011). Several studies have shown that grazing activities like trampling can break down soil aggregates and reduce the stability, relative abundance, and ultimately, the amount of C in each soil fraction (Li et al., 2013; Wang et al., 2020; Wen et al., 2016). Further, studies have shown that grazing intensity has a variable effect on SOC in grasslands (Bork et al., 2020; Hewins et al., 2018; Wang et al., 2020) including the amount of carbon held in different fractions (Mujuru et al., 2013; Tieszen et al., 1983; Zimmermann et al., 2007). Canadian prairies are primarily used for cattle grazing (Bailey et al., 2010). Identifying cattle management systems that increase C storage within more stable fractions could improve our ability to mitigate climate change.

Cattle grazing influences SOC stocks in different soil fractions through various grazing activities like vegetation defoliation, trampling, and nutrient input (Dong et al., 2022; Hewins et al., 2018). Several types of grazing management systems such as continuous grazing, where livestock are in a single pasture for the entire grazing season, or rotational grazing, where livestock rotates through multiple pastures during the grazing season, are implemented in grasslands depending on climate and resource availability. Adaptive multi-paddock (AMP)

grazing is a specialized system where cattle are grazed within individual pastures for short periods at high densities before being moved (Briske et al., 2014; Savory and Butterfield, 2016). AMP grazing can enhance soil water-holding capacity, increase soil fertility, reduce nutrient loss from the soil and alter plant communities (Döbert et al., 2021; Grenke et al., 2022; Khatri-Chhetri et al., 2022; Stanley et al., 2018; Teague et al., 2011). Further, AMP grazing is argued to enhance C storage in soil (Rasmussen et al., 2016; Stanley et al., 2018) by altering the soil microbial community (Khatri-Chhetri et al., 2022).

Soil microbial community activity can change SOC concentration and stocks via soil organic matter decomposition and changes to nitrogen (N) availability (Bhattacharyya et al., 2022; Cao et al., 2019). On the one hand, microorganisms metabolize and respire organic carbon, while also building SOC stocks through the formation of microbial necromass and the formation of stabilized residues in association with minerals within soil aggregates (Bhattacharyya et al., 2022). Specifically, microbial indicators such as the abundance of fungi, arbuscular mycorrhiza fungi (AMF), fungal to bacterial (F: B) ratio, and gram-positive to gram-negative (GP: GN) ratio, are considered indicators of C storage in soil (Fanin et al., 2019; Malik et al., 2016) and are responsive to grazing systems (Khatri-Chhetri et al., 2022). Understanding the association between these microbial indicators and SOC stocks in different soil C fractions remains an important knowledge gap. Further, while many studies on livestock management effects on soil C have accounted for stocking rate (Bork et al., 2020; Dong et al., 2022; Soares et al., 2022), none have assessed the relationship of other grazing management variables like stocking density for their effect on soil C within different fractions. Thus, it is essential to understand the relationship between soil microbial indicators and C pools in soil to better characterize the net impact of grazing management system on soil C and its stability in grassland ecosystems.

Our main objective is to understand the effect of grazing management systems, using a variety of specific grazing metrics on SOC and N concentrations and stocks in size and density fractions of soil. Additionally, we examined whether the effects of grazing management on the distribution of soil carbon in different fractions varied along an environmental gradient and among the associated microbial communities. Knowledge of the distribution of C and N among different size and density fractions can provide an understanding of how grazing systems and management practices affect the quality and long-term stability of SOC in grassland soils.

4.2 Materials and Methods

4.2.1 Study Area

We sampled soils from 24 ranches located along a climatic gradient in Alberta, Canada (Table S1). Mean annual temperatures for the sites ranged from 2.2 °C to 4.14 °C, with annual precipitation varying from 326 mm to 551 mm and aridity ranged from 24. 4 – 4.3 based on 30-year climate normals (1989-2018) (Table S1). The sites represented three major soil orders: Chernozemic (n=16), Luvisolic (n=6), and Regosolic (n=2) (Government of Canada, 1998). Most grasslands were dominated by agronomic grasses such as smooth brome (*Bromus inermis* L.), meadow brome (*Bromus biebersteinii* Roem and Schult.), crested wheatgrass (*Agropyron cristatum* L. Gaertn), timothy (*Phleum pretense* L.), orchard grass (*Dactylis glomerata* L.) and creeping fescue (*Festuca rubra* L.), along with legumes such as alfalfa (*Medicago sativa* L.), Cicer milkvetch (*Astragalus cicer* L.), sweet clover (*Melilotus officinalis* (L.)Pall.) and alsike clover (*Trifolium hybridum* L.). A few sites were dominated by native grasses, such as rough fescue (*Festuca campestris* Rydb.).

4.2.2 Sampling design and soil sampling

The 24 ranches that we sampled consisted of 12 pairs. Each pair included one ranch practicing AMP grazing (for at least five years) and the other ranch in the pair was a nearby ranch (within 5 km distance) practicing conventional grazing (varying from continuous grazing to rotational grazing strategies) hereafter named "conventional" ranches. Both ranches within a pair were located on a similar ecosite to minimize the effects of environmental variability within pairs. The ranches in this study are a subset of sites reported in Bork et al. (2021), which thoroughly describes management differences between AMP and conventional grazing groups. The two grazing treatments could be distinguished from each other based on stocking density, which is a standard measure of the number of animal units (AU) per unit area for a single pasture and expressed as AU ha^{-1} (AMP: mean ± SE: 32.3 ± 9.1 AU ha^{-1}; conventional: 3.2± 0.9 AU ha^{-1}) and rest-to-grazing ratio (AMP: 26.2± 7.6; conventional: 2.55± 1.6 AU ha^{-1}). The rest-to-grazing ratio was calculated as the number of days of rest prior to subsequent re-grazing and standardized per day of active grazing during the early phase of the growing season (before August 1) (Bork et al., 2021). Stocking rate, the standard measure of the number of animal units per unit area for a time period and expressed as AUM ha^{-1} (AUM: animal-unit-months), was not significantly different between the two grazing treatments (AMP: 3.47± 0.6 AUM ha^{-1}; conventional: 2.92± 0.8 AUM ha^{-1}) (Tables S1 & S2). To characterize the climate at each ranch, we used an aridity index known as the annual heat: moisture (AHM) index, derived from mean annual precipitation and temperature, obtained from ClimateAB 3.21 software package (Alberta Environment, 2005; Mbogga et al., 2010) .

4.2.3 Soil physical and chemical analyses

From each ranch, 5 soil cores (2.54 cm diameter) were randomly collected from 0-15 cm depth within a 10-ha area in June 2017. Cores were combined and transported to the lab for further analysis. Soil samples were first air-dried and passed through a 2 mm sieve to remove large fragments and plant roots. Soil organic C and N concentrations of the bulk soils were analyzed by dry combustion methods where sub-samples were oven-dried at 60 °C for 48 hr, ground to a fine powder in a ball grinder (Retsch MM400 Mixer Mill, Retsch, Haan, Germany) (Hewins et al. 2018), and then analyzed for total organic C and total N using a Flash 2000 CHNS elemental analyzer (Thermo Scientific, America). To convert C and N concentrations to stocks (Mg ha^{-1}), soil bulk density and depth of soil were used (Baah-Acheamfour et al., 2015). Bulk density was measured using additional separate soil samples collected with a metal core (3.81 cm diameter × 15cm deep) that was then dried and sieved (2 mm). Soil material less than 2 mm in diameter was dried and weighed, and the volume of coarse material (> 2 mm) measured by water displacement, and then subtracted from the core volume (Blake and Hartge, 1986). Soil texture was determined using the hydrometer method (Kroetsch and Wang, 2008); all samples were pretreated for organic matter using 30% hydrogen peroxide (H_2O_2) (Jensen et al., 2017), and samples with pH > 6.5 were treated with hydrochloric acid (HCl) to remove carbonates (Francis and Aguilar, 1995; Kroetsch and Wang, 2008). Soil pH was measured in a 1:5 mix of soil and deionized water solution after being shaken for 30 minutes (Kalra, 1995) with a digital pH meter (Model Accumet AB150 pH/mV Meter, Fisher Scientific, America). Soil microbial indicator data were obtained from previously published research, where the abundance of fungi, AMF, and F: B ratios were quantified using phospholipid fatty acid

analysis, with detailed extraction and analysis provided in Khatri-Chhetri et al. (2022). However, the number of ranches with the data is lower (n=16; 8 pairs).

4.2.4 Particle size fractionation

Quantification of C in different size-fractions can be accomplished through physical, chemical, and biological techniques (Christensen, 2001; Six et al., 2002). Among these techniques, physical fractionation of whole soil, based on the size of a particle is more common because the method is chemically less destructive and can be related to the function of soil C (Arevalo et al., 2012; Creamer et al., 2011). Soil samples were separated into fine- (<53 µm, silt, and clay), medium- (53-250 µm, microaggregates), and coarse- (>250 µm, macroaggregates) fractions by wet-sieving (Kong *et al.*, 2005; Baah-Acheamfour *et al.*, 2014). Air-dried subsamples were passed through a 2 mm sieve and 50 g of soil was weighed into 250 mL Nalgene bottles to which 100 mL of deionized water was added. The soil sample was shaken on a horizontal shaker for 30 minutes and then carefully washed under slowly running water through a set of 53 µm and 250 µm sieves to isolate the three size fractions (Shang *et al.*, 2014). This procedure yielded three soil fractions (<53 µm, 53-250 µm, and >250 µm) that were dried in an oven at 60 °C for 72 hours. After oven-drying, the fractions were weighed and then prepared for elemental analysis following the procedure described in the bulk soil section above.

4.2.5 Density fractionation

Soil density fractionation was performed to assess less complex organic matter like free or occluded light fractions and various heavy mineral fractions (Moni et al., 2012; Viret and Grand, 2019). Density fractionation was performed to separate soil organic matter into light (<1.85 g cm^{-3}) and heavy (>1.85 g cm^{-3}) fractions based on their density (Crow et al., 2007). Air-dried (< 2 mm sieved) soil samples were used for density fractionation using the sodium polytungstate

(SPT) method (Zimmermann et al., 2007). The SPT solution was prepared by adding 741 g of SPT to 859 mL of ultra-pure water to produce 1.6 g cm^{-3} of SPT solution. A hydrometer was used to verify the density of SPT solution. Ten grams of soil sample was mixed with 30 mL of the SPT solution (Crow+ et al., 2007) in a 50 mL conical centrifuge tube and shaken for 1 minute in a horizontal shaker. Then the tubes were centrifuged at 1000 g for 15 minutes (Zimmermann et al., 2007). The resulting supernatant was collected through a Whatman filter (0.7 µm pore size) in a Buchner's funnel using vacuum pressure and rinsed five times with deionized water to remove the SPT (Baah-Acheamfour et al. 2015). The collected fraction contained the light fraction (LF). Next, the remaining soil sample and newly added 30 mL of SPT solution were shaken for 1 minute and centrifuged at 1000 g for another 15 minutes and the supernatant collected and rinsed with deionized water five times. This process was repeated for three times to ensure all light fraction was collected, after which the material collected was combined to form all the light fraction. The remaining soil in the bottom of the tube is the heavy fraction (HF), which was filtered in a Buchner's funnel using vacuum pressure, then rinsed with deionized water five times. Both light and heavy fractions were dried in an oven at 60 °C for 72 hours, then weighed and prepared for elemental analysis following the procedure described above.

4.2.6 Statistical analyses

To evaluate the grazing treatment effect on soil properties like pH, texture, and bulk density, mixed-effects models were used where grazing treatment was used as a fixed factor and ranch pair was a random effect. Models were estimated using the "lmer" function in "lme4" (Bates et al., 2015). Similarly, the relationship between microbial indicators and SOC stocks within different fractions were examined using the same model mentioned above; however, a subset of the data for which we had data on the microbial community was used for the analysis. To test

grazing treatment effects on the soil mass distribution of different soil fractions we used multivariate analysis of variance (MANOVA), with mineral particle size and density fractions run separately. The different soil fractions comprised dependent variables, and MANOVA allows a comparison of mean differences on the dependent variables simultaneously. Further, to test the effect of grazing treatment on SOC concentrations, SOC stocks, and soil properties, we used a linear mixed-effects model where grazing treatment was a fixed effect and ranch pair was a random effect. Annual heat moisture (AH: M) was used as a co-variate in the mixed model to describe variation in the physical environment across sites. Specific grazing management metric effects on SOC concentrations and SOC stocks in bulk soils and fractions were tested with a mixed-effects model where stocking rate, stocking density and rest-to-grazing ratio were used as fixed effects, and ranch pair was used as a random effect. Response variables included each of the three size fractions and two density fractions. We used a P value of < 0.10 to identify significant effects to reduce the probability of type II error because study sites were distributed across a wide range of geographic locations (400 km) with a high degree of variation in environmental characteristics and management practices. All statistical analyses were performed in R 4.0.0 (R Development Core Team, 2020).

4.3 Results

4.3.1 Soil properties

Grasslands within the two grazing treatments differed in soil textures (Sand: F=5.1, Df.res= 11, P= 0.04; Clay: F=3.3, Df.res=11, P= 0.09), with soils under AMP grazing having more clay (AMP: 26.1 ± 3.8 %; Conventional: 21.4 ± 2.8 %) but less sand (AMP: 37.1 ± 6.1 %; Conventional: 45.0 ± 4.4 %). There were no significant effects of the grazing treatments on silt, soil pH, or bulk density (Table 4.1 & S3).

4.3.2 Percent mass distribution of soil size and density fractions

The average recovery of soil samples during size fractionation was >90 % and density fraction was >93 % for both AMP and conventional grazing (Table 4.2). With greater than 90 % soil recovery on most sites for both size and density fractionation, the overall size and density fraction recovery rates were considered to be very good (Viret and Grand, 2019). The recovery rate on size fractions ranged from 82 to 97.7 %, with 14 of 24 sites having a recovery greater than 90%, while the recovery rate on density fractions ranged from 78 to 99.8 %, with 21 of the 24 sites having greater than 90 % recovery. Low recovery could be associated with the loss of dissolved and colloidal particles during rinsing, which is an unavoidable step of the method. In density fractionation, clay particles might have been lost during the filtration step and some of the soluble organic matter might have dissolved in the STP solution leading to low recovery (Baah-Acheamfour et al., 2015; Viret and Grand, 2019). Soil mass under both grazing systems was dominated by coarse size (>250 µm), followed by fine (<53µm), and then by medium (53-250 µm) fractions (Table 4.2). Similarly, heavy fractions rather than light fractions dominated soils from both grazing systems (Table 4.2). The mass of the medium size fraction ($F=3.25$, $df= 1$, $P= 0.08$) and heavy density fraction ($F= 3.13$, $df= 1$, $P= 0.09$) were both greater in AMP grazed grassland soils than under conventional grazing (Table 2 & S4). Stocking density affected mean mass (%) of macroaggregate ($F= 3.57$, df.res= 14.66, $P= 0.07$), where the relative mass of macroaggregates decreased with increasing stocking density (Figure 4.1). However, stocking rate and rest-to-grazing ratio did not affect the soil mass distribution of size and density fractions (Table S5).

4.3.3 Grazing system effect on SOC and total N in bulk and fractionated soil

Grazing treatment did not significantly affect soil organic carbon (SOC) concentration (g SOC kg^{-1} dry soil) or stock (Mg ha^{-1}) in bulk soils despite trending 5- 11% greater for both under AMP grazing (Table 4.3). However, the concentration of carbon (F= 3.6, df.res= 10, *P*= 0.08) and SOC stock (F= 3.4, df.res= 10, *P*= 0.09) in fine fractions were both greater in AMP than in conventional grazing (Table 4.3 & Table S6). Further, there were significant grazing treatment by AHM interaction effects on SOC within the fine fractions (Table S6). Specifically, SOC concentration and stock decreased with increasing AHM (aridity) under conventional grazing, but did not change under AMP grazing (Figure S1) in the fine fractions. We did not detect an effect of grazing system on SOC within the coarse, medium, or density (light and heavy) fractions (Table S6). However, SOC concentration and stock decreased with increasing AHM (*P*<0.05) within each of the bulk, medium, coarse, and heavy fractions (Figure S1). In soils subject to AMP grazing, the largest SOC stock was in the fine fraction, which was 1.1 times and 1.7 times higher than in the coarse and medium size fractions, respectively (Table 4.3). In contrast, in soils under conventional grazing the coarse fraction held the largest SOC stock, which was more than 2.1 and 1.6 times higher than that in the medium and fine fractions on average (Table 3). Similarly, the distribution of SOC concentrations and stocks was higher in light fractions than in heavy fractions under both grazing treatments (Table 4.3).

Soil total N concentration and stock in bulk soil were not significantly affected by grazing treatments (Tables 4.3 & S7). Similar to soil C patterns however, the largest total N stock under AMP grazing was within the fine fraction, whereas in conventional grazing more N was held in the coarse fraction (Table 4.3). The interaction of grazing and AHM affected total N concentration in the fine fractions (Tables 4.3 & S7), where N stock decreased with increasing

AHM under conventional grazing but increased under AMP grazing (Figure S3). Total N concentration and stock in the coarse, medium, light, and heavy fractions were not affected by the grazing treatments or their interaction with AHM (Table S7). Similar to SOC, total N concentration and stock decreased with increasing aridity (P <0.01) in bulk soil as well as the coarse, medium, and heavy fractions (Table S7 & Figure S4).

Soil C: N ratios were affected by grazing and its interaction with AHM in bulk soil and coarse fractions (Table S8). Soil C: N ratio was higher in bulk soil of AMP grazed grasslands than those conventionally grazed (F= 4.8, df.res= 10, P= 0.0003), and increased with AHM under AMP grazing but decreased with AHM in conventional grazing (Table 4.3). In contrast, the C: N ratio of the coarse fraction was higher (F=28.6, df.res= 10, P <0.001) in conventionally grazed grasslands than in AMP ranches (Table 4.3). The opposite trend occurred for C: N in the coarse fraction of bulk soil, where the ratio decreased with increasing AHM under AMP grazing but increased with increasing AHM under conventional grazing (Figure S3).

4.3.4 Grazing management metrics effect on SOC and total N in bulk and fractionated soil

Grazing management factors like stocking rate and stocking density affected SOC and N concentration in coarse and medium fractions of soils, while the rest-grazing ratio affected C: N ratios in light fractions of the soils (Tables S9 & S10). Soil organic C and N concentrations in coarse (SOC: F= 4.9, df.res= 9.9, P= 0.05; N: F=4 .6, df.res= 10.9, P= 0.05) and medium (SOC: F=11.5, df.res= 8.2, P= 0.008; N: F=6.38, df.res= 8.7, P= 0.02) fractions were affected by the interaction of stocking rate and stocking density, where SOC and total N concentrations at high stocking rates decreased with increasing stocking density (Figure 4.2A- 4.2D). Additionally, SOC and total N concentrations tended to be higher at low stocking rates and low stocking density. Further, C: N ratios within light (F= 3.7, Df.res=16.7, P= 0.09) fractions decreased with

increasing stocking density, while SOC in medium fractions first increased and then decreased with increasing rest-to-grazing ratios (Table S9- S11 & Figure 4.3A-4.3B). In contrast, SOC and N stock were not affected by any of the management factors in bulk soil, nor the size class or density fractions (Table S9 & S10).

4.3.5 Microbial indicator association with SOC stock in different fractions

Abundance of fungi (F= 6.1, df.res= 10.9, *P*= 0.03) and F: B ratio (F= 6.1, df.res= 10.9, *P*= 0.03) were associated with SOC stock in fine fractions (Table S12), where SOC stock increased with increasing F: B ratios. SOC concentrations in coarse fractions decreased with increasing GP: GN ratio (F= 4.4, df.res= 10.5, *P*= 0.05) (Figure 4.4). There was no relationship between the microbial indicators and SOC in bulk soil and the medium size fraction or density fractions.

4.4 Discussion

4.4.1 AMP grazing promotes microaggregates distribution

Grazing altered the physical structure of the soil, where AMP grazing promoted an increase in the relative abundance of microaggregates (<250 μm) in grassland soil compared to conventional grazing. A higher proportion of microaggregates in AMP grazing might be the result of heavy trampling by cattle due to the high stocking density, which destroys soil macroaggregates by mechanical stress leading to higher microaggregate (Hiltbrunner et al., 2012). In contrast, there was a higher proportion of macroaggregates in grassland soils under conventional grazing than under AMP grazing, which could be due to lower stocking densities practiced in these pastures, which led to less disruption of macroaggregates through mechanical forces like trampling (Pinheiro et al., 2004; Warren et al., 1986). A higher proportion of

macroaggregates indicates that soil carbon storage was higher in the coarse fraction under conventional grazing than in AMP grazed grassland. Soil textures were different under two grazing systems, where AMP grazing led to more clay texture whereas, conventionally grazed ranches were more sandier. Higher clayey texture in AMP grazing might be due to higher soil protection from litter and live vegetation which deters clay particle loss through natural erosion (Chartier and Rostagno, 2006). Further, AMP grazing improved water infiltration which consequently protects clay particles in soil (Döbert et al., 2021). This preservation of fine particles, especially clay, through reduced erosion also could explain the greater amount of heavy density soil fractions under AMP grazing, because it increases C stability and deters the mineralization process (Soares et al., 2022). However, sandier soil with reduced microaggregates and fine- silt-and-clay in soils under conventional grazing might indicate greater rates of natural erosion or soil loss due to lower soil protection from litter and live vegetation (Chartier and Rostagno, 2006). These changes in aggregate distribution due to grazing systems are important because they might ultimately affect soil carbon storage.

4.4.2 AMP grazing increases SOC in fine fractions

The amount of C and N held in the fine fraction was greater in soils under AMP grazing compared to conventional grazing. Higher amounts of C and N in soil fine fraction under AMP grazing indicates potentially longer-term C sequestration because these are more recalcitrant carbon pools (Six et al., 2002). Generally, SOC stability is influenced by three main mechanisms: 1) occlusion within soil aggregates (Jones and Edwards, 1998; Six et al., 2002); 2) association with soil mineral particles such as clay and silt; and 3) formation of decomposition-resistant molecular structures (e.g., humic substances). These mechanisms create physical/chemical protection of SOC against microbial decomposers, enzymes, and oxygen

(Assunção et al., 2019; Jones and Edwards, 1998; Six et al., 2002). Higher SOC stocks in fine fractions under AMP grazing may be due to a higher percentage of clay particles, which increase the physical protection of SOC within aggregates (Six et al., 2002; Yang et al., 2021). Further, clay particles have a higher surface area for C absorption, and SOC stability increases with increasing clay content in soil (Arrouays et al., 2006; Feller and Beare, 1997; Yu et al., 2019). Conventionally grazed grasslands stored higher SOC concentrations and stocks within coarse fractions, suggesting comparatively short-term C storage because of the potential for faster organic matter decomposition and mineralization in coarse fractions compared to fine fractions (Hassink, 1997; Six et al., 2002). Higher SOC stocks in coarse fractions of the conventional grazing might be reflective of the higher relative abundance of coarse fractions (macroaggregates) due to the low stocking density practiced in conventional grazing compared to AMP grazing. Further, a higher C: N ratio in the coarse fraction under conventional grazing indicates more organic material is likely undecomposed or partially decomposed. Hence, AMP grazing promotes C within fine fractions by enhancing clay particles which can protect C and may promote long-term SOC storage and stabilization (Arrouays et al., 2006; Assunção et al., 2019; Jones and Edwards, 1998; Six et al., 2002).

Higher SOC in the fine fraction also might also be related to soil microbial community living and dead (necromass) and physical soil attributes like microporosity (Buckeridge et al., 2022; Oades, 1984; Soares et al., 2022; Wang et al., 2021). Moreover, this concept is supported by our finding where SOC stock increases with an increasing F: B ratio. Generally, bacteria are located in the micropores within the microaggregates, whereas fungi are restricted to larger pores within or between macroaggregates (Ranjard et al., 2003). Furthermore, higher C stocks in fine fractions also could be attributed to microbial necromass trapped in fine soil aggregates (Wang et

al., 2021). Microbial necromass contributes to long-term SOC stabilization by forming mineral-associated C by physical or chemical adsorption to clay particles and hydroxide (Cotrufo et al., 2019; Sokol et al., 2019). Moreover, the C: N ratio decreased with increasing GP: GN in the coarse fraction because a higher GP: GN ratio indicates more labile plant-derived C (Fanin et al., 2019). Furthermore, gram-negative bacteria comprised of rapidly growing form dominate at the early stage of decomposition targeting labile C. In contrast, later stages of decomposition are dominated by gram-positive bacteria (Francisco et al., 2016) that excel at decomposing complex organic C compounds (Fanin et al., 2019; Herman et al., 2012). Hence, it is likely that soil organic C in different fractions was associated with different microbial communities.

Although there was a higher SOC stock and concentration in bulk soil under AMP grazing, the effect of grazing was non-significant, likely due to the low sample size. However, there was a shift to more stable C under AMP grazing, demonstrated through higher C in fine fraction and availability of higher soil microaggregates vs. higher macroaggregates and SOC in coarse fractions under conventional grazing. Those results indicate that under AMP grazing more fine/clay particles could be protected from being lost through erosion; whereas, under conventional grazing more fine/clay particles could be eroded over time due to lower soil protection from litter and live vegetation, leading to lower microaggregates and clay contents in soil (Chartier and Rostagno, 2006). Hence, AMP might be building more C over time, but the increase was not large enough to detect in bulk soil.

4.4.3 Management metrics affect C and N concentration in different fractions

Soil organic C and N in coarse and medium fractions were influenced by stocking rate and stocking density. Ranches practicing high stocking rates and high stocking density led to decreased SOC concentration in coarse and medium fractions of the soil, which might be due to

reduced plant residue and destruction of soil aggregates (Soares et al., 2022). Livestock trampling under higher stock density can physically destroy soil aggregates (Abdalla et al., 2018), while a heavy stocking rate may reduce plant residues on the soil surface (Krzic et al., 2006). In contrast, low to medium stocking rates and a low to medium stocking density can increase SOC concentrations within coarse and medium soil fractions, thereby supporting the expectation that a low stocking density leads to less physical disturbance of soil aggregates (Wang et al., 2020). This pattern is supported by previous studies where low to medium stocking rates increased SOC in grassland soil (Abdalla et al., 2018; Han et al., 2008; Ganjegunte et al., 2005). Hence, heavily grazed grasslands, as compared to moderately grazed grasslands, have a lower SOC concentration in coarse and medium fractions, which is likely due to a breakdown of macroaggregates (Wang et al., 2020).

4.4.4 Climate and grazing interactively affect SOC in different fractions

Climate is an important driver of SOC storage across large geographical regions (Hewins et al., 2018); we found that SOC concentrations in bulk soils and within different size and density fractions decreased with warmer and drier climatic conditions. This pattern is consistent with many previous studies that demonstrate SOC concentrations increase from warmer and drier climates to cooler and wetter conditions (Doetterl et al., 2015; Hewins et al., 2018; Throop et al., 2021; Zeng et al., 2021). Tentatively, this pattern raises concerns about SOC storage in grassland soil as the climate becomes more arid with climate change (Parton et al., 1995). Warmer and drier climates decrease plant productivity and the input of organic carbon to the soil (Zeng et al., 2021). Further, the lower SOC concentrations in warmer, drier environments may relate to the lower soil fungal diversity (Khatri-Chhetri et al., n.d.). Aridity influences the microbial decomposition of SOC (Davidson and Janssens, 2006; von Lützow and Kögel-Knabner, 2009;

Zeng et al., 2021) and higher temperature may increase decomposition rates (Six et al., 2002; von Lützow et al., 2007; Zeng et al., 2021).

In the present study, the effect of grazing treatment on soil C and N properties depended on aridity; however, increases in SOC stock under AMP grazing decreases with increasing aridity like in conventional grazing when extremely arid sites (one pair) were removed, indicating that more studies are required to understand the trend. Thus, we suggest that a larger sample size, spanning a greater range of climate conditions, will be needed to verify the effect of AMP grazing on SOC across broader geographical regions.

4.5 Conclusion

Grazing affected the distribution of soil C, both based on particle size and density fractionation assessments. Our finding suggests that both the overall grazing treatment and grazing management metrics, such as stocking density, affected soil aggregate distribution. Further, grazing treatment and stocking density directly influence SOC and N stocks and concentrations within different soil pools. Macroaggregates was higher under conventional grazing, whereas microaggregates and fine soil fractions were higher in soil subject to AMP grazing, which may be due to the effect of high animal impacts breaking down vegetation residues through trampling. Furthermore, AMP grazing promoted SOC stocks in the fine fractions and potentially enhanced C stability in association with clay particles and F: B ratios. Stocking rate and stocking density were also important management metrics that influenced SOC concentrations in different soil fractions. This study also highlighted the relationship between soil C and soil microbial community composition, suggesting more research is required to better understand their contribution to soil carbon sequestration within different pools. Our study also suggests that the

adoption of AMP grazing could potentially help sequester more C by enhancing soil C stability, which could aid efforts to offset greenhouse gas emissions in the long-term.

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Tables

Table 4. 1**:** Summary (mean± SE) of physical and chemical properties at 12 paired locations. Ranches either practiced Adaptive multi-paddock grazing or conventional approaches (Conventional). Mixed effect model was used to test the grazing effect on soil properties, where grazing system was used as fixed factor and ranch pair was random effect. Bold text with * indicates significant effects ($P \leq 0.1$).

Soil properties	AMP	Conventional
pH	6.2 (0.1)	6.4 (0.1)
Bulk Density (g cm-3)	0.75 (0.04)	0.74 (0.05)
Sand (%)	**37.1 (6.1)**	**45.0 (4.4)***
Silt (%)	36.8 (3.7)	33.6 (3.0)
Clay (%)	**26.1 (3.8)**	**21.4 (2.8)***

Table 4. 2: Summary of mean (±SE) fractional soil mass (%) and total soil recovered after segregation of different particle size and density fraction from soils under adaptive multipaddock grazing (AMP) and conventional grazing system. *P* value derived from MANOVA tests evaluating grazing system effect on soil mass distribution (%) on the abundance of three separate particle size fractions and two separate density fractions. A separate analysis was done for size and density fractions. Bold text with * indicates significant effects ($P \leq 0.1$).

Soil Fraction Type	Fraction (%)	AMP	Conventional
Size Fraction	Coarse (>250µm)	35.2 (2.7)	40.5 (3.0)
	Medium (53-250 µm)	**25.3 (1.5)**	**21.6 (1.3) ***
	Fine (<53µm)	30.6 (2.2)	28.0 (3.1)
	Total soil recovered	91.1 (1.3)	90.1 (0.8)
Density Fraction	Light	1.9 (0.3)	2.8 (0.7)
	Heavy	**94.8 (1.7)**	**90.9 (1.4) ***
	Total soil recovered	96.7 (1.7)	93.7 (1.2)

Table 4. 3: Summary of mean (±SE) concentration and stock of soil organic carbon, total nitrogen and C: N under Adaptive Mulitpaddock grazing (AMP) and conventional grazing system in different size and density fractions and bulk soil. Bold text with * indicates significant effects ($P \leq 0.1$).

Soil Type	Grazing system	SOC concentration ($g\ kg^{-1}$)	SOC stock ($Mg\ ha^{-1}$)	N concentration ($g\ kg^{-1}$)	N stock ($Mg\ ha^{-1}$)	C:N
Size Fraction						
Coarse	AMP	53.6 (6.4)	19.8 (2.9)	4.1 (0.5)	1.5 (0.2)	**11.9 (1.2)***
(>250µm)	Conventional	63.6 (7.9)	26.9 (3.9)	4.7 (0.6)	2.0 (0.3)	**13.8 (0.5)***
Medium	AMP	45.5 (0.3)	12.7 (1.5)	3.5 (0.3)	0.98 (0.1)	13.2 (0.4)
(53-250 µm)	Conventional	56.8 (0.7)	12.5 (1.7)	4.4 (0.8)	0.97 (0.1)	13.1 (0.3)
Fine	AMP	**63.6 (7.6)***	**21.7 (3.7)***	5.3 (0.4)	1.7 (0.1)	11.9 (0.8)
(<53µm)	Conventional	**57.2 (7.6)***	**16.4 (2.5)***	5.1 (0.6)	1.5 (0.2)	10.9 (0.3)
Density Fraction						
Light	AMP	184 (20.5)	4.03 (0.8)	12.3 (1.9)	0.3 (0.06)	15.6 (0.7)
	Conventional	163 (8.4)	4.77 (0.9)	10.4 (0.6)	0.3 (0.07)	15.9 (0.5)
Heavy	AMP	37.6 (3.3)	39.9 (4.3)	3.1 (0.3)	3.2 (0.3)	12.4 (0.4)
	Conventional	50.1 (7.7)	47.6 (6.8)	3.9 (0.6)	3.8 (0.5)	12.6 (0.2)
Bulk Soil	AMP	64.3 (9.2)	70.1 (8.9)	5.0 (0.7)	5.4 (0.6)	**13.2 (0.6)***
	Conventional	58.8 (9.5)	61.1 (9.5)	4.7 (0.8)	4.9 (0.7)	**12.5 (0.7)***

Figures

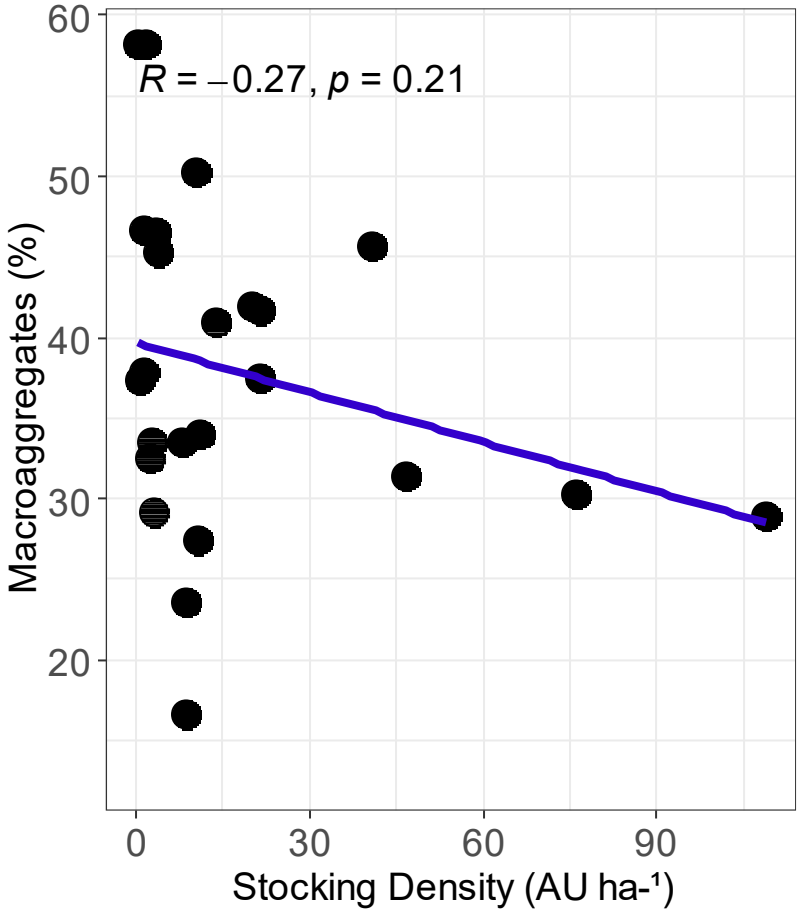

Figure 4. 1: The relationship between macroaggregates distribution (%) and stocking density (AU ha^{-1}).

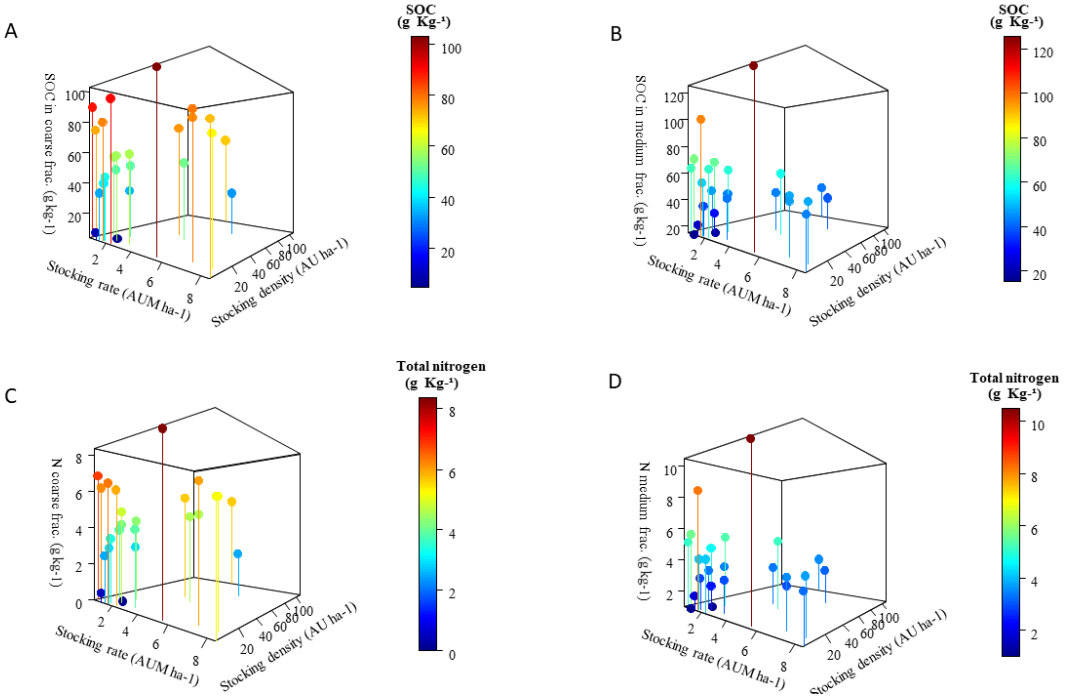

Figure 4. 2: The relationship between SOC and total N concentration and stocking density and stocking rate in coarse and medium fractions.

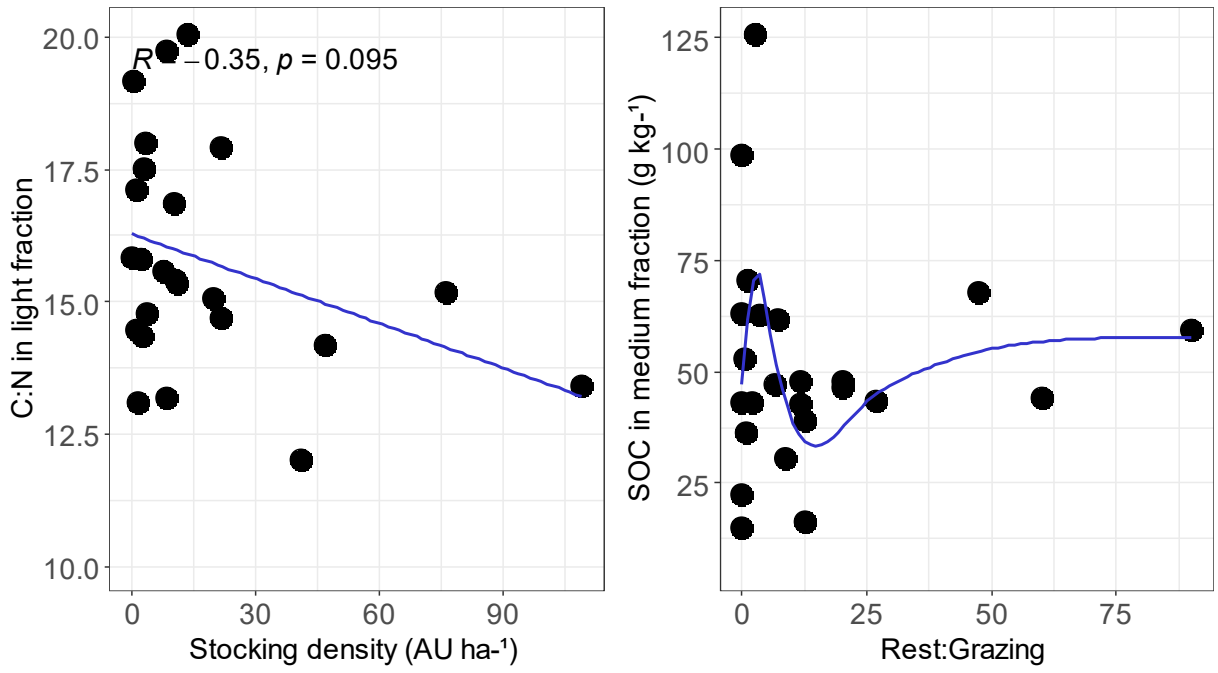

Figure 4. 3: The relationship between **A.** C: N in light fraction and stocking density and **B.** soil organic carbon in medium fraction and rest-to-grazing ratio.

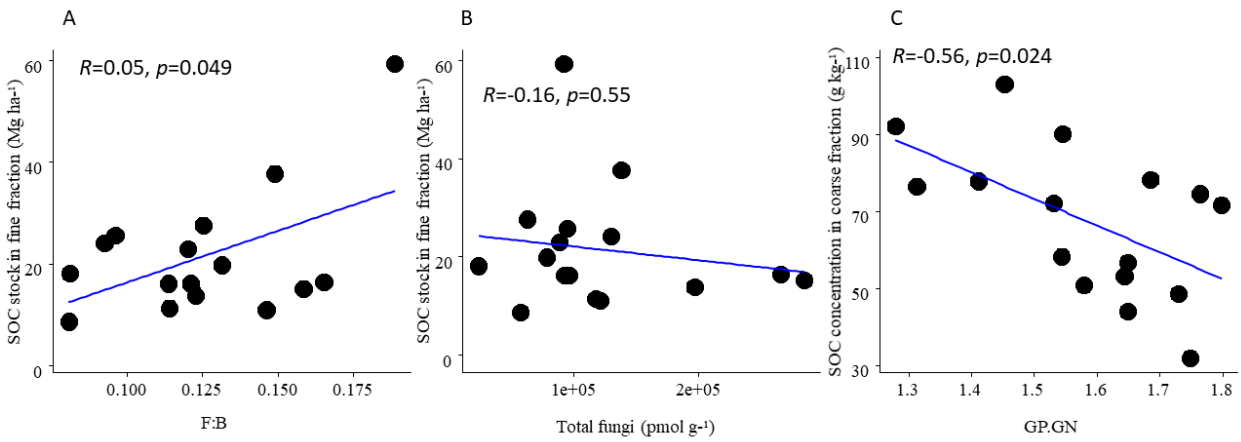

Figure 4. 4: The relationship between **A.** soil organic carbon stock and soil F:B ratio in fine fraction; **B.** soil organic carbon stock and fungal abundance in fine fraction; **C.** soil organic carbon concentration and gram positive: gram negative (GP: GN) ratio in coarse fraction

Chapter 5. Conclusions and implications for future research

5.1 Overview of the study

Agriculture, particularly cattle production is responsible for the emission of large volumes of greenhouse gases (GHG) that contribute to climate change. Grasslands are known to hold large amounts of carbon in their soil and are used extensively for grazing livestock. Livestock grazing can affect the soil microbial community below ground. Soil microbes are a vital component of grassland ecosystems, play a major role in soil C storage, and affect greenhouse gas emissions from soil. Soil microbial response and subsequent effects on soil C storage at different pool sizes to different grazing systems are understudied. In order to evaluate the benefits of a grazing system for storing soil C and eventually reducing atmospheric greenhouse gases, I have examined the soil microbial community and related this data to measurements of carbon pools.

The objectives of my thesis were to 1) to understand the overall grazing system effect (AMP vs. conventional grazing) on soil microbial community, (2) to assess how specific management metrics such as stocking rate, stocking density, and rest periods mediate in shaping microbial community under different grazing systems, and (3) to gain a comprehensive understanding of how grazing system and specific management metrics shape SOC at different size and density fractions. To accomplish these objectives, I have collected soil samples from 38 ranches across a large area of the northern Great Plains in the Canadian prairie provinces of Alberta, Saskatchewan, and Manitoba from paired designed ranches where one ranch practices AMP grazing whereas the other ranch in the pair practices non-AMP, which is known as conventional grazing in this study. These ranches were practicing specific management systems for at least 5 years. To address the first and second objectives, I have used multiple molecular

and microbial techniques, which provided me with overall soil microbial community measures from domain to species level.

This thesis research provided a more complete understating of grazing system effects on soil microbial community and SOC held in different fractions under AMP and conventional grazing systems in Canadian prairie. This project uses pairwise design to compare two different grazing systems (AMP vs. conventional way of grazing), while previous studies were mostly conducted either grazed vs. non-grazed or continuous vs. rotational grazing. This approach is important for two reasons. First, there has been criticism that researchers cannot replicate the adaptive management conducted by experienced ranchers within controlled experiments (Savory and Butterfield, 2016). The ranchers participating in this study all had at least 10 years of experience with their grazing system ensuring that we measured "real world" results. Secondly, not all AMP ranchers conduct AMP ranching in the same way, the variation among ranchers allowed me to identify what aspects of their grazing management contributed to the outcomes (Bork et al., 2021). This study was unique because it employed a large number of commercial ranches in contrast to many previous studies that have used either a limited number of study sites or employed small experimental plots only. In this study, conventional grazing includes all types of practiced grazing systems ranging from continuous to rotational grazing, which enable a comparison of AMP grazing to typical practices and provided ranch level quantification of the benefits of different management practices on carbon storage.

5.2 Research result summary and implication to management

Overall AMP grazing enhance bacterial and fungal richness, fungal diversity and B:F ratios that are expected to increase soil C. AMP grazing alters microbial communities by altering soil's physical and chemical properties. I found that AMP grazing reduces F:B, GP:GN, and MBC: MBN ratio than conventional grazing by promoting soil nutrients. Labile nutrients like dissolved organic C were higher in AMP grazing soil, which promotes bacterial abundance in the system, which can take over the early phase of the soil organic C decomposition process. Further, this study demonstrated that AMP grazing increases network complexity, microbial richness evenness, and diversity by altering soil's properties like pH. Fungal diversity can promote plant diversity and productivity because soil microbes provide different resources to vegetation (Van Der Heijden et al., 2008). Higher fungal diversity reduces soil organic matter decomposition and can offset CO_2 emissions (Yang et al., 2016). Thus, the higher diversity and co-occurrence of members of microbial communities under AMP grazing may be indicative of greater ecological balance and complexity of the microbiome, which can be more resilient to environmental stresses (Banerjee et al., 2019).

Management metrics such as stocking rate, stocking density, and rest periods were equally involved in shaping the microbial community. Stocking rate reduced microbial biomass carbon to nitrogen ratio, but increased bacterial and fungal richness and network complexity, indicating the presence of a more resilient microbial community when pastures were intensely grazed. Similarly, stocking density reduces F:B ratio, likely because the heavy trampling associated with high stocking density creates a physical disturbance that destroys fungal hypha (Bardgett et al., 2001; Kaur et al., 2010). In contrast, a long rest period was beneficial for fungal richness and diversity. The positive association of rest-to-grazing and fungal richness and

diversity indicates the importance of rest duration specifically for the fungal community. Overall, specific grazing management metrics represnet different components of the ecosystem highlighting the complex interactions between grazing and soil communities and processes.

Grazing system and stocking density affected the distribution of the different sizes of soil aggregates, and AMP grazing enhanced the concentration of C within silt-size microaggreagtes. In particular, AMP increased clay particles which can protect C and may foster long-term SOC sequestration and stabilization (Arrouays et al., 2006; Assunção et al., 2019; Jones and Edwards, 1998; Six et al., 2002). However, aridity is equally an important driver of SOC across large geographical regions, where it influences the effect of grazing treatment on soil carbon and nitrogen properties. My study demonstrated that AMP can stabilize clay-protected C within silt-size microaggreagtes by influencing soil F: B and GP: GN ratio. Thus, my study supports the idea that AMP grazing is generally beneficial for increased soil carbon storage and resilient soil microbiome which can help to meet climate change mitigation goals.

Considering the global demand to address climate change; increasing C storage and retention in the soil through biological means would be a significant and sustainable way to reduce GHG emissions and meet climate change mitigation goals. Beef production is one of the largest revenue-generating agriculture industries in Canada which contribute $21.8 billion to Canada's GDP. While beef production is largely criticized for its contribution to atmospheric GHGs through enteric methane production by cattle, it is not recognized for the ability of grazing systems to be used as a tool to increase soil carbon stores. Given the vast extension of Canadian prairies which are primarily used for grazing beef cattle (Bailey et al., 2010) they could be a significant C sink if managed properly. This may , help Canada in reducing its net C emission. Based on the findings from this thesis research, AMP grazing increases biological indicators of

soil C storage both in quantity and stability. My finding demonstrated how AMP grazing store SOC for a long duration by promoting C in fine fractions could be as stabilized clay-protected C (Keiluweit et al., 2015; Yang et al., 2021) and enhance fungal diversity and richness, which potentially can slow down the decomposition process and CO_2 emission from soil (Yang et al., 2016). Additionally, AMP grazing could lead to a potentially more resilient and resistant soil ecosystem than the conventional type of grazing by altering soil properties like pH and texture and by generating complex microbial networks, higher microbial richness, and diversity. Consequently, the adoption of AMP grazing should generally have a positive impact on grassland soil carbon storage in long run. However, to reach the full potential of AMP grazing to mitigate climate change, specific management metrics should be considered. While most previous studies examining livestock management on soil accounted for stocking rate only and not stocking density and rest periods, which may contribute to the confusion in the literature on how grazing ultimately affects SOC. My findings highlighted that stocking density and rest periods are equally important management metrics besides stocking rate in structuring microbial community and C storage in soil. Given both enhance microbial diversity and complexity and stable C sequestration potential of AMP grazing, my finding supports the idea that AMP grazing is generally increase soil carbon storage and resiliency of rangelands.

5.3 Future research needs

This thesis has generated additional questions that could be addressed in future studies. Grazing management system effects assessed by different managements metrics on soil microbial community was evaluated in detailed by various molecular and microbial techniques. However, how various environmental factors including vegetation, litter and root shape soil biota under different grazing system is missing. Future studies can assess the environmental factors

such as surface litter, soil type, vegetation type and plant root biomass effects on soil microbial community to gain insights into the functioning of soil ecosystems. In the future, more efforts should be given to explore the spatial heterogeneity of microbial communities by soil depth, which can provide a better understanding of how microorganisms respond to grazing. Further, future studies should investigate the temporal stability of soil microbial communities by collecting soil samples at temporal intervals over a period of time and analyze changes in microbial composition and activity. Temporal stability is an important aspect of microbial community dynamics and can help us understand the resilience and stability of soil ecosystems. Additionally, detailed microbial involvement in different C pools and necromass quantification is lacking, future studies need to investigate how the microbial community changes lead to different pools of C in different soil fractions and under what conditions/mechanisms the soil microbial community forms stable SOC pools under different grazing systems. Further, this study suggests investigating the direct relationship between soil microbial community and GHG emission under both grazing systems by targeting functional genes (e.g., protein-encoding genes). My findings suggested stocking density and rest periods are equally important management metrics besides stocking rate, highlighting the need to identify regional-specific grazing metrics that best suited for C storage.

5.4 Summary

In summary, my study aids inthe understanding the effect of the cattle grazing system and specific grazing managements such as stocking rate, stocking density, and rest-to-grazing ratio on soil microbial communities and subsequent effects on SOC storage. Overall, it appears that AMP grazing is beneficial for soil carbon storage. To our knowledge, this is the first study to report in detail soil microbial diversity, network complexity, and keystone taxa for AMP grazing

in Northern Great Plains. Maintaining AMP grazing with high stocking density and long rest periods may enhance soil microbial richness and diversity, promoting C sequestration and stability (Malik et al., 2016; Waring et al., 2013). I recommend incentivizing the adoption of AMP grazing as one of the important steps to help meet climate change mitigation goals by raising C sequestration and reducing GHG emissions. Thus, the findings of this study have improved our understanding of soil microbial community response to grazing management and have provided important guidelines for ranchers and policymakers to encourage practices AMP that has the potential to offset atmospheric climate change by storing more C in soil and slow down C cycling. Thus, this study highlights a potential opportunity for the beef industry and Canada to fight climate change. This is likely doubly beneficial for producers, as AMP grazing also increases above ground forage production (Grenke et al., 2022) and reduces drought risk (Döbert et al., 2021), although support may be needed to enable adoption because of increased fencing, watering, and labour needs. For Canada, such innovation can help the country to meet its GHG reduction targets and goals.

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Appendix A. Chapter 2: Supplementary data

Supplementary Table 1: Summary of sampling site detail. Predictor variables are grouped by grassland management treatment (Multi-paddock and conventional) and grazing metrics (SR= Stocking rate (AUM ha $^{-1}$), SD= Stocking density (AU ha $^{-1}$), R:G= rest to grazing ratio), as well as climate. BT: Boreal Transition; AP: Aspen parkland; FG: Fescue grasslands; MG: Mixedgrass; Conve: Conventional grazing; Elevation = Average elevation of a ranch (m); MAT = Mean annual temperature (deg C), MAP = Mean annual precipitation (mm), AHM = Annual heat: moisture index derived from MAT and MAP (AHM= (MAT+10)/ (MAP/1000))

			Grassland Management					Climate		
RanchID	**Ecoregion**	**Soil order**	**Grazing**	**SR** (AUM ha $^{-1}$)	**SD** (AU ha $^{-1}$)	**R:G**	**Elevation** (m)	**MAT** (^{0}C)	**MAP** (mm)	**AHM**
AB02AMP	BT	Luvisolic – Gray	Multi-padd	4.50	40.87	90.00	981	3.15	515.87	25.92
AB02nAMP	BT	Luvisolic – Gray	Conve.	3.12	7.78	2.11	985	3.14	516.33	25.88
AB03AMP	AP	Chernozemic – Black	Multi-padd	1.70	8.43	6.43	1123	3.30	469.70	29.03
AB03nAMP	AP	Chernozemic – Black	Conve.	0.33	2.96	0.00	1132	3.29	469.60	29.04
AB05AMP	BT	Luvisolic – Gray	Multi-padd	3.72	46.80	60.00	739	3.00	456.43	29.32
AB05nAMP	BT	Luvisolic – Gray	Conve.	8.39	1.19	0.00	717	2.96	455.63	29.26
AB08AMP	AP	Chernozemic – Black	Multi-padd	5.78	75.98	12.50	839	3.14	464.43	28.85
AB08nAMP	AP	Chernozemic – Black	Conve.	1.50	1.61	0.00	840	3.14	464.20	28.86
AB13AMP	AP	Chernozemic – Black	Multi-padd	3.76	108.78	11.67	672	2.22	389.27	32.69
AB13nAMP	AP	Chernozemic – Black	Conve.	1.56	2.72	0.84	672	2.20	388.53	32.74
MB32AMP	AP	Regosolic – Black	Multi-padd	3.32	8.28	40.00	439	3.11	513.30	26.99
MB32nAMP	AP	Regosolic – Black	Conve.	2.34	0.52	0.00	440	3.12	513.33	27.00

MB34AMP	AP	Chernozemic – Black	Multi-padd	5.33	15.09	80.00	409	2.61	518.07	25.25
MB34nAMP	AP	Chernozemic – Black	Conve.	4.07	0.74	0.00	420	2.57	518.30	25.19
MB35AMP	AP	Chernozemic – Black	Multi-padd	2.87	15.66	21.67	465	3.20	533.60	25.51
MB35nAMP	AP	Chernozemic – Black	Conve.	2.15	1.98	1.67	469	3.24	532.03	25.66
SK14AMP	FG	Chernozemic – Brown	Multi-padd	2.85	13.04	18.75	891	4.51	433.17	35.08
SK14nAMP	FG	Chernozemic – Brown	Conve.	3.43	4.11	0.80	877	4.60	430.70	35.49
SK20AMP	BT	Chernozemic – Black	Multi-padd	2.18	13.69	20.00	521	1.56	461.80	26.13
SK20nAMP	BT	Chernozemic – Black	Conve.	4.38	5.74	0.00	516	1.57	460.97	26.17
SK21AMP	AP	Chernozemic – Black	Multi-padd	2.64	25.72	30.00	618	2.40	428.50	30.32
SK21nAMP	AP	Chernozemic – Black	Conve.	1.26	1.57	2.63	641	2.28	438.70	29.31
SK24AMP	AP	Chernozemic – Black	Multi-padd	0.78	25.66	45.00	611	1.40	473.40	25.05
SK24nAMP	AP	Chernozemic – Black	Conve.	1.89	2.67	2.50	649	1.44	470.63	25.29
SK28AMP	AP	Chernozemic – Black	Multi-padd	5.32	45.35	60.00	622	2.58	509.30	25.78
SK28nAMP	AP	Chernozemic – Black	Conve.	2.80	2.22	3.43	616	2.59	508.30	25.85
SK29AMP	AP	Chernozemic – Black	Multi-padd	9.01	142.86	105.00	656	2.74	528.70	25.40
SK29nAMP	AP	Chernozemic – Black	Conve.	1.99	0.37	0.00	659	2.74	528.90	25.39
SK17AMP	MG	Chernozemic – Brown	Multi-padd	1.79	11.67	90.00	845	4.67	364.77	42.99
SK17nAMP	MG	Chernozemic – Brown	Conve.	1.86	1.11	0.00	861	4.63	367.10	42.61

Supplementary Table 2: Summary of grazing management practices employed at 15 paired locations. Ranches either practiced multi-paddock grazing, or were neighboring ranches using conventional approaches (Conventional).

Management practices	**Multi-paddock**		**Conventional**		**p-value**
	mean	**sd**	**mean**	**sd**	
Stocking Density (AU ha $^{-1}$)	39.9	40.1	2.5	2.1	**0.002**
Stocking Rate (AUM ha $^{-1}$)	3.70	2.1	2.7	1.9	0.186
Rest to Grazing	46.1	32.8	0.9	1.2	**0.02**

Supplementary Table 3: PLFA identification key based on Microbial Identification Inc (MIDI) system.

Microbial Group			Specific PLFA markers	Reference
Total Bacteria	Total Gram positive	Gram positive	14:0 iso; 14:0 anteiso; 15:0 iso; 15:0 anteiso; 15:1 iso w9c; 15:1 iso w6c; 15:1 anteiso w9c 16:0 iso; 16:0 anteiso; 17:0 iso;17:0 anteiso 17:1 iso w10c; 17:1 iso w9c; 17:1 anteiso w9c; 17:1 anteiso w7c; 18:0 iso; 19:0 iso; 20:0 iso	O'Leary 1988; Zelles, 1999; Kaur et al., 2005; Kaiser et al., 2010; Ruess and Chamberlain, 2010; Buyer and Sasser, 2012; Feng et al., 2003
		Actinomycetes	16:0 10-methyl; 17:0 10-methyl; 17:1 w7c 10-methyl; 18:0 10-methyl; 18:1 w7c 10-methyl; 19:1 w7c 10-methyl; 20:0 10-methyl	Edlund et al, 1985; O'Leary and Wilkinson 1988; Kroppenstedt 1992; Zelles 1999
	Gram negative		14:1 w5c; 14:1 w7c; 14:1 w9c; 15:1 w5c; 15:1 w6c; 15:1 w7c; 15:1 w9c; 16:1 w6c; 16:1 w7c; 16:1 w8c, 16:0 2OH; 17:1 w8c; 17:0 cyclo w7c; 17:0 iso 3OH; 18:1 w5c; 18:1 w6c; 18:1 w7c; 19:1 w6c; 19:1 w8c; 19:0 cyclo w7c; 19:0 cyclo w9c; 20:1 w4c; 20:1 w6c; 20:1 w8c; 20:1 w9c	Zelles, 1999; Feng et al., 2003; Buyer and Sasser, 2012; Kaiser; Mathew et al., 2012; Quideau et al. 2016
	Anaerobe		12:0 DMA, 13:0 DMA, 14:1 w7c DMA, 14:0 DMA, 15:0 iso DMA, 15:0 DMA, 16:2 DMA, 17:0 DMA, 16:1 w9c DMA, 16:1 w7c DMA, 16:1 w5c DMA, 16:0 DMA, 18:2 DMA, 18:1 w9c DMA, 18:1 w7c DMA, 18:1 w5c DMA18:0 DMA, 19:0 cyclo 9,10 DMA	White et al, 1999
Total Fungi	AM Fungi		16:1w5c	Federle 1986; Frostegård and Bååth (1996); Quideau et al. 2016
	Fungi		18:2w6c	Federle 1986; Frostegård and Bååth (1996); Quideau et al. 2016

Supplementary Table 4: Contribution (in percentage) of soil variables to each of seven principal components (PCs) resulting from the PCA analysis. The primary contributors to each PC are in bold.

Metric	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Sand: Clay	4.6	3.5	23.6	61.8	1.3	4.7	0.5
BD	9.0	45.1	0.7	1.1	15.2	28.7	0.2
pH	3.0	14.9	39.9	28.1	0.0	13.8	0.1
N	31.1	0.8	3.1	0.4	2.0	8.8	53.8
C	29.1	0.6	4.4	2.5	5.4	12.7	45.3
Water-extractable C	13.6	8.7	24.6	1.4	21.1	30.6	0.0
Water-extractable N	9.5	26.4	3.7	4.7	54.9	0.7	0.1

Supplementary Table 5: Coordinate (direction) of soil variables for all seven principal components (PC) resulting from the PCA analysis.

Metric	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Sand:Clay	0.37	0.20	0.51	-0.73	0.09	-0.12	-0.01
BD	0.51	0.74	-0.09	0.10	0.30	0.29	-0.01
pH	0.30	-0.43	-0.67	-0.50	0.01	0.20	-0.01
N	-0.95	-0.10	0.18	-0.06	0.11	0.16	-0.12
C	-0.92	-0.09	0.22	-0.15	0.18	0.20	0.11
Water-extractable C	-0.63	0.33	-0.52	-0.11	0.35	-0.30	0.00
Water-extractable N	-0.52	0.57	-0.20	-0.20	-0.57	0.05	0.01

Supplementary Table 6: Results of the ANOVA test (mixed effect model) for the effect of grazing system and AHM on each soil characteristic. Bold indicates significant effects ($p<0.05$)

Metric		Intercept	Grazing	AHM	Metric		Intercept	Grazing	AHM
DOC	F	47.748	6.514	0.53	**pH**	F	59.097	0.016	0.507
	Df.res	13.277	13.993	13.11		Df.res	13.301	13.995	13.085
	Pr(>F)	0.000	**0.023**	0.48		Pr(>F)	0.000	0.900	0.489
DON	F	6.328	6.584	0.04	**Bulk Density**	F	14.604	0.037	0.524
	Df.res	13.395	13.997	13.05		Df.res	13.515	13.998	13.039
	Pr(>F)	0.025	**0.022**	0.84		Pr(>F)	0.002	0.850	0.482
Total Carbon	F	31.071	3.167	1.69	**Clay**	F	5.053	3.885	0.003
	Df.res	13.328	13.996	13.07		Df.res	13.270	13.992	13.138
	Pr(>F)	0.000	0.090	0.22		Pr(>F)	0.042	0.069	0.956
Total Nitrogen	F	7.624	1.716	0.23	**Silt**	F	3.075	6.494	0.867
	Df.res	13.309	13.995	13.08		Df.res	13.273	13.993	13.116
	Pr(>F)	0.016	0.211	0.64		Pr(>F)	0.103	**0.023**	0.369
C:N	F	463.284	1.177	12.65	**Sand: Clay**	F	10.570	0.036	0.536
	Df.res	13.694	13.998	13.03		Df.res	13.272	13.993	13.122
	Pr(>F)	0.000	0.296	**0.00**		Pr(>F)	0.006	0.852	0.477

Supplementary Table 7: Results of the ANOVA test (mixed effect model) for the effect of grazing system and AHM on individual and total soil microbial PLFA functional group abundance.

PLFA Group		Intercept	Grazing	AHM
Gram positive	F	10.93	0.18	3.9
	Df.res	13.34	13.99	13.07
	Pr(>F)	0.005	0.67	0.69
Actinomycetes	F	2.15	0.005	0.64
	Df.res	13.37	13.99	13.05
	Pr(>F)	0.16	0.93	0.43
Gram negative	F	2.33	0.28	3.39
	Df.res	13.56	13.99	13.03
	Pr(>F)	0.14	0.60	0.08
Anaerobic	F	359.14	0.24	3.04
	Df.res	13.27	13.99	13.12
	Pr(>F)	0.001	0.62	0.10
Total Bacteria	F	1.28	0.02	2.94
	Df.res	13.39	13.99	13.05
	Pr(>F)	0.27	0.87	0.10

Supplementary table 8: Results of model selection accomplished through dredge function in the MuMIn package in R software. Models tested individual grazing management practices (stocking density (SD), stocking rate (SR) and rest to grazing ratio (R:G)) for each microbial marker (Fungi to Bacteria ratio (F:B); gram positive bacteria (GP); gram negative bacteria (GN); gram positive to gram negative bacteria (GP: GN); microbial biomass carbon (MBC); microbial biomass nitrogen (MBN)) across 15 ranch pairs. Ranch pair was specified as a random effect to address geographic variation in climate and soils. Bold indicates the model with the lowest AICc (model within 2 AIC are considered equally plausible).

Response	Model	AICc	Delta AICc	log-Likelihood	AICc weight
MBC	**Null**	**42.13**	**0.00**	**-17.60**	**0.91**
	SR	46.96	4.83	-18.68	0.08
	SD	53.82	11.69	-22.11	0.00
	R:G	54.68	12.55	-22.54	0.00
	SD+SR	60.08	17.95	-23.79	0.00
	R:G+SR	60.42	18.28	-23.96	0.00
	R:G+SD	66.93	24.80	-27.21	0.00
	R:G+SD+SR	73.50	31.37	-28.92	0.00
MBN	**Null**	**28.20**	**0.00**	**-10.64**	**0.98**
	SR	35.87	7.67	-13.13	0.02
	SD	42.51	14.31	-16.45	0.00
	R:G	42.54	14.34	-16.47	0.00
	SD+SR	49.60	21.40	-18.55	0.00
	R:G+SR	50.31	22.11	-18.90	0.00
	R:G+SD	56.48	28.28	-21.99	0.00
	R:G+SD+SR	63.88	35.68	-24.11	0.00

MBC:MBN	**Null**	**53.74**	**0.00**	**-23.41**	**0.77**
	SR	**56.25**	**2.52**	**-23.33**	**0.22**
	SD	63.81	10.07	-27.11	0.01
	R:G	64.51	10.77	-27.45	0.00
	SD+SR	68.74	15.00	-28.12	0.00
	R:G+SR	68.84	15.10	-28.17	0.00
	R:G+SD	76.23	22.49	-31.86	0.00
	R:G+SD+SR	81.45	27.71	-32.90	0.00
GP:GN	**Null**	**-14.48**	**0.00**	**10.70**	**0.99**
	SR	**-5.09**	**9.39**	**7.35**	**0.01**
	R:G	-0.84	13.64	5.22	0.00
	SD	0.63	15.11	4.48	0.00
	R:G+SR	8.32	22.80	2.09	0.00
	SD+SR	9.65	24.13	1.42	0.00
	R:G+SD	12.44	26.92	0.03	0.00
	R:G+SD+SR	21.80	36.28	-3.07	0.00
AMF	**SR**	**320.79**	**0.00**	**-155.59**	**0.48**
	SD+SR	323.10	2.31	-155.30	0.15
	R:G+SR	323.12	2.33	-155.31	0.15
	Null	324.27	3.49	-158.67	0.08
	R:G	325.76	4.97	-158.08	0.04
	SD	325.78	5.00	-158.09	0.04
	R:G+SR+SR	325.99	5.20	-155.17	0.04
	R:G+SD	328.41	7.62	-157.95	0.01
Fungi	**SR**	**333.46**	**0.00**	**-161.93**	**0.40**
	SD+SR	**334.36**	**0.90**	**-160.93**	**0.26**

	Null	336.53	3.07	-164.80	0.09
	R:G+SR	336.64	3.18	-162.07	0.08
	R:G+SD+SR	336.67	3.20	-160.51	0.08
	SD	337.41	3.95	-163.91	0.06
	R:G+SD	339.47	6.01	-163.49	0.02
	R:G	339.69	6.22	-165.04	0.02
Total Fungi	**SR**	**338.35**	**0.00**	**-164.37**	**0.43**
	SD+SR	**339.40**	**1.05**	**-163.45**	**0.26**
	R:G+SR	340.96	2.62	-164.23	0.12
	R:G+SD+SR	342.12	3.78	-163.24	0.07
	SD	342.63	4.28	-166.51	0.05
	Null	342.78	4.44	-167.93	0.05
	R:G	344.83	6.49	-167.62	0.02
	R:G+SD	345.15	6.81	-166.33	0.01
F: B	**Null**	**-113.43**	**0.00**	**60.18**	**0.94**
	SD	-107.39	6.04	58.50	0.05
	SR	-104.84	8.59	57.22	0.01
	R:G	-96.04	17.39	52.82	0.00
	SD+SR	-94.43	19.00	53.47	0.00
	R:G+SD	-90.30	23.13	51.40	0.00
	R:G+SR	-86.22	27.21	49.36	0.00
	R:G+SD+SR	-77.06	36.36	46.36	0.00
GP	**SR**	**371.20**	**0.00**	**-180.80**	**0.40**
	SD+SR	**372.64**	**1.44**	**-180.07**	**0.20**
	R:G+SR	**372.71**	**1.51**	**-180.10**	**0.19**
	R:G+SD+SD	373.96	2.76	-179.16	0.10
	Null	375.24	4.04	-184.16	0.05
	R:G	376.77	5.57	-183.59	0.02

	SD	377.00	5.80	-183.70	0.02
	R:G+SD	378.11	6.91	-182.80	0.01
Actinomycetes	**Null**	**21.93**	**0.00**	**-7.51**	**0.98**
	SR	29.91	7.98	-10.16	0.02
	SD	35.42	13.48	-12.91	0.00
	R:G	35.79	13.86	-13.10	0.00
	SD+SR	43.23	21.30	-15.37	0.00
	R:G+SR	43.74	21.81	-15.62	0.00
	R:G+SD	48.64	26.70	-18.07	0.00
	R:G+SD+SR	56.68	34.74	-20.51	0.00
GN	**SR**	**364.67**	**0.00**	**-177.53**	**0.42**
	SD+SR	**366.18**	**1.51**	**-176.84**	**0.20**
	R:G+SR	**366.42**	**1.75**	**-176.96**	**0.18**
	R:G+SD+SR	367.92	3.26	-176.14	0.08
	Null	368.59	3.92	-180.83	0.06
	R:G	370.35	5.68	-180.37	0.02
	SD	370.39	5.72	-180.40	0.02
	R:G+SD	371.93	7.26	-179.72	0.01
Anaerobic	**Null**	**27.33**	**0.00**	**-10.20**	**0.98**
	SR	35.25	7.92	-12.82	0.02
	SD	40.91	13.58	-15.65	0.00
	R:G	41.21	13.88	-15.80	0.00
	SD+SR	48.65	21.32	-18.08	0.00
	R:G+SR	49.03	21.70	-18.27	0.00
	R:G+SD	53.86	26.54	-20.68	0.00
	R:G+SD+SR	61.82	34.49	-23.08	0.00
Total Bacteria	**Null**	**16.61**	**0.00**	**-4.84**	**0.98**

SR	24.89	8.28	-7.65	0.02
R:G	30.50	13.89	-10.45	0.00
SD	30.65	14.04	-10.52	0.00
SD+SR	38.60	21.99	-13.05	0.00
R:G+SR	38.78	22.16	-13.14	0.00
R:G+SD	44.08	27.47	-15.79	0.00
R:G+SD+SR	52.26	35.64	-18.30	0.00

Supplementary Table 9: Results of model selection accomplished through dredge function in the MuMIn package of R software. Final global model with soil principal component (PC1), AH: M and at least one best grazing management practice derived from supplementary Table 5 were used as fixed effects and ranch pair was specified as a random effect to address geographic variation in climate and soils.

	Model	AICc	Delta AICc	log Likelihood	AICc weight
MBC	**PC1**	**41.64**	**0.00**	**-16.02**	**0.52**
	Null	**42.13**	**0.49**	**-17.60**	**0.41**
	SR	46.96	5.32	-18.68	0.04
	PC1+SR	47.66	6.02	-17.58	0.03
	AHM	50.20	8.56	-20.30	0.01
	AHM+PC1	50.34	8.70	-18.92	0.01
	AHM+SR	54.88	13.25	-21.19	0.00
	AHM+PC1+SR	56.20	14.56	-20.28	0.00
MBN	**PC1**	**27.40**	**0.00**	**-8.90**	**0.57**
	Null	**28.20**	**0.79**	**-10.64**	**0.38**
	AHM+PC1	34.16	6.76	-10.83	0.02
	AHM	34.84	7.44	-12.62	0.01
	PC1+SR	35.75	8.35	-11.63	0.01
	SR	35.87	8.47	-13.13	0.01
	AHM+SR	42.67	15.27	-15.08	0.00
	AHM+PC1+SR	42.71	15.30	-13.53	0.00
MBC:MBN	**Null**	**53.74**	**0.00**	**-23.41**	**0.66**
	SR	56.25	2.52	-23.33	0.19
	AHM	57.81	4.07	-24.11	0.09

	PC1	59.55	5.81	-24.97	0.04
	AHM+SR	61.13	7.39	-24.32	0.02
	PC1+SR	62.33	8.59	-24.91	0.01
	AHM+PC1	64.06	10.33	-25.78	0.00
	AHM+PC1+SR	67.71	13.97	-26.03	0.00
GP:GN	**Null**	**-70.30**	**0.00**	**38.61**	**0.99**
	PC1	-60.77	9.53	35.18	0.01
	SR	-58.98	11.32	34.29	0.00
	AHM	-58.10	12.20	33.85	0.00
	PC1+SR	-49.19	21.10	30.85	0.00
	AHM+PC1	-48.43	21.86	30.47	0.00
	AHM+SR	-46.62	23.68	29.56	0.00
	AHM+PC1+SR	-36.67	33.62	26.16	0.00
AMF	**AHM+PC1+SR**	**316.01**	**0.00**	**-150.18**	**0.43**
	PC1+SR	**316.79**	**0.78**	**-152.14**	**0.29**
	AHM+PC1	319.19	3.18	-153.35	0.09
	AHM+SR	319.82	3.81	-153.66	0.06
	PC1	319.97	3.96	-155.18	0.06
	SR	320.79	4.77	-155.59	0.04
	AHM	323.31	7.30	-156.86	0.01
	Null	324.27	8.26	-158.67	0.01
Fungi	**AHM+PC1+SR**	**319.68**	**0.00**	**-152.01**	**0.43**
	AHM+PC1+SD+SR	**321.22**	**1.54**	**-151.06**	**0.20**
	AHM+PC1	321.85	2.17	-154.67	0.15
	AHM+SR	323.29	3.62	-155.40	0.07
	AHM+SD+SR	323.79	4.11	-154.07	0.06
	AHM+PC1+SD	323.80	4.12	-154.07	0.05
	AHM	325.78	6.10	-158.09	0.02

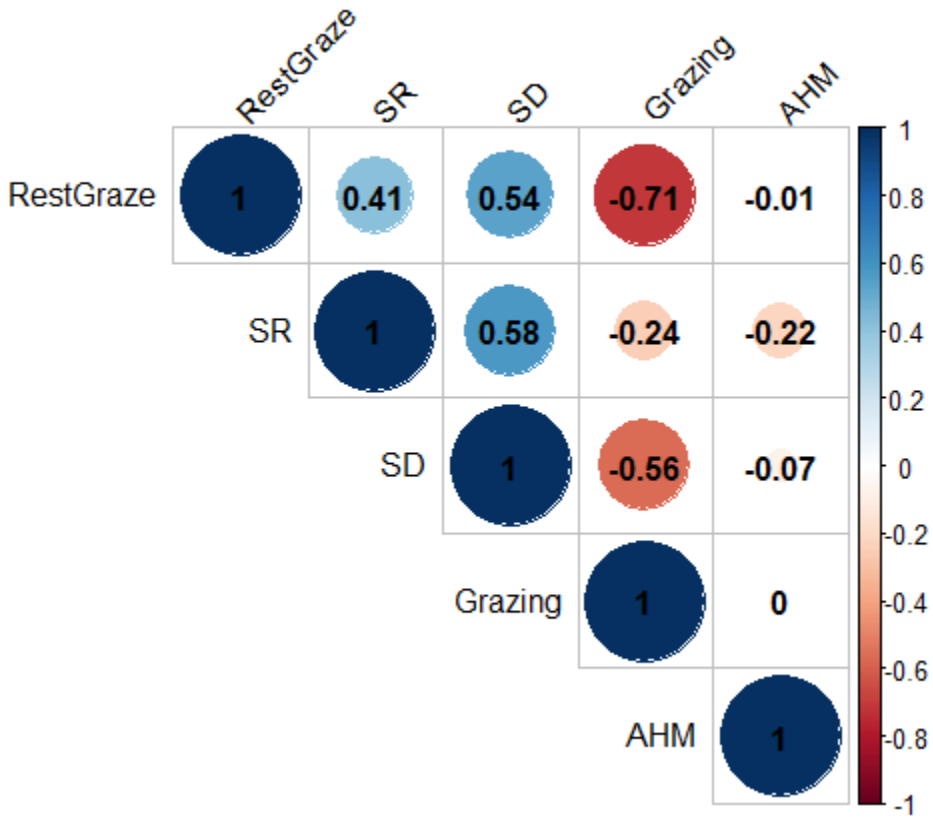
	AHM+SD	326.69	7.01	-157.10	0.01
	PC1+SR	328.62	8.94	-158.06	0.00
	PC1+SD+SR	330.69	11.02	-157.52	0.00
	PC1	331.37	11.69	-160.88	0.00
	SR	333.46	13.79	-161.93	0.00
	PC1+SD	333.47	13.79	-160.48	0.00
	SD+SR	334.36	14.69	-160.93	0.00
	Null	336.53	16.85	-164.80	0.00
	SD	337.41	17.74	-163.91	0.00
Total Fungi	**AHM+PC1+SR**	**329.95**	**0.00**	**-157.15**	**0.46**
	AHM+PC1+SD+SR	331.98	2.03	-156.45	0.17
	AHM+PC1	333.29	3.34	-160.40	0.09
	PC1+SR	333.48	3.53	-160.49	0.08
	AHM+SR	334.33	4.38	-160.91	0.05
	AHM+PC1+SD	334.67	4.72	-159.51	0.04
	AHM+SD+SR	335.32	5.37	-159.83	0.03
	PC1+SD+SR	335.49	5.54	-159.92	0.03
	PC1	337.61	7.66	-164.00	0.01
	AHM	337.97	8.02	-164.19	0.01
	AHM+SD	338.21	8.26	-162.86	0.01
	SR	338.35	8.40	-164.37	0.01
	PC1+SD	338.65	8.70	-163.08	0.01
	SD+SR	339.40	9.45	-163.45	0.00
	SD	342.63	12.67	-166.51	0.00
	Null	342.78	12.83	-167.93	0.00
F:B	**Null**	**-113.43**	**0.00**	**60.18**	**0.91**
	SD	-107.39	6.04	58.50	0.04
	PC1	-107.16	6.27	58.38	0.04
	AHM	-99.49	13.94	54.54	0.00

	PC1+SD	-97.97	15.46	55.24	0.00
	AHM+SD	-92.98	20.45	52.74	0.00
	AHM+PC1	-92.97	20.46	52.73	0.00
	AHM+PC1+SD	-83.34	30.09	49.49	0.00
GP-soil and climate	**AHM+PC1**	**364.36**	**0.00**	**-175.93**	**0.86**
	AHM	368.76	4.40	-179.58	0.10
	PC1	370.53	6.17	-180.46	0.04
	Null	375.24	10.88	-184.16	0.00
GP-Management and climate	**AHM+SR**	**364.92**	**0.00**	**-176.21**	**0.41**
	AHM+R:G+SR	**366.54**	**1.63**	**-175.44**	**0.18**
	AHM+SD+SR	**366.66**	**1.74**	**-175.50**	**0.17**
	AHM+R:G+SD+SR	368.17	3.26	-174.54	0.08
	AHM	368.76	3.84	-179.58	0.06
	AHM+R:G	370.50	5.58	-179.00	0.03
	AHM+SD	370.77	5.85	-179.13	0.02
	SR	371.20	6.29	-180.80	0.02
	AHM+R:G+SD	372.02	7.11	-178.18	0.01
	SD+SR	372.64	7.72	-180.07	0.01
	R:G+SR	372.71	7.79	-180.10	0.01
	R:G+SD+SR	373.96	9.05	-179.16	0.00
	Null	375.24	10.32	-184.16	0.00
	R:G	376.77	11.86	-183.59	0.00
	SD	377.00	12.08	-183.70	0.00
	R:G+SD	378.11	13.19	-182.80	0.00
Actinomycetes	**Null**	**21.93**	**0.00**	**-7.51**	**0.95**

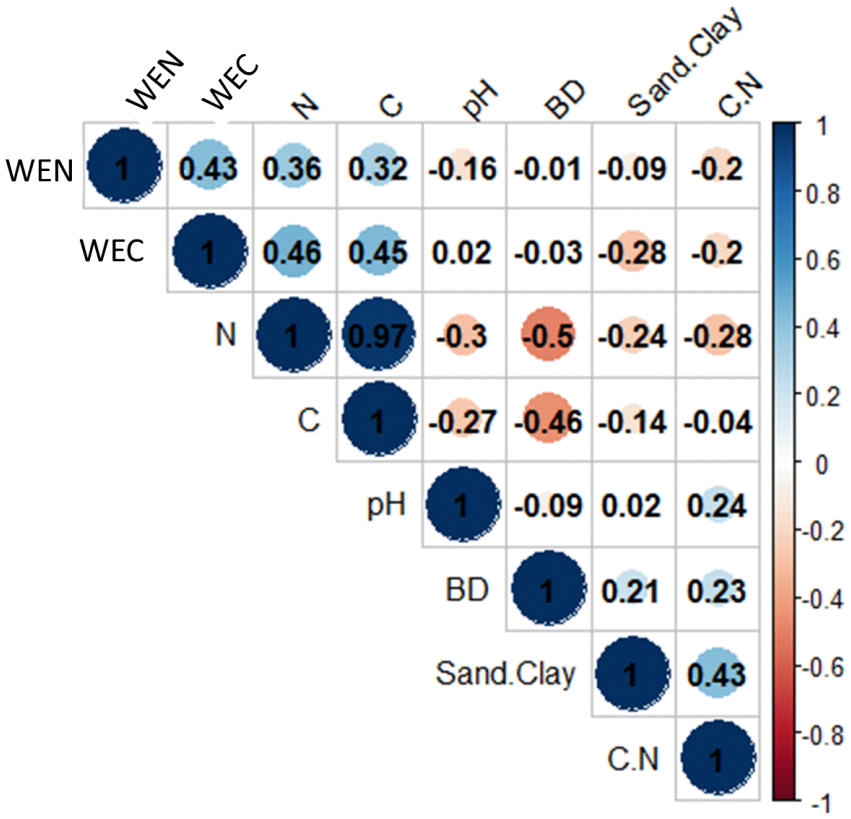
	PC1	29.38	7.44	-9.89	0.02
	SR	29.91	7.98	-10.16	0.02
	AHM	30.40	8.47	-10.40	0.01
	PC1+SR	37.55	15.61	-12.52	0.00
	AHM+PC1	38.07	16.14	-12.79	0.00
	AHM+SR	38.44	16.50	-12.97	0.00
	AHM+PC1+SR	46.33	24.39	-15.34	0.00
GN-soil and climate	**AHM+PC1**	**358.95**	**0.00**	**-173.22**	**0.83**
	AHM	363.08	4.13	-176.74	0.10
	PC1	364.17	5.22	-177.28	0.06
	Null	368.59	9.65	-180.83	0.01
GN-management and climate	**AHM+SR**	**359.38**	**0.00**	**-173.44**	**0.42**
	AHM+SD+SR	**361.22**	**1.85**	**-172.78**	**0.17**
	AHM+R:G+SR	361.42	2.04	-172.88	0.15
	AHM	363.08	3.70	-176.74	0.07
	AHM+R:G+SD+SR	363.30	3.92	-172.10	0.06
	SR	364.67	5.29	-177.53	0.03
	AHM+R:G	365.09	5.71	-176.30	0.02
	AHM+SD	365.12	5.74	-176.31	0.02
	SD+SR	366.18	6.81	-176.84	0.01
	R:G+SR	366.42	7.04	-176.96	0.01
	AHM+R:G+SD	366.96	7.58	-175.65	0.01
	R:G+SD+SR	367.92	8.55	-176.14	0.01
	Null	368.59	9.22	-180.83	0.00
	R:G	370.35	10.97	-180.37	0.00
	SD	370.39	11.02	-180.40	0.00

	R:G+SD	371.93	12.56	-179.72	0.00
Anaerobic	**AHM+PC1+SR**	**297.90**	**0.00**	**-141.11**	**0.46**
	AHM+PC1	299.50	1.65	-143.51	0.20
	AHM+SR	300.30	2.39	-143.88	0.14
	PC1+SR	301.50	3.58	-144.48	0.07
	AHM	302.10	4.25	-146.26	0.05
	PC1	303.10	5.24	-146.76	0.03
	SR	304.00	6.13	-147.21	0.02
	Null	305.90	7.98	-149.47	0.01
Total Bacteria	**Null**	**16.61**	**0.00**	**-4.84**	**0.93**
	AHM	23.35	6.74	-6.87	0.03
	PC1	24.26	7.65	-7.33	0.02
	SR	24.89	8.28	-7.65	0.01
	AHM+PC1	31.25	14.64	-9.38	0.00
	AHM+SR	31.82	15.21	-9.66	0.00
	PC1+SR	32.73	16.12	-10.12	0.00
	AHM+PC1+SR	39.95	23.34	-12.15	0.00

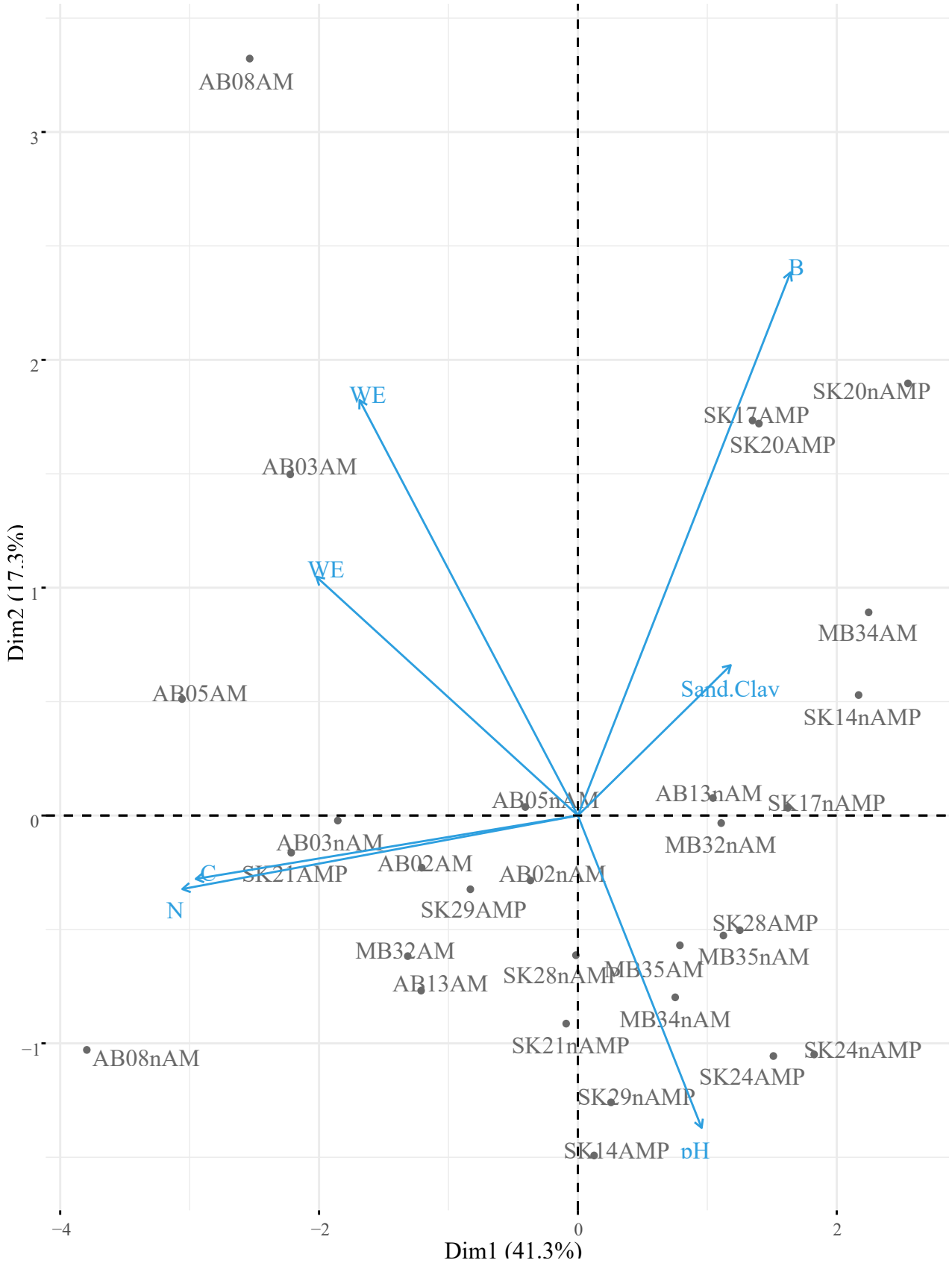
Note: SD=stocking density; SR=stocking rate; R: G= rest to grazing ratio; MBC= microbial biomass carbon; MBN= microbial biomass nitrogen; F: B=Fungi to Bacteria ratio; GP= gram positive bacteria; GN= gram negative bacteria; GP: GN= gram positive to gram negative bacteria; soil PC1= soil principal component1; AHM=annual heat moisture.



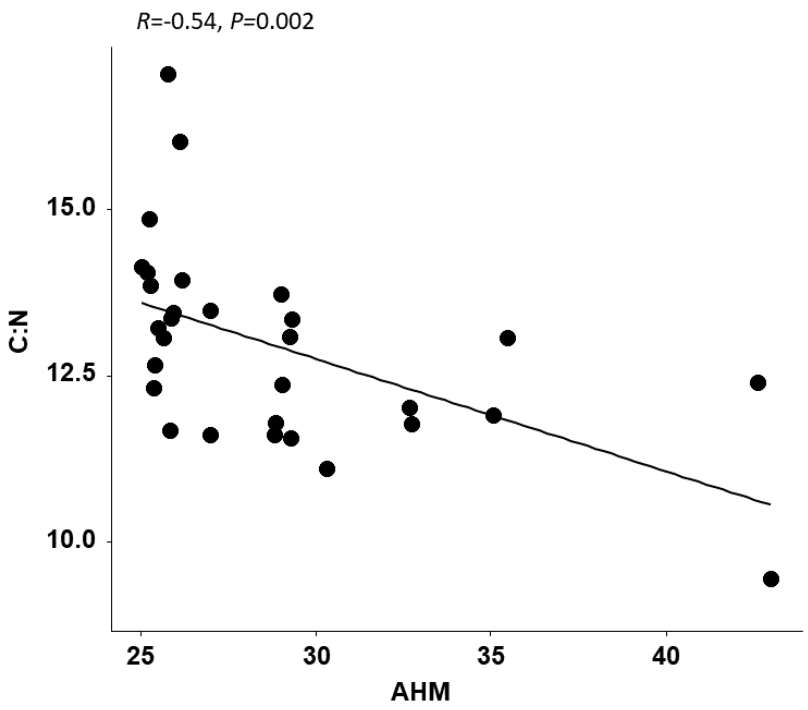
Supplementary Figure 1: Correlation (Pearson coefficients) matrix assessing collinearity among grazing systems and management practices among five grassland management variables. Grazing includes adaptive multi-paddock grazing and those neighboring ranches (Conventional), RestGraze = rest-to-grazing ratio, SR = stocking rate, SD = animal unit density and AHM=Annual Heat moisture (climatic factor).



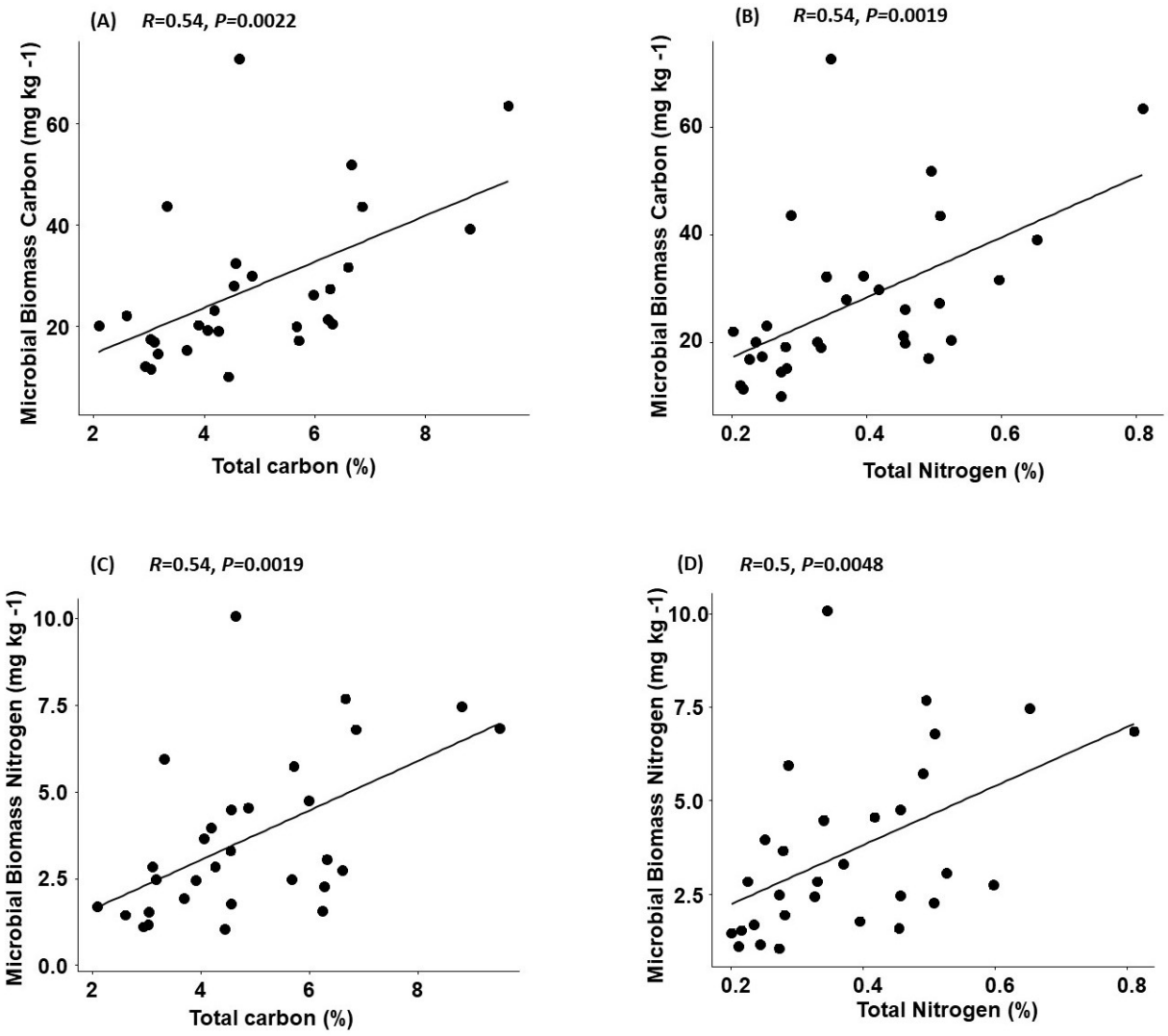
Supplementary Figure 2: Correlation matrix (Pearson coefficients) assessing collinearity among eight biophysical variables: Water-extractable nitrogen (WEN) and water-extractable carbon (WEC), total nitrogen (N), total carbon (C), pH, soil bulk density (BD), soil texture (Sand: Clay ratio) and C: N ratio.


AB08AM
B
WE
SK20nAMP
SK17AMP
SK20AMP
AB03AM
WE
MB34AM
AB05AM
Sand.Clav
SK14nAMP
AB05nAM
AB13nAM
SK17nAMP
AB03nAM
MB32nAM
C
SK21AMP
AB02AM
AB02nAM
N
SK29AMP
SK28AMP
MB32AM
MB35nAM
SK28nAMP
MB35AM
AB13AM
MB34nAM
SK21nAMP
SK24nAMP
AB08nAM
SK24AMP
SK29nAMP
SK14AMP
pH
Dim2 (17.3%)
Dim1 (41.3%)
3
2
1
0
−1
−4
−2
0
2

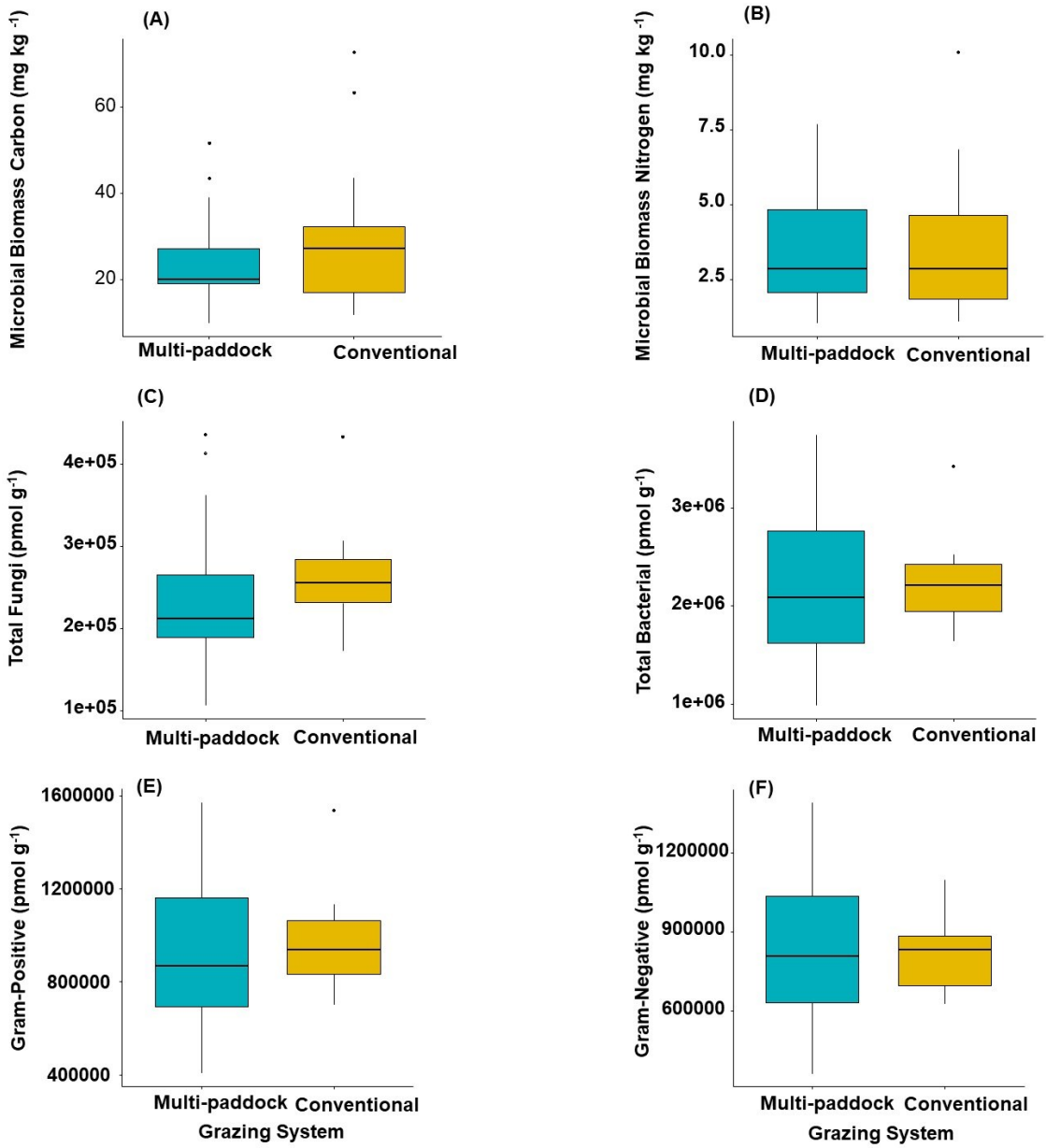
Supplementary Figure 3: PCA biplot of soil properties included pH, bulk density, C, N, water-extractable carbon (WEC), water-extractable nitrogen (WEN) and the ratio of sand: clay (i.e., soil texture).



Supplementary Figure 4: Relationship between carbon: nitrogen ratio to AHM.



Supplementary Figure 5: Relationship between MBC and MBN to total carbon and total nitrogen.



Supplementary Figure 6: Boxplot showing Microbial biomass C and N, total fungal, total bacterial, Gram-positive, and Gram-negative PLFA abundance under AMP and conventional grazing

Appendix B. Chapter 3: Supplementary data

Supplementary Table 1: Summary of sampling site detail (n=26). Predictor variables are grouped by grassland management treatment (Adaptive Multi-paddock and conventional) and grazing metrics (SR= Stocking rate (AUM ha $^{-1}$), SD= Stocking density (AU ha $^{-1}$), R:G= rest to grazing ratio), as well as climate. BT: Boreal Transition; AP: Aspen parkland; FG: Fescue grasslands; MG: Mixedgrass; Conve: Conventional grazing; Elevation = Average elevation of a ranch (m); MAT = Mean annual temperature (deg C), MAP = Mean annual precipitation (mm), AHM = Annual heat: moisture index derived from MAT and MAP (AHM= (MAT+10)/ (MAP/1000))

				Grassland management				Climate			
					SR						
					SR	level	SD	R:G	Elevation	MAT	MAP	AHM
RanchID	PairID	Ecoregion	Soil order	Grazing	(AUM ha -1)		(AU ha -1)		(m)	(oC)	(mm)	
AB02nAMP	AB02	BT	Luvi	Conv.	3.1	High	7.8	2.1	985	3.1	516.3	25.9
AB02AMP	AB02	BT	Luvi	AMP	4.5	High	40.9	90.0	981	3.1	515.9	25.9
AB05AMP	AB05	BT	Luvi	AMP	3.7	High	46.8	60.0	739	3.0	456.4	29.3
AB05nAMP	AB05	BT	Luvi	Conv.	8.4	High	1.2	0.0	717	3.0	455.6	29.3
AB08nAMP	AB08	AP	Cherno	Conv.	1.5	Low	1.6	0.0	840	3.1	464.2	28.9
AB08AMP	AB08	AP	Cherno	AMP	5.8	High	76.0	12.5	839	3.1	464.4	28.8
AB13nAMP	AB13	AP	Cherno	Conv.	1.6	Low	2.7	0.8	672	2.2	388.5	32.7
AB13AMP	AB13	AP	Cherno	AMP	3.8	High	108.8	11.7	672	2.2	389.3	32.7
MB32nAMP	MB32	AP	Rego	Conv.	2.3	Low	0.5	0.0	440	3.1	513.3	27.0
MB32AMP	MB32	AP	Rego	AMP	3.3	High	8.3	40.0	439	3.1	513.3	27.0
MB34nAMP	MB34	AP	Cherno	Conv.	4.1	High	0.7	0.0	420	2.6	518.3	25.2
MB34AMP	MB34	AP	Cherno	AMP	5.3	High	15.1	80.0	409	2.6	518.1	25.3

MB35nAMP	MB35	AP	Cherno	Conv.	2.1	Low	2.0	1.7	469	3.2	532.0	25.7
MB35AMP	MB35	AP	Cherno	AMP	2.9	Low	15.7	21.7	465	3.2	533.6	25.5
SK14AMP	SK14	FG	Cherno	AMP	2.8	Low	13.0	18.8	891	4.5	433.2	35.1
SK14nAMP	SK14	FG	Cherno	Conv.	3.4	High	4.1	0.8	877	4.6	430.7	35.5
SK16AMP	SK16	MG	Cherno	AMP	3.0	Low	370.6	90.0	907	4.8	384.6	40.6
SK16nAMP	SK16	MG	Cherno	Conv.	3.9	High	1.1	0.0	900	4.8	383.1	40.9
SK17AMP	SK17	MG	Cherno	AMP	1.8	Low	11.7	90.0	845	4.7	364.8	43.0
SK17nAMP	SK17	MG	Cherno	Conv.	1.9	Low	1.1	0.0	861	4.6	367.1	42.6
SK20AMP	SK20	BT	Cherno	AMP	2.2	Low	13.7	20.0	521	1.6	461.8	26.1
SK20nAMP	SK20	BT	Cherno	Conv.	4.4	High	5.7	0.0	516	1.6	461.0	26.2
SK24AMP	SK24	AP	Cherno	AMP	0.8	Low	25.7	45.0	611	1.4	473.4	25.0
SK24nAMP	SK24	AP	Cherno	Conv.	1.9	Low	2.7	2.5	649	1.4	470.6	25.3
SK28nAMP	SK28	AP	Cherno	Conv.	2.8	Low	2.2	3.4	616	2.6	508.3	25.9
SK28AMP	SK28	AP	Cherno	AMP	5.3	High	45.3	60.0	622	2.6	509.3	25.8

Supplementary Table 2**:** Summary of grazing management practices employed at 13 ranch paired locations (n=26). Ranches either practiced Adaptive multipaddock grazing (AMP grazing, or were neighboring ranches using conventional approaches (Conventional). *P* values derived from mixed effect model where grazing system was used as fixed effect and ranch pair as random effect on each grazing management factors. Bold text indicates significant effects ($p \leq 0.05$)

Management practices	**AMP**		**Conventional**		**p-value**
	mean	**SE**	**mean**	**SE**	
Stocking Density (AU ha $^{-1}$)	60.9	19.14	2.58	0.41	**0.05**
Stocking Rate (AUM ha $^{-1}$)	3.47	0.29	3.18	0.36	0.64
Rest to Grazing	49.2	6.09	0.87	0.23	**0.00001**

Supplementary Table 3: Primers of total bacteria and total fungi used for the quantative polymerase chain reactions (qPCR) and next-generation sequencing (NGS).

Method	Target	Primers sequence (5'-3')	Common name	Reference
qPCR	Total Bacteria	ACT CCT ACG GGA GGC AGC AG ATT	338f	Fierer et al., 2005
		ACC GCG GCT GCT GG	518r	
	Total Fungi	TTA GCA TGG AAT AAT RRA ATA GGA	nu-SSU-0817	Borneman and Hartin,
		TCT GGA CCT GGT GAG TTT CC	nu-SSU-1196	2000
NGS	Total Bacteria	CCT ACG GGN GGC WGC AG GAC TAC	341F	Klindworth et al.,
		HVG GGT ATC TAA TCC	805R	2013
	Total Fungi	CTT GGT CAT TTA GAG GAA GTA A GCT	ITS1	Orgiazzi et al., 2012
		GCG GTT CTT CAT CGA TGC	ITS2	
Illumina adapters were added to primers	Forward	TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG		Illumina protocol
	Reverse	GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G		

Supplementary Table 4: Results of the ANOVA test (mixed effect model) of grazing system and AHM on each total bacterial and fungal gene abundance (n=26). Bold text indicates significant effects ($p \leq 0.05$).

Gene Abundance, *gene copies g-1 soil, dry weight*		Intercept	Grazing	AHM
Total Bacteria	F	3.5	3.5	0.02
	Df.res	11.0	12.0	11.0
	Pr(>F)	0.1	0.08	0.9
Total Fungi	F	0.001	1.0	0.1
	Df.res	11.0	12.0	11.0
	Pr(>F)	0.9	0.3	0.7

Supplementary Table 5: Results of the ANOVA test (mixed effect model) of grazing system and aridity effect on each soil properties. Bold text indicates significant effects ($p \leq 0.05$).

	F	Df	P	F	Df	P
		soil pH			C:N	
Grazing	5.4	1	**0.03**	1.6	1	0.2
AHM	1.4	1	0.2	13.8	1	**0.003**
Grazing*AHM	6.1	1	**0.03**	2.1	1	0.1
		Bulk Density			Sand	
Grazing	2.9	1	0.1	0.1	1	0.7
AHM	0.003	1	0.97	1	1	0.3
Grazing*AHM	3.2	1	0.1	0.3	1	0.5
		Carbon			Silt	
Grazing	0.2	1	0.6	0.19	1	0.6
AHM	2.2	1	0.1	0.46	1	0.5
Grazing*AHM	0.3	1	0.5	0.48	1	0.4
		Nitrogen			Clay	
Grazing	2.1	1	0.1	0.06	1	0.8
AHM	0.006	1	0.9	0.9	1	0.3
Grazing*AHM	2.8	1	0.1	0.1	1	0.7

Supplementary Table 6: Results of the ANOVA test (mixed effect model) of grazing management factors like stocking rate (SR), stocking density (SD) and rest to grazing ratio (RG) effect on each soil properties. Bold text indicates significant effects ($p \leq 0.05$).

	F	Df	P	F	Df	P
		soil pH			C:N	
Stocking rate	0.2	1	0.6	1.3	1	0.23
Stocking density	9.1	1	**0.01**	8.1	1	**0.01**
Rest:Grazing	4.9	1	0.05	3.4	1	0.08
SR:SD	10	1	**0.009**	8.5	1	**0.01**
SR:RG	1.5	1	0.25	13	1	**0.003**
SD:RG	4.7	1	0.05	4.7	1	**0.01**
SR:SD:RG	7.2	1	**0.02**	2.3	1	0.1
		Bulk Density			Sand	
Stocking rate	0.9	1	0.3	1.6	1	0.2
Stocking density	0.03	1	0.8	0.002	1	0.9
Rest:Grazing	0.5	1	0.4	0.8	1	0.3
SR:SD	0.0001	1	0.9	0.3	1	0.5
SR:RG	0.004	1	0.9	2.3	1	0.1
SD:RG	0.04	1	0.8	0.7	1	0.4
SR:SD:RG	0.09	1	0.7	0.2	1	0.6
		Carbon			Silt	
Stocking rate	0.6	1	0.4	0.1	1	0.7
Stocking density	0.01	1	0.9	0.2	1	0.6
Rest:Grazing	0.004	1	0.9	0.3	1	0.5
SR:SD	0.05	1	0.8	0.02	1	0.8
SR:RG	0.6	1	0.4	3.09	1	0.09
SD:RG	0.04	1	0.8	0.1	1	0.6

SR:SD:RG	0.08	1	0.7	0.8	1	0.3
	Nitrogen			Clay		
Stocking rate	1.4	1	0.2	17.1	1	**0.0006**
Stocking density	1	1	0.3	1.2	1	0.2
Rest:Grazing	0.9	1	0.3	0.8	1	0.3
SR:SD	1.9	1	0.1	3.2	1	0.08
SR:RG	0.3	1	0.5	0.3	1	0.5
SD:RG	0.5	1	0.4	1.2	1	0.2
SR:SD:RG	0.9	1	0.3	1.5	1	0.2

Supplementary Table 7: Alpha diversity indices of the microbial communities under different grazing systems. Values were calculated from sequencing data and are expressed as mean ± standard deviation (n=26).

	Index of diversity by community							
	Bacteria				Fungi			
Grazing system	Richness	Shannon Index	Simpson Index	Evenness Index	Richness	Shannon Index	Simpson Index	Evenness Index
AMP	3944 ±409	6.50±0.3	0.99±0.005	0.78±0.03	628±98	4.4±0.4	0.96±0.03	0.68±0.06
Conventional	3783±573	6.54±0.3	0.99±0.003	0.79±0.02	591±87	3.9±0.6	0.92±0.05	0.61±0.08

Table 8: Results of the ANOVA test (mixed effect model) of grazing system and AHM on each soil microbial diversity indices. Bold text indicates significant effects ($p \leq 0.05$).

		Bacteria			Fungi		
Diversity Index		Intercept	Grazing	AHM	Intercept	Grazing	AHM
Richness	F	57	3.56	0.07	25.02	1.18	1.26
	Df.res	11	12	11	11	12	11
	Pr(>F)	1.12E-05	0.08	0.79	0.0004	0.29	0.28
Shannon Index	F	600.15	7.5	0.05	77.79	10.74	1.51
	Df.res	11	12	11	11	12	11
	Pr(>F)	5.96E-11	**0.01**	0.81	0.000003	**0.006**	0.24
Simpson Index	F	43274.46	5.64	0.0007	531.03	7.73	0.7
	Df.res	11	12	11	11	12	11
	Pr(>F)	<2e-16	**0.03**	0.97	1.152E-10	**0.01**	0.42
Evenness Index	F	836.64	8.7	0.02	69.1	11.4	1.8
	Df.res	11	12	11	11	12	11
	Pr(>F)	9.78E-12	**0.01**	0.87	0.000007	**0.006**	0.21

Supplementary Table 9: PERMANOVA results table for grazing system and aridity effect on bacterial and fungal beta diversity (weighted UniFrac). Df: degrees of freedom; SS: sum of squares; MS: mean sum of squares (n=26). Bold text indicates significant effects ($p \leq 0.05$)

	Df	SS	F	R2	P
Fungal					
Grazing	1	0.39	1.08	0.04	0.16
AHM	1	0.72	2	0.07	**0.001**
Grazing: AHM	1	0.36	1	0.03	0.48
Bacterial					
Grazing	1	0.16	1.13	0.04	0.21
Aridity	1	0.37	2.6	0.09	**0.007**
Grazing: AHM	1	0.07	0.5	0.02	0.91

Supplementary Table 10: Results of the ANOVA test (mixed effect model) of stocking rate, stocking density and rest to grazing ratio effect on each soil microbial diversity indices. Bold text indicates significant effects ($p \leq 0.05$).

	Stocking Rate (AUM ha^{-}1)			Stocking Density (AU ha^{-}1)			Rest: Grazing ratio		
	F	Df.res	Pr(>F)	F	Df.res	Pr(>F)	F	Df.res	Pr(>F)
Bacteria									
Richness	3.9	22.8	**0.05**	0.07	13.4	0.8	0.9	14.4	0.34
Shannon	1.5	21.3	0.2	1.3	18.9	0.3	0.21	15.2	0.65
Simpson	0.1970	21.8	0.6	1.1109	18.8	0.31	2.7	14.7	0.11
Evenness	0.43	22.03	0.5	1.9	19.4	0.2	1.13	15.3	0.3
Fungi									
Richness	3.5	23.7	0.07	0.6	22.9	0.4	6.4	15.7	**0.02**
Shannon	0.005	23.8	0.95	0.5	22.7	0.5	4.6	15.9	**0.04**
Simpson	0.2	23.7	0.69	1.2	23.1	0.3	3.5	17.6	0.07
Evenness	0.009	23.8	0.9	0.7	20.6	0.4	3.4	15.2	0.08

Supplementary Table 11: Results of the ANOVA test (mixed effect model) of grazing system effect on each soil microbial phylum. Bold text indicates significant effects ($p \leq 0.05$).

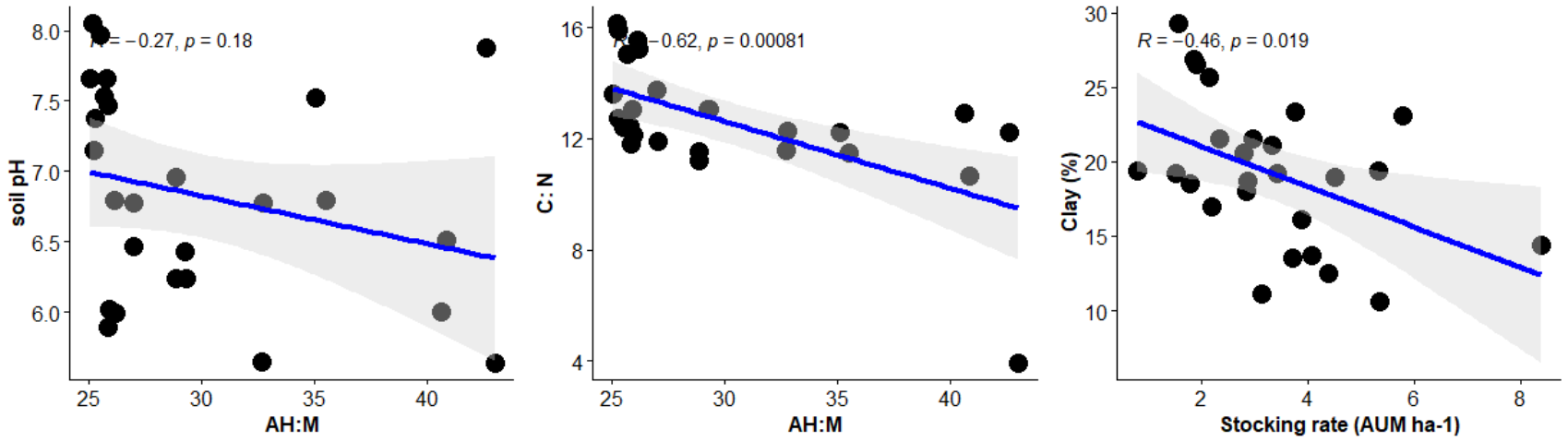
Bacteria				Fungi			
Phylum		Intercept	Grazing	Phylum		Intercept	Grazing
Proteobacteria	F	1051	0.01	Ascomycota	F	593.9	0.3
	Df.res	12	12		Df.res	12	12
	Pr(>F)	4.69E-13	0.9		Pr(>F)	1.37E-11	0.5
Acidobacteria	F	397.7	1.4	Basidomycota	F	46.9	0.4
	Df.res	12	12		Df.res	12	12
	Pr(>F)	1.44E-10	0.3		Pr(>F)	1.76E-05	0.6
Actinobacteria	F	380.9	4.6	Blastocladiomycota	F	4.7	3.4
	Df.res	12	12		Df.res	12	12
	Pr(>F)	1.85E-10	**0.053**		Pr(>F)	0.05	0.08
Bacteroidetes	F	192.8	2.5	Chytridiomycota	F	63.5	3.2
	Df.res	12	12		Df.res	12	12
	Pr(>F)	9.37E-09	12		Pr(>F)	3.91E-06	0.09
Chloroflexi	F	111.2	1.2	Glomeromycota	F	18.5	0.2
	Df.res	12	12		Df.res	12	12
	Pr(>F)	2.02E-07	0.3		Pr(>F)	0.001	0.6
Cyanobacteria	F	325.6	4	Microsporidia	F	1.7	1.7
	Df.res	12	12		Df.res	12	12

	Pr(>F)	4.62E-10	0.06
Fibrobacteres	F	45.9	1.1
	Df.res	12	12
	Pr(>F)	1.98E-05	0.3
Firmicutes	F	58	6.2
	Df.res	12	12
	Pr(>F)	6.18E-06	**0.02**
Gemmatimonadetes	F	78.3	0.09
	Df.res	12	12
	Pr(>F)	1.32E-06	0.76
Planctomycetes	F	261.2	1.2
	Df.res	12	12
	Pr(>F)	1.65E-09	0.3
Verrucomicrobia	F	25.7	8.4
	Df.res	12	12
	Pr(>F)	0.0003	**0.01**
Candidate_div WPS1	F	104.3	3.7
	Df.res	12	12
	Pr(>F)	2.85E-07	0.07
Armatimonadetes	F	53.7	0.1
	Df.res	12	12
	Pr(>F)	9.06E-06	0.71
	Pr(>F)	0.21	0.21
Rozellomycota	F	20.61	0.14
	Df.res	12	12
	Pr(>F)	0.0006	0.7
Unknown	F	30.9	0.6
	Df.res	12	12
	Pr(>F)	0.0001	0.4
Zygomycota	F	24	5.6
	Df.res	12	12
	Pr(>F)	0.0004	**0.04**

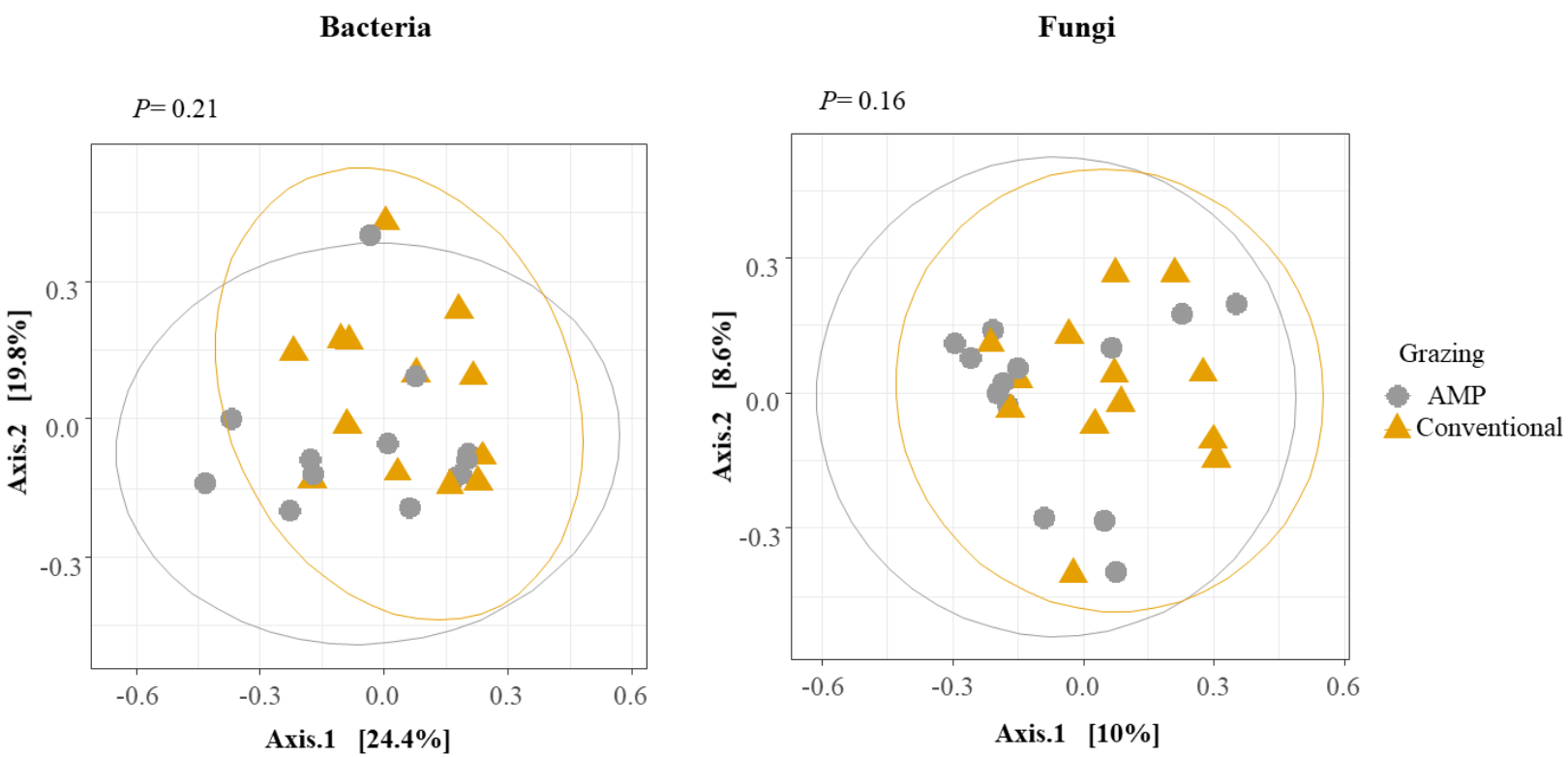
Other Phyla	F	267.1	4.4
	Df.res	12	12
	Pr(>F)	1.45E-09	**0.05**

Supplementary Table 12: Topological properties of the empirical OTUs to OTUs correlation networks of microbial (bacterial and fungal) communities and their associated random network under adaptive multipaddock grazing, conventional grazing system, and high and low stocking rate.

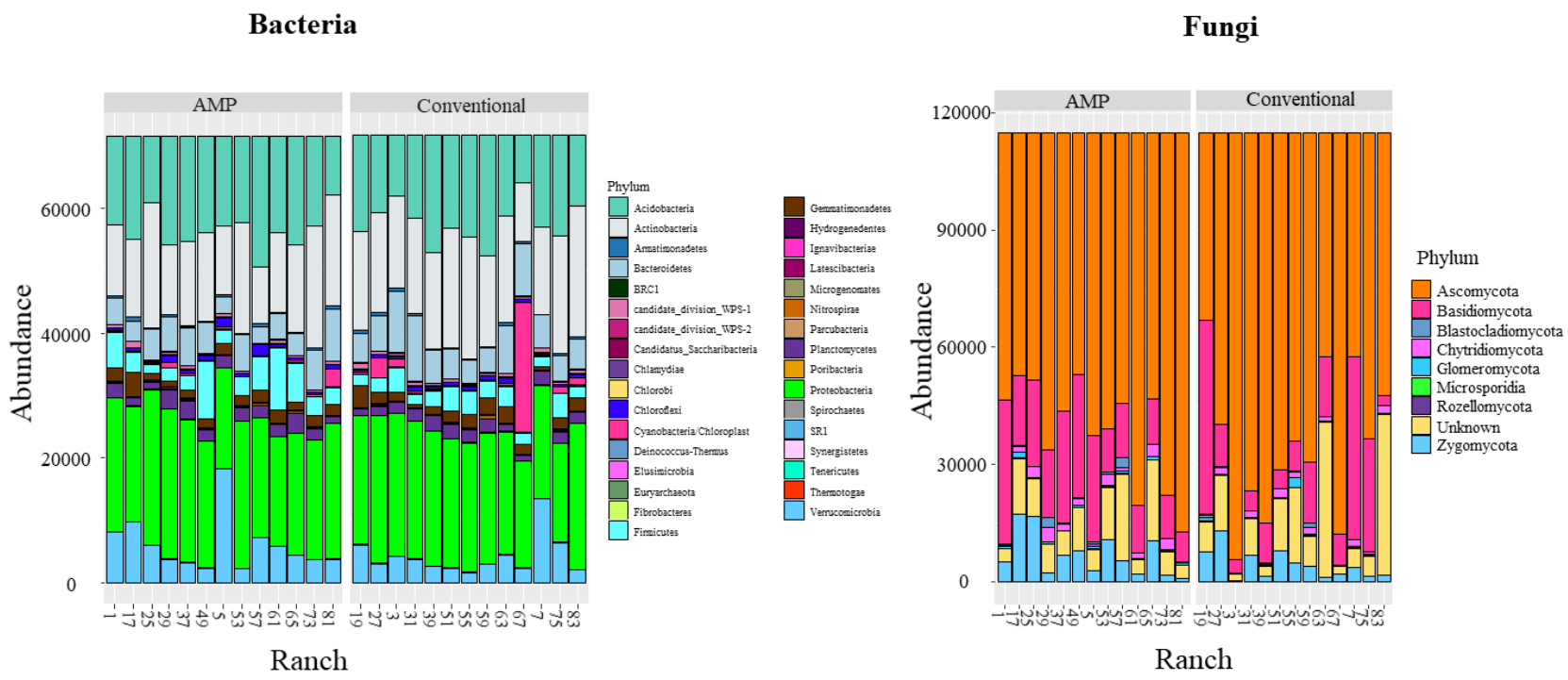
Network properties	AMP grazing	Conventional grazing	Low stocking	High stocking
No. of nodes	390	325	332	444
No. of edges (correlations)	709	355	486	810
Positive correlations	614	315	451	695
Negative correlations	95	40	35	115
Diameter	20	23	24	18
Average Degree	3.6	2.2	2.9	3.6
Average clustering coefficient	0.36	0.39	0.36	0.46
Average path length	5.6	7.5	8.9	5.7
Modularity value	0.68	0.9	0.8	0.73
Random network				
Average degree	9.5	8.2	1.7	2.1
Diameter	8	9	8	13
Average clustering coefficient	0.024	0.024	0.001	0.004
Average path length	2.7	2.8	2.3	3.7



Supplementary Figure 1: Relationship between A. soil pH and B. C: N ratio and aridity (AHM). Regression lines are linear fits of microbial richness and diversity to grazing management factors. Regression lines are linear fits of microbial richness and diversity to grazing management factors.



Supplementary Figure 2: Community structure of bacterial (16S rRNA) and fungal (ITS) communities under AMP and conventional grazing system assed by beta diversity patterns using principal coordinate analysis (PCoA) performed on weighted UniFrac distance matrix. *P* values derived from interaction effect of grazing and aridity on beta diversity using PERMANOVA. Ellipses indicate 95% confidence interval.

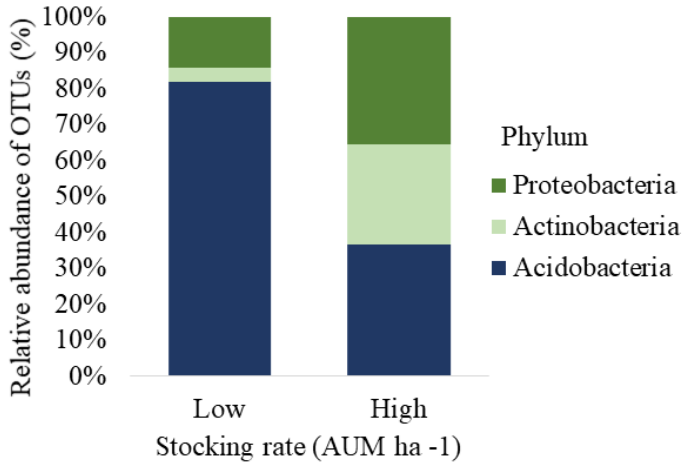


Supplementary Figure 3: Bacterial (16S rRNA) and fungal (ITS) community composition under AMP and conventional grazing measured by the relative abundance of phyla in each ranch (site)

A

Keystone taxa: *Genus* (Phylum)	
Low stocking rate	High stocking rate
Gp6 (Acidobacteria)	*Gp6* (Acidobacteria)
Gp5 (Acidobacteria)	*Gp3* (Acidobacteria)
Gp22 (Acidobacteria)	*Gp1* (Acidobacteria)
Gemmatimonas (Proteobacteria)	*Gaiella* (Actinobacteria)
Nitrosospira (Proteobacteria)	Conexibacter (Actinobacteria)
Thiohalobacter (Proteobacteria)	*Acidisoma* (Proteobacteria)
Deferrisoma (Proteobacteria)	*Anderseniella* (Proteobacteria)

B



Supplementary Figure 4: Keystone taxa and their relative abundance under low and high stocking rates (AUM ha-1). **A.** List of keystone taxa; stack diagram showing relative abundance of keystone at **B.** phylum level. Values are the average relative abundance of 13 samples in low and high stocking rates.

Appendix C. Chapter 4: Supplementary data

Supplementary Table 1: Summary of sampling site detail. Predictor variables are grouped by grassland management treatment (Adaptive Multi-paddock (AMP) and conventional (conv.) and grazing metrics (SR= Stocking rate (AUM ha $^{-1}$), SD= Stocking density (AU ha $^{-1}$), R:G= rest to grazing ratio), as well as climate. BT: Boreal Transition; AP: Aspen parkland; FG: Fescue grasslands; MG: Mixedgrass; Conve: Conventional grazing; Elevation = Average elevation of a ranch (m); MAT = Mean annual temperature (deg C), MAP = Mean annual precipitation (mm), AHM = Annual heat: moisture index derived from MAT and MAP (AHM= (MAT+10)/(MAP/1000)), Luvi:Luvisolic; Cherno: Chernozemic; and Rego; Regosolic.

					Climate				Grazing management		
					Elevation	MAT	MAP		SR	SD	R:G
RanchID	PairID	Ecoregion	SoilOrder	Grazing	(m)	(° C)	(mm)	AHM	(AUM ha $^{-1}$)	(AUha $^{-1}$)	
AB01AMP	AB01	BT	Luvi	AMP	999	3.2	543.8	24.8	6.3	21.6	11.7
AB01nAMP	AB01	BT	Luvi	Conv.	1001	3.2	550.8	24.4	2.3	1.3	3.5
AB02AMP	AB02	BT	Luvi	AMP	981	3.1	515.9	25.9	4.5	40.9	90.0
AB02nAMP	AB02	BT	Luvi	Conv.	985	3.1	516.3	25.9	3.1	7.8	2.1
AB03AMP	AB03	AP	Cherno	AMP	1123	3.3	469.7	29.0	1.7	8.4	6.4
AB03nAMP	AB03	AP	Cherno	Conv.	1132	3.3	469.6	29.0	0.3	3.0	0.0
AB04AMP	AB04	AP	Cherno	AMP	1320	3.7	540.9	26.7	2.0	19.9	7.0
AB04nAMP	AB04	AP	Cherno	Conv.	1304	3.7	535.3	26.8	0.6	3.4	1.0
AB05AMP	AB05	BT	Luvi	AMP	739	3.0	456.4	29.3	3.7	46.8	60.0
AB05nAMP	AB05	BT	Luvi	Conv.	717	3.0	455.6	29.3	8.4	1.2	0.0

AB06AMP	AB06	FG	Cherno	AMP	1270	4.0	521.3	28.1	1.4	13.7	47.5
AB06nAMP	AB06	FG	Cherno	Conv.	1255	4.0	517.5	28.4	5.4	3.7	2.7
AB07AMP	AB07	AP	Cherno	AMP	916	3.1	474.9	28.0	7.0	11.1	20.0
AB07nAMP	AB07	AP	Cherno	Conv.	919	3.1	474.9	28.0	8.0	10.7	20.0
AB08AMP	AB08	AP	Cherno	AMP	839	3.1	464.4	28.8	5.8	76.0	12.5
AB08nAMP	AB08	AP	Cherno	Conv.	840	3.1	464.2	28.9	1.5	1.6	0.0
AB10AMP	AB10	MG	Rego	AMP	714	4.1	326.4	45.3	1.9	10.2	12.5
AB10nAMP	AB10	MG	Rego	Conv.	711	4.1	326.2	45.3	1.0	0.2	0.0
AB11AMP	AB11	AP	Cherno	AMP	661	2.2	384.0	33.1	1.8	21.5	26.8
AB11nAMP	AB11	AP	Cherno	Conv.	661	2.2	385.2	33.1	1.3	0.7	0.0
AB12AMP	AB12	AP	Cherno	AMP	704	2.6	383.5	34.3	1.9	8.6	8.6
AB12nAMP	AB12	AP	Cherno	Conv.	704	2.6	383.5	34.4	1.5	2.2	0.5
AB13AMP	AB13	AP	Cherno	AMP	672	2.2	389.3	32.7	3.8	108.8	11.7
AB13nAMP	AB13	AP	Cherno	Conv.	672	2.2	388.5	32.7	1.6	2.7	0.8

Supplementary Table 2: Summary (mean ± SE) of grazing management metrics employed at 12 paired ranch locations. Ranches either practiced Adaptive multi-paddock grazing (AMP) or were neighboring ranches using conventional approaches (Conventional). Differences in grazing management factors under AMP and conventional grazing was evaluated using ANOVA (mixed effect model) where grazing system was used as fixed factor and ranch pair was random effect. Bold text indicates significant effects ($P \leq 0.1$).

Management metrics	AMP	Conventional	P-value
Stocking Density (AU ha -1)	32.30 (9.1)	3.20 (0.9)	**0.008**
Stocking Rate (AUM ha -1)	3.47 (0.6)	2.92 (0.8)	0.490
Rest to Grazing Ratio	26.20 (7.6)	2.55 (1.6)	**0.012**

Supplementary Table 3: Analysis of variance (mixed effect model) of the effect of grazing system on soil properties. Significant *P* values (*P* <0.1) are in bold font.

Soil	Grazing System		
Properties	F	Df.res	Pr(>F)
pH	1.55	11	0.283
Bulk density	<0.01	11	0.946
Sand	5.19	11	**0.043**
Silt	0.75	11	0.403
Clay	3.34	11	**0.094**

Supplementary Table 4: Results of MANOVA tests evaluating effect of grazing system on soil mass distribution (%) on the abundance of three separate particle size fractions and two separate density fractions. A separate analysis was done for size and density fractions. Bold text indicates significant effects ($P \leq 0.1$).

		Grazing System		
Soil Fraction Type	Fraction	df	F	Pr(>F)
Size Fraction	Size fraction			
	Coarse (>250µm)	1	1.65	0.21
	Medium (53-250 µm)	1	3.25	**0.08**
	Fine (<53µm)	1	0.45	0.50
Density Fraction				
	Density fraction			
	Light	1	1.30	0.2
	Heavy	1	3.13	**0.09**

Supplementary Table 5: Analysis of variance (mixed effect model) of the effect of management metrics: stocking rate, rest to grazing, and stocking density on soil mass distribution (%) on the abundance of three separate particle size fractions and two separate density fractions. Significant P values ($P< 0.1$) are in bold font.

	Stocking rate			Stocking density			Rest to Grazing		
Fraction	df.res	F	Pr(>F)	df.res	F	Pr(>F)	df.res	F	Pr(>F)
Size fraction									
Coarse (>250µm)	21.99	0.31	0.58	14.66	3.57	**0.07**	16.33	0.03	0.85
Medium (53-250 µm)	18.64	0.43	0.51	20.09	0.56	0.46	20.43	0.24	0.62
Fine (<53µm)	21.89	0.48	0.49	16.8	0.34	0.56	17.26	0.0001	0.99
Density fraction									
Light	18.63	0.24	0.63	19.99	0.93	0.34	20.43	0.25	0.61
Heavy	18.63	2.51E-01	0.62	19.98	9.33E-01	0.34	20.44	2.60E-01	0.61

Supplementary Table 6: Analysis of variance (mixed effect model) of the effect of grazing system, AHM and the interaction of system and AHM on soil organic C concentration and stock in bulk soil and particle-size and density fractions. Significant P values ($P< 0.1$) are in bold font.

	SOC concentration (g kg^{-1})			SOC stock (Mg ha^{-1})		
Fractionation type		Grazing	AHM	Grazing:AHM	Grazing	AHM	Grazing:AHM
Coarse (>250 µm)	F	1.70	27.2	1.15	0.70	19.3	0.30
	Df.res	10	10	10	10	10	10
	Pr(>F)	0.22	**0.0003**	0.3	0.40	**0.001**	0.60
Medium (53-250 µm)	F	0.56	28.33	0.34	0.005	11.6	0.0001
	Df.res	10	10	10	10	10	10
	Pr(>F)	0.47	**0.0003**	0.57	0.90	**0.006**	0.90
Fine (<53 µm)	F	3.57	2.64	4.34	3.40	5.6	4.70
	Df.res	10	10	10	10	10	10
	Pr(>F)	**0.08**	0.14	**0.06**	**0.09**	**0.04**	**0.05**
Light fraction	F	0.056	0.44	0.14	0.3	0.09	0.20
	Df.res	10	10	10	10	10	10
	Pr(>F)	0.82	0.51	0.71	0.60	0.7	0.60
Heavy fraction	F	1.19	25.67	0.64	0.06	49.4	0.20
	Df.res	10	10	10	10	10	10
	Pr(>F)	0.29	**0.0004**	0.44	0.8	**3.57E-05**	0.60

Bulk Soil C	F	0.04	13.51	0.08	0.0007	21.2	0.03
	Df.res	10	10	10	10	10	10
	Pr(>F)	0.85	**0.004**	0.78	0.9	**0.0009**	0.80

Note: A separate analysis was done for size and density fractions and bulk soil

Supplementary Table 7: Analysis of variance (mixed effect model) of the effect of grazing system and AHM interactions on total nitrogen concentration and stock on bulk soil and particle-size and density fractions. Significant P values (P<0.1) are in bold font.

		Total N concentration (g kg^{-1})			Total N stock (Mg ha^{-1})		
Fractionation type		Grazing	AHM	Grazing:AHM	Grazing	AHM	Grazing:AHM
Coarse (>250 µm)	F	1.34	22.1	0.94	0.60	19.50	0.30
	Df.res	10	10	10	10	10	10
	Pr(>F)	0.27	**0.0008**	0.35	0.43	**0.001**	0.60
Medium (53-250 µm)	F	0.4	23.14	0.24	0.001	14.70	0.0001
	Df.res	10	10	10	10	10	10
	Pr(>F)	0.54	**0.0007**	0.63	0.90	**0.003**	0.90
Fine (<53 µm)	F	3.26	0.95	3.74	1.20	1.30	1.70
	Df.res	10	10	10	10	10	10
	Pr(>F)	0.10	0.35	**0.08**	0.30	0.70	0.20
Light fraction	F	0.03	0.27	0.10	0.40	0.07	0.30
	Df.res	10	10	10	10	10	10
	Pr(>F)	0.86	0.61	0.74	0.50	0.80	0.60
Heavy fraction	F	0.86	19.07	0.45	0.20	47.70	0.50
	Df.res	10	10	10	10	10	10
	Pr(>F)	3.75	**0.001**	0.52	0.6	**4.14E-05**	0.51
Bulk Soil C	F	0.04	11.59	0.07	0.005	21.68	0.04
	Df.res	10	10	10	10	10	10
	Pr(>F)	0.85	**0.006**	0.80	0.90	**0.0009**	0.80

Note: A separate analysis was done for size and density fractions and bulk soil.

Supplementary Table 8: Analysis of variance (mixed effect model) of the effect of grazing system and AHM interactions on C:N ratio on bulk soil and particle-size and density fractions. Significant P values (P<0.1) are in bold font.

		C:N		
Fractionation type		Grazing	AHM	Grazing:AHM
Coarse (>250 µm)	F	28.6	9.8	34.4
	Df.res	10	10	10
	Pr(>F)	**0.0003**	**0.01**	**0.0001**
Medium (53-250 µm)	F	0.006	0.4	0.002
	Df.res	10	10	10
	Pr(>F)	0.9	0.5	0.9
Fine (<53 µm)	F	0.1	2.2	0.3
	Df.res	10	10	10
	Pr(>F)	0.74	0.17	0.6
Light fraction	F	0.0002	0.02	0.006
	Df.res	10	10	10
	Pr(>F)	0.9	0.8	0.9
Heavy fraction	F	2.05	0.05	1.6
	Df.res	10	10	10
	Pr(>F)	0.2	0.8	0.2
Bulk Soil C	F	4.8	0.7	6.3
	Df.res	10	10	10

	Pr(>F)	**0.05**	0.42	**0.03**

Supplementary Table 9: Analysis of variance (mixed effect model) of the effect of grazing management factors such as (stocking density (SD), stocking rate (SR) and rest to grazing ratio (R:G)) for soil organic C concentration and stock on bulk soil, particle-size and density fractions across 12 ranch pairs. Bold text indicates significant effects ($P \leq 0.1$).

Fractionation type		Carbon (g kg^{-1})			Carbon (Mg ha^{-1})		
		F	Df.res	Pr(>F)	F	Df.res	Pr(>F)
Coarse	SR	0.31	14.2	0.5	0.09	16.9	0.7
(>250 µm)	SD	3.6	11.3	**0.08**	0.02	15.2	0.8
	RG	4.1	14.8	0.05	1.7	15.7	0.2
	SR:SD	4.9	9.9	**0.05**	0.2	14.8	0.6
	SD:RG	0.7	12.4	0.4	2.7	11.7	0.1
	SR:RG	0.9	12.1	0.5	0.2	10.7	0.6
Medium	SR	1.09	12.1	0.3	1.3	14	0.2
53-250 µm	SD	4.3	10.1	**0.06**	0.7	11.2	0.4
	RG	4.4	14.9	**0.05**	1.2	14.8	0.2
	SR:SD	11.5	8.2	**0.008**	0.09	9.7	0.7
	SD:RG	1.7	14.1	0.2	1.6	12.5	0.2
	SR:RG	0.1	14	0.7	0.07	12.2	0.7
	SR	0.03	14.9	0.8	0.1	16	0.7
Fine	SD	0.02	16.7	0.8	0.07	16.9	0.7
<53 µm	RG	0.5	16.8	0.4	0.5	16.5	0.4
	SR:SD	0.04	16.6	0.8	0.1	16.9	0.7
	SD:RG	0.0001	11.1	0.9	0.01	11.3	0.8
	SR:RG	0.02	9.2	0.8	0.02	9.7	0.8
	SR	0.1	14.9	0.7	0.005	16.7	0.9
	SD	0.2	16.7	0.5	0.03	16.5	0.8

Light fraction	RG	2.3	16.8	0.1	0.0005	16.2	0.9
	SR:SD	0.1	16.6	0.7	0.33	16.5	0.5
	SD:RG	1.1	11.1	0.3	1.6	11.5	0.2
	SR:RG	0.4	9.2	0.5	0.6	10.1	0.4
	SR	0.1	14.7	0.6	0.07	16.5	0.7
	SD	0.7	11.7	0.4	0.006	13.9	0.9
	RG	1.1	14.9	0.2	0.1	15.3	0.6
Heavy fraction	SR:SD	1.8	10.4	0.1	0.1	13.2	0.7
	SD:RG	0.2	12.2	0.6	0.3	11.8	0.5
	SR:RG	0.1	11.9	0.6	0.01	11.1	0.9
	SR	0.1	16.9	0.6	0.1	16.7	0.6
	SD	0.008	16	0.9	0.01	14.4	0.8
	RG	0.6	16	0.4	0.5	15.5	0.4
	SR:SD	0.5	15.8	0.4	0.01	13.8	0.9
Bulk Soil C	SD:RG	0.8	11.6	0.3	0.2	11.8	0.6
	SR:RG	0.4	10.4	0.5	0.06	10.9	0.8

Supplementary Table 10: Analysis of variance (mixed effect model) of the effect of grazing management factors such as (stocking density (SD), stocking rate (SR) and rest to grazing ratio (R:G)) for soil N concentration and stock and C:N ratio on bulk soil and particle-size and density fractions across 12 ranch pairs. Bold text indicates significant effects ($P \leq 0.1$).

Fractionation type		Nitrogen (g kg-1)			Nitrogen Mg ha-1)			C:N		
		F	Df.res	Pr(>F)	F	Df.res	Pr(>F)	F	Df.res	Pr(>F)
Coarse	SR	0.2	15.1	0.6	0.01	16.9	0.8	0.7	14.9	0.3
(>250 µm)	SD	3	12.1	0.1	0.06	15.7	0.7	0.07	16.7	0.7
	RG	2.4	14.9	0.1	1.1	15.9	0.3	1	16.8	0.3
	SR:SD	4.6	10.9	**0.05**	0.2	15.5	0.5	0.2	16.6	0.5
	SD:RG	0.5	12.1	0.4	2.4	11.6	0.1	0.7	11.1	0.3
	SR:RG	0.6	11.7	0.4	0.1	10.5	0.7	0.03	9.2	0.8
Medium	SR	0.06	12.8	0.4	0.7	14	0.4	0.09	14.1	0.7
53-250 µm	SD	2.5	10.4	0.1	1.3	11.2	0.2	2.3	11.2	0.1
	RG	1.06	14.9	0.3	0.7	14.8	0.4	0.9	14.8	0.3
	SR:SD	6.38	8.7	**0.02**	0.5	9.7	0.4	2.3	9.8	0.1
	SD:RG	0.8	13.3	0.3	1.1	12.5	0.2	0.7	12.5	0.4
	SR:RG	0.07	13.1	0.7	0.1	12.2	0.7	0.9	12.2	0.3
	SR	0.05	14.9	0.8	0.1	15.6	0.7	0.003	14.9	0.9
Fine	SD	0.02	16.7	0.8	0.09	16.9	0.7	0.07	16.7	0.7
<53 µm	RG	0.7	16.8	0.4	0.7	19.7	0.4	0.002	16.8	0.9
	SR:SD	0.09	16.8	0.7	0.1	16.9	0.7	0.004	16.6	0.9
	SD:RG	0.1	11.1	0.6	0.2	11.2	0.6	1.6	11.1	0.2
	SR:RG	0.3	9.2	0.5	0.01	9.5	0.9	0.9	9.2	0.3
	SR	0.3	14.9	0.5	0.005	16	0.9	0.1	16.5	0.7
	SD	0.2	16.7	0.6	0.03	16.9	0.8	3.7	16.7	**0.06**

Light fraction	RG	0.7	16.8	0.4	0.03	16.5	0.8	1.5	16.3	0.2
	SR:SD	0.06	16.6	0.8	0.4	16.9	0.5	2	16.7	0.1
	SD:RG	3.2	11.1	0.1	2.2	11.3	0.1	1.9	11.5	0.1
	SR:RG	0.05	9.2	0.8	0.5	9.7	0.4	0.5	10	0.4
	SR	0.1	14.5	0.6	0.05	16.5	0.8	0.02	13.6	0.8
	SD	0.9	11.5	0.3	0.001	13.8	0.9	0.3	10.9	0.5
	RG	1.2	14.9	0.2	0.1	15.3	0.7	0.004	14.8	0.9
Heavy fraction	SR:SD	2.1	10.2	0.1	0.08	13.1	0.7	0.00001	9.3	0.9
	SD:RG	0.2	12.3	0.6	0.3	11.8	0.5	0.04	12.7	0.8
	SR:RG	0.2	12	0.6	0.003	11.1	0.9	0.01	12.5	0.9
	SR	0.07	16.9	0.7	0.2	16.9	0.6	0.01	16.6	0.9
	SD	0.005	15.9	0.9	0.009	15.7	0.9	0.1	14.1	0.6
	RG	0.5	16	0.4	0.4	15.9	0.5	0.4	15.4	0.5
	SR:SD	0.6	15.7	0.4	0.0004	15.5	0.9	0.04	13.4	0.8
Bulk Soil C	SD:RG	0.8	11.6	0.3	0.2	11.6	0.6	0.1	11.8	0.7
	SR:RG	0.7	10.4	0.4	0.2	10.5	0.6	0.1	11	0.6

Supplementary Table 11: Summary of the most parsimonious linear mixed models for SOC and N in bulk soil and fractions. The model included management metrics: stocking rare (SR), stocking density (SD), and rest-to-grazing ratio (RG) as fixed effects, and ranch pair was specified as a random effect to address geographic variation in climate and soil type. To account for model selection uncertainty and to the understand interaction effect we report unconditional standard errors (SE). Standardized coefficients (ω_p^2) were used to determine effect size with 95% confidence intervals as a measure of significance provided; bold variables have *P*-value <0.1 and confidence intervals that do not overlap 0.

Fractionation		Carbon (g kg-1)					Nitrogen (g kg-1)				
type		Estimate	Std.Error	p	ω_p^2	95%	Estimate	Std.Error	p	ω_p^2	95%
Coarse	Intercept	57.5	8.39	0.00	0	[0.00, 0.00]	4.39	0.65	0.00	0	[0.00, 0.00]
(>250 µm)	SR	-1.4	2.43	0.55	-0.14	[-0.69, 0.22]	-0.1	0.2	0.60	-0.13	[-0.67, 0.40]
	SD	**0.49**	**0.24**	**0.07**	**0.52**	**[-0.01, 0.97]**	**0.03**	**0.02**	**0.08**	**0.52**	**[-0.08, 1.13**
	RG	-0.68	0.31	0.48	-0.61	[-1.13, -0.02]	-0.04	0.02	0.12	-0.5	[-1.14, 0.14]
	SR:SD	**-0.366**	**0.15**	**0.05**	**-0.6**	**[-1.23, -0.26]**	**-0.03**	**0.01**	**0.04**	**-0.65**	**[-1.26, -0.03]**
	SD:RG	0.02	0.02	0.39	0.4	[-0.37, 1.73]	0.001	0.001	0.44	0.36	[-0.62, 1.32]
	SR:RG	0.19	0.27	0.48	0.27	[-0.74, 1.01]	0.01	0.02	0.42	0.31	[-0.50, 1.13]
Medium	Intercept	49.05	9.28	0.00	0	[0.00, 0.00]	1.24	0.19	0.00	0	[0.00, 0.00]
53-250 µm	SR	-2.3	2.11	0.28	-0.23	[-0.69, 0.22]	-0.03	0.047	0.42	-0.18	[-0.65, 0.29]
	SD	**0.43**	**0.2**	**0.05**	**0.48**	**[-0.01, 0.97]**	0.007	0.004	0.12	0.39	[-0.12, 0.89]

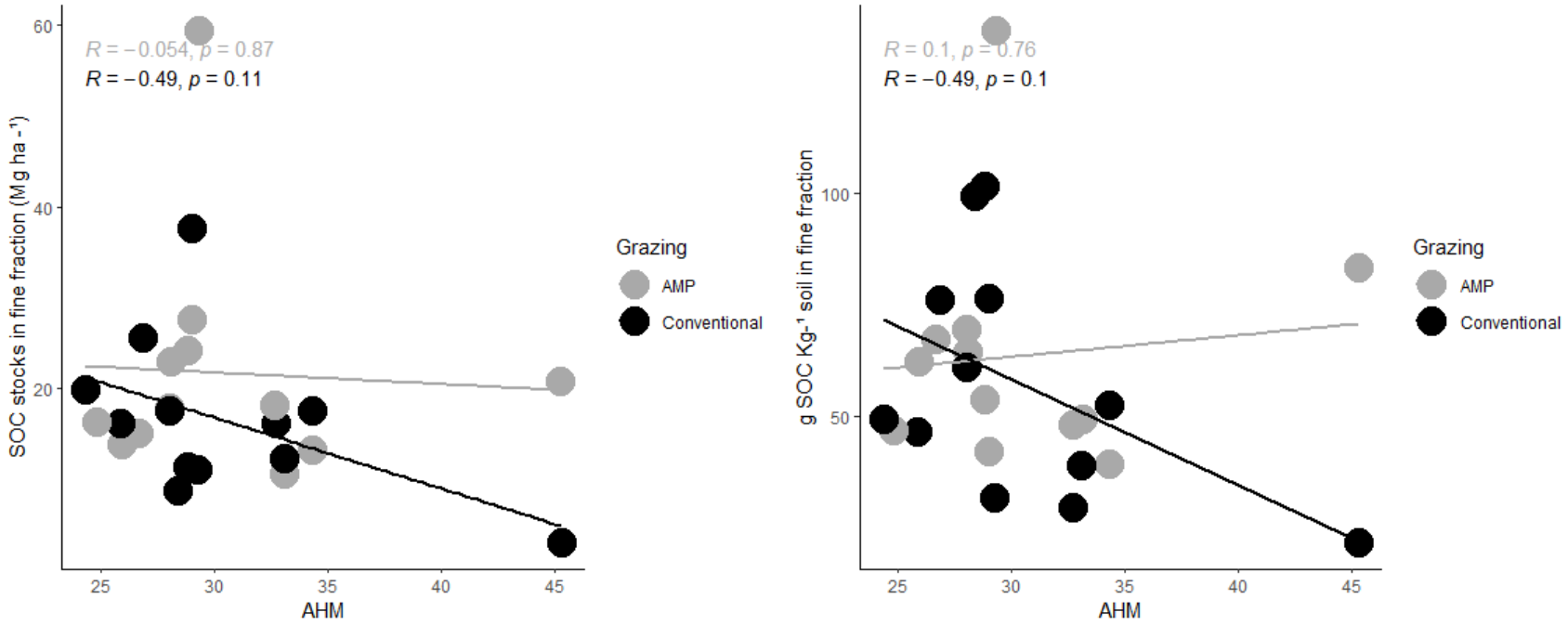
	RG	**-0.62**	**0.28**	**0.04**	**-0.58**	**[-1.13, -0.02]**	-0.006	0.006	0.29	-0.29	[-0.85, -0.28]
	SR:SD	**-0.43**	**0.12**	**0.007**	**-0.74**	**[-1.23, -0.26]**	**-0.007**	**0.002**	**0.02**	**-0.61**	**[-1.12, -0.11]**
	SD:RG	0.03	0.02	0.18	0.68	[-0.37, 1.73]	0.005	0.0005	0.34	0.46	[-0.55, 1.47]
	SR:RG	0.09	0.29	0.73	0.14	[-0.74, 1.01]	0.001	0.006	0.77	0.11	[-0.72, 0.95]
Fine	Intercept	4.02	0.12	0.00	0	[0.00, 0.00]	1.62	0.1	0.00	0	[0.00, 0.00]
<53 μm	SR	-9.75	0.05	0.85	-0.06	[-0.67, 0.55]	-0.01	0.04	0.80	-0.08	[-0.70, 0.55]
	SD	1.09	0.005	0.85	0.07	[-0.68, 0.82]	0.0008	0.004	0.86	0.06	[-0.70, 0.82]
	RG	5.8	0.007	0.43	0.31	[-0.49, 1.11]	0.005	0.006	0.37	0.35	[-0.45,1.16]
	SR:SD	-8.93	0.003	0.81	-0.09	[-0.85, 0.67]	-0.001	0.003	0.72	-0.13	[-0.90, 0.64]
	SD:RG	3.87	0.0003	0.99	0.41	[-0.85, 0.86]	-0.0001	0.0003	0.68	-0.18	[-1.09, 0.73]
	SR:RG	7.06	0.003	0.86	0.06	[-0.63, 0.74]	0.002	0.003	0.56	0.2	[-0.55, 0.95]
Light fraction	Intercept	5.08	0.058	0.00	0	[0.00, 0.00]	2.31	0.06	0.00	0	[0.00, 0.00]
	SR	0.01	0.02	0.67	0.1	[-0.38, 0.57]	0.01	0.02	0.53	0.13	[-0.29, 0.55]
	SD	-0.001	0.003	0.54	-0.17	[-0.75, 0.41]	0.001	0.002	0.58	0.14	[-0.38, 0.66]
	RG	0.005	0.003	0.12	0.48	[-0.13, 1.10]	0.003	0.003	0.37	0.24	[-0.31, 0.80]
	SR:SD	0.0007	0.001	0.67	0.12	[-0.47, 0.71]	-0.0005	0.001	0.78	-0.07	[-0.60, 0.46]

	SD:RG	0.0002	0.0001	0.28	0.35	[-0.32, 1.03]	**0.003**	**0.0001**	**0.08**	**0.53**	**[-0.06, 1.12]**
	SR:RG	-0.001	0.002	0.50	-0.18	[-0.73, 0.38]	-0.0004	0.001	0.82	-0.05	[-0.53, 0.42]
Heavy fraction	Intercept	6.39	0.58	0.00	0	[0.00, 0.00]	1.81	0.16	0.00	0	[0.00, 0.00]
	SR	-0.08	0.17	0.66	-0.12	[-0.69, 0.45]	-0.02	0.04	0.64	-0.12	[-0.68, 0.43]
	SD	0.01	0.01	0.39	0.26	[-0.38, 0.90]	0.005	0.004	0.31	0.31	[-0.32, 0.93]
	RG	-0.02	0.02	0.27	-0.36	[-1.05, 0.32]	-0.007	0.006	0.26	-0.36	[-1.04, 0.31]
	SR:SD	-0.01	0.01	0.17	-0.43	[-1.08, 0.22]	-0.004	0.003	0.15	-0.45	[-1.08, 0.18]
	SD:RG	0.001	0.001	0.59	0.27	[-0.79, 1.33]	0.0002	0.0005	0.59	0.27	[-0.79, 1.33]
	SR:RG	0.008	0.02	0.68	0.17	[-0.71, 1.06]	0.002	0.005	0.63	0.2	[-0.68, 1.08]
Bulk Soil C	Intercept	68.85	10.01	0.00	0	[0.00, 0.00]	5.43	0.81	0.00	0	[0.00, 0.00]
	SR	1.66	3.76	0.66	0.13	[-0.47, 0.72]	0.09	0.3	0.77	0.09	[-0.52,0.69]
	SD	-0.04	0.4	0.10	-0.03	[-0.75, 0.68]	0.002	0.03	0.93	0.03	[-0.70, 0.75]
	RG	0.44	0.5	0.39	0.31	[-0.44, 1.06]	0.31	0.04	0.45	0.28	[-0.49, 1.04]
	SR:SD	-0.21	0.26	0.42	-0.28	[-1.01, 0.45]	-0.01	0.02	0.38	-0.31	[-1.05, 0.43]
	SD:RG	-0.03	0.03	0.35	-0.41	[-1.37, 0.54]	-0.002	0.002	0.36	-0.42	[-1.38, 0.55]
	SR:RG	0.23	0.32	0.49	0.25	[-0.55, 1.04]	0.02	0.03	0.40	0.31	[-0.49, 1.12]

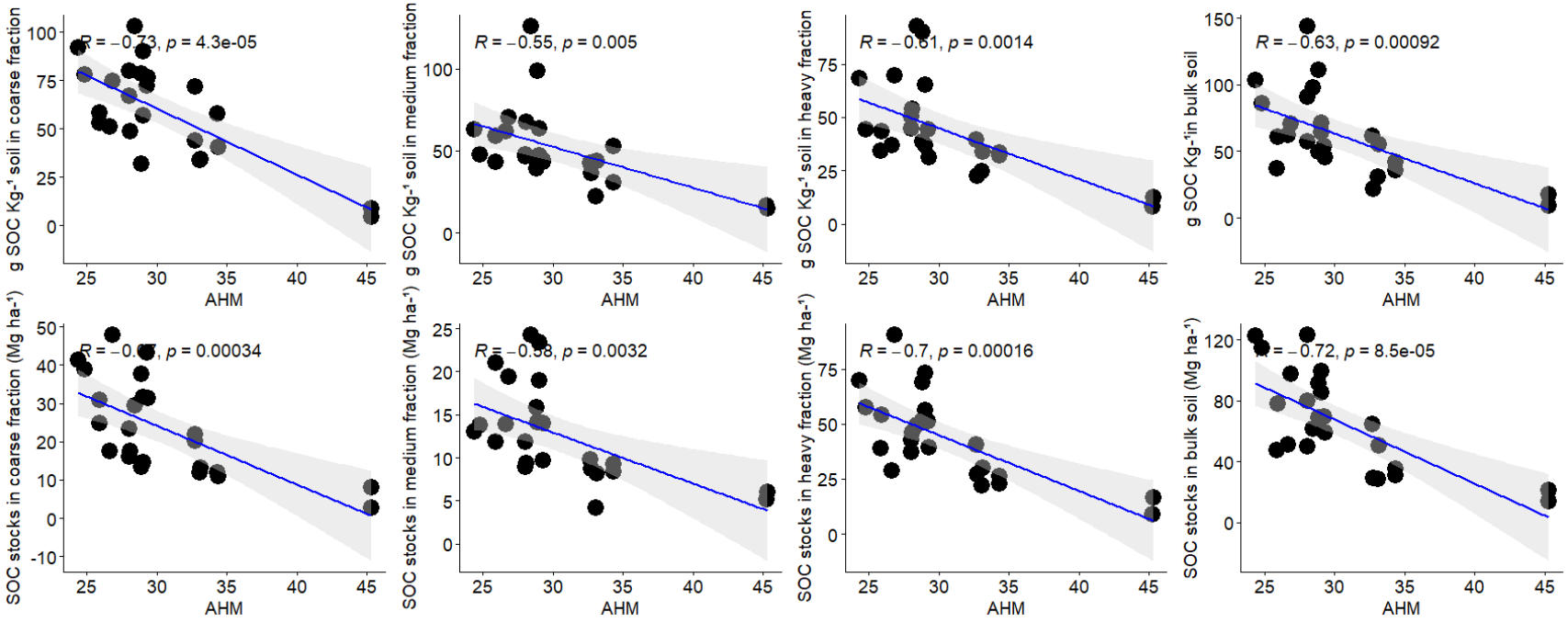
Supplementary Table 12: Analysis of variance (mixed effect model) of the effect of soil microbial indicators (Arbuscular Mycorrhiza Fungi (AMF), fungi, fungi: bacteria (F: B) and gram-positive to gram-negative (GP: GN)) for soil SOC concentration and stock on bulk soil and particle-size and density fractions across 8 ranch pairs (n=16). Bold text indicates significant effects ($P \leq 0.1$).

Fractionation type		SOC concentration (g kg-1)			SOC stock (Mg ha-1)		
		F	Df.res	Pr(>F)	F	Df.res	Pr(>F)
Coarse	AMF	0.03	5.3	0.8	0.6	5.3	0.4
(>250 µm)	Fungi	0.1	10.9	0.7	0.01	10.9	0.8
	F:B	0.3	9.9	0.5	0.4	9.9	0.5
	GP:GN	4.4	10.5	**0.05**	1.5	10.5	0.2
Medium	AMF	0.4	5.3	0.5	0.008	4.8	0.9
53-250 µm	Fungi	0.7	10.9	0.4	0.4	9.8	0.5
	F:B	0.8	9.8	0.3	1.2	8.8	0.2
	GP:GN	0.8	10.3	0.3	1	8.9	0.3
Fine	AMF	0.1	5.3	0.7	0.7	5.3	0.4
<53 µm	Fungi	0.0002	10.9	0.9	6.1	10.9	**0.03**
	F:B	0.08	9.9	0.7	4.4	9.9	**0.06**
	GP:GN	0.04	10.5	0.8	0.4	10.5	0.5
Light fraction	AMF	0.1	5.3	0.7	0.02	5.3	0.8
	Fungi	0.05	109.9	0.8	0.07	10.9	0.7
	F:B	0.09	9.9	0.7	0.2	9.9	0.6
	GP:GN	0.004	10.5	0.9	0.3	10.5	0.5

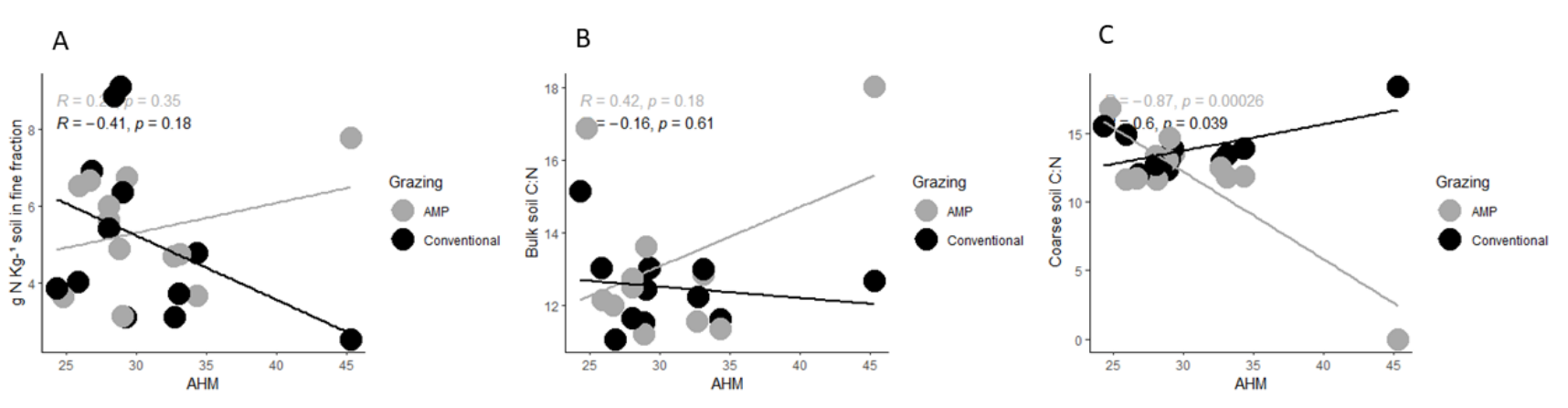
Heavy fraction	AMF	0.1	5.3	0.7	0.1	5.2	0.7
	Fungi	0.2	10.9	0.6	0.05	10.9	0.8
	F:B	1	9.9	0.3	0.2	9.8	0.6
	GP:GN	0.9	10.5	0.3	0.1	10.3	0.7
Bulk Soil C	AMF	1.9	5.1	0.2	0.4	4.3	0.5
	Fungi	0.8	10.7	0.3	0.09	6.6	0.7
	F:B	0.04	9.5	0.8	0.1	6.5	0.7
	GP:GN	0.3	9.9	0.5	0.6	6.2	0.4



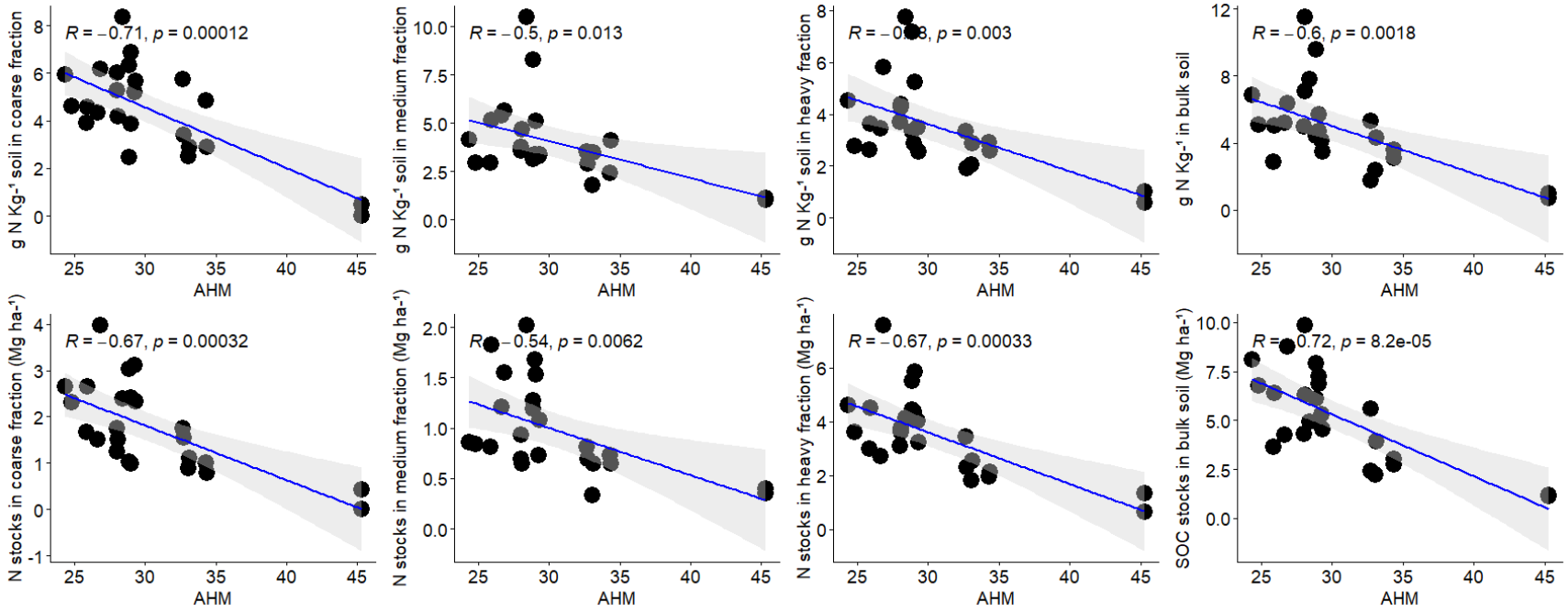
Supplementary Figure 1: Interaction between soil organic C stock and concentration in fine size fraction and aridity under AMP and conventional grazing.



Supplementary Figure.2. The relationship between SOC concentration and stock and AHM in bulk soil and soil fractions across both grazing systems.



Supplementary Figure 3: Interaction between **A.** N in fine size fraction; **B**. C: N in bulk and **C.** C: N in coarse fraction and aridity under AMP and conventional grazing.



Supplementary Figure 4. The relationship between N concentration and stocks and AHM in bulk soil and fractions across both grazing systems.