IT'S THE LITTLE THINGS: RANGELAND SOIL HEALTH INDICATORS AND MICROBIAL COMMUNITY RESPONSE TO DEFERRED GRAZING IN THE INTERMOUNTAIN WEST

by

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ABSTRACT

Rangelands of the Intermountain West express unpredictable climate and environmental factors, causing plants in this region to be particularly sensitive to grazing early in the growing season. Soil microbial communities are foundational to soil health, mediating key processes that regulate vegetation productivity, nutrient cycling, organic matter formation, and ecosystem resiliency. In the extensive rangeland systems of the Intermountain West that support livestock grazing, the roles of the soil microbial community remain poorly understood. Well-managed and sustainable grazing practices on rangelands that allow for rest periods, can reduce pressure during critical growing periods, optimizing vegetation productivity through their impact on microbial biomass, species diversity, enzyme activity, and other functional traits such as carbon and nitrogen sequestration. Despite the global importance of rangelands, knowledge gaps exist regarding the impacts of specific grazing management approaches on microbial communities and soil health indicators. This review synthesizes the literature to clarify the influence of rangeland grazing management on four microbially-mediated soil health indicators: microbial biomass carbon (MBC), extracellular enzyme activity (EEA), soil organic carbon (SOC) pools, and microbial community structure and function. By clarifying existing patterns, key research gaps are identified and are accompanied by specific management recommendations to incorporate microbial ecology into rangeland and soil health assessments.

Keywords: *Carbon sequestration, grazing management, microbial ecology, soil health, soil microbes, sustainability*

CHAPTER ONE

GENERAL INTRODUCTION AND REVIEW OF LITERATURE

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Introduction

Rangelands of the Intermountain West are shaped by low and variable precipitation, strong seasonal temperature shifts, heterogenous low-quality forage resources, and plant-soil mosaics that create spatially patchy nutrient distribution and variable productivity (DelCurto et al., 2023; Sandhage-Hofmann, 2023). While environmental conditions, such as the semi-arid climate of the Intermountain West, heavily influence these factors, microorganisms in the soil are the base drivers of ecosystem function. Soil microbial communities regulate nutrient availability, drive carbon and nitrogen cycling, influence soil organic matter (SOM) formation, and support plant resilience to environmental stressors and grazing pressure (Evans et al., 2017). Plants are closely associated with microorganisms in the rhizosphere that strongly influence the growth and health of vegetation (Berendsen et al., 2012). Consequently, managing rangelands to improve soil biotic integrity may offer downstream benefits including enhanced forage production, water retention, and greater ecosystem resilience to climate variability. Although microbial-mediated metrics such as microbial biomass carbon (MBC), soil organic carbon (SOC) pools, extracellular enzyme activity (EEA), and microbial community composition, structure, and function are increasingly recognized as vital indicators of soil health, there is a lack of research within microbial ecology that informs soil health management in rangeland grazing systems (Bargali, 2024; Ren et al., 2018; Stott, 2019; Uwituze et al., 2022).

Livestock grazing is one of the most prevalent land use systems in the Intermountain West, yet its effects on soil microbial processes vary widely across studies. Factors such as grazing intensity, duration, and timing relative to plant phenology, rest periods, and interactions with soil chemical and physical properties and plant community structure all influence microbial responses. Evidence demonstrates that moderate grazing can stimulate root exudation, microbial

activity, and nutrient turnover, whereas heavy grazing reduces SOC inputs, microbial biomass, and enzymatic activity through defoliation, reduced soil cover, and increased soil compaction (Henry et al., 2024; Zhou et al., 2017). Timing of grazing introduces an additional layer of complexity where early season grazing may alter plant allocation of nutrients and rhizosphere inputs in the soil that may have downstream effects on the soil microbiome. While these insights are well established, the literature lacks an integrated synthesis that clarifies findings and what these patterns mean for soil health assessments and adaptive rangeland management.

This review synthesizes current knowledge on how grazing management practices affect microbially mediated soil health indicators focusing on four key indicators that link microbial function to ecosystem processes: MBC, EEA, SOC pools, and microbial community structure and functional traits. By comparing findings across studies, we identify consistent patterns, highlight areas of disagreement, and evaluate ecological mechanisms. Thus, identifying the soil microbial community's response to grazing pressure and environmental stress may improve our understanding of rangeland biological integrity and sustain agricultural productivity by guiding future management decisions.

To guide monitoring and management decisions we pose the following questions:

1.) How do grazing intensity, duration, and timing influence soil microbial biomass, SOC pools enzymatic activity, and community structure in semi-arid rangelands?
2.) Which microbial indicators are most reliable and feasible for monitoring while being sensitive to management changes?
3.) What ecological mechanisms explain patterns or discrepancies among studies and how do these mechanisms inform adaptive management?

By addressing these questions, we aim to identify microbial soil health indicators with the greatest potential for monitoring soil health in the Intermountain West and provide a synthesis that connects microbial ecology with practical rangeland management strategies.

Rangeland Grazing Impacts on Soil Microbial Ecology

Rangelands of the Intermountain West encompass extensive, low-productivity landscapes where water availability, vegetation structure, and soil properties vary substantially across space and time. This inherent heterogeneity shapes the distribution of soil resources and amplifies the influence of soil microbial communities on nutrient retention, SOM formation, and plant resilience. As a prevalent management practice, livestock grazing is a dominant force shaping the soil microbiome and biogeochemical cycling (Fig. 1). These impacts are context dependent, so adaptability is paramount in restoration efforts on rangelands due to the vast landscape diversity within the ecotype and the interactions between grazing intensity, duration, and timing (Davis et al., 2014; Wei et al., 2022). Livestock actively affect vegetation and soil by defoliation through grazing, soil compaction through trampling, and cycling of nutrients through the deposition of manure and urine (Alkemade et al., 2013; Osmond, 2007). Grazing can reduce fuel for wildfires, shift nutrient deposition and distribution, alter biodiversity, disrupt biological soil crusts, and change water resource dynamics at landscape scales (Pieper et al., 1994). Because these plant and soil structural changes directly influence nutrient inputs and microhabitats, they ultimately determine how soil microbes respond to grazing. Properly managed grazing regimes with light to moderate grazing can stimulate plant growth, promote root exudation, stimulate extracellular enzyme activity, and optimize nutrient cycling; thus, improving grassland productivity. In contrast, heavy grazing can reduce litter inputs, limit root development, and increase soil surface exposure leading to lower moisture retention and higher soil temperatures (Zhang et al., 2018;

Zhou et al., 2017). The magnitude and direction of these responses depend on many complex factors, so rangeland management must be adaptive to accommodate unpredictable environmental factors, policy changes, economic demands, and ecosystem services (DelCurto et al., 2023; Sandhage-Hofmann, 2023). By adjusting variables such as stocking rate, timing of grazing, and animal species, range condition can be fine-tuned for optimal ecological and economic sustainability (Westoby et al., 1989; Williams et al., 2022).

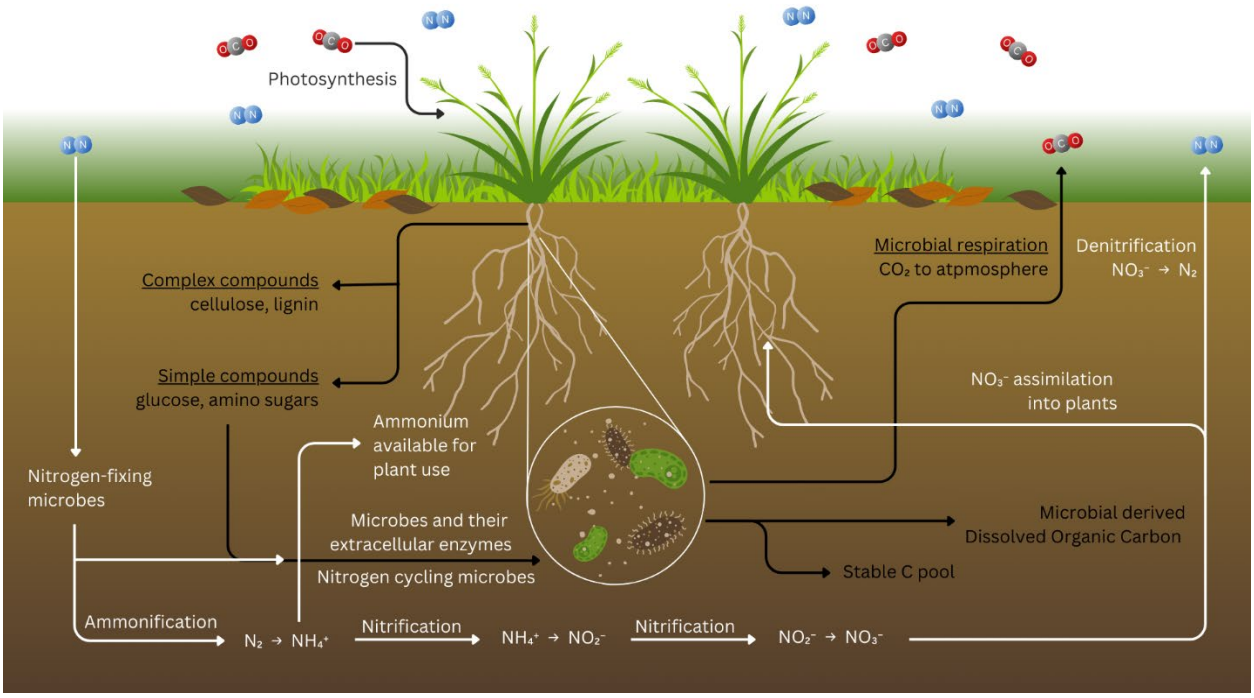

Figure 1. Microbially mediated biogeochemical cycling with respect to carbon and nitrogen.

Microbial processes are particularly sensitive to moisture availability in semi-arid systems where irregular rainfall events and drought periods regulate microbial activity, nutrient availability, and soil and plant function (Evans et al., 2017). As a result, the capacity of rangeland soils to maintain hydrologic function and biotic integrity is tightly coupled with microbial

responses to both climate and management. Manipulation of livestock grazing systems can either benefit or impair these delicate ecosystems.

Timing of grazing relative to plant phenology is a valuable management tool, but its impact on soil health is less understood. Due to the harsh climate and unpredictability of environmental factors, plants in the Intermountain West are particularly sensitive to grazing in the early growing season from floral initiation through seed development (Davis et al., 2014; DelCurto et al., 2023; Osmond, 2007). During this critical period, plant demands for energy and nutrients are high, regrowth potential declines with decreasing soil moisture, and grazing pressure decreases plants' ability to produce both root and shoot, often resulting in the allocation of energy towards aboveground growth at the expense of root development (Blaser, 1986; DelCurto et al., 2023). Furthermore, as energy is concentrated toward the stem, leaves, and flower, less nutrients are excreted through the root system, limiting inputs that support the rhizosphere (Bai & Cotrufo, 2022; Zhou et al., 2017). Wei et al. (2022) studied the effects of grazing during the early growing season on rangeland vegetation community characteristics in the alpine grasslands of northwestern China, finding that vegetation height, coverage, and biomass were lower in grasslands that were grazed during the growing period. Grazing during this time can also facilitate plant diversity through the consumption of competitively dominant plant species (Bagchi & Ritchie, 2010; Olff & Ritchie, 1998; Wei et al., 2022). The timing of grazing also plays a crucial role in shaping biodiversity across different ecological groups. For example, in a study by Davis et al. (2014), conducted in the high-elevation grasslands of southwestern Montana, grazing earlier in the growing season reduced arthropod diversity and influenced plant biomass, height, and regrowth potential. Although several studies demonstrate reductions in vegetation and microbial activity under early season grazing, others report neutral

or mixed responses depending on the system's environment, underscoring the need to interpret microbial indicators related to soil health.

Efforts to assess soil health in rangelands increasingly recognize the contribution of the soil microbiome to biogeochemical cycling for proper ecosystem function. However, quantitatively measuring the physical, chemical, and biological indicators of soil health has proven difficult (Fierer et al., 2021). The United States Department of Agriculture, Natural Resource Conservation Service (USDA NRCS) proposed several standardized soil health indicators with the goal of providing a framework of methods that are effective and sensitive to changes, ready for commercial use, cost-effective for producers, precise and repeatable, and easily interpretable for agricultural management decisions (Stott, 2019). However, the Soil Management Assessment Framework (SMAF) was largely developed for croplands and may not fully capture the spatial and temporal variability intrinsic to semi-arid rangelands. This highlights the need for context-specific interpretations of soil health indicators that are ecologically relevant, sensitive to management, and practical for producers to monitor. Together, these ecological and management considerations establish a foundation for evaluating the influence of grazing on microbial-mediated biogeochemical processes in rangeland systems. The following sections synthesize evidence for four microbial soil health indicators: SOC, MBC, EEA, and microbial community structure.

Soil Organic Carbon

Rangeland soil serves as an integral component of carbon cycling. Estimated to contain one-third of the global SOC stock, they are a valuable resource for increasing carbon sequestration (Reeder & Schuman, 2002; Schuman et al., 2002). Carbon sequestration is the process by which atmospheric CO_2 is removed and stored in the stable C pool (Bai & Cotrufo,

2022; Reeder & Schuman, 2002). This process is mediated by both plants, through photosynthesis, and soil microbes, through the decomposition and mineralization of complex carbon compounds. In other ecosystems, such as forests or cropping systems, a major portion of organic C is stored in aboveground plant biomass (Abraha et al., 2018; Reeder & Schuman, 2002). In contrast, the distribution of carbon in grasslands is unique, where less than 1% of the ecosystem's organic C is stored in aboveground plant biomass while 9% is stored belowground in the root system. The remaining 90% of the total rangeland ecosystem C lies in SOM (Reeder & Schuman, 2002). Soil organic carbon is closely associated with critical soil functions such as nutrient composition, pH buffering, water retention, soil aggregate stability, and mitigating effects of atmospheric carbon (Sandhage-Hofmann, 2023).

SOC pools represent longer-term carbon dynamics and reflect both plant and microbial derived inputs (Evans et al., 2017; Henry et al., 2024). As part of the carbon pool, microbial derived SOM can be partitioned into two fractions: particulate organic matter (POM) and mineral associated organic matter (MAOM; Lavallee et al., 2020). POM is more labile and closely tied to recent plant inputs while MAOM represents a more stable and persistent pool formed through microbial processing (Kleber et al., 2021). These pools differ in their chemical properties, persistence in the soil, sensitivity to grazing, and ecological significance (Bai & Cotrufo, 2022; von Lützow et al., 2007; Whalen et al., 2024).

Grazing influences plant productivity and litter inputs which in turn, more directly affects particulate organic matter response and accumulation. Moderate grazing may maintain or slightly increase POM due to sustained plant production, whereas heavy grazing commonly decreases POM by reducing aboveground biomass and root turnover. In contrast, MAOM represents longer-term carbon stabilization and is primarily formed from microbial necromass rather than

plant residues (Camenzind et al., 2023). MAOM is slower to change in dryland systems, particularly those characterized by moisture limitation and low productivity such as semi-arid rangelands. As a result, many studies often find subtle or undetectable changes in MAOM over short time scales.

Research has shown that varied livestock grazing intensities significantly alter total soil organic carbon (SOC). For example, prolonged periods of high intensity grazing leads to net SOC loss and soil degradation while decreasing grazing pressure and integrating rotational grazing results in SOC increases, though by smaller increments (Bagchi & Ritchie, 2010; Bai & Cotrufo, 2022; Henry et al., 2024; Ingram et al., 2008; Liu et al., 2012). Heavy grazing intensity can result in a reduction of aboveground forage biomass, soil moisture, and soil microbial biomass resulting in an overall decrease in SOM and C inputs (Holt, 1997; Ingram et al., 2008). Nevertheless, the long-term effects of grazing on SOC are also dependent on environmental conditions, soil type, soil depth, and herbivore type (Bai & Cotrufo, 2022; Zhou et al., 2017). Overall, management that maintains plant cover, enhances root biomass, and supports microbial activity can promote the formation of SOC. Because SOC pools integrate ecological processes across extensive time scales, they can serve as indicators of long-term soil health, but may be less responsive to short-term management changes.

Attempts have been made to use trait-based frameworks to link microbial community traits to specific SOC cycling processes. Much of this work has focused on microbial carbon use efficiency (CUE) which often correlates to total SOM carbon, but some studies have demonstrated this relationship does not translate to specific SOM pools such as MAOM where the correlation may be positive, negative, or neutral (Domeignoz-Horta et al., 2020; Whalen et al., 2024). Other approaches have attempted to describe the relationship between SOM and the

soil microbial community in terms of resource acquisition strategies, high growth yield, or stress tolerance (Malik et al., 2020). These findings challenge binary trade-off models and highlight the importance of synergistic traits in promoting SOM function. All these approaches are limited by the complexity of the tripartite plant-soil-microbe interactions and while new frameworks have provided valuable insight in forests (Whalen et al., 2024), they remain to be empirically validated across different ecosystems.

Recent studies have highlighted the complex interplay of microbial processes that govern SOC formation and stabilization (Camenzind et al., 2023; Fierer et al., 2021; Malik et al., 2020). These processes include microbial anabolism and catabolism, community turnover rates, competitive interactions, necromass production and recycling, and the formation of POM and MAOM through microbial processing (Bai & Cotrufo, 2022; Camenzind et al., 2023; von Lützow et al., 2007; Whalen et al., 2024). Monitoring SOC is challenging because of the lack of studies monitoring baseline dynamics on a long-term time scale (Henry et al., 2024). Soil organic carbon is slow to react to management changes, with responses not typically observed for at least 3 to 5 years in sub-humid temperate climates and even longer under more arid conditions (Stott, 2019). Additionally, carbon and mineralization assays in the lab have extensive procedures, making them time intensive for high-throughput labs and lab analysis may not reflect true rates in the field (Fierer et al., 2021; Stott, 2019). These challenges are exacerbated by the global diversity of rangeland systems where soil carbon storage is constrained by soil characteristics and climatic factors (Henry et al., 2024). Future research needs to account for this microbial multifunctionality, where physiological, biochemical, and morphological traits interact synergistically to influence SOM across functional pools (Camenzind et al., 2023; Whalen et al., 2024).

Microbial Biomass Carbon

The rhizosphere, or area around plant roots, is another important component of soil health and plant productivity. The rhizosphere is a nutrient-rich zone of high microbial biomass, biological activity, and improved water-holding capacity due to soil aggregation (Evans et al., 2017). Plant interspaces tend to have less organic matter inputs and less biological activity. Thus, rangelands exhibiting more dense plant cover tend to have healthier soils, increased soil microbial diversity and activity, and are at reduced risk for erosion (Evans et al., 2017; Holt, 1997). Soil MBC is sensitive to changes in land management and responsive to organic matter deposition from plant litter and root exudates. Thus, it can be a useful metric to estimate C and N pools, root exudation, and biological activity (Stott, 2019). Because MBC reflects the size of the active microbial community, it responds relatively quickly to changes in grazing pressure and plant resource allocation. Additionally, its rapid response to moisture and management changes makes MBC a useful metric for detecting potential indications of the effectiveness and sustainability of land management strategies (Holt, 1997).

Across studies, rangelands grazed by wild ungulates or with a low-intensity grazing strategy (seasonal or rotational grazing) can enhance carbon sequestration (SOC storage) and nitrogen dynamics because grazing only occurs for short periods (Bagchi & Ritchie, 2010; Bai & Cotrufo, 2022; Reeder & Schuman, 2002; Zhou et al., 2017). Reeder and Schuman (2002) found that non-grazed pastures had lower SOC compared to lightly grazed pastures due to the higher production of weedy plants and immobilization of C in aboveground plant litter. Grazing at heavier stocking rates tends to reduce SOC storage (Bai & Cotrufo, 2022). Zhao et al. (2024) demonstrate that this effect disrupts the plant-soil-microbe system that drives carbon accumulation where at higher stocking rates, C inputs from the rhizosphere decline significantly.

This reduces substrates available for microbial growth, in turn, leading to lower microbial biomass and necromass formation. Soil compaction may further reduce aeration, water holding capacity, and resource availability; thus, suppressing microbial activity (Henry et al., 2024; Zhao et al., 2024).

Early growing season grazing may limit C flow to the rhizosphere and depress MBC during critical growth periods. However, mixed responses exist in the context of timing of grazing. In some systems lower microbial biomass is reported under early season grazing due to reduced soil moisture and root exudation (Blaser, 1986; Davis et al., 2014). But, some research also reports that early season grazing may have neutral effects on MBC when precipitation is higher in the spring causing reduced grazing and resource competition. Because MBC integrates recent plant inputs and environmental conditions, it is a useful short-term indicator of grazing effects, but may be difficult to compare across different systems. Limitations also exist in that microbial biomass carbon does not inform which taxa are present in a particular system, more biomass is not necessarily more desirable, and more biomass cannot always be equated to more biological activity (Fierer et al., 2021).

Linking MBC and SOC: Microbial Pathways of Carbon Stabilization in Soils

MBC and SOC pools are tightly interconnected, forming a continuum of carbon cycling and stabilization that links short term microbial processes to long term soil health outcomes. MBC represents the active cycling pool of microbial cells that metabolize plant-derived carbon, while SOC pools, especially MAOM, represent the accumulation of microbial necromass and mineralized carbon that persist in the soil. The formation and persistence of MBC and SOC is determined by complex interactions between microbial processes, soil

physicochemical properties, and climatic factors including microsites beneath vegetation, bare soil, nutrient deposition, and moisture accumulation which may change the spatial distribution of MBC and SOC (Kleber et al., 2021; Tao et al., 2023; Zhou et al., 2023). Understanding how grazing affects microbial activity, turnover, and carbon allocation pathways provides insight into both short-term function and long-term C sequestration, which is critical for designing grazing management strategies.

Soil Microbial Extracellular Enzyme Activity

Most biochemical activity in the soil is predominantly driven by microorganisms and their enzymes; although, a fraction of enzymes in the soil are excreted by plant roots (Dick & Burns, 2011; German et al., 2011). Enzymes are produced and secreted by microbes in the soil to target specific compounds (carbohydrates, proteins, amino sugars, phosphates, etc.) and catalyze the decomposition of SOM (Allison & Vitousek, 2005; Stott, 2019). Because enzyme production is energy and N expensive, microbial enzymatic stoichiometry predicts that enzyme activity is constrained by substrate availability in the soil (Acosta-Martínez et al., 2019; Allison & Vitousek, 2005; Mori et al., 2021). Consequently, management practices such as grazing, which influence plant inputs, soil structure, and nutrient availability, can strongly regulate enzyme activity.

Several enzymes have been identified as soil health indicators due to their role in C and N cycling and responsiveness to management practices. β-glucosidase is a vital enzyme involved in decomposing cellulose, the most abundant complex polysaccharide on Earth (Acosta-Martínez et al., 2019; Beeson et al., 2015). It acts by catalyzing the hydrolysis of a β-1,4-glycosidic bond in cellulose, producing the simple, plant-available sugar, glucose (Das & Varma, 2011). β-glucosidase has been shown to respond quickly to changes in soil management (Knight & Dick,

2004) and is sensitive to shifts in SOM inputs while remaining relatively stable with seasonal changes (Acosta-Martínez et al., 2019; Knight & Dick, 2004).

β-1, 4-N-acetyl-glucosaminidase (NAG) is involved in the hydrolytic degradation of chitin, subsequently releasing N-acetyl glucosamine units (amino sugars) that are readily mineralized in soils (Uwituze et al., 2022). Thus, NAG can function as an indicator of both C and N cycling. It has been correlated with potentially mineralizable nitrogen, SOC, MBC, and phospholipid-derived fatty acids (Sainju et al., 2022) but is sensitive to soil pH (Daughtridge & Margenot, 2024; Uwituze et al., 2022). Furthermore, studies have confirmed that certain management practices such as no-tillage and crop rotation can increase NAG activity (Acosta-Martínez et al., 2019; Sainju et al., 2022) but less is known about NAG response to different management practices in rangeland systems. This lack of context-specific research represents a barrier to the adoption of C and N cycling enzyme monitoring in rangeland management.

Extracellular enzyme activity measurements have been most commonly used in cropping systems; however, there is growing interest in their applicability for rangeland ecosystems (Holt, 1997). Enzyme activity can be used to determine the biogeochemical reactions occurring in soils, and how management, such as grazing, influences nutrient cycling (Nannipieri et al., 2018). For example, intense heavy grazing may reduce enzyme activity through depletion of plant cover and soil compaction while light to moderate grazing can stimulate microbial enzyme activity and nutrient cycling by promoting root exudation and microbial access to labile substrates (Holt, 1997). Generally, soil enzymes are highly sensitive and responsive to changes in management and are readily denatured by increased temperatures and extreme pH (Tabatabai, 1994). Beyond grazing, crop rotation, fertilization, tillage, pollution, and climate can affect enzyme activity by altering soil structure, pH, and organic matter (Stott, 2019).

The power of EEA, especially in rangeland systems, lies in assessing microbial nutrient limitation using enzyme stoichiometry. Because enzyme production is resource-intensive, microbes often produce enzymes that target the most limiting nutrient in their microenvironment (Acosta-Martínez et al., 2019; Allison & Vitousek, 2005). Calculating the ratio of C-acquiring enzymes (β-glucosidase or β-galactosidase), N-acquiring (NAG), or P-acquiring (Phosphomonoesterases) may allow managers to determine whether their system is constrained by C, N, or P availability (Acosta-Martínez & Ali Tabatabai, 2011; Stott, 2019; Uwituze et al., 2022). Despite their sensitivity, there is still much debate over whether elevated or reduced enzyme activity indicates a strong nutrient cycling capacity or microbial nutrient limitation and the relationship between enzyme activity and nutrient acquisition in soils is not entirely understood (Acosta-Martínez et al., 2019; Allison & Vitousek, 2005; Knight & Dick, 2004; Stott, 2019). This ambiguity means that EEA must be interpreted in conjunction with other soil chemical properties such as C:N:P pools and pH. Furthermore, the few enzymes most commonly measured are only a small subset of the total activity in the system and some may not be associated with viable cells (Nannipieri et al., 2018). Considerably less research has examined how grazing strategies, particularly grazing timing, affect specific enzyme pathways in rangelands, representing a gap in understanding microbial-mediated nutrient cycling under different management regimes.

Microbial Community Composition, Structure, & Function

Methodology advances in microbiology – particularly high-throughput sequencing of bacterial 16S rRNA genes and fungal ITS regions – have expanded our ability to link microbial community composition to soil processes and metabolic functions in response to management (Daughtridge & Margenot, 2024; Doran & Parkin, 1994; Evans et al., 2017; Franzluebbers,

2018; Jackson et al., 2007). Rather than aiming for a single ideal microbial community indicative of healthy soil, current frameworks emphasize identifying taxa or functional genes associated with key soil processes (i.e. nitrification, denitrification, methane production, and cellulose degradation; Fierer et al., 2021; Guo, 2019). Grazing-induced shifts in microbial communities often reflect cascading effects of defoliation, plant inputs, disturbance, and nutrient availability.

Microbial diversity is commonly assessed using alpha-diversity (within sample) and beta-diversity (between sample dissimilarity). The Shannon diversity index incorporates both richness and evenness, offering insight into how management alters the structure of microbial communities. Light to moderate grazing has been shown to maintain or increase α-diversity by supporting plant functional diversity, increasing SOC turnover and activity in the rhizosphere (Ma et al., 2022; Xun et al., 2018; Zhang et al., 2019). It's important to consider that diverse microbial community composition is not always better and there is a paucity of evidence available about the benefits of specific species. Additionally, the current methods don't differentiate between DNA from intact cells and "relic" DNA in the soil (Fierer et al., 2021). Overgrazing often reduces α-diversity through compaction, loss of vegetative cover, and reduced moisture (Xun et al., 2018). Beta diversity provides insight into how grazing or other management alters community composition across a landscape. In rangelands, β-diversity often increases under light or moderate grazing due to heterogenous distribution and land use by cattle, which creates microsites with distinct soil biotic conditions. Heavier grazing may homogenize soil conditions and lower β-diversity.

Changes in the relative abundance of specific taxa can be indicative of functional responses to grazing. Lighter intensity grazing strategies have been linked to increases in taxa associated with cellulose degradation, nitrification, and root carbon utilization (Ma et al., 2022;

Ma et al., 2019). This functional change is often due to an altered SOC turnover and enhanced microbial activity in the rhizosphere. Conversely, heavy grazing may decrease the abundance of oligotrophs (slow growing taxa that can thrive in nutrient poor environments) and increase disturbance-tolerant groups associated with reduced SOC and soil stability. These taxonomic shifts should be interpreted in ecological and site-specific context and should be combined with functional or network-based metrics.

Shotgun metagenomics and functional profiling tools have enabled researchers to infer the metabolic potential of environmental microbes. Similar to diversity metrics and relative abundance, grazing can influence the abundance of genes associated with certain ecological functions such as carbon compound degradation, nitrogen cycling, secondary metabolite production, and signal transduction (Guo, 2019; Nautiyal et al., 2010). These trait-based approaches – where microbes are classified by their trophic status, reproductive strategies, metabolic functions, or stress tolerance – provide further ecological insight, helping link microbial responses directly to mechanistic biogeochemical pathways in the soil.

Network analysis highlights how land management affects the interactions, relationships, and stability of microbial communities. Taxa that comprise a large portion of the community and are highly connected in the community are considered keystone taxa, often playing crucial roles in nutrient cycling and affecting soil health (Barberán et al., 2011). Rotational grazing strategies have been associated with higher connectivity between taxa, more positive relationships between taxa, tightly associated co-occurring taxa, and the presence of keystone species (Khatri-Chhetri et al., 2024). Higher stocking rates may also increase network complexity to a certain degree, but overgrazing may limit complexity, and other environmental factors should also be considered when evaluating the network. Network structure provides deeper understanding to the structure

of microbial communities that are not evident from diversity metrics or relative abundance of taxa alone. Together, microbial community composition, functional profiling, and network analysis provide complementary tools for evaluating how grazing management affects the biotic integrity of rangeland soils.

Discussion

As the beef cattle industry evolves, the sustainability of rangeland grazing systems is becoming dependent on our understanding of management influence on ecosystem processes and soil health. Traditional rangeland management focuses primarily on stocking rate and grazing intensity with less emphasis on the timing of grazing or season of use relative to plant phenology. Although research has documented how the timing of grazing can alter the plant community composition, structure, and function, less is known about how it affects soil microbial communities, EEA, or microbial mediated nutrient cycling. Yet, these biological processes underpin SOM formation, nutrient availability to forages, ecosystem resilience to grazing pressure, and contributions to range condition.

A central challenge in rangeland health assessment is that microbial responses to grazing are influenced by multiple extraneous environmental factors including, but not limited to, spatial and temporal heterogeneity, moisture limitation, plant functional traits, and management history. Consequently, no single soil health indicator – microbially mediated or otherwise – fully captures a holistic picture of soil health. Instead, meaningful interpretation integrates multiple physical, chemical, and biological indicators in an evaluation within the ecological context of the system.

Producers should aim to monitor their soil for biological activity to obtain a base line and progression of soil health. At a base level, this should include conducting soil tests that focus on soil biology such as MBC and EEA for nutrient cycling (Fierer et al., 2021; Haney et al., 2018;

Sainju et al., 2022; Schimel et al., 2022; Stott, 2019). Microbial indicators provide insight into how the biotic integrity of rangeland soils responds to grazing management variables such as timing, intensity, duration, and stocking rate (Fierer et al., 2021).

Table 1. Limitations, monitoring, and management of four microbial soil health indicators: SOC, MBC, EEA, and community structure

Indicator	Limitation for Rangelands	Monitoring & Management
SOC	Lab analysis procedures are time intensive; slow to reflect management changes (Lavallee et al., 2020)	Required for long-term monitoring of stable C pool dynamics; POM and MAOM fractions for better functional insight
MBC	Does not reveal taxonomic identity; more biomass does not equate more biological activity; variable spatially and temporally (Bargali, 2024)	Useful for short-term monitoring of resource availability changes post-grazing
EEA	Only measures small subset of enzymes; activity can be high due to nutrient limitation or high availability; sensitive to pH (Daughtridge & Margenot, 2024)	Excellent indicator of process-level nutrient cycling; must be interpreted in the context of soil pH and nutrient stoichiometry
Community Structure (DNA/RNA)	Does not differentiate between DNA from viable cells and relic DNA; diverse community composition is not inherently "better" (Khatri-Chhetri et al., 2024)	Provides mechanistic and functional insights, but lacks immediate practicality for producer-level monitoring

Together, these indicators emphasize that avoiding overgrazing, maintaining ground cover, and ensuring adequate rest periods – especially during early growth stages when plants may be more vulnerable to environmental stress – are foundational for sustaining microbial mediated processes that improve soil health (DelCurto et al., 2023). Deferred grazing strategies that allow for an undisturbed early growing period may enhance microbial function in the soil, leading to increased MBC, EEA, increased plant available nutrients, and increased biodiversity. Alternative practices such as grazing substitute forages (i.e. cover crops, winter annuals, or crop

residue) can provide opportunities to rest rangelands while maintaining soil stability with minimal impacts on SOC (Franzluebbers & Stuedemann, 2008; Kelly et al., 2021).

As a result of landscape-scale variability, implementing a comprehensive framework that incorporates multiple soil health indicators provides a more robust and holistic assessment of soil health than employing a single metric. For example, EEA data interpreted alongside MBC and soil chemistry may help identify whether enzyme activity is reflective of healthy nutrient cycling or nutrient stress (Daughtridge & Margenot, 2024). Although current efforts to standardize soil health measurements are promising, they have primarily focused on row crop agricultural systems, highlighting the need for adaptive soil management relevant and calibrated to rangeland systems. Furthermore, as precision agriculture tools become more prevalent, rapid, cost-effective, and comprehensive soil testing may become increasingly accessible (Fierer et al., 2021). The challenge lies in interpreting biological activity in the context of a rangeland system and understanding how this information can be used to make management decisions.

Advancing rangeland soil health assessment will require long-term, multi-scale field studies designed to link grazing management to microbial response. Many existing methods have been developed for research and are not entirely practical for real-world management. Bridging the gap between research methods and practical applications that are both ecologically meaningful and operationally feasible remains essential for developing soil health assessment in rangeland management. Research priorities should include identifying and quantifying microbial traits such as

- Carbon use efficiency, MBC, and SOC fractions
- Microbial multifunctionality and metabolic versatility
- Enzyme pools, nutrient mineralization, and substrate specialization

- Environmental stress tolerance and resilience to disturbance

Using a holistic approach that links microbial processes to grazing management, future research can help translate complex biological activity into practical, sustainable, and economically viable applications for producers and stakeholders.

Sustaining rangeland health “from the ground up” requires monitoring programs and management strategies that incorporate adaptive stocking rates, graze with plant phenology in mind, enable periods of rest on rangelands, maintain plant cover, protect soil structure, and support biotic activity in the soil. These systems are most likely to support robust and resilient microbial communities and long-term soil function. overall ecosystem health could improve, starting from the ground up by building soil health. As producers increasingly seek sustainable practices, incorporating microbial indicators into monitoring frameworks can provide useful perspectives into the biological foundations of rangeland productivity.

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CHAPTER TWO

SOIL HEALTH AND MICROBIAL COMMUNITY RESPONSE TO TIMING OF RANGELAND GRAZING IN THE INTERMOUNTAIN WEST

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Manuscript Information

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Abstract

Rangelands of the Intermountain West are a fundamental landscape for cow-calf production. Due to the climate and unpredictability of environmental factors, plants in this region are particularly sensitive to grazing early in the growing season. Soil microbial communities regulate key processes that sustain rangeland productivity, yet their responses to grazing management are not fully understood in the rangeland ecosystems of the Intermountain West. This study aimed to evaluate microbial activity by substrate induced respiration (SIR) and bacterial community composition under four grazing treatments (ungrazed control, mid-May (early), early-June (mid), and late-June (late) turnout times) and implemented a 16 pasture (4 ha each) randomized complete block design. Pastures were grazed (2.5 ac/AUM) for three weeks at a time, then cattle were moved to the next set of pastures. Six soil moisture and temperature data loggers were deployed in each pasture along known gradients, and soil samples were collected in each pasture at five timepoints from May to August. Microbial biomass was estimated using the substrate-induced respiration (SIR) method. Substrate induced respiration did not change by treatment ($p = 0.11$), but increased significantly throughout the growing season (by timepoint; $p < 0.001$) across all treatments. Among the environmental variables measured, organic matter and sampling timepoint influenced SIR the most. Microbial and fungal diversity were assessed using 16S and ITS sequencing on the Illumina NextSeq platform. Bacterial community composition was dominated by the phyla *Actinomycetota* (averaged 65% relative abundance across treatments), *Pseudomonadota* (7.13%), and *Bacillota* (5.71%). Several genera were detected at significant levels ($p < 0.05$) in the control pastures, indicating that grazing may still alter the abundance of more rare taxa. *Ascomycota* and *Basidiomycota* dominated the fungal community. Shannon diversity indices were consistently high (>7.5) and showed modest variation by

treatment and significant variation by timepoint. Differences in community network strucuture and function were observed between treatments and sampling timepoints. The fungal networks were consistently less dense than the bacterial networks, but both exhibited a greater number and strength of positive interactions compared to negative. Soil phosphorus was the single strongest predictor of variance in microbial community structure followed by sampling timepoint and pH. Functional analysis revealed that the majority of the bacterial community participated in biosynthesis of secondary metabolites (17.9%), followed by the metabolism of various substances. Saprotrophs were the most abundant fungal guild, but did not differ between treatments. Together, these results suggest that grazing timing influences microbial activity and functional dynamics more strongly than community composition, with potential implications for soil carbon cycling and rangeland resilience under adaptive grazing management.
Keywords*: grazing management, microbial ecology, soil health, soil microbiome, sustainability*

Introduction

Arid and semi-arid rangelands are a crucial resource for cow-calf production in the U.S., an industry that generates more cash receipts than any other agricultural commodity in the country (USDA-ERS, 2023). The importance of rangelands exceeds their function as a forage source for grazing livestock and wildlife and are a larger part of socioeconomic and environmental dynamics. Rangelands support biodiversity of plants and wildlife, sequester carbon in the soil, regulate water and nutrient cycles, and are foundational to rural economies (Godde et al., 2018; Milner, 2010). Despite its integral role nationwide, the beef cattle industry constantly receives pressure to increase the sustainability of management practices, promote biodiversity, and improve ecosystem function in an unpredictable environment. Sustaining these

ecosystems while maintaining productive grazing systems is a central challenge for producers and rangeland managers.

Rangeland health is typically quantified by evaluating hydrologic function, soil stability, and biotic integrity (Printz et al., 2014; Pyke et al., 2002). While environmental conditions, such as the semi-arid climate of the Intermountain West, heavily influence these factors, soil biology can also have significant impacts on overall ecosystem function (Van Der Heijden et al., 2008). Manipulating grazing variables such as stocking rate, timing, frequency, and animal species can enhance ecosystem services and support long-term productivity when properly managed (Westoby et al., 1989; Williams et al., 2022). Although grazing management has been studied extensively for its effects on plant community structure and function, far less is known about its influence on the composition, function, and resilience of soil microbial communities.

Timing of grazing plays a critical role because plants experience seasonal fluctuations in their ability to recover from defoliation, or grazing, but is a less understood management tool (Alkemade et al., 2013; Bailey, 2004; Mosley, 1997; Zhou et al., 2017). Due to the harsh climate and unpredictability of environmental factors, native rangeland plants in the Intermountain West are particularly sensitive to grazing in the early growing season (Davis et al., 2014; DelCurto et al., 2023; Osmond, 2007). In the Intermountain West, the critical stage of growth occurs from elongation of the culm through seed development (Holechek, 2004). During this time, plants have a higher energy demands, regrowth potential declines with decreasing soil moisture, and grazing pressure reduces plants' ability to produce both root and shoot, often resulting in the allocation of energy towards aboveground growth at the expense of root development (Blaser, 1986; DelCurto et al., 2023).

Microorganisms in the soil are the underlying drivers of ecosystem function, performing crucial functions for nutrient cycling, organic matter decomposition, plant development, and plant resiliency to environmental stressors such as harsh weather conditions, grazing pressure, and anthropogenic disturbances (Doran & Parkin, 1994). Despite its importance, the soil microbiome response to grazing, remains largely unexplored (Stott, 2019; Yang et al., 2018). Grazing can alter microbial biomass carbon (MBC) and microbial activity, along with the richness, diversity, function, and composition of soil microbial communities (Wu et al., 2022; Yang et al., 2018). Alpha- and beta-diversity metrics such as richness and relative abundance yield estimates of species composition and biodiversity, but do not account for functional roles or interactions between taxa. Microbial community functionality is complex with individual species maintaining positive or negative relationships creating a network of functions that are impacted by biodiversity (Barberán et al., 2011; Khatri-Chhetri et al., 2024). While these analytical methods are well established, there is a lack of research within microbial ecology that informs soil health management in rangeland grazing systems. Moreover, the effects of management variables such as timing, duration, and intensity of grazing on soil microbial traits remain poorly quantified. Thus, identifying the soil microbial community response to these environmental pressures may improve our understanding of rangeland biological integrity and sustain agricultural productivity by guiding future management decisions.

Microbial activity is responsive to management changes, making it a useful indicator of soil health (Bargali, 2024; Stott, 2019). Factors such as nutrient availability, texture, pH, and moisture content influence microbial activity in the soil, which in turn affect plant growth and ecosystem function (Nautiyal et al., 2010). Soil microbial biomass carbon represents the living component of soil organic matter, is sensitive to management changes, and is responsive to

organic matter deposition from plant litter and root exudates. Thus, it can be a useful metric to estimate C and N pools and biological activity in the soil (Holt, 1997; Stott, 2019).

Given the limited knowledge available regarding the effects of grazing timing on the soil microbiome and soil health, the objective of this research was to evaluate how timing of grazing during the growing season affects microbially-mediated soil health indicators. Specific objectives included understanding how the environment drives changes in the soil microbiome across both treatment effects and seasonality, evaluate how grazing deferment affects microbial activity (as measured by SIR) and biogeochemical processes as it relates to carbon cycling, and profile the soil microbial community's composition, structure, connectivity, and function in response to this grazing regime. We hypothesize that microbial activity will be greatest in the late season grazing treatment due to the longer undisturbed growing period similar to the ungrazed control. Additionally, we predict that soil moisture, temperature, and pH will be primary drivers of the microbial community as less stress-tolerant microbes may have negative responses to desiccation and pH will act as a regulator of microbial metabolism through enzyme activity. Further, we predict that the microbial community composition and structure will be most similar between the ungrazed control and late grazed pastures for similar reasons, but that the core microbiome, or keystone taxa, will remain consistent across all four treatments. By investigating these relationships, we seek to enhance understanding of how grazing management influences soil health and impacts rangeland sustainability.

Methods

Ethics Statement

All protocols and procedures were approved by the Montana State University Agricultural Animal Care and Use Committee (#2019-AA12). All animals used in this study were provided by the Montana Agriculture Experiment Station.

Study Site

This experiment was performed at Montana State University's Red Bluff Research Ranch, located near Norris in southwest Montana, USA (45.556984, -111.691292). There is minimal slope at the study location (2 – 8%) and the soil type is predominantly Varney clay loam, a deep well-draining soil formed in alluvium (Web Soil Survey, 2024*Web Soil Survey*). Field experiments were conducted from May through July in 2024 and 2025. In 2024 the mean annual temperature and mean annual precipitation (rain and snowfall) were 8.0 ^{o}C and 142 cm, respectively. In 2025 the mean annual temperature and precipitation were 7.6 ^{o}C and 105 cm, respectively (NOAA, 2025).

Experimental Design

This experiment evaluated the effect of timing of grazing throughout the growing season on the soil microbial community and microbial soil health indicators. The experiment was set up as a randomized complete block design (Fig.1, RCBD) with four replicates. Sixteen four-hectare pastures were divided into four 16 ha blocks with four pastures within each block randomly assigned to treatments. Grazing treatments were implemented as follows: Mid-May early season grazing (EG), early June mid-season grazing (MG), End of June late season grazing (LG), and ungrazed controls (UG). Four mature cannulated cows, stratified by weight, were allocated per

pasture at each treatment time and grazing treatments were applied for three weeks at a time. The grazing experiment was repeated for two years with identical treatments applied to each pasture across years.

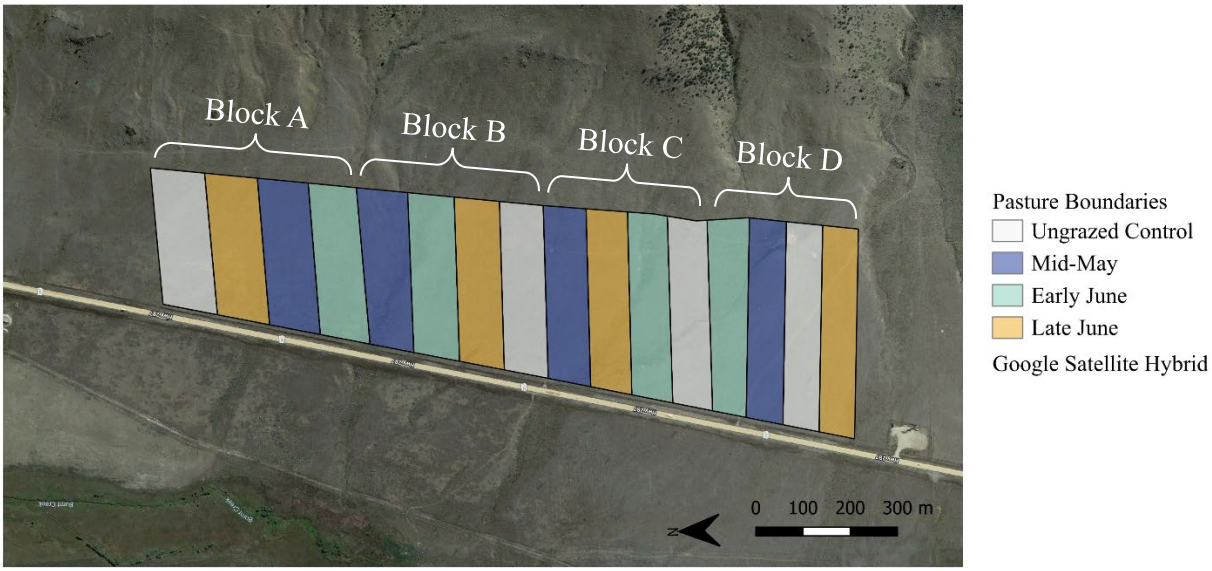


Figure 1. Randomized complete block design for the field experiment at Red Bluff Research Ranch (Norris, MT) with a total of sixteen pastures divided into four 40 ac blocks, each consisting of four 4 ha pastures randomized by treatment.

Soil sampling & preparation

Six sampling locations per pasture were stratified based on soil electrical conductivity (EC) from an EM38 Mk2 ground conductivity meter (Geonics, Mississauga, ON, Canada), soil moisture, and elevation on the site, measured by SWIR Sentinel satellite imagery (Fig. 2). HOBO MX (Onset, Bourne, MA) soil moisture and temperature data loggers were deployed in each pasture at the same locations that soil sampling would take place (Fig. 2). Each sensor was buried at a depth of 15 cm. Soil sampling was conducted during the second year of study (2025) to allow for system acclimation and to increase the likelihood of detecting a biological response. Sampling of all 16 pastures was performed every three weeks upon grazing rotation and three weeks after cattle were removed from the study site for a total of five sampling timepoints (mid-

May, early June, late June, mid-July, and early August). Using sterile technique with soil step probes (AMS, American Falls, ID) the top 15 cm of soil were sampled after each grazing treatment for a total of 480 samples (4 treatments x 4 replicates x 6 samples per treatment x 5 sampling dates). The samples were kept on ice until transported to the lab where they were immediately homogenized and any plant material removed by passing soil through a no. 10 sieve (2 mm). Samples were subset into 50 mL centrifuge tubes for microbial biomass carbon (MBC), extracellular enzyme activity (EEA). A smaller aliquot of each soil sample was subset into a 2 mL tube for DNA analysis. Samples were subsequently stored at -20ºC to prevent degradation or change until further analysis. Samples requiring long-term storage were kept at -80ºC.

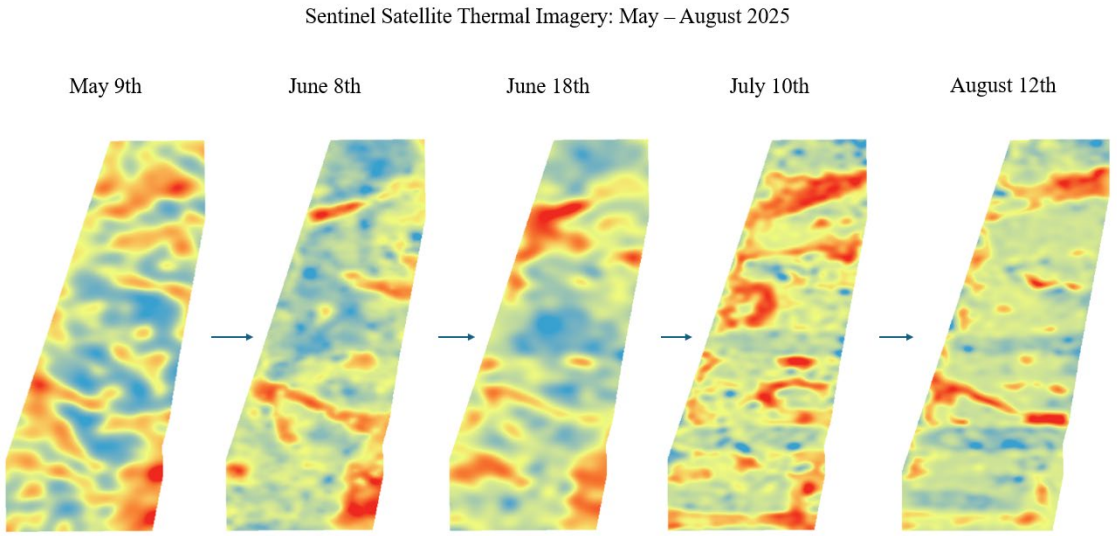


Figure 2. Change in percent soil moisture at the Red Bluff Research Ranch study site in southwestern Montana throughout the growing season measured by Sentinel satellite thermal imagery. Blue indicates wetter conditions and red, drier.

A subset of soil from the first sampling timepoint in mid-May was dried for 48 hours at 55 ºC and sent to Ward Laboratories, Inc. (Kearney, NE) for soil chemistry analysis. The

following physical properties, nutrients, and minerals were measured: texture, organic matter (%LOI), pH, nitrate nitrogen (ppm), Olsen phosphorus (ppm), sulfate sulfur (ppm), zinc (ppm), iron (ppm), manganese (ppm), copper (ppm), calcium (ppm), magnesium (ppm), sodium (ppm), boron (ppm), and cation exchange capacity sum of cations (me/100g).

Microbial Biomass Carbon

Microbial biomass determination was estimated using the substrate induced respiration (SIR) method to determine the effects of grazing timing (Fierer, 2003; West & Sparling, 1986). Approximately 5 g of fresh soil was weighed in a 50 mL centrifuge tube equipped with rubber septa caps for gas sampling. 10 mL of yeast solution (3g autolyzed yeast extract to 250mL ddH_2O) was added to each tube. Tubes were then sealed and shaken horizontally at 20^oC for the duration of the assay. After 10-20 minutes of shaking, a 1 mL headspace gas sample was removed and injected into a Q-S151 Infrared CO_2 Analyzer (Qubit Systems, Kingston, ON, Canada). This was the T0 time point. The peak of the resulting respiration curve and time of injection were recorded. Concentration of CO_2 (ppm) was recorded two more times at approximately 2 hours and 4 hours after the initial T0 time point, each with a 1 mL sample. The respiration rate (μg C-CO_2/g soil/hour) was calculated.

DNA and Metagenomic Preparation

DNA was extracted from a 250 mg soil subsample using the Qiagen DNeasy PowerSoil Pro Kit (Qiagen Inc., Germantown, MD, USA), adhering to the manufacturer's protocol. Library preparation and amplicon sequencing of the bacterial V4-V5 variable region of the 16S rRNA gene and fungal ITS2 region were performed by Novogene (Sacramento, CA) on the Illumina NovaSeq 6000 platform using the NovaSeq Reagent Kit (500 cycles).

Sampling for metagenomic analysis occurred at two timepoints in late June and early August in all pastures. Following sample collection and homogenization, 2 g subsamples were preserved in 16 mL Qiagen LifeGuard Soil Preservation reagent to prevent RNA degradation. RNA and DNA were coextracted using the ZymoBIOMICS DNA/RNA Miniprep Kit and submitted to Novogene (Sacramento, CA) for library preparation and metagenomic sequencing on the Illumina NextSeq (150+150 bp PE) platform.

Quality filtering, chimera removal, and assembly of reads into error-corrected amplicon sequence variants (ASVs) were performed using dada2 (Callahan et al., 2016). Taxonomic assignments were performed on the Silva 138.2 database for bacterial 16S amplicons and the Unite fugal taxonomy database for ITS amplicons. Taxa were filtered with a 0.25% minimum relative abundance threshold to remove spurious sequences (Reitmeier et al., 2021).

Statistical analysis

Statistical analyses for microbial community composition and microbial biomass carbon were conducted in RStudio, version 4.4.2. Taxa and operational taxonomic unit tables (OTU) were assembled using the *dada2* package and both the *phyloseq* and *microbiome* packages were applied to assess community richness, alpha diversity (Shannon diversity index), and evenness (Lahti, 2017; McMurdie & Holmes, 2013). Relative abundance of taxa (beta diversity) was calculated using the *microViz* package (Barnett, 2021). The g*gplot2* package was used for all data visualization and QGIS was used for mapping (QGIS Development *QGIS Geographic Information System*, 2025; Wickham, 2016).

Ordination analysis of community structure was performed on the Bray-Curtis dissimilarity matrix to visualize β-diversity, or variation in the microbial community structure, across all samples. Differences in community composition between grazing times were

determined at the phylum, family, and genus levels using a Kruskal-Wallis test with a significance threshold of $p < 0.05$ to identify differences in the soil microbiome between treatments.

Variance Partitioning was conducted using the *phyloseq* package (McMurdie & Holmes, 2013). Soil factors considered included volumetric water content (%VWC), temperature (ºC), nitrate (ppm), Olsen phosphorus (ppm), sulfate sulfur (ppm), organic matter (%LOI), and SIR. Effect summaries were filtered to include ASVs above a 5% threshold.

Co-occurrence network analysis was performed to determine how treatments affect the connectivity and robustness of microbial communities. Microbial community networks were visualized in Cytoscape (Shannon et al., 2003).

Differential abundance analysis was performed using Random Forest – Kruskal-Wallis rank sum test with the *microeco* package (Liu et al., 2021) to identify significant differences in the relative abundance of taxa and predicted functions.

Metagenomic sampling was done at the late June timepoint and the end of season timepoint, early August, in all pastures. Predictive functional profiling of the 16S metagenomic dataset was performed using *microeco* (Liu et al., 2021) and *file2meco* (Liu et al., 2022) packages, KEGG pathways were compared at Level.2. Ecological functions of the fungal ASVs (ITS amplicons) were predicted using the FUNGuild database and guilds were classified according to trophic models (Nguyen et al., 2016; Põlme et al., 2020).

Results

Soil physical & chemical properties

The soil properties of this site are generally characteristic of rangelands (Fig. 3). Soil pH was relatively neutral with an east-west gradient. Organic matter and Nitrate N followed a rough

north-south gradient, but along with K and S, these nutrient levels were normal for this site (Jones & Olson-Rutz, 2025). Phosphorus, however, was low and could present as a limiting nutrient in downstream analysis. Olson phosphorus should fall between 16 and 30 ppm. On average, phosphorus averaged 10.8 ppm on the site across treatments (Tab. 1). The ungrazed control pastures exhibited higher water content and lower temperature. The early June grazing treatment had the lowest SIR rates of the four treatments.

Table 1. Mean ± standard deviation of soil physical, chemical, and microbial activity properties according to treatment at the study site at Red Bluff Research Ranch, in southwestern Montana (data from 2025).

Soil Property	**Ungrazed Control**	**Mid-May**	**Early June**	**Late June**
pH	7.18 ± 0.49	7.05 ± 0.41	7.30 ± 0.54	7.09 ± 0.44
Organic Matter (%LOI)	4.10 ± 0.81	3.60 ± 0.92	3.88 ± 0.75	4.15 ± 0.75
Nitrate Nitrogen (ppm)	1.08 ± 0.69	0.89 ± 0.54	1.08 ± 0.59	0.86 ± 0.37
Olsen Phosphorus (ppm)	11.49 ± 8.83	10.15 ± 2.91	10.25 ± 3.83	11.27 ± 2.58
Potassium (ppm)	365.79 ± 127.70	351.62 ± 61.55	362.58 ± 86.79	347.38 ± 62.05
Sulfate Sulfur (ppm)	10.00 ± 1.27	9.32 ± 1.46	9.67 ± 1.11	10.23 ± 1.04
Volumetric Water Content (%)	18.41 ± 7.46	17.28 ± 6.58	15.58 ± 10.60	18.39 ± 6.32
Temperature (°C)	16.79 ± 3.70	18.16 ± 4.09	17.76 ± 3.69	17.55 ± 3.82
SIR (ug C CO2/kg soil)	13.03 ± 6.77	13.14 ± 6.75	11.40 ± 5.25	13.18 ± 7.42

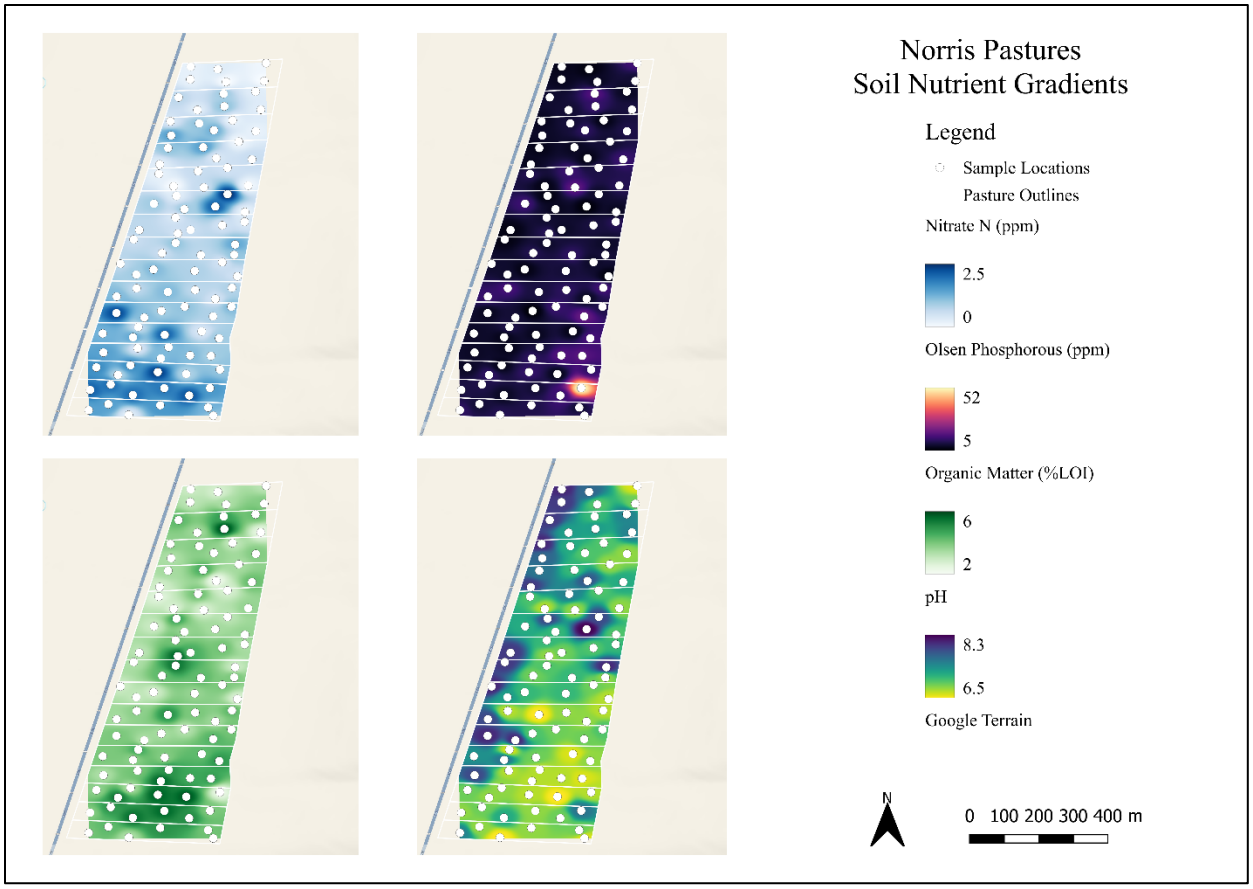


Figure 3. Interpolated maps of nutrient gradients across the study site at Red Bluff Research Ranch, in southwestern Montana, including nitrate N (ppm) and organic matter (%LOI), which have a North-South gradient, Olsen phosphorus (ppm), and pH, which has both a North-South and East-West gradient (data from 2025).

Soil Microbial Activity

Soil microbial activity, as measured by SIR, did not differ between grazing treatments. (Fig. 4; $p = 0.11$). However, the early June turnout treatment showed a tendency toward overall lower respiration rates and exhibited a tighter distribution of values compared to the other treatments.

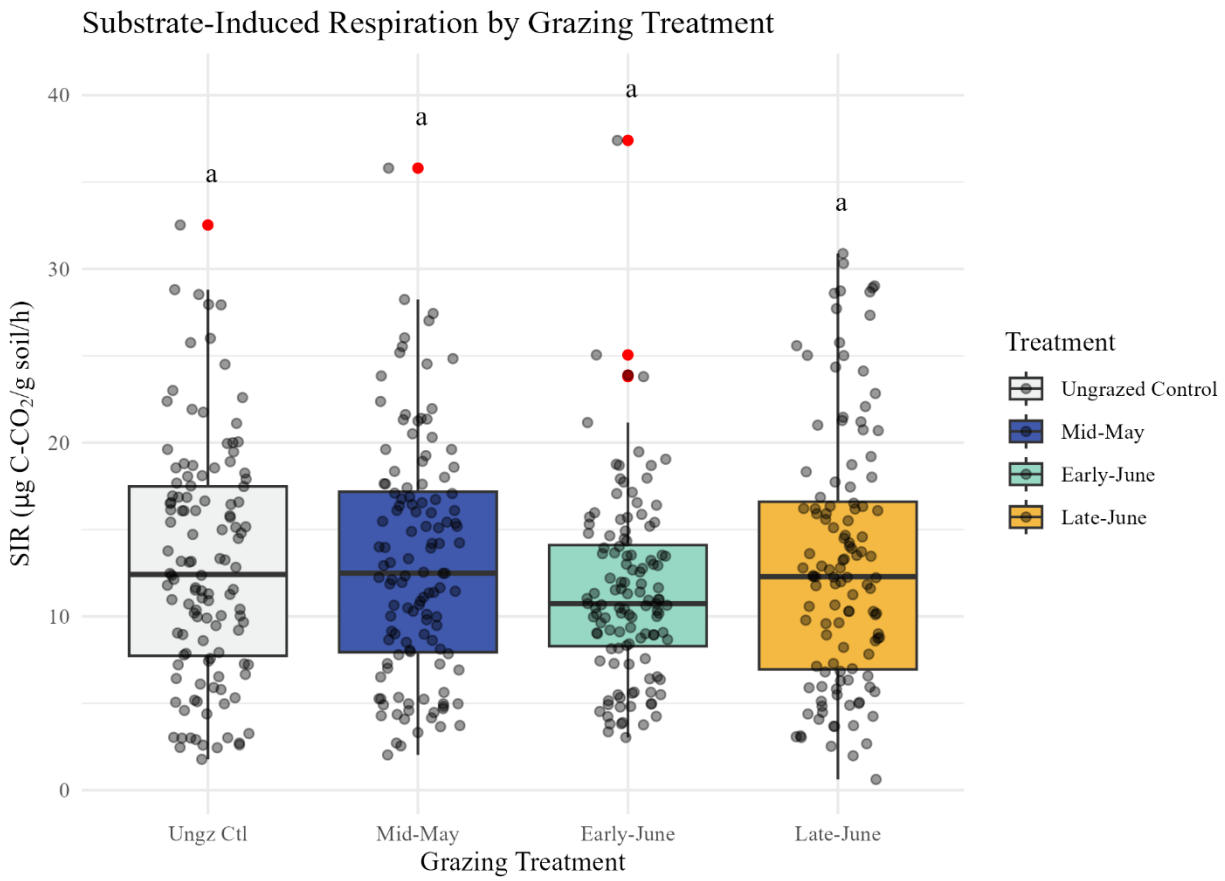


Figure 4. Substrate induced respiration according to timing of grazing in the 2025 season at Red Bluff Research Ranch in southwestern Montana. No significant differences were detected in SIR between grazing turnout times.

Substrate induced respiration increased gradually throughout the duration of the study regardless of treatment (Fig. 5). The lowest mean SIR was recorded at the first sampling point in mid-May (4.57 ug CO_2 C/kg soil/h ± 1.42), while the highest was observed at the fourth timepoint in mid-July (17.5 ug CO_2 C/kg soil/h ± 5.70) before decreasing slightly in early August (16.7 ug CO_2 C/kg soil/h ± 6.58), three weeks after cattle had been removed from the study site. This progressive increase likely reflects seasonal trends in moisture and temperature that stimulate microbial metabolism. The early June treatment displayed the most distinct response in that the SIR rates consistently remained the lowest among treatments across all sampling time points. By the final timepoint, SIR values across treatments converged, indicating that the initial

timing of grazing effects did not lead to persistent differences in microbial activity later in the season when plants became dormant.

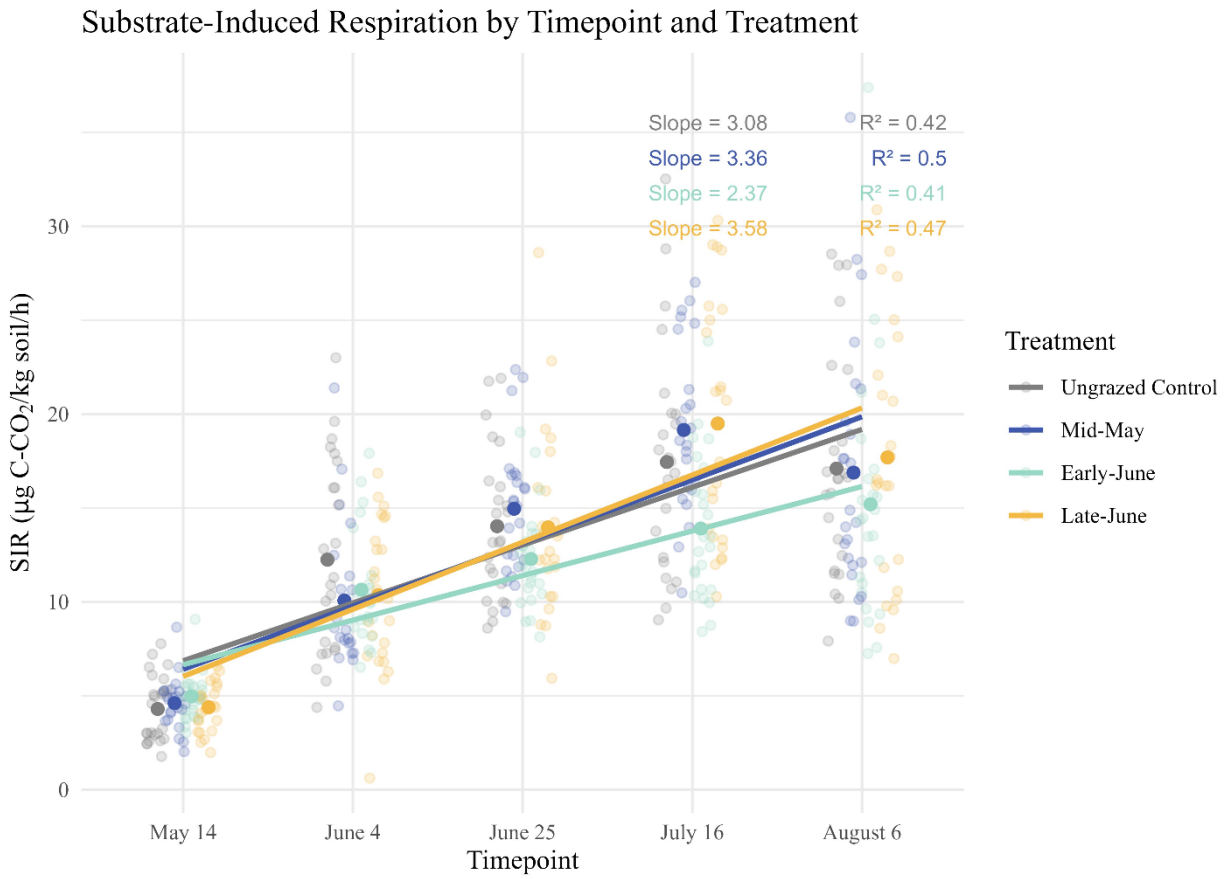


Figure 5. Linear fit of SIR according to timing of grazing and sampling timepoint throughout the 2025 season at Red Bluff Research Ranch in southwestern Montana. Timepoint had a significant impact on respiration values, increasing progressively throughout the study period.

Linear modeling indicated that several environmental factors significantly correlated with SIR (Tab. 2). Organic matter content and sampling timepoint had a strong positive effect on SIR ($p < 0.0001$). Soil pH also impacted SIR ($p = 0.010$). These findings are consistent with the understanding that soil organic carbon is a primary metabolite for microbial communities and that pH can also influence microbial activity and metabolism. When VWC and temperature were not included in the model, SIR displayed a significant treatment by timepoint interaction ($p = 0.0031$). Soil N, P, and K were not significant predictors of SIR.

Table 2. Type III Analysis of Variance Table with Satterthwaite's method modeling SIR as a function of soil physical and chemical properties at Red Bluff Research Ranch in southwestern Montana (2025).

	Den df	F value	Pr(>F)
Treatment	452.14	4.9868	0.002058 **
Timepoint	451.91	177.3249	< 2.2e-16 ***
Organic Matter (%LOI)	453.10	37.5141	1.971e-09 ***
pH	444.38	6.6132	0.010447 *
Nitrate (ppm N)	414.52	0.4294	0.512657
Phosphorus (ppm P)	453.59	1.6452	0.200263
Potassium (ppm K)	453.95	2.1552	0.142780
Treatment:Timepoint	451.91	2.5281	0.003115 **

Microbial Community Composition

A total of 14,021,951 bacterial (16S rRNA) and 12,473,206 fungal (ITS) raw sequence reads were generated from 480 soil samples, providing robust sequencing depth for downstream diversity analysis. Bacterial and fungal community evenness and richness were determined using the Shannon diversity index and observed diversity. The Shannon diversity index did not differ significantly among grazing treatments for either bacterial or fungal communities (Fig. 6ab). Although values fluctuated slightly throughout the season, no consistent patterns attributable to grazing treatment were observed. This indicates that grazing treatment did not influence within-sample microbial richness or evenness during the 2025 grazing season.

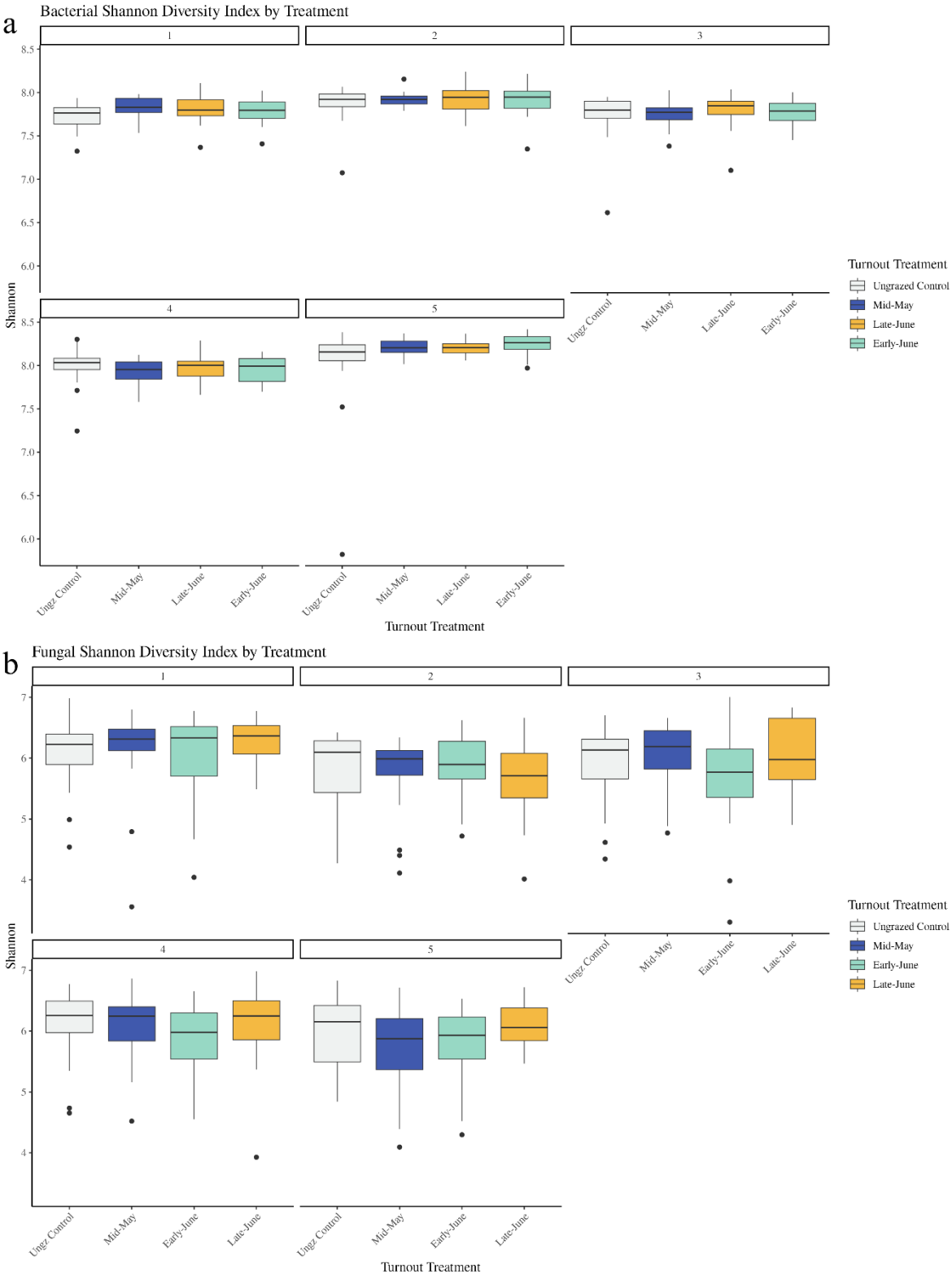


Figure 6. Shannon diversity index among treatments (timing of grazing) and sampling timepoints for 16S (a) and ITS (b) datasets at Red Bluff Research Ranch in southwestern Montana. Sampling timepoints occurred on May 14th (1), June 4th (2), June 25th (3), July 16th (4), and August 6th (5) 2025. Shannon diversity indices did not differ significantly.

In contrast, pairwise comparisons indicated that there was a significant difference in the observed diversity of the bacterial community temporally, throughout the growing season, with the first timepoint in mid-May expressing the lowest value and the third sampling timepoint in late June expressing the highest (Figure 7a). Both the first and third sampling time points were significantly different from the other three time points ($p < 0.001$), suggesting that the communities experienced distinct seasonal shifts in richness at the beginning and peak of the growing season. Sampling timepoint three, in late June, had the highest median observed diversity, significantly higher than timepoints in early June, mid-July, and early August ($p < 0.001$). Sampling timepoints in mid-July and early August formed a statistically homogenous group exhibiting a lower median diversity ($p = 0.039$), suggesting that the observed diversity stabilized at a lower level following the peak. Fewer differences were observed in the fungal community, but the second and fourth timepoints had lower observed diversity than the first, third, and fifth timepoints ($p < 0.05$; Fig. 7b). The first timepoint, in mid-May was not statistically significant from the peak ($p = 0.825$), suggesting a transitional state when grazing began.

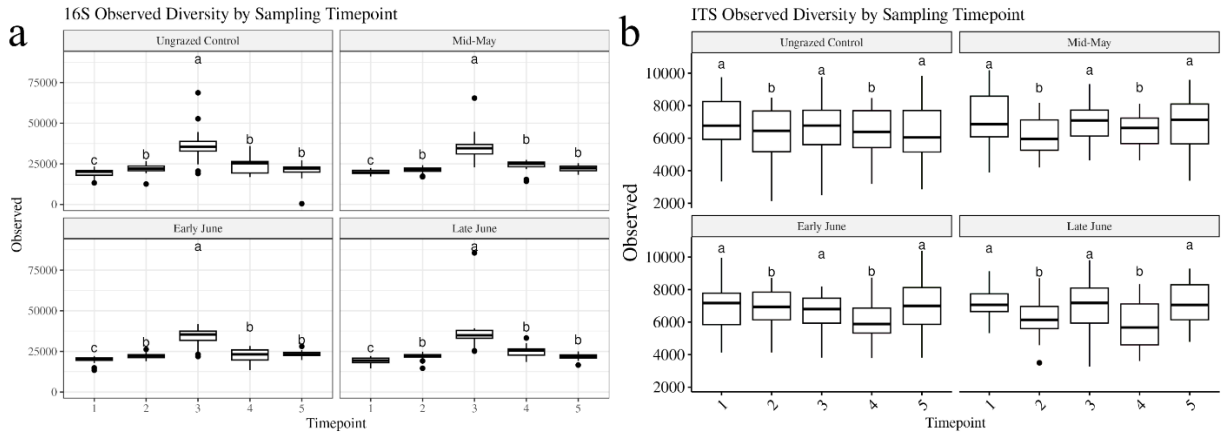


Figure 7. Observed diversity, or number of unique taxa, in the 16S (a) and ITS (b) communities across sampling timepoints at Red Bluff Research Ranch in southwestern Montana (2025). Observed diversity significantly changed across sampling timepoints, regardless of treatment.

In all locations, bacterial communities were predominated by the phyla *Actinomycetota* and *Pseudomonadota* (formerly *Proteobacteria*), and *Bacillota* (Fig. 8a). *Pseudonocardia*, *Solirubrobacter*, *Microlunatus*, *Niallia*, and *Nocardioides* were among the most abundant bacterial genera (Fig. 8c). The prevalence of *Actinomycetota* and *Pseudomonadota* suggests a community adapted to complex organic matter degradation and high soil C/N ratios (Bao et al., 2021).

The fungal community was primarily composed of the phyla *Ascomycota* and *Basidiomycota* (Fig. 8b). Genera from the *Ascomycota* phylum along with *Cladosporium*, *Penicillium*, and the *Pleosporales* order were the most abundant fungal taxa (Fig. 8d). *Ascomycota* is a terrestrial root-associated fungi responsible for cell wall degredation669 and *Basidiomycota* is a key decomposer of lignin (Manici et al., 2024).

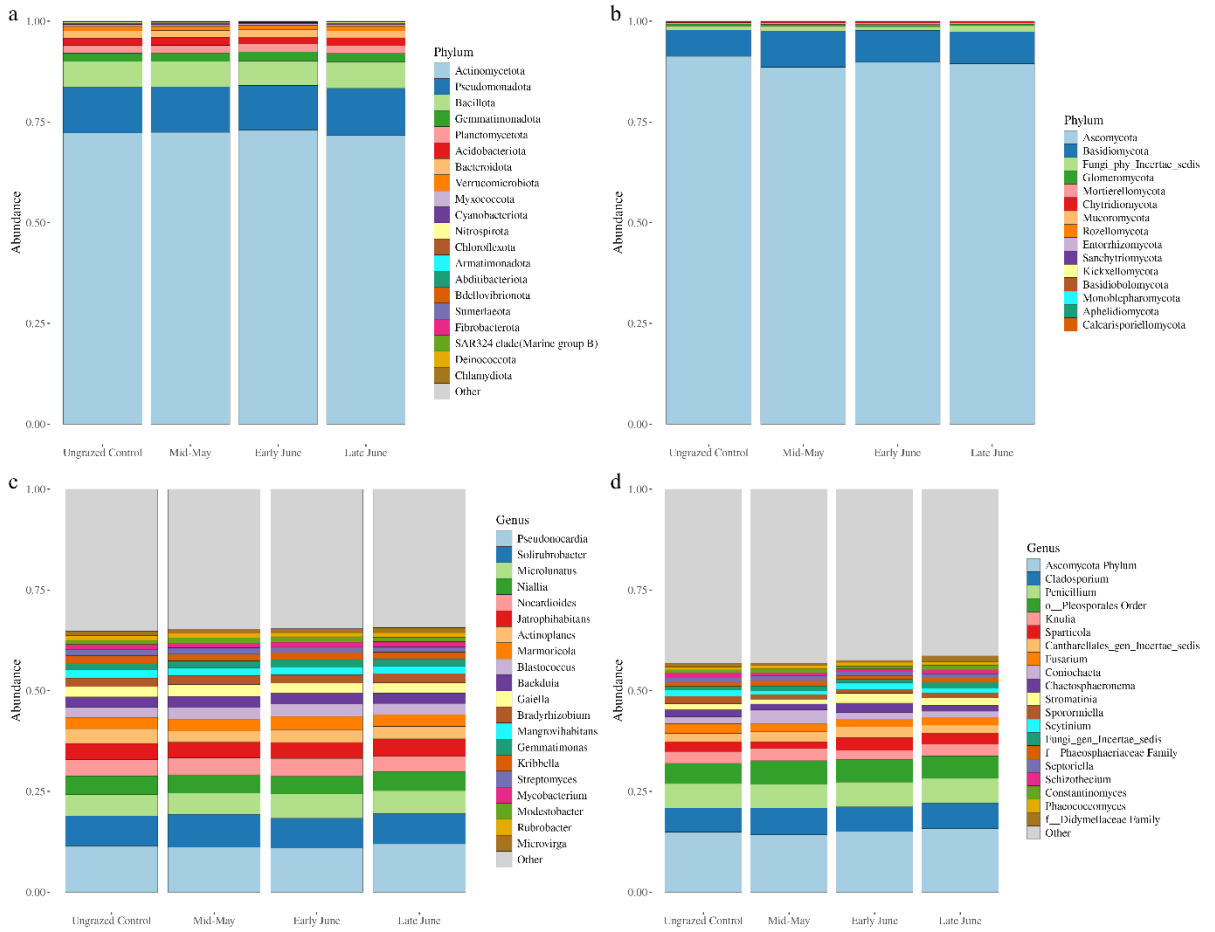


Figure 8. Relative abundance of bacterial (a) and fungal (b) phyla and relative abundance of bacterial (c) and fungal (d) genera grouped by grazing treatment at Red Bluff Research Ranch in southwestern Montana (2025).

Principal Co-ordination Analysis

The principal co-ordination analysis (PCoA) based on Bray-Curtis dissimilarities revealed shifts in the microbial community structure across treatments (Fig. 9). While the plots show a high degree of overlap, the 16S axes explained 11.7% and 5.7% of the total variation respectively (Fig. 9a) and the ITS axes explained 26.2% and 6.8% of variation respectively (Fig. 9b). The PERMANOVA results confirmed that timing of grazing had a significant impact on overall community composition (16S: $R^2 = 0.013$, $F = 2.1126$, $p = 0.001$; ITS: $R^2 = 0.012$, $F = 1.9932$, $p = 0.004$). Post-hoc pairwise PERMANOVA tests indicated that bacterial community

structure differed significantly among nearly all treatment groups. The early and mid-treatment groups showed the strongest differentiation from other treatments, particularly when compared to each other ($F = 2.86$, $p = 0.006$). As hypothesized, the late treatment did not differ from the ungrazed control ($p = 0.064$). In contrast, the only significant differences in the fungal community occurred between the mid and late grazing treatments ($F = 2.67$, $p = 0.024$). While differences in treatments were significant, R^2 values were low for both models across all treatments, explaining a small portion of total variation among samples.

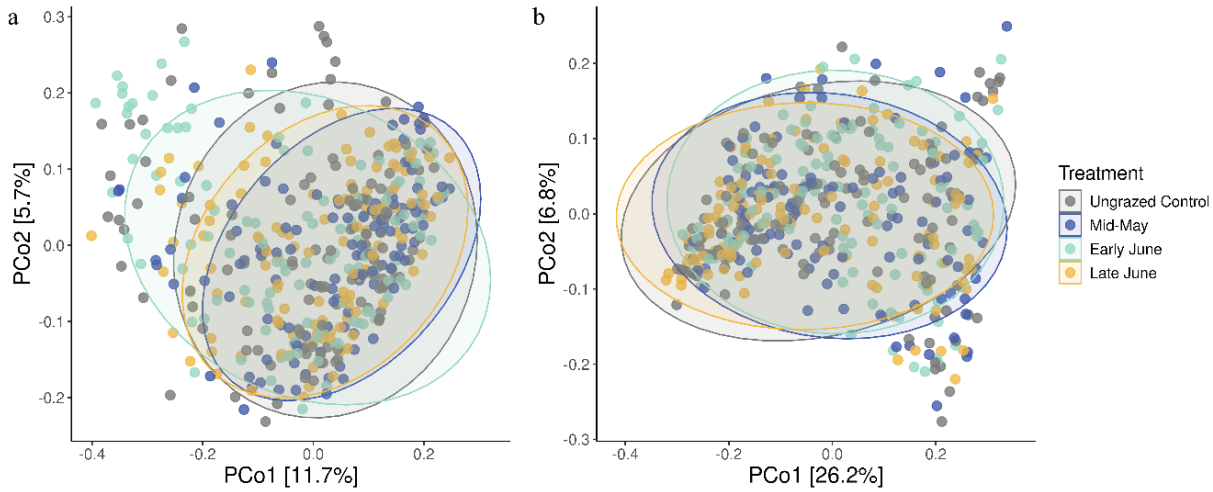


Figure 9. Principal co-ordination plots based on Bray-Curtis dissimilarities for (a) bacterial 16S rRNA and (b) fungal ITS communities at Red Bluff Research Ranch in southwestern Montana (2025). Each point represents an individual sample colored by treatment group. Percentages on the axes represent a proportion of total variation explained by each principal coordinate. Bacterial communities were significantly influenced by treatment (PERMANOVA: $R^2 = 0.013$, $p = 0.001$), with 11.7% of the total variance explained by the primary axis. Fungal communities exhibited a higher variance explained by the primary axis (26.2%) and were also significantly affected by treatment (PERMANOVA: $R^2 = 0.012$, $p = 0.004$).

Constrained Ordination Analysis

The dbRDA results show that while both 16S and ITS communities are structured by environmental and soil chemistry variables, the bacterial community was more strongly constrained by these factors ($R^2 = 0.278$, $p = 0.001$), with the first two axes accounting for 75.7%

of that constrained variance (Fig. 10ac; dbRDA1 = 47.8%; dbRDA2 = 27.9%). In contrast the environmental variables explained a much smaller portion of the fungal community variation (Fig. 10bd; $R^2 = 0.088$, $p = 0.001$). This suggests that soil bacteria are more sensitive to soil chemistry while soil fungal communities may be more driven by stochastic processes.

Vector fitting of the bacterial community indicated that phosphorus availability (Olsen P; $R^2 = 0.51$, $p = 0.001$), microbial activity (SIR; $R^2 = 0.47$, $p = 0.001$), potassium (K; $R^2 = 0.34$, $p = 0.001$), and pH ($R^2 = 0.24$, $p = 0.001$) were the most influential drivers of community composition (Fig. 10a). Samples from the ungrazed control treatment clustered slightly separate from the early, mid, and late treatments along the dbRDA1 axis, driven by higher pH and VWC in the grazed pastures. The high level of overlap may suggest a higher level of community resilience. The fungal community showed similar directional responses to the same environmental drivers, though clustering was more distinct than that observed in bacteria (Fig. 10b). While pH ($R^2 = 0.56$, $p = 0.001$), phosphorus (Olsen P; $R^2 = 0.55$, $p = 0.001$), potassium (K; $R^2 = 0.38$, $p = 0.001$) and activity (SIR; $R^2 = 0.37$ $p = 0.001$) remained significant correlates (Fig. 10a), there was less overlap between treatments compared to bacteria. Temporal shifts were observed in both communities across the five sampling timepoints (Fig. 10cd). While community composition shifted progressively from mid-May (timepoint 1) to early August (timepoint 5), the substantial overlap of the confidence intervals suggests that treatment effects were more dominant that seasonal variation in defining the fungal community structure (Fig. 10bd) while the opposite is true for the bacterial community (Fig. 10ac).

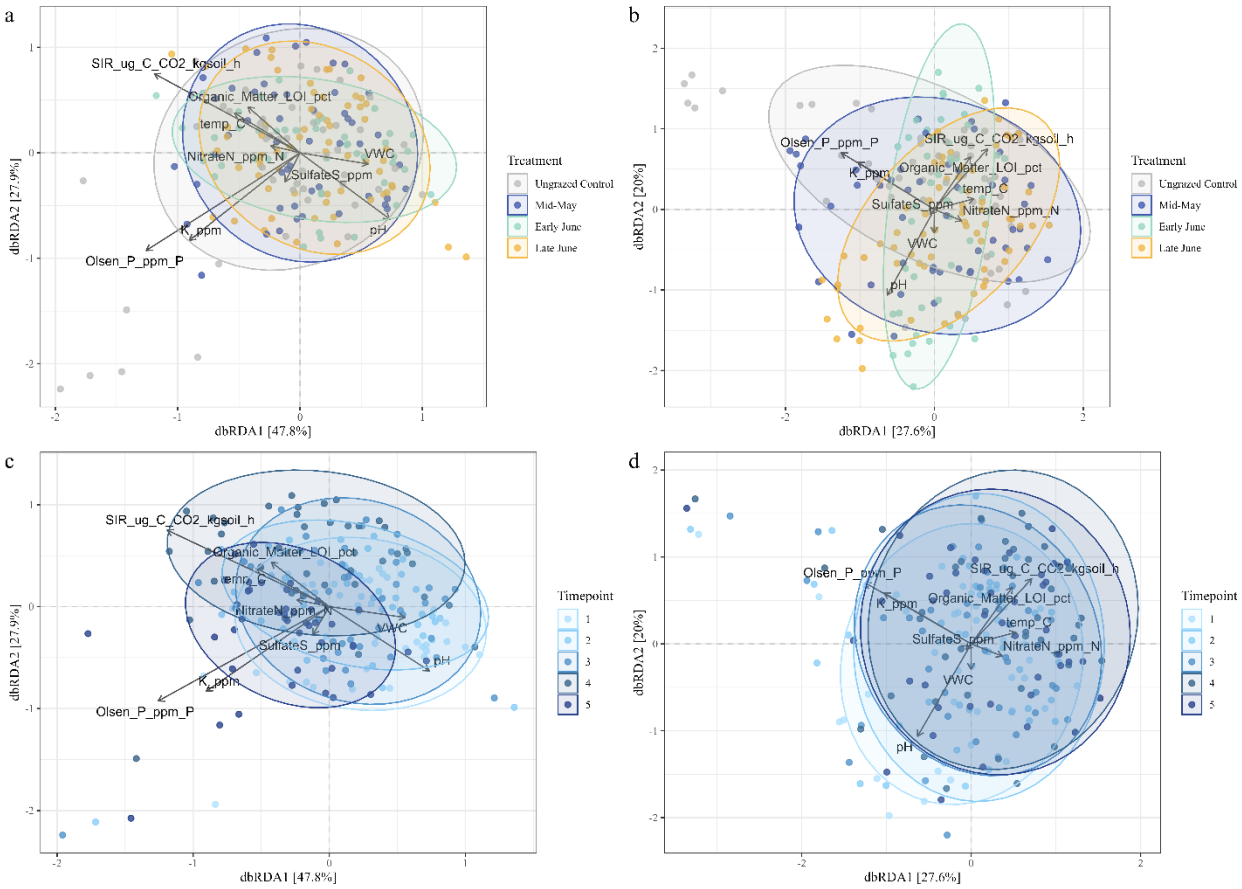


Figure 10. Constrained ordination, distance-based redundancy analysis (dbRDA) of microbial communities driven by environmental variables at Red Bluff Research Ranch in southwestern Montana (2025). The plots show the relationship between treatment and sampling timepoint on the variation in microbial community structure. The percentage of constrained variance explained by each axis is shown in brackets. Points indicate individual samples, ellipses show the 95% confidence interval around the centroid (mean) of the respective group (treatment or timepoint). Overlapping ellipses suggest no significant difference between the groups. Vectors represent environmental variables, scaled by their correlation with the ordination axes. The length and direction of the arrow indicate the strength and relationship of the variable to the community structure. a) Bacterial community grouped by treatment. The first two axes explained 75.7% of the community variation (dbRDA1 = 47.8; dbRDA2 = 27.9%). b) Fungal Community grouped by treatment. The overall model explains 8.8% of the community variation (dbRDA1 = 27.65%; dbRDA2 = 20%). c) Bacterial community grouped by sampling timepoint (1-5, corresponding to mid-May, early June, late June, mid-July, and early August. d) Fungal community grouped by sampling timepoint.

Variance Partitioning

Variance partitioning analysis was used to quantitatively assess the relative contributions of treatment, soil chemistry, key environmental variables, and microbial activity (SIR) to the

variation in amplicon sequence variants (ASVs). 472 ASVs were considered in the 16S bacterial model and 454 ASVs were considered in the ITS fungal model. Among the measured parameters, soil phosphorus (P) content emerged as the single strongest predictor of variance in microbial community structure accounting for 5.9% of variance in 34.7% of bacterial genera and 4.8% of variance in 26.7% of fungal genera (Fig. 11). The sampling timepoint (seasonal variation) was the second most important variable confirming the expected temporal dynamics in the bacterial community structure (Fig. 11a; 3.9% of 25.2% ASVs). For the fungal community, block explained 3.1% of the variation of 21.6% ASVs, suggesting that spatial variation has a greater impact on fungal communities compared to bacterial communities (Fig. 11b). Soil pH contributed the next largest fraction of total variance, explaining 3.5% of variance in 21.8% of bacterial ASVs and 2.8% of variance in 17.8% of fungal ASVs. Variance in ASVs explained by SIR was similar between bacterial and fungal analysis where SIR explained 2.7% of the variance in 16.7% of bacterial ASVs and 2.2% of the variance in 12.6% of fungal ASVs.

The four variables together accounted for 16% of bacterial variance and 12.9% of fungal variance. This implies that about 85.5% of the total variance remained unexplained (residuals) which can likely be attributed to unmeasured environmental factors such as spatial, topographical, and ecological processes. Lastly, treatment had a greater impact on the variance in fungal ASVs (10.6%) compared to bacterial ASVs (5.5%)

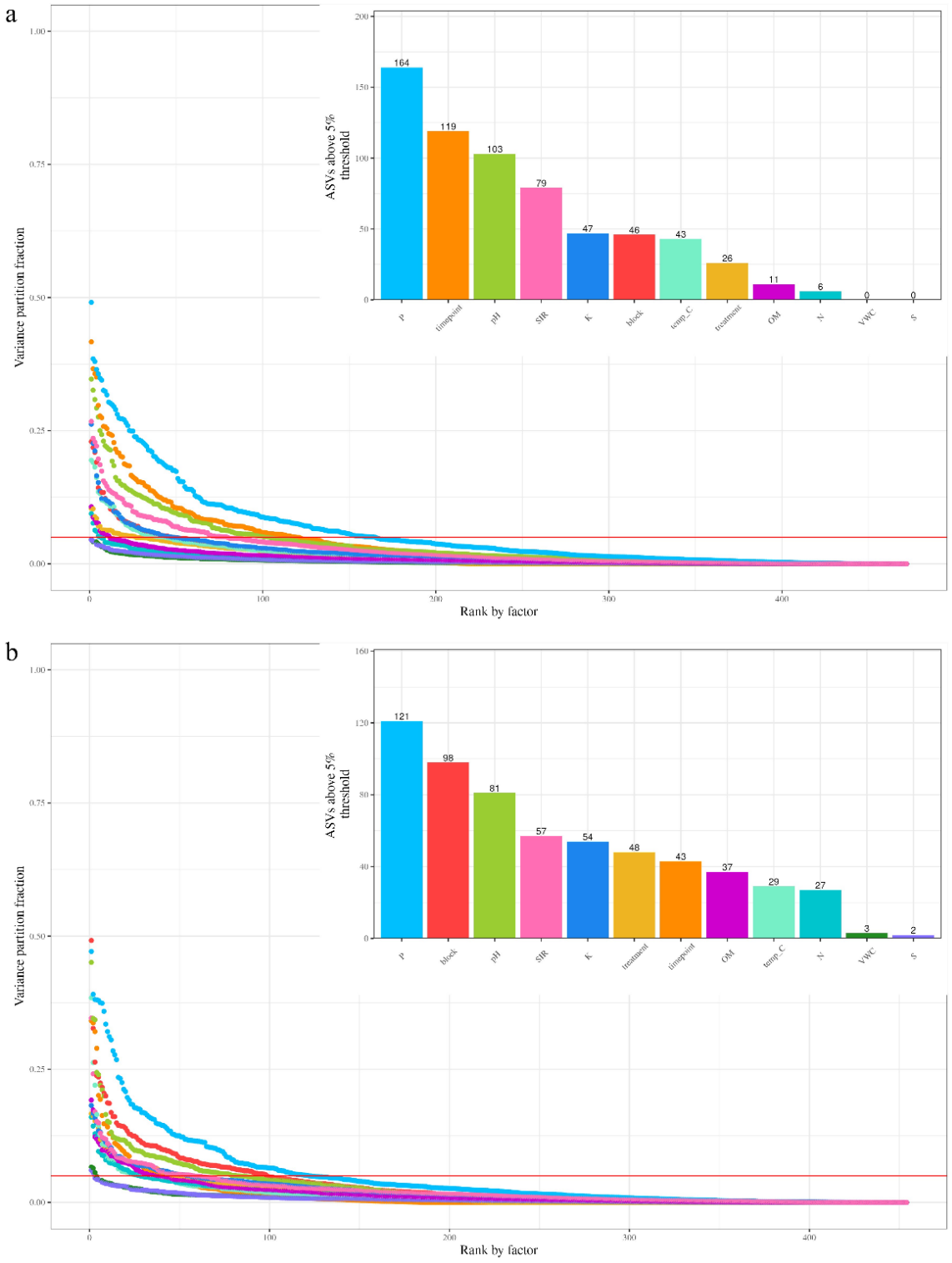


Figure 11. Variance partitioning analysis quantifying the relative contributions of environmental variables to the variation in the bacterial (a) and fungal (b) communities at Red Bluff Research Ranch in southwestern Montana (2025). Variance partitioning revealed that soil phosphorus, sampling timepoint, soil pH, and SIR explained 16 % of bacterial variance (a) and timepoint, block, soil pH, and SIR explained 12.9% of fungal variance (b).

Co-occurrence Network Analysis

An analysis of microbial community networks was applied to assess the complexity of the microbial community structure and the inter-taxa interactions grazing across treatments. The grazing treatments resulted in changes in the connectivity and strength of their interactions. When observing the 16S bacterial network (Fig. 12a), 20% of taxonomic families were shared between all treatments. This core set of taxa suggests that this site has a stable core bacterial microbiome. Treatments also shaped a unique community composition. The ungrazed control and mid-season grazing treatments had the highest percentage of unique taxa, 15% each. In contrast, early grazing and late grazing harbored fewer unique taxa, 5% and 10% respectively. When observing the ITS fungal network (Fig. 12b), only 3.3% of taxonomic families were shared between all treatment groups and there were more unique taxa per individual treatment group compared to the bacterial networks. The early and mid-season treatment groups each exhibited 16.7% unique families. The ungrazed control and late season treatments each exhibited 20% unique families and shared 10% making them the most similar in terms of shared fungal taxa, likely due to the similar time periods over the season they spent ungrazed.

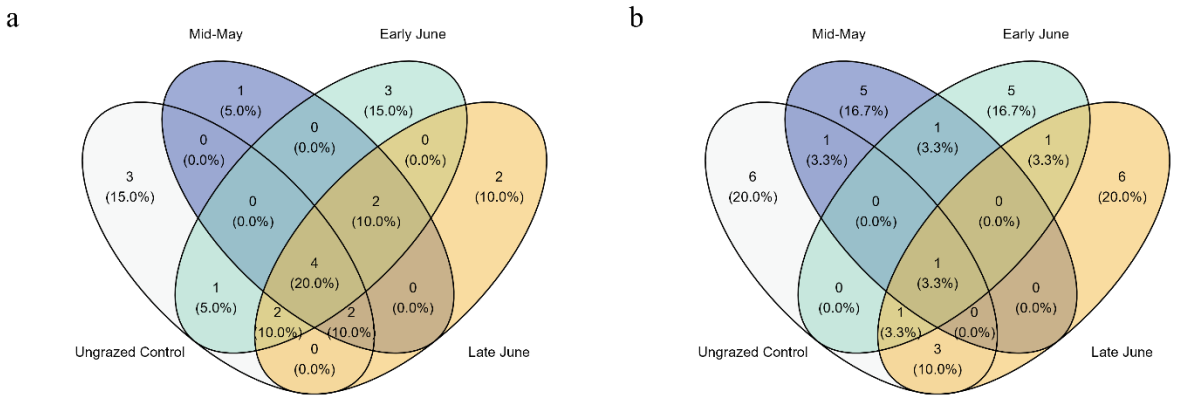


Figure 12. Venn diagrams depicting shared taxa between treatments at the Family level for (a) bacterial and (b) fungal communities at Red Bluff Research Ranch in southwestern Montana (2025).

Co-occurrence microbial network structures differed across grazing treatments, suggesting that timing of grazing acts as a filter on inter-taxa relationships (Fig. 13). In the 16S bacterial networks early June grazing treatment (mid-season grazing) exhibited the highest number of edges (connections) indicating a greater community complexity (Fig. 13ab).

The bacterial networks consistently exhibit a higher number of edges (connections) across all treatments compared to fungal networks indicative of a more complex and densely connected community structure (Fig. 13a). The fungal networks were consistently more sparse indicating a less interconnected community structure where the ratio of edges to nodes is lower, and the network topology appears more dispersed compared to the bacterial networks (Fig. 13b). Across all treatments, several keystone taxa were revealed indicating their roles in maintaining network integrity and stability (Tab. 3 and 4). These included the following bacteria (phylum, family, and genera) *Bacillota Bacillaceae Niallia, Actinomycetota Geodermatophilaceae Blastococcus, Actinomycetota Pseudonocardiaceae Pseudonocardia,* and *Pseudomonadota Xanthobacteraceae Bradyrhizobium* and fungi *Ascomycota Cladosporiaceae Cladosporium,*

Ascomycota Aspergillaceae Penicillium, Ascomycota Caliciaceae Amandinea, and *Basidiomycota.*

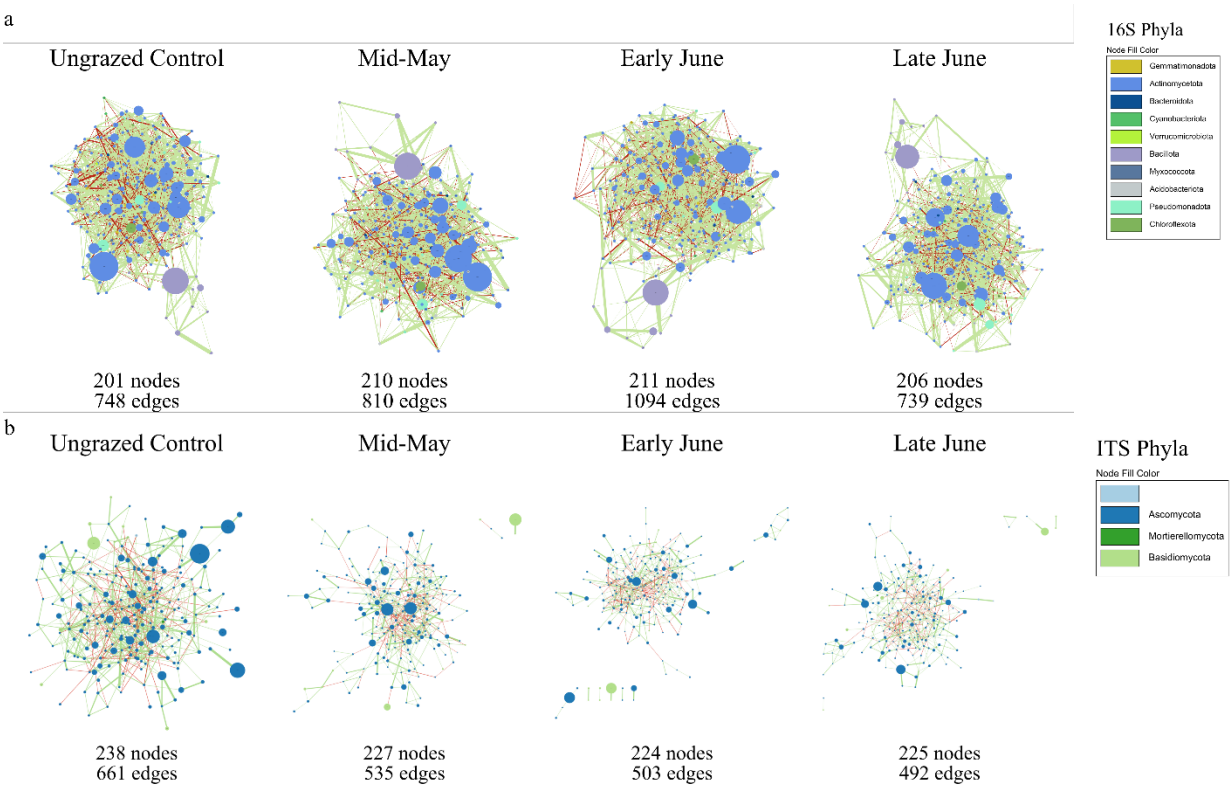


Figure 13. Co-occurrence networks of bacterial (a) and fungal (b) communities across four grazing turnout treatments at Red Bluff Research Ranch in southwestern Montana (2025). Bacterial community networks – color of node indicates phylum (keystone phyla include Blue = *Actinomyceota*; Purple = *Bacillota*; Cyan = *Pseudomonadota*; Green = *Chloroflexota*), size of node indicates abundance of taxa, green edges indicate a positive relationship while red edges indicate a negative relationship. Thickness of edges is indicative of strength of the relationship. Fungal community networks (b) color of node indicates phylum (keystone phyla include Blue = *Ascomycota*; Green = *Basidiomycota*), size of node indicates abundance of taxa, green edges indicate a positive relationship while red edges indicate a negative relationship. Thickness of edges is indicative of strength of the relationship.

A Chi-squared test revealed that all four treatments presented a higher number of total positive correlations than negative correlations (Fig. 14; $p = 0.02$). However, after fitting a generalized linear model and performing post-hoc comparisons using estimated marginal means, the early June treatment was only significantly different from the late June treatment in the

bacterial community ($p = 0.0094$) with the late June treatment producing a greater portion of positive edges when compared to the other treatments and control. The global Wilcoxon test revealed that the absolute strength of positive interactions (edges) was greater than the mean strength of negative interactions in all treatments across the entire bacterial community ($p < 0.0001$). This suggests that mutualistic relationships are not only more frequent but also stronger than competitive ones in the bacterial microbiome regardless of grazing timing.

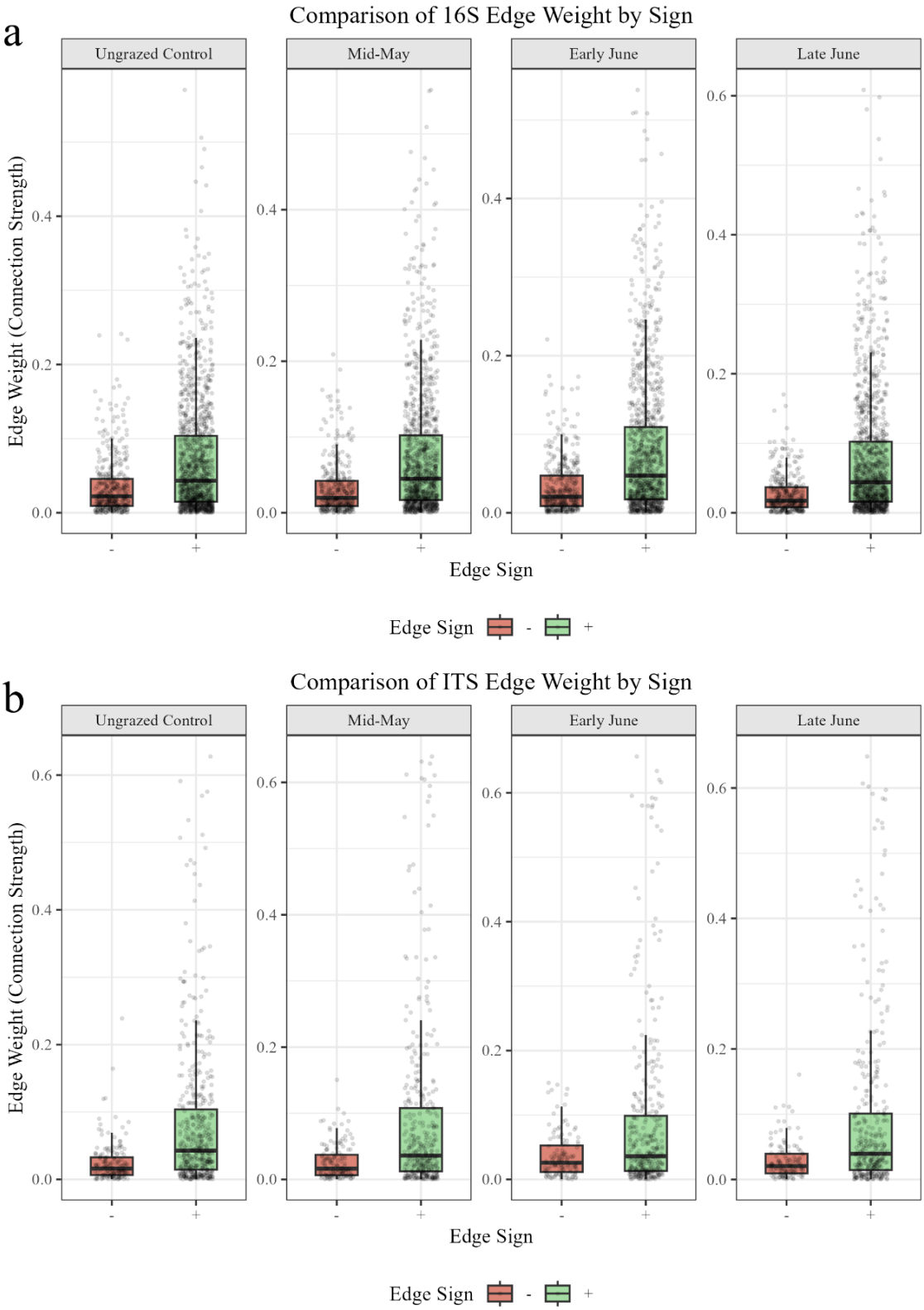


Figure 14. Comparison of network edge (connection) strength of positive (green) or negative (red) relationship direction between network taxa for both 16S (a) and ITS (b) communities at Red Bluff Research Ranch in southwestern Montana (2025).

Table 3. Top bacterial network taxa, grouped by phylum, across grazing treatments at Red Bluff Research Ranch in southwestern Montana (2025).

Phylum	Family	Genus	Ungrazed Control	Mid-May	Early June	Late June	No. Treatments
Acidobacteriota	Pyrinomonadaceae	Arenimicrobium	Absent	Absent	Present	Absent	1
Acidobacteriota	NA	NA	Absent	Absent	Absent	Present	1
Actinomycetota	Micromonosporaceae	Actinoplanes	Present	Absent	Present	Present	3
Actinomycetota	Nocardioidaceae	Marmoricola	Absent	Present	Absent	Present	2
Actinomycetota	Geodermatophilaceae	Modestobacter	Absent	Absent	Present	Absent	1
Actinomycetota	Nocardioidaceae	Nocardioides	Present	Absent	Absent	Absent	1
Actinomycetota	Pseudonocardiaceae	Pseudonocardia	Absent	Present	Present	Present	3
Actinomycetota	Micromonosporaceae	NA	Absent	Absent	Present	Present	2
Actinomycetota	NA	NA	Present	Absent	Present	Present	3
Bacillota	Bacillaceae	Niallia	Present	Absent	Present	Absent	2
Bacteroidota	Chitinophagaceae	Ferruginibacter	Present	Absent	Absent	Absent	1
Gemmatimonadota	Gemmatimonadaceae	Gemmatimonas	Absent	Absent	Absent	Present	1
Gemmatimonadota	Gemmatimonadaceae	Gemmatirosa	Present	Absent	Absent	Absent	1
Gemmatimonadota	Gemmatimonadaceae	NA	Absent	Present	Absent	Absent	1

Table 3. Continued

Pseudomonadota	Acetobacteraceae	Acidiphilium	Absent	Present	Absent	Absent	1
Pseudomonadota	Xanthobacteraceae	Bradyrhizobium	Absent	Absent	Present	Absent	1
Pseudomonadota	Beijerinckiaceae	Methylobacteriu	Absent	Absent	Absent	Present	1
Pseudomonadota	Beijerinckiaceae	Microvirga	Absent	Absent	Absent	Present	1
Verrucomicrobiota	Chthoniobacteraceae	Candidatus Udaeobacter	Present	Absent	Absent	Absent	1

Table 4. Top fungal network taxa, grouped by phylum, across grazing treatments at Red Bluff Research Ranch in southwestern Montana (2025).

Phylum	Family	Genus	Ungrazed Control	Mid-May	Early June	Late June	No. Treatments
Ascomycota	Pleosporaceae	Alternaria	Absent	Absent	Present	Present	2
Ascomycota	Caliciaceae	Amandinea	Present	Absent	Absent	Present	2
Ascomycota	Saccotheciaceae	Aureobasidium	Present	Absent	Absent	Present	2
Ascomycota	Cladosporiaceae	Cladosporium	Present	Present	Absent	Absent	2
Ascomycota	Nectriaceae	Fusarium	Absent	Absent	Absent	Present	1
Ascomycota	Trichomeriaceae	Knufia	Absent	Present	Absent	Absent	1
Ascomycota	Aspergillaceae	Penicillium	Present	Absent	Present	Present	3
Ascomycota	Physciaceae	Phaeophyscia	Absent	Present	Present	Absent	2
Ascomycota	NA	Pulvinella	Absent	Present	Absent	Absent	1
Ascomycota	Sporormiaceae	Sparticola	Absent	Absent	Absent	Present	1
Basidiomycota	Hygrophoraceae	Hygrocybe	Absent	Absent	Absent	Present	1
Basidiomycota	Filobasidiaceae	Naganishia	Absent	Absent	Absent	Present	1
Basidiomycota	Hydnodontaceae	Trechispora	Absent	Present	Absent	Absent	1
Basidiomycota	Bulleribasidiaceae	Vishniacozyma	Absent	Present	Absent	Absent	1

Table 4. Continued

Basidiomycota	NA	NA	Absent	Present	Absent	Absent	1

To evaluate the impact of timing of grazing on co-occurrence network interactions, properties for bacterial (Fig. 15a) and fungal (Fig. 15b) networks were analyzed. Bacterial networks remained relatively stable across treatments with no significant differences observed in betweenness centrality or clustering coefficient. The late June treatment was characterized by higher average shortest path length ($p < 0.0001$), closeness centrality ($p < 0.0001$), and degree ($p < 0.001$).

In contrast, fungal networks exhibited more profound structural shifts in response to grazing timing (Fig. 15b). Networks from the late June treatment were characterized by significantly lower degree and neighborhood connectivity ($p < 0.0001$) than the mid-May treatments, coupled with a significant reduction in average shortest path length ($p < 0.0001$). The presence of high betweenness centrality outliers also suggests that a few key taxa may play disproportionate roles as bridges within the community, potentially increasing the network's sensitivity. These changes indicate that late-season grazing may promote a more densely connected fungal network.

Taxa with a higher degree have the most direct connections, those with high betweenness centrality sit on paths between other nodes, and high closeness centrality is indicative of taxa that respond quickly to environmental shifts. In the control pastures, the 16S genera *Microlunatus* and *Bradyrhizobium* exhibited the highest betweenness centrality and degree while in the treatment pastures, the genera *Conexibacter*, *Rubrobacter*, *Pseudonocardia*, and *Nocardioides* had the greatest betweenness centrality, closeness centrality, and degree (Tab. 5). *Alternaria*, *Knufia*, *Pseudogymnoascus*, and unclassified ITS genera under the *Ascomycota* phylum had the highest degree among all treatments. Closeness centrality was dominated by *Trechispora* and the

Basidiomycota phylum, all present in the control pastures (Tab. 6). The *Basidiomycota* phylum also had the highest betweenness centrality in the late treatment, possibly connecting different functional groups as the plant community senesced.

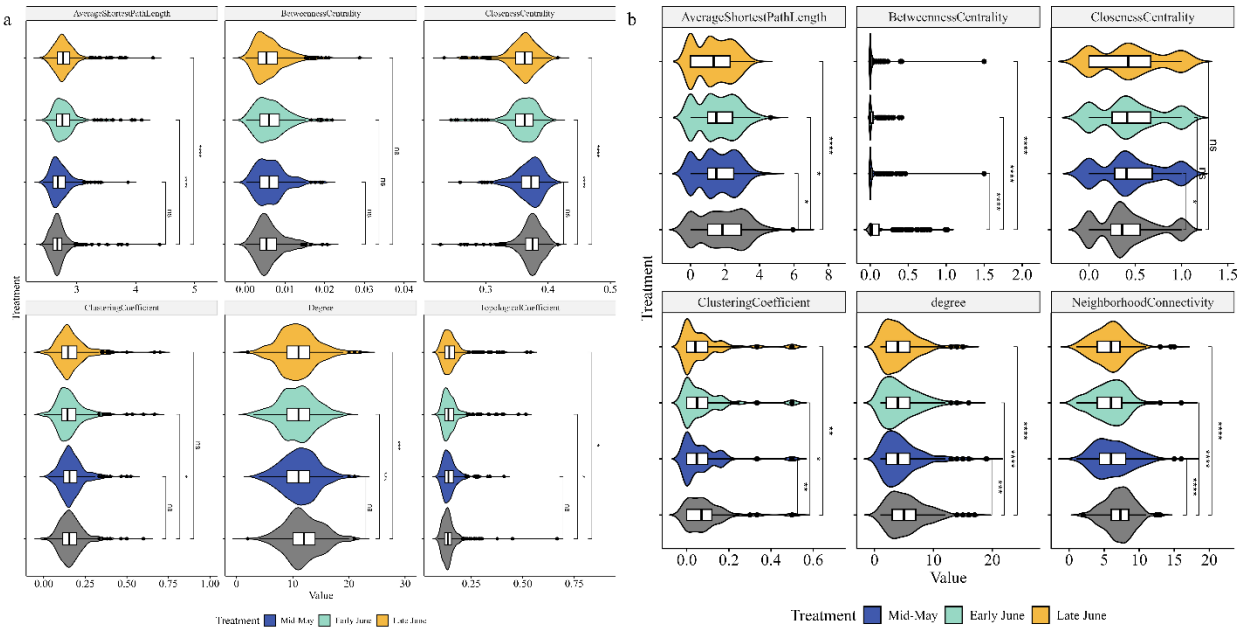


Figure 15. Network properties of bacterial (a) and fungal (b) networks at Red Bluff Research Ranch in southwestern Montana (2025). Average shortest path length, betweenness centrality, closeness centrality, clustering coefficient, degree, topological coefficient (16S), and neighborhood connectivity (ITS) were analyzed.

Table 5. Bacterial genera exhibiting the highest network parameters overall at Red Bluff Research Ranch in southwestern Montana (2025). Network values indicate the maximum observed values across all treatment-specific co-occurrence networks

Genus	**Treatment**	**Network value**
Average Shortest Path Length		
Niallia	Ungrazed Control	4.406
Sporosarcina	Late June	4.295
Sporosarcina	Early June	4.101

Table 5. continued

Betweenness Centrality		
Conexibacter	Late June	0.029
Rubrobacter	Early June	0.022
Pseudonocardia	Late June	0.021
Closeness Centrality		
Microlunatus	Ungrazed Control	0.416
Conexibacter	Late June	0.415
Nocardioides	Mid-May	0.413
Clustering Coefficient		
Bacillus	Early June	0.667
Sporosarcina	Late June	0.667
Domibacillus	Ungrazed Control	0.600
Degree		
Conexibacter	Late June	22.000
Bradyrhizobium	Ungrazed Control	21.000
Nocardioides	Mid-May	21.000
Topological Coefficient		
Niallia	Ungrazed Control	0.667
Crossiella	Late June	0.538
Sporosarcina	Late June	0.521

Table 6. Fungal genera exhibiting the highest network parameters overall at Red Bluff Research Ranch in southwestern Montana (2025). Network values indicate the maximum observed values across all treatment-specific co-occurrence networks

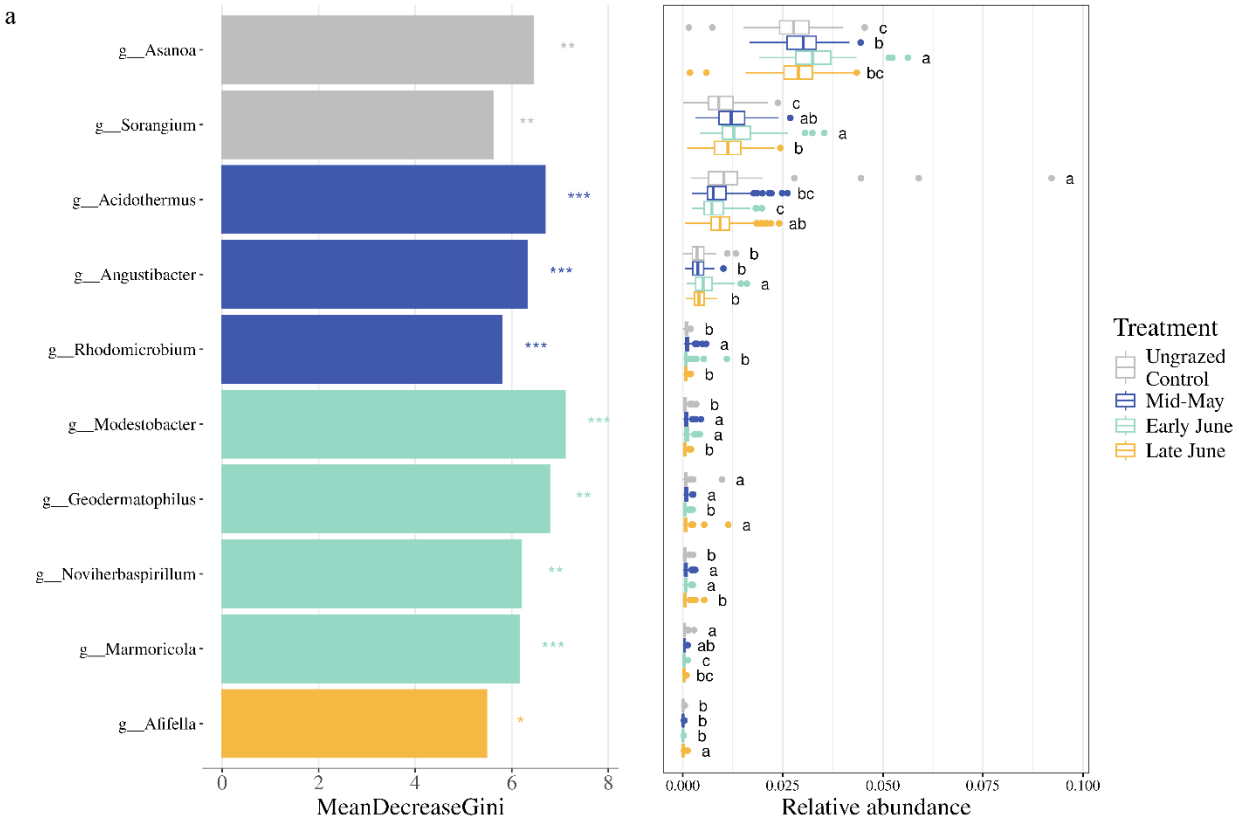
Genus	Treatment	Network value
Average Shortest Path Length		
Ascomycota Phylum	Ungrazed Control	5.938
Ascomycota Phylum	Ungrazed Control	5.097
Phaeococcomyces	Early June	4.638
Betweenness Centrality		
Darksidea	Mid-May	1.500
Basidiomycota Phylum	Late June	1.500
Paracladophialophoraceae Family	Ungrazed Control	1.015
Closeness Centrality		
Trechispora	Ungrazed Control	1.000
Trechispora	Ungrazed Control	1.000
Basidiomycota Phylum	Ungrazed Control	1.000
Clustering Coefficient		
Fusarium	Ungrazed Control	0.500
Ochroconis	Ungrazed Control	0.500
Phaeoclavulina	Mid-May	0.500
Neighborhood Connectivity		
Basidiomycota Phylum	Mid-May	16.000
Knufia	Early June	16.000
Chaetosphaeronema	Mid-May	15.000

Table 6. continued

Degree		
Alternaria	Mid-May	19.000
Knufia	Mid-May	19.000
Pseudogymnoascus	Ungrazed Control	17.000

Differential Abundance Analysis

Differential abundance analysis by the Random Forest method was conducted with center log ratio transformed data, Kruskal-Wallis rank sum test, and *p*-values FDR corrected using Holm's method (Fig. 16). The Mean Decrease Gini (MDG, a measure of importance from the Random Forest model) was also calculated. This indicated that several taxa were significantly important, predominately the bacterial genera *Modestobacter* (MDG = 7.16, $p < 0.001$), *Acidothermus* (MDG = 6.43, $p = 0.00020$), and *Angustibacter* (MDG = 6.31, $p = 0.00018$; Fig. 16a), and the fungal genera *Naganishia* (MDG = 6.49, $p = 0.00017$), *Lycoperdon* (MDG = 4.94, $p = 0.0050$), and *Nectriopsis* (MDG = 4.69, $p = 0.000078$; Fig. 16b).


a
g__Asanoa
g__Sorangium
g__Acidothermus
g__Angustibacter
g__Rhodomicrobium
g__Modestobacter
g__Geodermatophilus
g__Noviherbaspirillum
g__Marmoricola
g__Afifella
0
2
4
6
8
MeanDecreaseGini
0.000
0.025
0.050
0.075
0.100
Relative abundance
Treatment
Ungrazed
Control
Mid-May
Early June
Late June

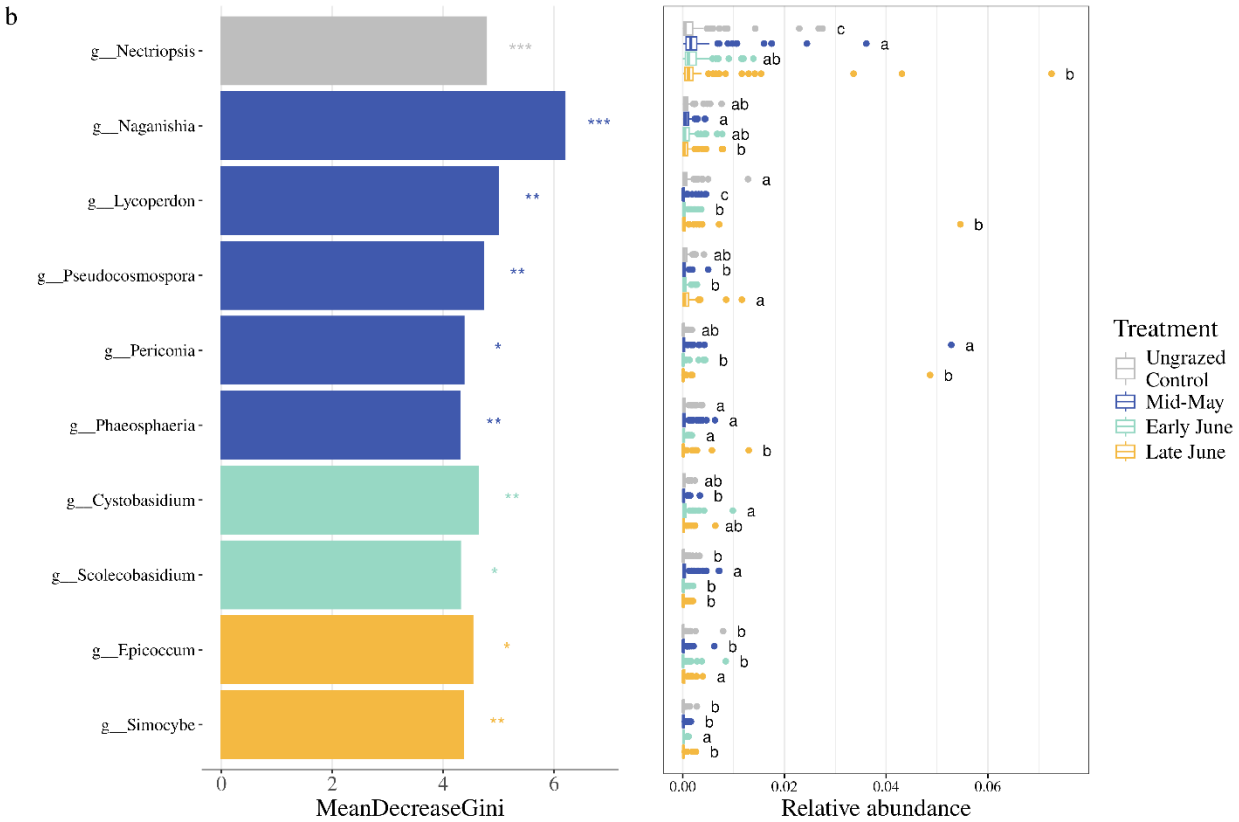

b
g__Nectriopsis
g__Naganishia
g__Lycoperdon
g__Pseudocosmospora
g__Periconia
g__Phaeosphaeria
g__Cystobasidium
g__Scolecobasidium
g__Epicoccum
g__Simocybe
0
2
4
6
MeanDecreaseGini
0.00
0.02
0.04
0.06
Relative abundance
Treatment
Ungrazed
Control
Mid-May
Early June
Late June

Figure 16. Differential abundance and importance of significant microbial taxa across treatments at Red Bluff Research Ranch in southwestern Montana (2025). a) Bacterial community: bar plots (left) show the Mean Decrease Gini (a measure of importance from the Random Forest model) for the top 11 bacterial genera that significantly distinguish the grazing treatments. Box plots (right) illustrate the relative abundance of these same genera across the four treatments. The color of the bar in the left panel corresponds to the treatment where that genus shows the highest mean relative abundance. b) Fungal community: Bar plots (left) and box plots (right) follow the same representation as in panel (a), displaying the top 10 significant fungal genera that distinguish the grazing treatments. Asterisks in the left panel indicate the statistical significance of the differential abundance test (Kruskal-Wallis): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Predictive Functional Profiling

Predictive functional profiling using the KEGG database and Differential abundance analysis of the predicted pathways with the Aldex2 method revealed highly consistent 16S functional structure across grazing treatments (Fig. 17). Biosynthesis of secondary metabolites comprised the greatest portion of functions (17.9%), followed by the metabolism of terpenoids and polyketides (16.5%), carbohydrates (9.74%), and xenobiotics (8.48%) – accounting for over half of the 16S functional profile. This implies functional redundancy and stability of bacterial functional potential in response to short-term grazing throughout the season.

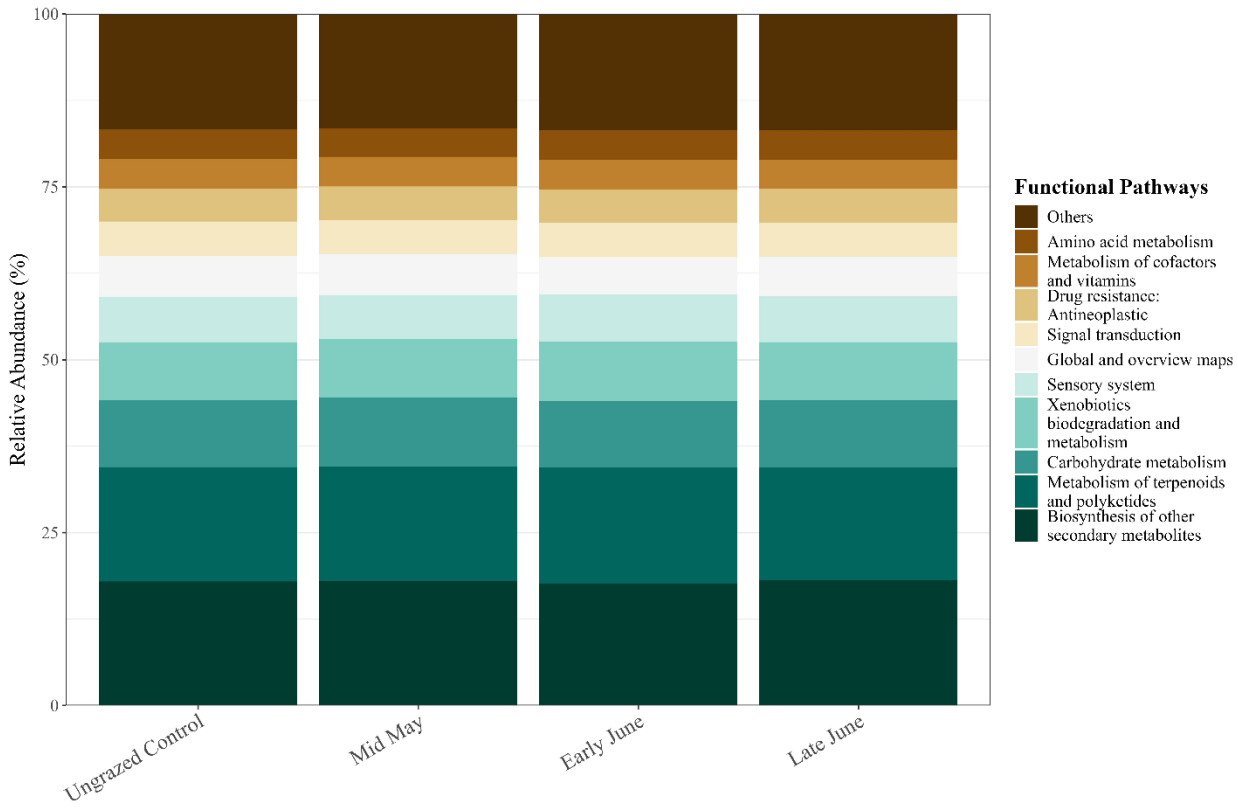


Figure 17. Relative abundance of 16S functional pathways using the KEGG database indicating that biosynthesis of secondary metabolites and metabolism of terpenoids, polyketides, carbohydrates, and xenobiotics comprise the majority of the bacterial functional profile at Red Bluff Research Ranch in southwestern Montana (2025).

Differential abundance analysis of the predicted pathways with the Aldex2 method revealed that fungal ecological guilds showed several treatment-dependent shifts in relative abundance (Fig. 18). Four ITS functional guilds showed significant differences across treatments: Animal Endosymbiont ($p = 0.00002$), Lichenized ($p = 0.0497$), Plant Parasite ($p = 0.0480$), and Symbiotroph ($p = 0.0476$). Tukey pairwise comparison showed that Animal Endosymbionts were most abundant later in the season. Specifically, abundance in early June grazing treatment pastures (0.0056%) saw a 62-fold increase compared to the control (0.00009%; $p < 0.0001$), and 3.7-fold increase from the mid-May treatment (0.0015%; $p = 0.00053$).

Lichenized fungi were most abundant in ungrazed control soils and tended to decline under grazing treatments, specifically from control (6.82%) to early grazing (4.47%; $p = 0.0497$). Plant parasites increased under early (0.0995%) and mid-season (0.991%) grazing relative to the control (0.703%), but were lowest in the late treatment (0.361%; $p = 0.0480$). Symbiotrophs were the most abundant guild and decreased modestly under the late grazing treatment (22.2%) relative to the control (25.4%; $p = 0.0476$)

Most other guilds, including Saprotrophs, Pathotrophs, and Ectomycorrhizal fungi, did not differ significantly among treatments, indicating that timing of grazing influenced a subset of functional guilds rather than the entire fungal community. However, saprotrophs, which use extracellular digestion to decompose organic matter, were one of the most abundant guilds across all treatments.

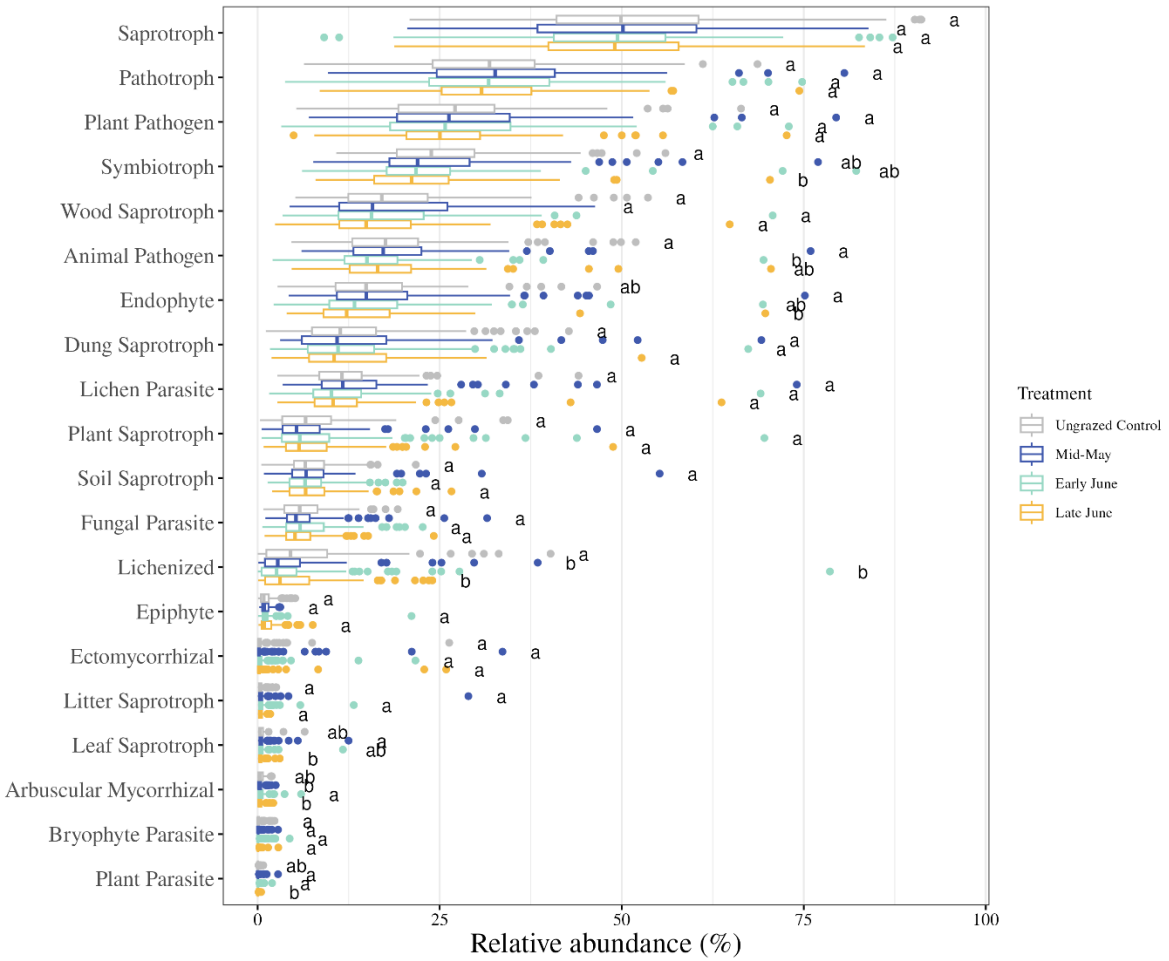


Figure 18. Differential abundance analysis of predicted fungal ecological guilds. Box plots illustrate the percent relative abundance of the top 10 most important functions across the four treatments at Red Bluff Research Ranch in southwestern Montana (2025). Saprotrophs are the most abundant functional guild, while animal endosymbionts, lichenized, plant parasite, and symbiotrophs were the most statistically different guilds between treatments.

Discussion

This study evaluated how deferred grazing on rangelands of the Intermountain West influences soil health indicators and the microbial community landscape using 16S and ITS sequencing along with metagenomic and soil chemical data for community, functional, and environmental profiling. The results of this study indicate that while seasonal progression

remains a dominant driver of microbial activity and community structure, the timing of grazing shapes microbial community dynamics, network interactions and specific functional guilds.

Microbial Biomass Carbon (Substrate Induced Respiration)

Substrate induced respiration provides an index of both microbial biomass and the active fraction of the microbial community capable of rapid metabolism when supplied with a readily available carbon substrate. Because SIR reflects microbial metabolic potential in response to recent carbon inputs, such as root exudates or fresh litter, it offers insight into how grazing management influences microbial activity, carbon turnover, organic matter formation, and carbon storage. Although no significant differences between treatments were detected, the early June (mid) treatment exhibited the most distinct response. During the peak green-up period and throughout the growing season, SIR rates for the early June turnout treatment consistently remained the lowest among all groups. This may suggest that turning out livestock early in the growing season, coinciding with peak plant growth and sensitivity to defoliation, may suppress microbial activity. This could be due to higher root exudation by plants or simply as a result of topsoil disturbance.

SIR did significantly increase overall throughout the growing season, implying that microbial activity in semi-arid systems is more strongly governed by seasonal drivers than by timing of grazing. Many perennial grasses and forbs characteristic of the Intermountain West increase carbon allocation belowground during mid to late growing season stages, with the roots still exuding carbon compounds even after stem, leaf, and seed growth slows, which can promote microbial activity (Zhou et al., 2017). Additionally, as the season progresses and litter deposition, root turnover, and grazing heterogeneity increase, the pool of organic substrates available to

microbes may increase, thus explaining the increase in SIR over the season. Lastly, despite declining soil moisture, increased temperatures can enhance some enzymatic reaction rates, amplifying microbial metabolism to substrates. The short, three-week grazing event for each treatment may not have imposed enough stress to alter the carbon pool accessible to microbes; thus, explaining the lack of treatment effect.

Increased SOC is often promoted as a rangeland health indicator as it has a strong influence on soil organic matter (SOM). However, SOC changes slowly and may not be an ideal soil health indicator when detecting changes from short term management. Microbial indicators, such as MBC and EEA, tend to react more quickly to changes in management. Thus, microbial indicators may provide producers insight into the effect of their management strategies much faster than traditional measurements like SOC.

Environmental Drivers and the Role of Phosphorus

Soil P (Olsen phosphorus) emerged as one of the strongest predictors of both 16S (R^2 = 0.51) and ITS (R^2 = 0.55) community structure. In some agroecosystems, P availability or limitation acts as a filter for microbial life with high P favoring fast-growing taxa while suppressing the growth of nutrient scavenging taxa, such as some mycorrhizal fungi or P-solubilizing bacteria like *Microlunatus*. The diverging vectors of P and pH (Fig. 10) reflect the relationship where P solubility is highly pH dependent (Barrow, 2017). For both microbial communities, the slight clustering of the control samples away from the treatment groups along with these vectors, may indicate that grazing alters the nutritional landscape of the soil, forcing a shift in the microbial equilibrium.

Variance partitioning further emphasizes the ecological complexity of soil chemical properties and the microbial community. Notably, phosphorus, pH, and SIR explained a large portion of variation in both bacterial and fungal communities. Treatment minimally affected variance among ASVs overall, but the fungal community was more sensitive to turnout times than the bacterial suggesting that bacterial and fungal assemblages differ in the extent to which they are regulated by edaphic gradients and disturbance. Across both 16S and ITS datasets, available P and pH explained the greater portion of compositional variation. Soil pH is a well-established metric for soil health and microbial ecology as it influences enzyme function, cell membrane stability, and competitive interactions (Daughtridge & Margenot, 2024). Available P played an influential role, consistent with the nutrient limitation characteristics of semi-arid rangelands where P availability may constrain microbial growth or activity, root productivity, and organic matter turnover (Abouguendia, 1997). Furthermore, fungal diversity may be increased while bacterial diversity may decrease as a result of microsites with lower levels of phosphorus (Evans et al., 2017; Martínez-García et al., 2011). Microbial activity (SIR) explained a moderate portion of variance across datasets, indicating that shifts in energy availability and nutrient cycling vary with compositional change. However, a large portion of variation remained unexplained by the measured variables. This is likely due to the spatial heterogenous microsites which characterize semi-arid rangelands (Fig. 3). Unmeasured plant-soil interactions, legacy effects from previous management, and chemical interaction pathways may also contribute to the unexplained variance. These patterns show that microbial assemblages are structured by substrate quality, availability, and stability.

Microbial Community Composition and Successional Trends

The bacterial (16S) community structure supported our hypothesis of late-season grazing exhibiting the least community response to grazing. While early and mid-season grazing treatments shifted the community away from the control, late-season community did not differ from the control. This suggests a degree of community resilience where the soil microbiome stabilizes or reverts toward a baseline state as plants reach senescence and soil moisture and temperature decline. In contrast, the PCoA revealed that fungi (primary axis variance: 26.2%) may be more sensitive to the downstream effects of grazing disturbance than soil bacteria (11.7%).

Microbial communities under early season grazing were largely similar across treatments, suggesting that grazing effects emerge only after sustained defoliation and soil disturbance. This seasonal progression reflects the coupling between plant phenology, root-associated nutrient supply, and microbial turnover – processes that intensify as the growing season advances and soil moisture declines (Evans et al., 2017). This temporal pattern suggests that the ecological effects of turnout time manifest progressively. The short, early turnout may not have produced an immediate community structure change, but the cumulative changes to plant carbon inputs, soil microclimate, and disturbance accumulate and may be detectable later in the season.

Grazing timing shaped microbial community composition through multiple interacting pathways, influencing β-diversity, network complexity, and the relative contributions of soil chemistry and environmental factors to the microbial community. Although 16S α-diversity remained stable across grazing treatments, clear shifts were evident in the community composition over time, and in the cooccurrence networks, indicating that grazing affects specific taxa and how they interact, rather than simply altering richness or evenness. The composition of

soil microbial community composition is influenced by environmental factors and soil properties often dependent on-site characteristics.

Network Complexity and Functional Roles of Keystone Taxa

Network analysis provided an additional layer of insight into how timing of grazing affects microbial community structure. Co-occurrence networks varied among treatments with some producing more highly connected and modular networks (early June) with a larger number of keystone taxa, while other treatments led to simpler less connected networks (late June for both 16S and ITS). More connected networks may reflect environments with sustained root-derived carbon inputs and diverse ecological niches, whereas simplified networks suggest reduced resource heterogeneity and weaker linkages between microbes. Greater network connectivity is often associated with enhanced community resilience and functional stability (Khatri-Chhetri et al., 2024). Fungal communities tended to respond more strongly to grazing and environmental factors, which may help explain why fungal networks and keystone taxa shifted more noticeably among treatments than bacterial networks.

Keystone taxa included the following phyla, families, and genera: *Actinomycetota Pseudonocardiaceae Pseudonocardia*, *Bacillota Bacillaceae Niallia*, *Actinomycetota Geodermatophilaceae Blastococcus*, and *Actinomycetota Solirubrobacteraceae Solirubrobacter*. Although not a keystone taxa, *Pseudomonadota Xanthobacteraceae Bradyrhizobium* is a nitrogen-fixer and may be indicative of N-cycling functions in the soil. The following predominant taxa across treatments gave insight into soil biotic function at the site regardless of treatment. Genus *Pseudonocardia* are soil bacteria that produce secondary metabolites to protect ants from fungal disease (Goldstein & Klassen, 2020). *Niallia*, a genus under the *Bacillota*

phylum, is involved in the decomposition of OM and nutrient cycling (N fixation and phospahte stabilization). Its PGPR properties of rhizobacteria promote phytohormones and may produce polysaccharides and biofilms that bind soil particles, aiding in aggregation. Specifically, it produces β-glucanases and other hydrolytic enzymes (Saxena et al., 2020). Due to its genomic plasticity, genera *Blastococcus* plays a role in stress tolerance such as drought, exhibits plant growth promoter (PGPR) traits, and can enhance soil fertility (Sbissi et al., 2025). *Solirubrobacter* may be more abundant in soils with lower SOC, shows PGPR characteristics, and may be a pioneer organism that enables microbiome development of plant rhizospheres (Jiang et al., 2023). *Actinomycetota 67-14* is an unclassified strain from the *Solirubrobacterales* order. The *Solirubrobacterales* order is often detected in drier environments, has potential PGPR characteristics, and is resilient to stress, specifically from UV radiation and desiccation (Jiang et al., 2023). The lesser influential genus, *Microlunatus,* consists of species that aerobically accumulate phosphate as intracellular polyphosphates – a form of stored phosphorus that can be released to plants (Kawakoshi et al., 2012). The strong prevalence of *Actinomycetota* and *Pseudomonadota* suggests a community adapted to complex organic matter degradation and high soil C/N ratios.

Ascomycota phylum is a terrestrial root-associated fungi and *Basidiomycota* phylum is a key decomposer. *Aspergillaceae Penicillium*, *Cladosporiaceae Cladosporium*, and *Nectriaceae Fusarium*, were all keystone families and genera under the *Ascomycota* phylum. *Aspergillaceae Penicillium* are saprotrophs that excel at enzymatically breaking down complex organic polymers, act as plant growth promoters, and solubilize phosphorus (Chandra et al., 2025; Radhakrishnan et al., 2014; Whitelaw et al., 1999). *Cladosporium spp.* are endophytic,

saprophytic fungi that play roles in organic matter decomposition, exhibit plant growth promoting characteristics, and enhance stress tolerance from heavy metal saturation (Răut et al., 2021). *Fusarium spp.* are notorious for being a plant pathogen causing vascular wilt, but it also often acts as a saprophyte living on senescent plant matter (Wakelin et al., 2008).

The constrained ordination analysis results highlight a divergence in how bacterial and fungal communities respond to soil nutrient properties. The higher explanatory power of the bacterial model ($R^2 = 0.278$) compared to the fungal model ($R^2 = 0.088$) suggests that bacteria are more tightly coupled to the immediate soil chemical environment. This is consistent with the "copiotroph-oligotroph" framework, where many soil bacteria – particularly those in disturbed systems – respond rapidly to fluctuations in labile nutrient pools like carbon, phosphorus, or potassium (Stone et al., 2023). Due to their growth form and ability to translocate nutrients across hyphal networks, fungal communities may exhibit degrees of environmental buffering, causing them to be less restricted by localized microsites of soil nutrients. The low R^2 may suggest that fungi are more governed by stochastic processes or biotic interactions such as host-specificity that were not captured by the variables measured in this study.

Management Implications and Soil Health

Together, the data show that the timing of grazing exerts seasonally dynamic effects on belowground communities. The ecological consequences of grazing turnout timing therefore depend not only on whether grazing occurs, but when it occurs relative to plant phenology and soil nutrient dynamics – a consideration that has important implications for monitoring microbial indicators of soil health and for designing adaptive rangeland management strategies. This experiment was conducted on rangeland of Southwestern Montana, but rangelands are diverse

with unique characteristics that vary spatially and temporally. Therefore, differing rangeland ecosystems may have varying responses to management and should be evaluated accordingly. In the context of highly variable rangeland landscapes, irregular distribution of rainfall both spatially and temporally places constraints on ecological functions, biological activity, and nutrient transport in the soil. Future research should aim to determine whether the microbial community responds more directly to grazing or environmental factors like soil moisture, temperature, and nutrient gradients. Further, studies should aim to address whether there is a greater microbial response mediated by plants rather than a direct response to grazing. Overall, applied research should aim to develop practical strategies for producers to monitor soil biological health in addition to the soil nutrient profile.

While most grazing management recommendations currently depend on grazing intensity or duration, timing is also an important factor that influences soil health and microbial activity. By resting pasture early in the growing season, forages have a better opportunity to establish, developing plants and soils are less stressed by grazing pressure, and microbes can enhance nutrient cycling. Soil microorganisms enhance plant development and resilience by breaking down complex polymers into plant-available compounds, cycling nutrients, and preventing disease all of which mitigate the impact of environmental stressors like extreme weather and grazing (Bai & Cotrufo, 2022). By delaying the turnout of cattle on range, pressure on rangelands could be reduced during critical growing periods, promoting microbial activity, enhancing range condition, increasing ecosystem biodiversity, and potentially improving cattle performance.

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CHAPTER THREE

CONCLUSION

Conclusions and Future Directions

While soil health is commonly monitored in cropping systems by nutrient analysis and soil carbon metrics, soil biology is receiving more attention and soil heath is becoming more prevalent in rangeland systems (Stott, 2019). Understanding how grazing management influences soil biological processes is critical for rangeland sustainability and productivity. Despite the growing recognition of the soil microbial community's role in nutrient cycling and the microbiome, relatively little is known about how grazing at different times relative to plant phenology affects the soil microbiome in terms of composition, structure, activity, and function. Furthermore, previous research has focused on the soil bacterial community's response to grazing while less emphasis is placed on the fungal community. This research addressed this gap by evaluating how deferred grazing and environmental factors influence microbial activity and community dynamics.

Overall, results from this study demonstrate that soil microbial responses to grazing are often subtle and reflect resilience to stress rather than insensitivity to environmental or management drivers. Across all grazing turnout times, environmental factors such as soil P, K, pH, SIR, and seasonal effects emerged as strong predictors of microbial community composition and structure. The timing of grazing relative to plant phenology and soil conditions appears to play a central role in determining soil biological outcomes. From a microbial ecology perspective, this work contributes to a growing body of evidence that soil microbial communities are resilient to stress and functionally responsive to management.

Fungal communities represent a particularly important and understudied component of this system, playing critical roles in complex organic matter decomposition, nutrient retention, and soil aggregation. Yet, their response to grazing remains unresolved. Results from this study indicate that fungal community composition and connectivity may be more sensitive to management and spatial variation while the bacterial community responds more readily to seasonal resource pulses throughout the growing season. Change in microbial community structure may initiate long-term shifts in soil function that are not immediately detected through common soil health metrics.

From a management perspective, these findings may precede a shift towards managing soils for biological function. Deferred grazing periods when vegetation is vulnerable to disturbance may allow microbes to develop along with the plant community under less stressful soil conditions. However, research is still needed to investigate optimal alternative forage sources to rangeland grazing such as grazing cover crops, winter annuals, fallow, or haying instead. While this study does not prescribe a single optimal grazing strategy, it provides evidence that aligning grazing timing with favorable plant phenology stages and environmental conditions may help maintain biological integrity and resiliency in rangeland soils.

Due to the spatial and temporal heterogeneous nature of rangeland systems, this study could benefit from repetition across multiple sites to verify results across different systems. Furthermore, many belowground soil processes, including carbon and nitrogen sequestration, soil organic matter accumulation, soil aggregation, and microbial community change, take extensive periods of time to respond to management changes. Therefore, long-term studies are required to determine whether microbial responses translate into sustained changes in soil health

metrics and ecosystem resilience. Additionally, improved characterization of the fungal community and its functional guilds would enhance understanding of how grazing affects the more understudied portion of the soil microbiome. Integrating microbial community metrics with soil physical and chemical properties and plant responses to grazing will be critical in developing management strategies that balance livestock production with soil health goals.

In conclusion, this research advances understanding of how grazing timing influences soil microbial communities, while also highlighting critical gaps in scientific knowledge, particularly regarding fungal community responses and functional roles in rangeland soils. The results emphasize that allowing periods of rest during critical periods of the growing season may promote microbial activity and stabilize soil processes that underpin nutrient cycling and carbon dynamics. Importantly, these findings underscore the need to move beyond single-metric assessments of soil health and toward more holistic approaches that incorporate soil physical, chemical, and biological functions. Such monitoring efforts can help producers and land managers make informed decisions to develop adaptive, sustainable grazing strategies that support soil health, downstream production goals, and rangeland productivity.

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