DISSERTATION

EXPLORING THE ROLE OF PLANNED AND UNPLANNED BIODIVERSITY IN THE SOIL HEALTH OF AGROECOSYSTEMS

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ABSTRACT

EXPLORING THE ROLE OF PLANNED AND UNPLANNED BIODIVERSITY IN THE SOIL HEALTH OF AGROECOSYSTEMS

Agricultural management influences above and belowground biodiversity, which in turn alters agronomic outcomes and broader ecosystem health. Specifically, the manipulation of disturbance regimes, organic matter inputs, and nutrient management can drastically alter soil biological communities. Alterations to biodiversity, whether direct or indirect, have cascading implications for ecosystem processes like productivity, nutrient cycling, and resilience. Direct manipulations of biodiversity often take the shape of crop or variety selection for inclusion in agricultural systems, with indirect biodiversity effects on soil fauna and other associated organisms that are related to changes in management practices. This dissertation explores the varied ways in which cropping system management practices that manipulate biodiversity interact with key components of soil health, leading to the regulation of processes critical for sustainability. Utilizing research station, on-farm, and greenhouse experiments, I analyzed the effects of: 1) crop rotation diversity on soil macroinvertebrates; 2) grazing of diverse cover crop mixtures on soil health and wheat yields; and 3) wheat genotype diversity on soil microbiomes and nutrients cycling.

Managing crop diversity and biomass production over time can alter the size and structure of soil food webs that depend largely dead and decaying plant residues. Intensification of dryland crop rotations increases the frequency and amount of plant residues returned to the soil, which may help support more abundant and diverse soil communities that can improve important aspects of soil functioning. Utilizing a 32-year field experiment in eastern CO, USA, I

explored how dryland cropping system rotations and intensification helped shape soil macroinvertebrate communities and associated water dynamics. I found a trend of increased macroinvertebrate abundance with more frequent cropping, with a related increase in water infiltration rates. This finding suggests that effective management of water in these dryland systems may depend on improved understanding of the mechanisms by which soil organisms regulate soil structure and hydraulic properties.

Diverse cover crop mixtures have the potential to improve multiple aspects of soil health and agroecosystem resilience, but compete with cash crops for water in dryland agroecosystems. By doubling down on diversity and integrating livestock to graze cover crops, producers may be able to offset the economic ramifications of cover crop water use while maintaining their ecological benefits. To explore this, I evaluated several aspects of soil health after one season of grazed cover crop mixtures across ten on-farm replicated experiments. I compared grazed cover crops to ungrazed cover crops and bare fallow, the standard practice implemented to store soil water, but with potential negative consequences for soil health. I found that grazing did not reduce soil health outcomes compared to ungrazed cover crops (i.e. no compaction), and that both cover crop treatments increased surface soil aggregate stability compared to summer fallow. While the bare fallow treatment had higher wheat yields the following season due to increased soil water availability, preliminary results indicate that livestock utilization of diverse cover crop mixtures may help offset the associated income loss and represents a promising approach for supporting soil health and stability in agroecosystems.

Within-species (genotypic) diversity may also play an important role in regulating belowground biodiversity and associated soil function. Crop breeding has led to significant changes to plant traits over time, but changes in belowground traits are often ignored.

Belowground plant traits, specifically belowground C allocation to root tissue and exudates, may alter belowground communities with implications for plant nutrient acquisition in different soil contexts. Plant roots exert control over root-associated microbial communities, which can alter microbially-mediated processes like nutrient cycling. I explored this relationship in winter wheat (*Triticum aestivum* L.) using twelve genotypes spanning from a wild relative, an early land race, early 20th century, and modern releases. I used stable isotope tracing of ^{13}C to quantify rhizodeposition in soil. I then surveyed the rhizosphere microbial community using 16S and ITS amplicon sequencing, and quantified enzyme activities as an indicator of nutrient cycling function. I coupled these measurements with quantification of residue-derived N into wheat tissue using ^{15}N labeling. I found that root morphology was related to rhizodeposition amount, which was actively assimilated by the microbial biomass. Wheat-derived C in the microbial biomass was positively related to N-cycling enzyme activity and residue-derived N uptake in wheat. However, the link between N-cycling activity and N uptake was unclear. Interestingly, there was a distinction between bacterial taxa associated with high wheat-C assimilation and those related to residue N uptake by wheat, suggesting that soil microbial communities associated with root-derived C are largely distinct from those that regulate N mineralization from decomposing residues. Our observed links between rhizodeposition, microbial stimulation and residue N uptake suggest that belowground C strategies with high rhizodeposition may perform better in soils with greater reliance on organic nutrient sources.

Genotype-level variation in belowground C allocation patterns may be particularly important in different soil health contexts. Utilization of organically-derived fertilizers via compost and cover crops provide nutrients for crops while building soil organic C. Increasing soil biological activity with increased levels of C input may change the relationship between

plant roots the microbial communities that regulate nutrient cycling. Using two different genotypes of winter wheat with contrasting belowground C allocation strategies, I assessed their response to different soil nutrient management legacies using direct root measurements and ^{13}C isotope tracing. I then related these responses to microbial community structure and function, and measured wheat uptake of N from ^{15}N-labelled plant residues. I observed significant genotype x soil legacy interactions for root mass fraction, N-cycling enzyme activity, and residue-derived N uptake; overall, the high-exudation genotype had greater relative root growth, exudation, and enzyme stimulation in the low-organic C soil. Additionally, the high-exudation genotype also demonstrated stronger selection for specific microbial taxa. I found that the high-exudation genotype was relatively better at accessing residue-derived N in soils with high organic C, but was less successful in the low-organic C soil. Based on these results, the two belowground strategies may be considered more acquisitive or conservative in their relationship with soil microbes, fostering a more facilitative/mutualistic relationship or a more competitive relationship. Importantly, these relative success of these two nutrient acquisition strategies depends on soil nutrient management legacy, suggesting that higher exudation may be more effective in a context of improved soil health. As more focus and resources are deployed to improve soil health, it will be important to consider the often-ignored belowground crop traits to improve synergy with soil communities and nutrient sources for agroecosystem success.

The work presented here highlights critical applications of planned and unplanned biodiversity to improving the resilience and function of agroecosystems in various contexts. Overall, I found that increasing the amount and diversity of crop inputs to soils feeds soil biological communities, which mediate soil functions like water infiltration, erosion control, soil organic C accumulation, and nutrient cycling. To meet the environmental demands of today,

agroecosystem diversity should be prioritized both above- and belowground to improve soil health, with cascading impacts to the well-being of the environment and society.

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CHAPTER 1: INTRODUCTION

Agricultural soils are under threat globally due to widespread degradation, resulting from erosion, unbalanced nutrient and organic matter fluxes, and a variety of disturbances (Pimentel, 2006). Along with physical and chemical degradation (e.g., compaction, soil nutrient and organic matter depletion), poor soil management disrupts soil biological communities, which mediate many critical soil functions (Bardgett and van der Putten, 2014; Lavelle et al., 2006). Agricultural soil function is critical to human health, via the provision of food as well as the regulation of ecosystem functions like water purification and provision, soil carbon (C) storage, and as an important reservoir for biodiversity (Orgiazzi et al., 2016).

While often overlooked, soil organisms influence multiple soil attributes important for agricultural ecosystem (agroecosystem) productivity and resilience (de Vries et al., 2013). For example, soil structure is influenced by large-bodied soil organisms via their burrowing and aggregation activities, while fungi and other microorganisms influence soil structure through the production of adhesive C-based compounds and hyphal enmeshment that hold aggregates together (Six et al., 2004). Soil macro-invertebrates can shred plant residues and move them deeper in the soil profile, while both fauna and soil microorganisms exert a large influence on decomposition and nutrient cycling, even in intensive agricultural systems (Wall et al., 2015; Yan et al., 2020).

Rainfed agriculture in semi-arid regions (dryland agriculture) faces a unique set of challenges due to limited and uncertain precipitation, often leading to marginal production (Dhuyvetter et al., 1996; Hansen et al., 2017). Drylands cover about 41% of global land area, of which about 14% is cropland (FAO, 2019). Increased pressure on water resources coupled with climate change will likely expand dryland agriculture, as has already been observed in parts of

the U.S. (Deines et al., 2020). Importantly, many of the challenges in dryland agriculture emerge from precipitation variability itself, and adequate soil functioning can help alleviate the consequences of extreme events like rainfall and drought (Stewart and Peterson, 2015). However, cropping practices designed to conserve water and stabilize yields, namely summer fallow in which the land is left unplanted for over a year, have resulted in significant loss of soil C and other metrics of soil health (Peterson and Westfall, 1996).

Soil health is generally regarded as the physical, chemical, and biological attributes of a soil that allow it to perform multiple functions relevant for agriculture and/or other contexts (Lehman et al., 2015; Stott and Moebius-Clune, 2017). Agricultural practices are a major driver of soil health in agroecosystems, though local environmental context plays a large role in determining functional potential and management priorities (Bünemann et al., 2018). Many improvements to soil health translate into improve economic outcomes, but the relationships and timelines for improvement are complex and regionally specific (Miner et al., 2020). To address soil health declines in dryland agriculture, there is a need for viable fallow replacement strategies that promote soil health and associated functions while still supporting farm economic viability (Nielsen et al., 2016).

One aspect of management with the potential to improve soil health as well as other elements of ecosystem function and resilience is biodiversity. Biodiversity may be planned, as in the planting and maintenance of crop populations, as well as unplanned via changes to soil communities, natural pollinators, and the natural plant populations. Planned diversity includes crop rotations, which link different species in the same space across time as well as the spatial integration of multiple plant species at the same time, such as cover crop mixtures planted specifically to improve soil and environmental health (Schipanski et al., 2014). Diversity in crop

rotations have been linked to higher and more stable crop yields, as well as increases in soil C and nitrogen (N), all important metrics of agroecosystem function (Bowles et al., 2020; Gaudin et al., 2015; McDaniel et al., 2014; Smith et al., 2008). Species mixtures, like cover crops grown in polyculture, can provide a broad range of agroecosystem functions and benefits if a range of plant functional groups are employed (Finney and Kaye, 2017).

Specific plant traits may have a particularly important effect on unplanned biodiversity in soil biological communities. Plants and their associated belowground traits, including root structure, exudation patterns, and rhizosphere biochemistry, can select for and support distinct soil communities, which can then feedback to affect plant fitness (Bardgett and Wardle, 2010; Zhou et al., 2016). Genotype-level differences in belowground C allocation strategies may lead to unplanned changes in belowground communities that mediate plant success in a specific soil environment (Schmidt et al., 2016). Thus, there are many levels of diversity which can be manipulated within an agroecosystem that alter functioning, and at a large scale, can substantially alter processes critical to human and ecosystem health.

This dissertation seeks to link the planned selection of biodiversity related to crop species or genotypes with soil and environmental health outcomes that are often mediated by unplanned changes to belowground biodiversity. I focus on systems incorporating winter wheat (*Triticum aestivum* L.) for its importance as a drought-tolerant global staple crop and local relevance to semi-arid agriculture in the High Plains region. While cropping systems have limits to the amount and types of diversity that can be added while maintaining agronomic viability, there are many opportunities for increased biodiversity and ecosystem health that need further exploration. In this dissertation, I investigate the ecological implications of managing aboveground diversity, focusing on direct effects on soil biological communities and their function, and link these

dynamics to associated soil health outcomes important for the resilience and sustainability of agroecosystems (Fig. 1.1).

This dissertation begins with an investigation of soil macroinvertebrate communities in response to long-term crop rotations, and their potential influence on soil structure and hydrologic function. Soil macroinvertebrates can engineer ecosystems, changing the structure, porosity, and water dynamics of soil through their tunneling actions and other activities (Lavelle et al., 1997). Increased water infiltration reduces potential soil erosion from runoff and results in greater water capture for subsequent crop use, increasing resilience to climate extremes (Stewart and Peterson, 2015). Crop rotations with differing frequency of fallows and crop diversity change plant litter inputs and soil organic C (SOC) levels (Shaver et al., 2002; Sherrod et al., 2018). The objectives of Chapter 2 are to: 1) determine the effect of fallow frequency on soil macroinvertebrate communities, and 2) explore links between water infiltration and associated soil functions.

The third chapter assesses a crop rotation that incorporates diverse cover crop mixtures as well as livestock to biotically and economically diversify a water-limited wheat-based cropping system. Cover crops are unharvested crops, often grown in polyculture as mixtures, with the express purpose of improving soil health. While many regions have reported a variety of economic and environmental benefits from cover crops, cover crop water use in semi-arid regions makes cover crops financially challenging (Nielsen et al., 2016). Careful livestock grazing of the mixtures may provide the economic return needed to make cover crops work, but it is unclear if cattle grazing is deleterious for soil health goals. Importantly, farm-scale studies are necessary to capture the range of possible outcomes and more rigorously test this practice for on-farm adoption. The objectives of this chapter are to: 1) quantify the short-term (one season)

influence of cover crops on key metrics of soil health compared to conventional fallow, 2) assess the impacts of grazing cover crops on soil health, and 3) compare wheat yield from the different treatments to begin understanding the economic outcomes of the different practices. Diversification of dryland cropping systems with cover crops and livestock may build resilience to climatic and market disruptions, while improving aspects of soil health.

In Chapter four, I turn my focus to within-species, or genotype-level, plant diversity within winter wheat and its effects on rhizosphere microbial communities. Different crop cultivars, developed through breeding selection, may differ in belowground morphology that was not intentionally selected, but nonetheless important for plant nutrient acquisition and crop success. Plants allocate C belowground as root structures or as rhizodeposits, which broadly include exudates and other root-derived organic compounds transferred to the soil. Rhizodeposits can support and select for specific microbial taxa and communities, which may regulate beneficial functions for the plant (Badri and Vivanco, 2009). An important function of root-associated microbes is the mineralization of organic N sources into plant-available N via microbial enzyme activity and the turnover of microbial biomass (Chapman et al., 2006). The objectives of chapter four are to: 1) assess the variation in belowground C allocation among cultivars of winter wheat, 2) explore the relationship between belowground C allocation and microbial community structure and function, and 3) link belowground nutrient cycling activities to wheat uptake of N derived from decomposing plant residues. I hypothesized that increased exudation would vary by genotype, but higher exudation would lead to increased N-mineralization activity, increasing residue-derived N uptake.

Chapter five builds off of the understandings gleaned in Chapter four to further explore different microbially-mediated nutrient acquisition strategies under distinct soil health contexts.

There is growing understanding in the agricultural community and larger society of the importance of soil health for farm resilience, human well-being and environmental sustainability. Delivery of crop nutrients to soil is an important tenant of soil health management, and many systems (and policies) are looking to increase reliance on organic sources like compost and N-fixing cover crops to meet crop nutrient demands. Addition of organic nutrient sources also increases SOC and provides the primary energy base for soil biological communities. Differences in background SOC and microbial activity may determine the effectiveness of different exudation strategies in plant acquisition of N. I selected two winter wheat genotypes with contrasting belowground C allocation strategies and compared genotype responses and strategies in different soil health contexts. The objectives of this study were to: 1) compare the differential genotype responses in belowground C allocation (i.e. roots and exudation) of two varieties growing in two soils with contrasting soil nutrient management history, 2) determine wheat genotype and soil legacy effects on soil microbial community structure and function, and 3) quantify N acquisition from fresh plant residues in the different soil and genotype combinations. I hypothesized that genotypes would respond differently to high SOC based on belowground C strategy. Additionally, these differences in C allocation would change microbial recruitment and N-cycling enzyme activity. I also hypothesized that a high-exudation strategy is more successful at acquiring fresh residue N in a high organic C context, as the higher background nutrient content would prevent short-term immobilization by the microbial community in response to wheat C.

Agricultural soil management is crucial for increasing the health of arable soils. Soils are responsible for a multitude of ecosystem processes that make the earth habitable. The work presented here will help inform more responsible management of belowground organisms,

leading to more resilient and multifunctional agricultural systems. Through explorations of several promising (and agronomically-viable) manipulations of biodiversity, I assess the relationships between planned aboveground communities and the belowground organisms and functions they help support. Soils underpin many of the ecosystem processes society relies on, and proper management of our soil resources is critical for the sustainability of our planet.

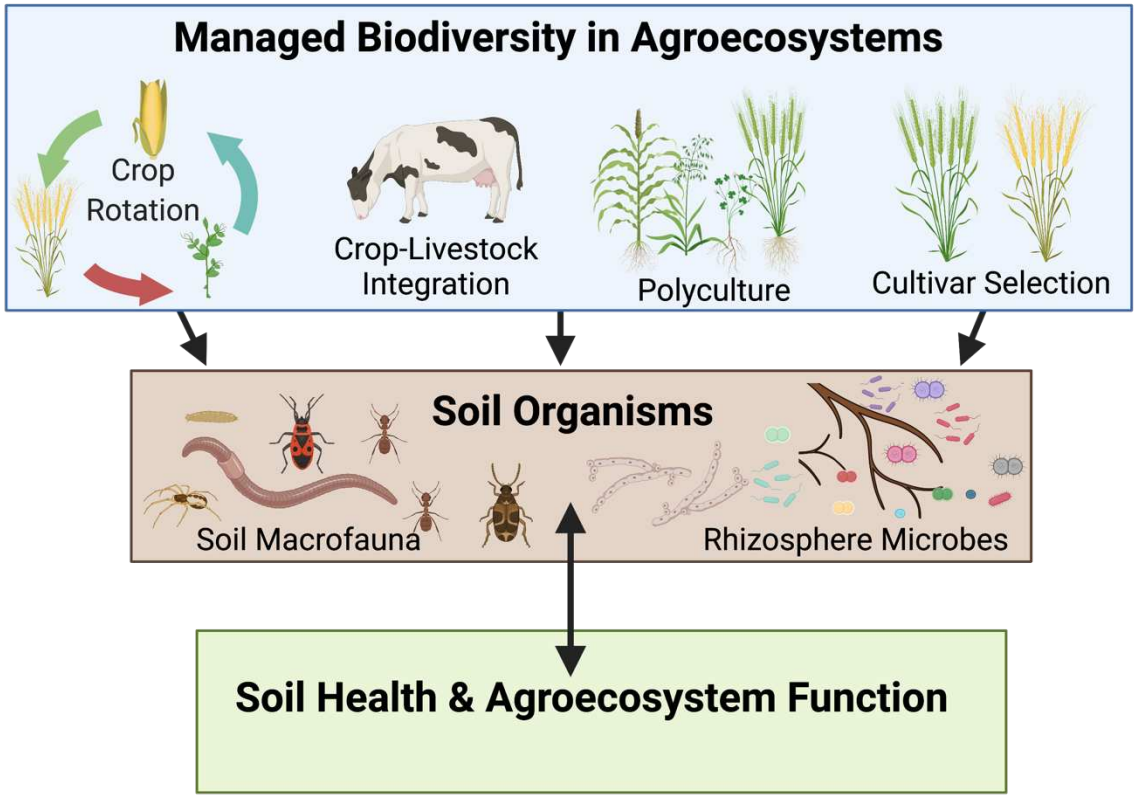

Figure 1.1. Conceptual diagram outlining the main themes and research areas addressed in this dissertation. Arrows indicate proposed linkages between aspects of agroecosystem diversity and overall soil health and agroecosystem function.

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CHAPTER 2: THE EFFECTS OF DRYLAND CROPPING SYSTEM INTENSITY ON SOIL FUNCTION AND ASSOCIATED CHANGES IN MACROFAUNA COMMUNITIES[1]

2.1 Introduction

Non-irrigated crop production in semi-arid areas, or dryland agriculture, represents a widespread and challenging context for farming (Singh et al., 1990). Yield is typically most limited by moisture in these regions and year to year variability is relatively high as it tracks with annual precipitation (Sherrod et al., 2014). Winter wheat (Triticum aestivum L.) is the most common crop in dryland systems of the Central and High Plains of the U.S. (Colorado, Kansas, Nebraska, western Oklahoma and northern Texas), where wheat planting is traditionally alternated with a 14-month bare (non-vegetated) fallow period, referred to as summer fallow, to store soil water and stabilize yields (Peairs & Armenta, 2010). While the summer fallow practice is successful at reducing annual yield variability (Dhuyvetter et al., 1996), soil water storage efficiency is low and over 75% of the precipitation that falls during the summer fallow is typically lost to evaporation (Peterson et al., 1996). Reliance on bare fallows has also been shown to contribute to the depletion of soil organic carbon (SOC) and topsoil erosion (Peterson & Westfall, 1996).

Adoption of no-till practices in the Central and High Plains regions of the U.S. has resulted in improved soil water storage through increased water capture and reduced evaporation, which makes fallow periods less critical for maintaining yields (Nielsen et al., 2005) and has allowed for more frequent cropping in dryland rotations. For example, adding corn (Zea mays

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L.) to a wheat-fallow (WF) rotation results in 3-year rotation (wheat-corn-fallow, WCF) and reduces fallow frequency to one summer fallow every three years. Rotations can be modified further by completely removing summer fallows from the system. Though wheat yields in continuously planted rotations are typically lower than those wheat crops following a fallow, overall system productivity (i.e., annualized yields) and profitability have been shown to be higher (McGee et al., 1997; Peterson et al., 1996; Rosenzweig et al., 2018). This is generally attributed to the greater number of crops grown, enhanced soil protection by crop residues on the soil surface, and the more efficient use of water by crops during the summer when evaporative demand is high (Farahani et al., 1998).

Increased cropping frequency has important implications for soil quality and function, largely due to higher organic matter inputs (above and belowground) returned to the soil. Transition to a continuously cropped system from wheat-fallow typically increases crop residue inputs by 70% (Shaver et al., 2002). SOC, aggregation, and porosity tend to increase when fallows are reduced or eliminated, likely due to added organic inputs (Rosenzweig et al., 2018; Shaver et al., 2002; Sherrod et al., 2005; Stromberger et al., 2007). While the evidence suggests improved biological activity, previous work has mainly focused on microbial communities (Rosenzweig et al., 2018; Stromberger et al., 2007) and impacts on other key soil biota remain poorly understood. Here we refer to the transition to less frequent fallows as a form of cropping system intensification, since more crop is grown per unit of area, over time. However, because vegetative cover is maintained for a greater portion of the year under these intensified rotations, soil functioning tends to improve, contrary to other forms of intensification (e.g., increased tillage, fertilization; Del Grosso et al., 2002; Rosenzweig et al., 2018).

Soil macrofauna represent an important, but often overlooked component of soil biological communities, especially in dryland systems where populations are thought to be rather low (de Bruyn & Conacher, 1990). Soil macrofauna is a general term for a group of invertebrates comprising various functional groups, including detritivores, herbivores, fungivores, and predators, as well as burrowing organisms, such as ants and earthworms, that are considered ecosystem engineers. Ecosystem engineers can be particularly important in building soil aggregates and macropores, altering soil organic matter and nutrient cycling (Brown et al., 2000; de Bruyn & Conacher, 1990; Lavelle, 1997), and regulating a suite of soil-based ecosystem services, including enhancing water infiltration and storage (Lavelle et al., 2006).

Macrofauna communities are largely supported by organic residues and detritus, and thus have been shown to benefit from reductions in disturbance, such as tillage (Briones & Schmidt, 2017) and increased organic inputs (Lavelle et al., 2001; Melman et al., 2019). Despite significant work relating soil macrofauna to soil processes, it remains unclear how intensification of dryland systems, i.e. reduced bare fallow frequency, affects soil macrofauna and the implications for diverse soil functions, specifically those related to soil water capture and storage. This is of particular relevance since soil macrofauna are poorly studied in the High and Central Plains region (e.g., Reynolds & Reeves, 2003) and largely overlooked by farmers and researchers as an indicator and regulator of soil quality, as faunal abundance is perceived to be low due to dry conditions and widespread cultivation. As many important agricultural areas experience moisture limitation and climate extremes, managing for organisms that enhance soil water capture and storage may be an important consideration for climate adaptation and overall agroecosystem resilience. For example, spring precipitation in Colorado has been consistently below average since 2000 and the number of extreme rainfall events are expected to increase

(Franson et al., 2017), making efficient water capture by soils an essential soil function for maintaining productivity

This study sought to examine the effects of fallow frequency and cropping intensity on soil chemical, physical and biological properties related to water capture. We sampled four different rotations of varying fallow frequency at two sites within a long-term, no-till dryland cropping system experiment in eastern Colorado. We hypothesized that rotations with reduced fallow frequency would support greater macrofauna populations, particularly ecosystem engineers, and these would in turn be related to enhanced soil structure (i.e. aggregation) and infiltration (Fig. 2.1).

2.2 Methods

2.2.1 Site descriptions and experimental design

The experimental sites considered in this study are part of the Dryland Agroecosystem Project, a multi-site, long-term cropping system experiment situated across a latitudinal gradient with increasing potential evapotranspiration (PET) from north to south (Peterson et al., 1993). The two sites considered in this study are located near Sterling (40°22'12"N, 103°7'48"W) (Logan County) and Stratton, Colorado (39°10'48"N, 102°15'36"W) (Kit Carson County). At each site, a field experiment was established in 1985 to evaluate the effects of cropping intensity and topography on productivity and soil properties in no-till dryland farming systems of the High Plains of Colorado. Each experimental plot crosses a topographical gradient and includes a summit, sideslope and toeslope position. All sites were under conventionally-tilled, wheat-fallow rotations for over 50 years prior to implementation of the experimental plots.

Precipitation at both sites averages 410 mm yr^{-1}, with the majority (about 75%) occurring as rain between April and September. The sites are located at roughly 1340 m in elevation, with temperatures ranging from an average low of -10°C in Jan to and average high of 32°C in July. Soils at both sites are classified as Mollisols in the Argiustoll soil subgroup with a loam texture. The toeslope sand, silt and clay content at the Sterling site is approximately 44%, 37% and 19%, and at the Stratton site 35%, 41% and 24%, respectively, and other soil properties have been reported previously (Sherrod et al. 2005).

At each site, we considered four different management treatments within the larger experiment that represent a gradient of fallow frequency. The treatments include: 1) wheat-fallow (WF), with bare summer fallow every other year (lowest intensity), 2) wheat-corn-fallow (WCF), with summer fallow 1 out of every 3 years (medium intensity), and 3) Continuous Cropping (CONT; referred to previously as opportunity cropping (OPP) (Peterson et al., 1993)), which does not follow a set rotation, and includes wheat and corn or other spring planted crops (millet, sorghum), but never has a summer fallow. Every phase of the set rotations is present each year, and the continuous rotation was planted to wheat for the 2015/2016 season. Additionally, both sites include a perennial grass treatment (GRASS) with permanent cover, seeded in 1986 with a native grass mixture including six different grasses, dominated by species of Agropyron and Bouteloua (Peterson et al., 1993), which receives no fertilizer and is left largely unmanaged. These strips are meant to simulate land managed under a Conservation Reserve Program (CRP), in which vulnerable soils are taken out of production and seeded back to native prairie to restore soil C and reduce erosion (Reeder et al., 1998). These four treatments encompass a gradient of agroecosystem management with increasing levels of vegetative cover and organic residue inputs. While we recognize that the GRASS system is not a true cropping

system, it reflects an upper limit for dryland agriculture in the region, in terms of potential soil cover and residue inputs. We note that the GRASS system also represents a widespread alternative management practice for vulnerable soils in the Great Plains, with millions of acres in conservation agreements (Farm Service Agency, 2019).

Treatment plots are 6.1 m wide with a length of approximately 300 m at Sterling and 200 m at the Stratton site. All four treatments are present in two replicate strips at each site, for a total of four replicates for each rotation treatment. Fertilizer N and P are typically added to plots at recommended rates at planting based on soil tests. Herbicides are applied as needed to control weeds and minimize associated soil water loss. Detailed descriptions of the agronomic management practices are presented in previous publications from these sites (eg., Sherrod et al. 2005).

2.2.2 Soil sampling and analysis

Soil sampling was conducted in late May and early June 2017 to evaluate a suite of soil biological, chemical, and physical parameters. All cropped plots had undergone wheat harvest the previous summer (July 2016) and so all were in wheat stubble after a winter fallow and in comparable stages of the rotation at the time of field sampling. All measurements considered here were conducted in the toeslope position, since previous observations suggested higher levels of soil organic matter (SOM) and associated biological activity in this part of the catena (Sherrod et al., 2005; Stromberger et al., 2007). To avoid unnecessary disruption of the long-term soil and yield monitoring area, macrofauna pits were excavated just upslope of the toeslope boundary.

2.2.3 Soil macrofauna communities

Soil macroinvertebrates were sampled using a modified Tropical Soil Biology and Fertility (TSBF) method (Anderson & Ingram, 1993). A 25 x 25 cm soil pit was excavated to a

depth of 20 cm along with surface residues and hand-sorted for visible macroinvertebrates (>2 mm). Two macrofauna pits were collected in each treatment plot, separated by approximately 20 m. Data were collected from each of the two pits separately, and then the values averaged to obtain a single plot-level population estimate that was used for all analyses. Specimens were stored in 70% ethanol and returned to the lab for identification. Macrofauna were generally classified to family (Dindal, 1990; Arnett et al., 2002; Arnett & Thomas, 2000), excepts for spiders (Araneae) which were only identified to order, and ants (Hymenoptera: Formicidae), which were classified to genus (Fisher & Cover, 2007; Gregg, 1963). Richness was calculated as the total number of different identifiable taxa at any level. Earthworms were rinsed and patted dry for determination of fresh biomass. As the majority of earthworms collected were juveniles, comprehensive classification was not possible; the few whole adult specimens that were collected were identified to species using the key of Schwert (1990), but earthworms as a whole were considered as a single taxa in all analyses.

2.2.4 Soil chemical analyses

A representative subsample of soil from the 20 cm-deep macrofauna sampling pit was collected after macrofauna removal, air-dried, and passed through a 2 mm sieve. Total Carbon (C) and nitrogen (N) was determined using a LECO CHN-1000 auto analyzer (St. Joseph, MI). Inorganic C was assessed using a modified pressure-calcimeter method (Sherrod et al., 2002), and the difference between total and inorganic C used to determine total SOC. Permanganate oxidizable C (POXC), thought to represent a relatively labile or active C pool, was determined according to Weil et al. (2003). Electric conductivity (EC) and pH were measured in a 1:2 soil to water mixture, and Olsen P was determined with a 0.5 M sodium bicarbonate ($NaHCO_3$) extracting solution. Potentially mineralizable N (PMN) was determined on air-dried soil using a

7-day anaerobic incubation (Drinkwater et al., 1996). Briefly, two sub-samples (8 g) of the 2 mm-sieved air-dried soil were prepared, one for immediate extraction and another for incubation. Incubated soils were submerged in water and the headspace flushed with N_2 gas for 1 minute before capping and incubating at 30°C for 7 days. Soils in each group were extracted with 2M KCl by shaking for 1 hour, then analyzed for NH_4^+ -N colorimetrically (Kandeler & Gerber, 1988).

2.2.5 Soil physical properties

Bulk density of surface soils (0-5 cm) was evaluated at four points in each plot using a sharpened metal cylinder (7 cm diameter). The cylinder was inserted vertically into the soil by hand and excavated. Soil within each cylinder was removed, placed in a plastic bag and transported back to the lab in a protective sleeve. Samples were weighed and gravimetric water content determined by oven-drying a subsample (~40g) at 105°C to calculate bulk density. The two cores were then pooled, gently broken apart by hand along natural planes of weakness to pass through an 8 mm sieve and air-dried. A 40 g sub-sample of the 8 mm sieved, air-dried soil was evaluated for aggregate stability using a wet-sieving method after Elliott (1986). Briefly, soil was submerged in deionized water on top of a 2 mm sieve for a 5 min. slaking period. The sieve was repeatedly submerged 50 times over a 2 min. period. This was repeated on successively smaller sieves (250 μm, 53 μm) and the soil remaining on each sieve was collected, dried and weighed to generate four aggregate size classes (>2000 μm, 250-2000 μm, 53-250 μm, < 53 μm). Aggregate stability was evaluated using the mean weight diameter (MWD; van Bavel, 1950), calculated as the fraction of soil present in an aggregate size class multiplied by the average diameter of aggregates in that size class.

Shortly after soil macrofauna sampling (1-2 weeks) and at least two days after a rain event, potential infiltration was measured near each macrofauna sampling point (1-1.5 m) using a Cornell sprinkle infiltrometer (Ogden et al., 1997). This method uses a portable rainfall simulator to apply water to the soil surface at a constant rate and allows for calculation of infiltration, runoff, and sediment production over time. In short, a stainless-steel ring (24 cm dia. x 11.8 cm long) was inserted into the soil surface to a depth of 7 cm and the infiltrometer was placed on the ring. Capillary tubes at the base of the infiltrometer emit water drops roughly 5 cm above the soil surface at a controlled rate of approximately 6 mm min^{-1} (14 in. hr-1). A hole (3 cm dia.) in the side of the steel ring positioned at the soil surface prevented excessive ponding and allowed for outflow and collection of runoff. The amount of water applied and lost as runoff was recorded every min. for the first 6 min., and every 3 min. after for a total water application period of 30 min. This duration was long enough to reach steady state infiltration in all plots. All runoff water was collected, homogenized in a large plastic container, and a ~750 ml aliquot taken for determination of runoff sediment, as an indicator of erosion potential. The time at which runoff was first observed was recorded as a metric of infiltration. Steady state infiltration was determined by calculating the average infiltration rate in the final 9 min. of water application. Total infiltration was calculated as the difference between the total water applied and runoff collected over the 30 min trial. Multiple soil cores were taken at the time of infiltrometer readings using a 2.5 cm hand-held soil probe to 10 cm depth for determination of gravimetric soil moisture.

2.2.6 Statistical analysis

For all soil chemical, biological, and infiltration parameters the two sub-samples in each plot were averaged to obtain a single plot-level value that was used for statistical tests, resulting

in two data points per treatment per site. Two-way analysis of variance (ANOVA) was used to evaluate the effect of crop rotation treatment and site on each measured variable using R version 3.5.1 (R Core Team, 2018), though the interaction was not considered due to low degrees of freedom. Data was log transformed as needed to meet the assumptions of ANOVA. Differences between cropping systems were tested using a post hoc Tukey test. In all analyses, a probability of obtaining our result due to random chance of less than 10% ($p = 0.10$) was used as the alpha level for determining significant results. We also explored bivariate relationships of soil chemical, physical and biological variables by Pearson correlation.

Additional analyses were conducted to understand multivariate differences in soil macroinvertebrate communities and their relation to soil physical and chemical parameters. All multivariate analyses were completed using the vegan package for R (Oksanen et al., 2018). Principal component analysis (PCA) was conducted on soil chemical and physical variables after removing highly colinear variables and standardizing by converting to *Z* scores (Vu, 2011). Multivariate analysis of soil macrofauna communities was completed with taxa data aggregated to order level and taxa appearing in fewer than three samples removed. The taxa table was standardized using the Wisconsin double standardization method (Legendre & Gallagher, 2001) and Bray-Curtis dissimilarities were used for comparison of community composition. Homogeneity of dispersion between groups (similar to assumptions of equal variance in ANOVA) was verified and differences in group composition were tested using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations (Anderson, 2001). Canonical correspondence analysis (CCA) was used to determine whether soil macroinvertebrate communities are structured by two soil properties related to water infiltration: aggregate stability (MWD) and initial infiltration rate (Legendre et al., 2011; McCune et al., 2002). Our constrained

approach allowed us to test the hypothesis that overall macrofauna community composition would be related to water infiltration; the ordination diagram lets us view the relationship between faunal communities and infiltration parameters, while filtering out other variability in the community data.

2.3 Results

2.3.1 Soil Macrofauna

Thirty-four different taxa were identified from all treatments in the two sites, including seven families of Coleoptera (beetles) and seven genera of ants (Hymenoptera, Formicidae). A total of 886 macroinvertebrate specimens was collected from the two sites (32 sampling monoliths), of which 333 were ants and 323 were earthworms. The few identifiable mature (i.e., clitellate) earthworms were identified as the European introduction *Aporrectodea turgida* (Eisen), a common species of the region (Reynolds, 2017). Earthworm populations were highly variable at both sites, with densities ranging from 74 to 340 individuals m^{-2} (Table 2.1). Among the ants collected, the dominant groups were the genera Lasius (147 individuals), followed by Ponera (109 individuals). A total of nine different ant taxa were identified, with three of the taxa found exclusively in the GRASS treatment (Table S2.1). Coleoptera (beetles) were the next most abundant group, with a total of 55 adults and 25 larvae collected from seven families across both sites (Table 2.1; Table S2.1).

There was an overall trend toward higher total macrofauna densities with greater cropping system intensity ($p = 0.002$), and the perennial grass (GRASS) treatment consistently outperformed the cropped systems as a habitat for large and diverse soil macrofauna communities (Table 1). The perennial grass treatment had significantly greater total macrofauna

abundance than the lowest intensity rotation (wheat-fallow, WF), with four times as many arthropods (post hoc Tukey $p = 0.04$; Fig. 2.2A). Earthworms were overall more abundant in Stratton vs. Sterling ($p = 0.04$), though there was higher earthworm biomass at Sterling ($p = 0.05$; Table 2.1). Also, a greater number of mature earthworms were found at Sterling (11) compared to Stratton (4) and individuals were generally larger at Sterling, but there were no significant treatment differences. Across both sites, there were on average 5-18 times more ants in the GRASS treatment (508 ± 182 individuals m-2) and overall ants exhibited a response to treatment ($p = 0.07$; Table 2.1). Pairwise comparison by treatment indicates that GRASS had significantly more ants than WCF. While densities were low and formal comparisons not conducted, we note that Hemiptera (true bugs) were only found in the CONT and GRASS treatments (Table 2.1). Also, while not significant, adult Coleoptera tended to be more abundant in the treatments with fallows (WF and WCF). Taxonomic richness was elevated in the GRASS plots ($p = 0.06$; Fig. 2.2B).

2.3.2. Soil physical and chemical properties

Total infiltration volume and time to runoff was generally higher in the GRASS plots ($p = 0.075$), and cropping system treatment had a significant effect on time to first runoff ($p = 0.011$; Table 2.2). Pairwise comparisons showed that GRASS had significantly greater total infiltration than WF, and a longer runoff time than all cropped treatments. Steady state infiltration rate did not differ consistently between treatments. Aggregate stability was significantly higher in the GRASS treatment, with a more than three-fold increase in MWD compared to WF ($p = 0.03$, Fig. 2.2D). Bulk density and SOC were both affected by cropping system treatment ($p = 0.033$; Table 2.1). GRASS had significantly lower bulk density and higher SOC than WCF ($p = 0.03$; Fig.

2.2B). Sediment load in the runoff water was not significantly different between treatments, but on average was lowest in the GRASS and highest in WCF (Table 2.2). Electrical conductivity was significantly elevated in the CONT treatment ($p = 0.026$), and N mineralization was significantly greater in the GRASS and WF treatments compared to the others ($p = 0.003$; Table 2.2). While POXC showed only marginally significant differences between treatments ($p = 0.09$), there was a trend towards higher POXC in the CONT and GRASS treatments (Table 2.2).

2.3.3 Relationships between soil biological, physical and chemical properties

Soil macrofauna were correlated with soil health indicators across management treatments. Earthworm abundance was positively correlated with time to first runoff ($r = 0.62, p = 0.011$; Table 2.3). Total arthropod populations were positively correlated with both total water infiltrated (R=0.63, $p = 0.01$) and MWD ($r = 0.68, p < 0.01$), and negatively correlated with bulk density ($r = -0.57, p = 0.02$). Arthropod abundance was also positively correlated with potentially mineralizable N ($r = 0.61, p = 0.01$).

Time to first runoff was positively related to SOC ($r = 0.65, p = 0.007$) and MWD ($r = 0.65$, p=0.007). Total infiltration was also positively correlated with SOC ($r = 0.52, p = 0.04$) and MWD ($r = 0.65, p = 0.007$), but negatively related to bulk density ($r = -0.71, p = 0.002$). Aggregate stability was correlated with SOC ($r = 0.66, p = 0.005$) and negatively correlated with bulk density ($r = -0.74, p = 0.001$). Steady state infiltration rate was highly related to total infiltration ($r = 0.95, p < 0.001$) and followed much the same relationship as total infiltration, except was also positively correlated with N mineralization ($r = 0.52, p = 0.04$) and showed a stronger relationship to arthropod abundance ($r = 0.75, p < 0.001$).

2.3.4 Multivariate trends

Principal component analysis (PCA) of selected soil variables revealed that GRASS separates strongly from the cropping system treatments, mainly along PC1, which is associated with BD, MWD, SOC and infiltration (Fig. 2.3). Agroecosystem management treatment was a significant predictor of soil characteristics (PERMANOVA pseudo $F = 22.1$, $p < 0.001$).

Constrained ordination using canonical correspondence analysis (CCA) suggests that macrofauna community structure (at the order level) is distinct within the different rotation treatments when looking specifically at the variability associated with aggregation (MWD) and time to runoff (Fig. 2.4). CCA was chosen in order to examine community structure that is directly related to specific environmental variables, i.e., it ignores community structure unrelated to the variables in the model and constrains it to the variables of interest. This allows us to test whether functional attributes of the soil important in a dryland agroecosystem, namely structure and infiltration, were related to the soil macrofauna community. In contrast to the unconstrained ordination of soil physical and chemical properties (Figure 2.3), there is a distinct grouping of macrofauna community by rotation, and they clearly separate along a gradient of fallow frequency (Fig. 4). Constrained axes cumulatively explained 20% of inertia (similar to variance). ANOVA-style permutation test of the CCA model with 999 permutations additionally shows that the CCA model is marginally significant (Macrofauna community structure ~ MWD + Runoff.Time; $p = 0.070$), though the axes were not significant ($p = 0.158$ and 0.285, respectively).

2.4 Discussion

2.4.1 Soil macrofauna and soil function in agroecosystems

The main objective of this study was to determine if long-term differences in land use and cropping system, namely distinguished by summer fallow frequency and organic residue input, in no-till, dryland agroecosystems leads to shifts in soil macrofauna communities, and to what extent these communities are associated with improved soil structure and hydraulic properties. The study sites employ several practices often categorized as 'conservation agriculture', which are widespread in the region (zero-tillage and direct-drill seeding) and have been shown to promote soil macrofauna (Briones & Schmidt, 2017; Edwards & Lofty, 1982). Ants and earthworms dominated soil macrofauna communities at both sites, together accounting for 74% of all individuals collected. Both of these groups are generally considered ecosystem engineers due to their ability to move and reshape the soil at multiple scales (Folgarait, 1998; Hastings et al., 2007; Orgiazzi et al., 2016) and can have important effects on soil structure and hydraulic properties.

Our findings indicate that soil macrofauna populations are linked to important soil functions in these dryland agroecosystems. The soil arthropods collected in our study (dominated by ants) were most related to infiltration dynamics (total water infiltrated and steady state infiltration rate). Ants build extensive tunnels and have been shown to dramatically increase water infiltration in a semi-arid pasture (Eldridge, 1993) and to fundamentally change soil structure (de Bruyn & Conacher, 1990). A recent study by Evans et al. (2011) directly tested the effects of soil macrofauna in a dryland wheat cropping system of western Australia and found that deep soil moisture and crop yields were significantly increased in the presence of the ants and termites. The work by Evans et al. highlights the potential importance of non-earthworm macrofauna in

soil modifications within actively cropped soil that contribute to critical hydraulic functions in water-limited systems. We note that other studies from comparable dryland systems in Australia suggest a similar diversity of ants, but fewer earthworms than found at our sites (Lobry de Bruyn, 1993; Wilson-Rummenie et al., 1999). In our study, potential N mineralization was also found to be associated with increased arthropod populations (Table 2.3), which may be due to ant activity, as it has been documented that certain ant populations, particularly in the *Lasius* genus, can increase microbial biomass and N mineralization in their mounds (Dauber & Wolters, 2000).

The effect of land use was clearly demonstrated with arthropod populations, which increased almost four-fold from WF to the GRASS treatments. Ant populations showed a marked increase in the perennial grass strips (average 508 individuals m^{-2}) compared to the low input wheat-fallow system (average 28 individuals m^{-2}; Table 2.1). The perennial plant presence is likely a key factor explaining this pattern, as it provides a more consistent food source, temperature regulation and greater habitat stability due to complete lack of mechanical disturbance associated with field operations (Sanabria et al., 2016). Additionally, greater plant species diversity in the grass treatments likely contributes to greater habitat complexity that, in turn, supports more active and diverse macrofauna communities (Laossi et al., 2008). While only a slight trend of increased arthropods with cropping intensity was observed, we acknowledge that our single time-point sampling does not capture the high seasonal and annual variability in macrofauna populations (Berg & Bengtsson, 2007; Doblas-Miranda et al., 2007). For example, we might expect different results if plots were sampled with active crop growth, especially compared to summer fallow. We note that our sampling method is specifically optimized for the sampling of earthworms and other endogenic fauna, while ants and other soil dwelling invertebrates are often

surveyed by assessing the density of mounds in a defined area (Briese & Macauley, 1977) and through buried pitfall traps (MacFadyen, 1962). However, we believe our method is appropriate for our purposes of capturing relative differences between treatments, and we intentionally sampled in spring (typically wet) for maximum activity, though absolute abundances should be interpreted with caution.

The earthworms collected in this study appear to largely belong to endogeic functional groups, which make shallow burrows that can contribute to water infiltration, porosity and soil structure, as well as to cycling of soil organic matter (Lee, 1985). The casting activities of endogeic earthworms are known to form aggregates that are enriched in C and highly stable (Fonte et al., 2007; Six et al., 2004). Significant increases in water infiltration have been associated with earthworm activities in semi-arid pasture and agricultural soils (Lee & Foster, 1991). Though the worms encountered here do not form large, continuous vertical burrows like those of anecic species that channel water deep into the soil (Andriuzzi et al., 2015), their contributions to soil structure are still likely to be important. Earthworms may improve infiltration and water-capture by increasing overall porosity and maintaining stable aggregates at the soil surface, which are resistant to rupture and associated crusting of surface soils (Blanchart et al., 2004). Our findings support the idea that earthworm presence was related to higher initial infiltration. The amount of time it took for water to begin running off increased from less than 1 minute to over 3 minutes in the plots with lowest to highest earthworm abundance (Table 2.1; Table 2.2). Recent work from nearby irrigated sites in Colorado also found a correlation between infiltration and shallower-burrowing endogeic earthworm populations (Deleon, 2017). We note that our results are correlative, and a direct documentation of earthworm burrows or manipulation of earthworm densities would be necessary to definitively attribute infiltration differences to earthworm

presence. It is important to note that more complex system feedbacks may also help explain our results. For example, soil structure and infiltration may be improved by increased plant cover directly, and soil macrofauna populations are also responding to increased aeration and moisture from plant growth, in addition to the greater availability of organic matter.

We expected a positive correlation between earthworms and cropping system intensity, as greater organic inputs in the more intense rotations are likely to support the energy requirements of earthworms and other soil macrofauna (Fonte et al., 2009; Lavelle et al., 2001). Earthworm abundance ranged from 74 individuals m^{-2} in the WF plots at Sterling to 340 individuals m^{-2} in the GRASS plots at Stratton. These densities are similar to those observed in pastures and irrigated corn systems of Colorado (Hurisso et al., 2011; Melman et al., 2019), indicating that earthworms are relatively tolerant to dryland cropping system conditions of the high plains. However, the lack of a significant cropping system effect on earthworm abundance is likely due to the high spatial variability common in earthworm abundance data (Whalen, 2004), the low number of replicates considered (two per treatment), and narrowness of our treatment plots. This finding also suggests that other factors, in addition to management, influence earthworm abundance in these systems. For example, the apparent discrepancy between earthworm abundance and biomass between the two sites suggest that life stages were not synchronized between the two sites. Nearby weather stations reported that the Stratton site received 151 mm of precipitation between April and June of the sampling year, whereas Sterling received about a quarter of that during the same time period (40 mm), which may have contributed to the high proportion of small juveniles collected at that site and highlights the temporal sensitivity of the soil-dwelling fauna.

Multivariate analyses considering entire soil macrofauna communities link soil biological activity with water dynamics. When macrofauna communities are visualized in ordination space in relation to soil aggregation (MWD) and runoff time, macrofauna communities separated by cropping treatment (Fig. 2.4). When the macrofauna communities were plotted in this way, the treatments not only grouped together as having similar community composition, but groups separated along the gradient of organic matter input intensity. That is, macrofauna communities associated with low aggregation and infiltration were more similar to each other and tended to be from low-intensity rotations. Our results corroborate the idea of soil macrofauna activity as a potential driving force of the changes to soil function seen with intensified dryland rotations (and associated increases in organic matter inputs). The challenge of more extreme drought and precipitation events will only be overcome through careful management of the soil, including soil-dwelling fauna that can support multiple soil functions (Lavelle et al., 2006). Due to the widespread nature of climate change and the large global extent of rain-fed cropping systems, functional soil outcomes in these systems have the potential to impact crop productivity and resilience across multiple scales.

2.4.2 Soil properties and agroecosystems

We found that fallow frequency affected soil structure and infiltration parameters (i.e., total infiltration, time before runoff; Table 2.2), with a trend of increased infiltration with cropping system intensity and significant gains seen in the perennial grass treatments. Assessment of soil hydrologic function at these sites 12 years into this same experiment found intensified rotations to have higher porosity and lower bulk density, though there was no rotation effect on soil sorptivity, which is similar to ponded water infiltration (Shaver et al., 2002). Though the results of this study are not directly comparable to those previous results because of differences in

sample collection, the trend toward increased hydraulic function with greater plant cover seen 19 years previously appears to have continued. Poor infiltration can result in runoff, which leads to the soil erosion and water loss from crop fields (Bronick & Lal, 2005). Water that can infiltrate into the soil and is stored deep in the soil profile is more protected from evaporation and can substantially increase crop yields in dryland cropping systems (Bonfil et al., 1999).

A recent meta-analysis of alternative crop management practices on infiltration found that even under no-till, continuous presence of roots (via perennial crops or inclusion of cover crops in rotation) was necessary to improve water infiltration (Basche & DeLonge, 2019). Similarly, we found a trend towards increased infiltration with higher cropping intensity, but it was only under conversion to a perennial system that significant increases in infiltration were observed. Though our study did not test the use of cover crops specifically, replacement of fallow with crops similarly increases soil cover and total plant inputs, resulting in improvements to soil water dynamics. Given that cover crops are difficult to incorporate into dryland rotations due to their use of limited water resources (Nielsen & Vigil, 2017; Unger & Vigil, 1998), intensification offers an alternative means of increasing organic inputs while maintaining economic viability.

Surface aggregation and SOC can also contribute to enhanced water provision for crops by contributing to higher porosity, lower bulk density and soil water holding capacity (Franzluebbers, 2002; Prove et al., 1990). We found increased water stable aggregation as fallow frequency decreased (Fig. 2.2). Increased residue inputs and rooting activity in the high-input treatments likely increase soil aggregation through a variety of mechanisms, including soil enmeshment by roots and fungal hyphae, exacerbated wet-dry cycles near growing roots, root exudation and stimulation of microbial activity, and by fostering the activity of soil macrofauna (Six et al., 2004). We found that soil aggregate stability in the top 0-5 cm was positively

correlated with initial infiltration ($r = 0.65$; Table 2.3) but not total infiltration, suggesting that aggregation at the soil surface does not necessarily translate to porosity deeper in the soil. Though our observed SOC values did not fit a consistent trend, numerically the GRASS and CONT treatments had the highest SOC. We expected to find higher SOC in these higher-input treatments, as this has been found in other dryland rotations across Colorado (Rosenzweig et al., 2018; Sherrod et al., 2005; Sherrod et al., 2018). We suspect that the sampling design and the location of samples on a slight slope resulted in the smaller response seen in our data.

There is a substantial body of literature documenting the increase in annualized yields in intensified dryland cropping systems, with increased water use efficiency generally used to explain the phenomenon (Farahani et al., 1998; Peterson & Westfall, 2004). Subsequent studies on the soil physical properties have suggested that increased soil structure and porosity may be helping to store more water and stabilize yields in intensified rotations without a fallow (Rosenzweig et al., 2018; Shaver et al., 2002). Our research further supports the idea that changes in overall soil functioning (i.e., water capture), together with altered timing of crop growth, is responsible for higher annualized yields in intensified rotations.

It is also important to note that the majority of the soil function metrics measured here showed a trend with increased cropping intensity, but significant differences were only realized with conversion to a long-term perennial grass system. Whereas we have noted above the limitations of the study design on our statistical inference, it is meaningful to emphasize that soil functional goals, including increased soil C storage, hydraulic function, and biodiversity, generally has limited potential in annual cropping systems compared with perennial systems. These trends have been found across several systems (Basche & DeLonge, 2017; Paustian et al., 1997), and further demonstrate the soil-improving potential of approaches that increase perennial vegetation

on farm landscapes, i.e. through integration of perennial pastures, increased landscape heterogeneity and transition to perennial-based crops. These options require further study and innovation, especially for semi-arid drylands, and are areas of active research (e.g. de Oliveira et al., 2019).

2.5 CONCLUSIONS

Findings from this study suggest that soil macrofauna communities are related to soil structure and hydraulic properties critical for dryland cropping systems. Increased plant cover and organic matter input appears to be one way to support these soil biota and is associated with increases in soil hydrologic function. Contrary to what may be expected from the regional climate and land use, we show that both earthworms and ants are present in appreciable populations at these sites, and that they each may contribute to enhancing infiltration in different capacities. Arthropods may also positively affect nutrient cycling and increase N provision. While soil macrofauna responses to cropping system intensity were not always significant, notable trends suggest that management practices with high organic matter return may benefit soil macrofauna populations. Soil macrofauna communities may confer soil functions that are important to agroecosystem productivity in dry areas, which could amplify the positive effects of organic inputs related to intensification of crop rotations, and thereby lessening the agronomic penalty of fallow replacement.

Increasing soil resilience to climate variability is a key management goal for increasing agricultural sustainability (Lipper et al., 2014). Soil macrofauna should be considered in management decisions in dryland contexts, as their activity likely contributes to important functions related to water capture. Therefore, as other agricultural challenges arise, it may be important to consider macrofauna communities when evaluating management options. For

example, herbicide resistant weeds present a complex and growing challenge, particularly for no-till farmers. While soil macrofauna population in our study site appear fairly robust despite use of conventional herbicides, including glyphosate, the use of even harsher herbicides and the reintroduction or increased use of tillage, which are increasingly employed to combat weeds, will likely have detrimental effects on macrofauna populations (Briones & Schmidt, 2017; Rose et al., 2016), and effects to soil function should be further evaluated.

Our results highlight the important ecosystem services and soil functions that are associated with perennial grass systems, as these systems consistently demonstrated high functionality and support of large and diverse soil macrofauna populations. As we look to our agroecosystems to provide services beyond food provision, it is critical to consider the benefits of conversion back to perennial grasslands (i.e. Conservation Reserve Program (CRP)) and conservation of native rangelands. Also, the development of future cropping systems might better incorporate features of these perennial grasslands, for example, by seeking to maintain more permanent soil cover and/or incorporate perennial grains. While improving intensification and management of cropping systems for enhanced water use efficiency remains a critical goal for dryland crop production, perennial grasslands should not be overlooked in helping to shape the agroecosystems of tomorrow or for their role as a refuge for soil biodiversity and reservoirs of important ecosystem functions across the landscape.

CHAPTER 2 TABLES AND FIGURES

Table 2.1. Abundance and richness of soil macrofauna within four long-term agroecosystem management treatments in field experiments near Stratton and Sterling, Colorado sampled in early June, 2017. Values represent the mean ± SE of each taxon per treatment (n = 2). P-values for two-way ANOVA results for overall cropping system intensity treatment and site effects are to the right of the means, with values in bold being significant at the $p < 0.10$ level. Interaction terms were not considered due to low degrees of freedom. Statistical comparisons were not conducted for Araneae, Hemiptera, or Lepidoptera due to low abundances.

	Sterling				**Stratton**				***p*-values**	
	WF[a]	**WCF**	**CONT**	**GRASS**	**WF**	**WCF**	**CONT**	**GRASS**	**Treatment**	**Site**
	Abundance (Ind. m^{-2})				Abundance (Ind. m^{-2})					
Hymenoptera	56 ± 32	40 ± 40	80 ± 64	600 ± 392	0 ± 0	40 ± 16	100 ± 100	416 ± 168	**0.070**	0.354
Coleoptera adults	60 ± 28	24 ± 16	12 ± 12	0 ± 0	24 ± 8	44 ± 4	20 ± 4	20 ± 4	0.246	0.922
Coleoptera larvae	0 ± 0	4 ± 4	24 ± 16	32 ± 8	4 ± 4	20 ± 12	8 ± 8	8 ± 0	0.343	0.487
Araneae	8 ± 8	8 ± 0	4 ± 4	4 ± 4	0 ± 0	8 ± 8	4 ± 4	4 ± 4	-	-
Hemiptera	0 ± 0	0 ± 0	0 ± 0	4 ± 4	0 ± 0	0 ± 0	16 ± 0	0 ± 0	-	-
Lepidoptera	4 ± 4	0 ± 0	4 ± 4	0 ± 0	0 ± 0	4 ± 4	0 ± 0	0 ± 0	-	-
Total arthropods	128 ± 8	80 ± 56	124 ± 60	660 ± 380	48 ± 16	124 ± 20	136 ± 112	468 ± 164	**0.014**	0.589
Earthworms	74 ± 66	120 ± 56	78 ± 14	116 ± 16	144 ± 56	124 ± 4	222 ± 34	340 ± 144	0.328	**0.038**
Earthworm biomass (g m^{-2})	24.7 ± 0.7	26.7 ± 10.8	28.7 ± 5.5	21.2 ± 0.2	11.3 ± 2.6	10.4 ± 4.9	28.1 ± 20.6	17.2 ± 7.8	0.738	**0.051**
Richness (taxa $plot^{-1}$)[b]	3.5 ± 0.5	2.8 ± 1.3	3.3 ± 0.3	4.8 ± 0.8	2.0 ± 0.0	4.3 ± 0.3	2.5 ± 1.0	5.0 ± 0.5	**0.061**	0.819

[a] WF, Wheat-Fallow; WCF, Wheat-Corn-Fallow; CONT, continuously cropped; GRASS, native perennial grass. [b]Richness was calculated using the total number of different taxa at the finest resolution attained for each group.

Table 2.2. Measured soil physical and chemical properties under four management treatments in a 34-year dryland no-till cropping system experiment in Stratton and Sterling, Colorado sampled in early June, 2017. Values represent treatment means ± SE. Each value is the average of two replicates per site. Two-way ANOVA P-values for the overall cropping system effects and site effects are to the right of the variable values, with values in bold being significant at the $p < 0.1$ level. Treat x Site interaction terms were not tested due to insufficient degrees of freedom. All variables are 0-20 cm deep from macrofauna sampling pit except BD and MWD which are from 0-5 cm.

	Sterling				**Stratton**				***P*-values**	
	WF[a]	**WCF**	**CONT**	**GRASS**	**WF**	**WCF**	**CONT**	**GRASS**	**Treat-ment**	**Site**
MWD[b] (µm)	826 ± 75.7	937 ± 62.0	1055 ± 20.0	2377 ± 57.3	496 ± 94.3	399 ± 42.5	692 ± 143.7	1907 ± 344.3	**< 0.01**	**< 0.01**
pH	6.3 ± 0.03	7.5 ± 0.62	7.2 ± 0.31	6.7 ± 0.06	7.6 ± 0.59	7.8 ± 0.33	7.8 ± 0.29	7.8 ± 0.06	0.210	**0.006**
EC (µS cm^{-3})	30 ± 10.0	75 ± 42.5	119 ± 53.8	33 ± 12.5	80 ± 15.0	89 ± 16.3	138 ± 7.5	69 ± 13.8	**0.026**	0.100
BD (g cm^{-3})	1.21 ± 0.08	1.13 ± 0.01	1.16 ± 0.03	0.98 ± 0.01	1.21 ± 0.05	1.31 ± 0.01	1.22 ± 0.03	1.16 ± 0.02	**0.033**	**0.008**
SOC (g kg^{-1})	11.6± 0.47	11.9 ± 0.95	12.4 ± 1.37	14.8 ± 0.80	11.4 ± 2.41	8.2 ± 1.09	13.0 ± 1.15	14.0 ± 0.74	**0.033**	0.270
POX C (mg kg^{-1})	427 ± 7.9	427 ± 29.3	456 ± 39.5	483 ± 3.6	356 ± 20.1	359 ± 8.7	494 ± 104.7	464 ± 9.5	**0.090**	0.322
Total N (g kg^{-1})	1.37 ± 0.00	1.3 ± 0.05	1.5 ± 0.14	1.6 ± 0.0	1.3 ± 0.17	1.1 ± 0.08	1.5 ± 0.02	1.3 ± 0.03	**0.036**	**0.040**
N min. (mg kg^{-1} wk^{-1})	333 ± 41.9	164 ± 49.9	170 ± 93.2	322 ± 40.5	232 ± 12.5	130 ± 11.2	134 ± 27.0	306 ± 41.4	**0.003**	0.150
Available P (ppm)	3.1 ± 0.41	1.8 ± 0.30	2.6 ± 0.87	0.9 ± 0.01	5.2 ± 2.95	3.3 ± 0.14	5.3 ± 1.54	1.5 ± 0.41	**0.074**	**0.051**
Runoff Sed. (mg L^{-1})	0.36 ± 0.05	0.94 ± 0.16	0.87 ± 0.42	0.35± 0.21	0.94 ± 0.52	0.91 ± 0.18	0.40 ± 0.14	0.27 ± 0.06	0.250	0.99
Tot. Infil. (mm)	43 ± 9.3	79 ± 9.3	63 ± 11.3	75 ± 25.4	25 ± 1.0	14 ± 2.0	26 ± 0.8	73 ± 11.0	**0.075**	**0.011**
Runoff Time (s)	54 ± 4.0	92 ± 9.0	79 ± 37.0	125 ± 35.5	83 ± 18.3	66 ± 20.5	94 ± 15.3	197 ± 16.5	**0.011**	0.220
SS Infil. Rate (mm min^{-1})	0.9 ± 0.10	2.3 ± 0.32	1.6 ± 0.45	2.5 ± 1.06	0.5 ± 0.12	0.3 ± 0.04	0.4 ± 0.16	2.3 ± 0.55	0.100	**0.060**

[a]WF, Wheat-Fallow; WCF, Wheat-Corn-Fallow; CONT, continuously cropped; GRASS, native perennial grass. MWD, mean weight diameter; BD, bulk density; SOC, soil organic carbon; N, nitrogen; Avail. P, Olsen phosphate; EC, electric conductivity; POX C, permanganate-oxidizable C; Tot. Infil, total water infiltrated over the 30 minute study period; Runoff Time, time after starting water application that runoff was detected; SS Infil. Rate, steady state infiltration rate; Runoff Sed., concentration of soil sediment in runoff water.

Table 2.3. Correlation table of biological, chemical and physical parameters under four management treatments in a 34-year dryland, no-till cropping system experiment in Stratton and Sterling, Colorado sampled in early June, 2017. Values are Pearson correlation coefficients (R), and bolded texts indicates significant ($p < 0.05$) correlation.

	Worm Ab.	Worm BM	Arth. Ab.	Rich-ness	MWD	pH	EC	BD	SOC	POX C	TN	N Min.	Avail. P	Runoff Sed.	Tot. Infil.	Runoff Time
Worm Ab.[a]	-															
Worm BM	0.04	-														
Arth Ab.	0.09	-0.23	-													
Richness	0.25	-0.40	**0.69**	-												
MWD	0.33	0.17	**0.68**	**0.59**	-											
pH	0.48	0.06	-0.23	-0.30	-0.25	-										
EC	0.30	0.28	-0.32	-0.39	-0.35	**0.69**	-									
BD	0.03	-0.13	**-0.57**	-0.27	**-0.74**	0.42	0.27	-								
SOC	0.23	0.00	0.42	0.30	**0.66**	-0.40	-0.27	**-0.69**	-							
POX C	0.25	-0.11	0.45	0.42	0.45	-0.27	-0.03	-0.41	**0.66**	-						
TN	-0.19	0.26	0.25	0.03	0.41	-0.5	-0.14	**-0.55**	**0.79**	**0.54**	-					
N Min.	-0.03	-0.28	**0.61**	**0.05**	**0.51**	**-0.64**	**-0.78**	-0.44	**0.51**	0.29	0.29	-				
Avail. P	-0.13	-0.31	-0.38	-0.41	**-0.64**	0.02	0.33	0.33	-0.04	-0.12	0.06	-0.28	-			
Runoff Sed.	-0.26	-0.01	-0.30	-0.28	-0.41	0.43	0.39	0.32	**-0.66**	-0.46	-0.49	**-0.51**	-0.07	-		
Tot. Infil	-0.01	0.15	**0.63**	0.42	**0.65**	-0.30	-0.33	**-0.71**	**0.52**	0.43	0.36	0.49	**-0.61**	-0.13	-	
Runoff Time	**0.62**	-0.16	0.37	0.44	**0.65**	0.06	-0.27	-0.34	**0.65**	0.43	0.19	0.36	-0.27	**-0.59**	0.38	
SS Infil. Rate	-0.10	0.04	**0.75**	0.45	**0.60**	-0.26	-0.39	**-0.63**	0.44	0.32	0.34	**0.52**	**-0.55**	-.011	**0.95**	0.34

[a]Worm Ab, earthworm abundance; Worm BM, earthworm biomass; Arth Ab., arthropod abundance; MWD, mean weight diameter; EC, electric conductivity; BD, bulk density SOC, soil organic carbon; POX C, permanganate-oxidizable C; TN, total nitrogen; N Min., potentially mineralizable nitrogen; Avail. P, Olsen phosphate (ppm); Runoff Sed., concentration of soil sediment in runoff water; Tot. Infil, total water infiltrated over the 30 minute study period; Runoff Time, time after starting water application that runoff was detected; SS Infil. Rate, steady state infiltration rate; Runoff Sed., concentration of soil sediment in runoff water.

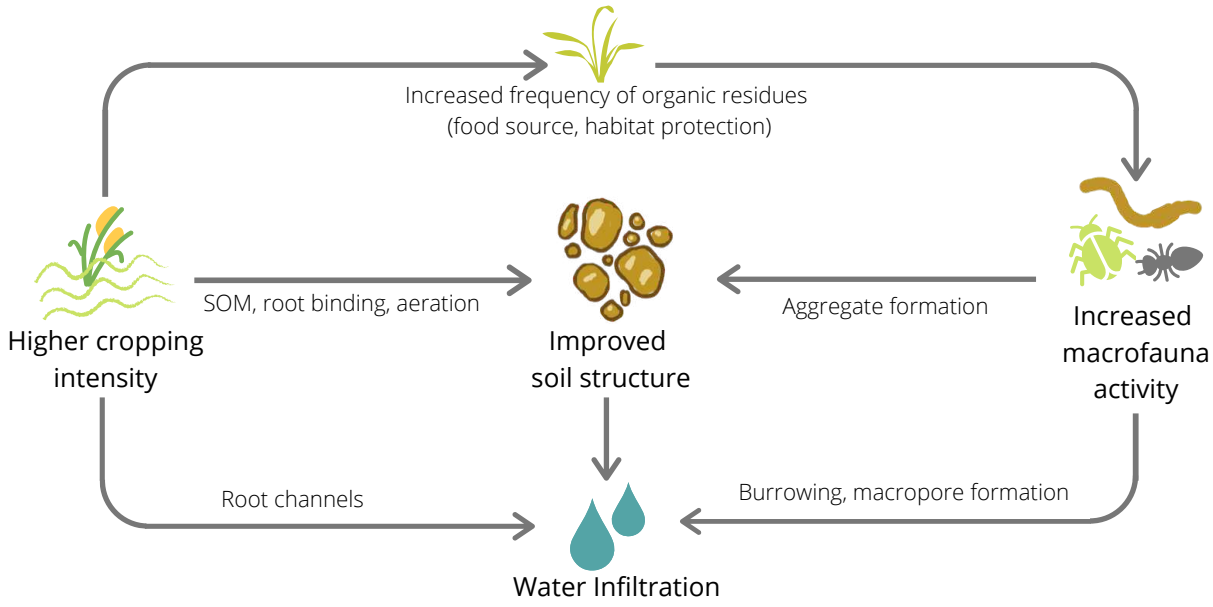

Figure 2.1. Schematic diagram of proposed soil biological and physical interactions in no-till agroecosystems, highlighting hydraulic function investigated in this study. Arrow labels indicate proposed mechanisms for interactions. SOM, soil organic matter.

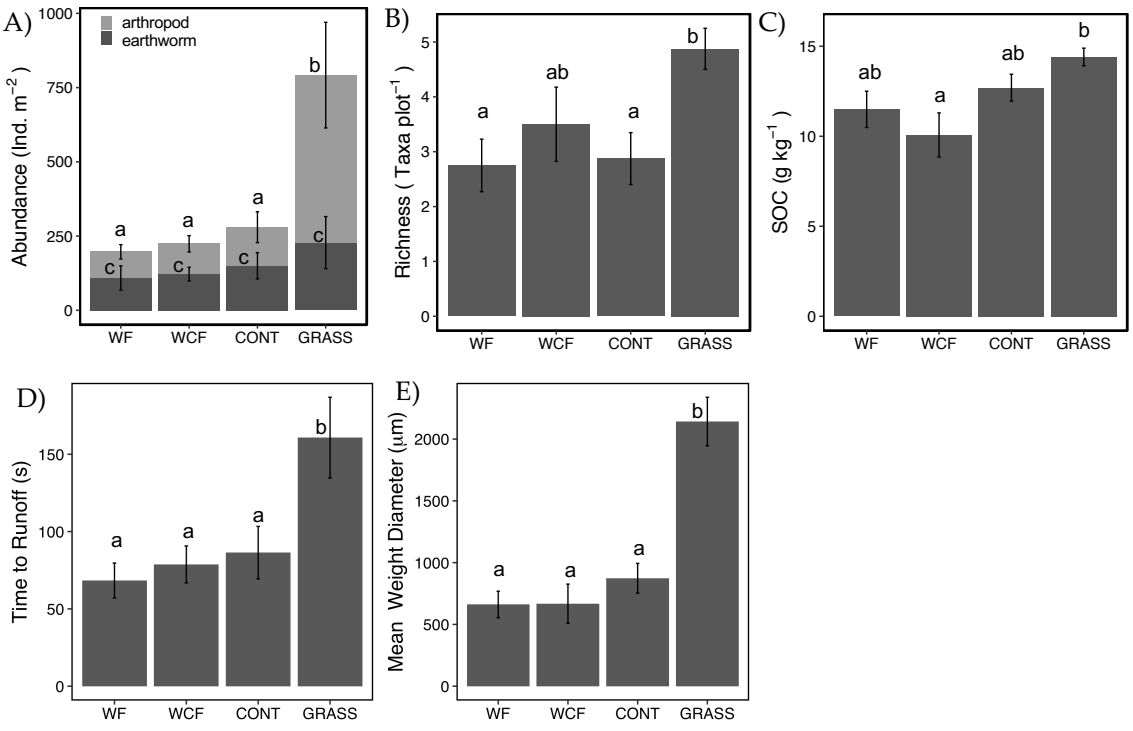

Figure 2.2. Select soil biological, physical and chemical properties by management treatment in a 32-year dryland no-till cropping system experiment in eastern Colorado, USA. The values presented represent treatment means across two sites, each with the following treatments: WF, Wheat-Fallow; WCF, Wheat-Corn-Fallow; CONT, continuously cropped; GRASS, perennial grass. Error bars represent 1 standard error of the mean. Different letters correspond to statistically significant (post hoc Tukey $p < 0.1$) differences between treatments across both sites ($n = 4$). (A) arthropod and earthworm abundance; (B) taxonomic richness of macrofauna orders (C) Soil organic carbon (SOC) concentration in the top 0-20 cm; (D) time after beginning rainfall simulation that runoff was detected; (E) mean weight diameter of water-stable aggregates in top 0-5 cm.

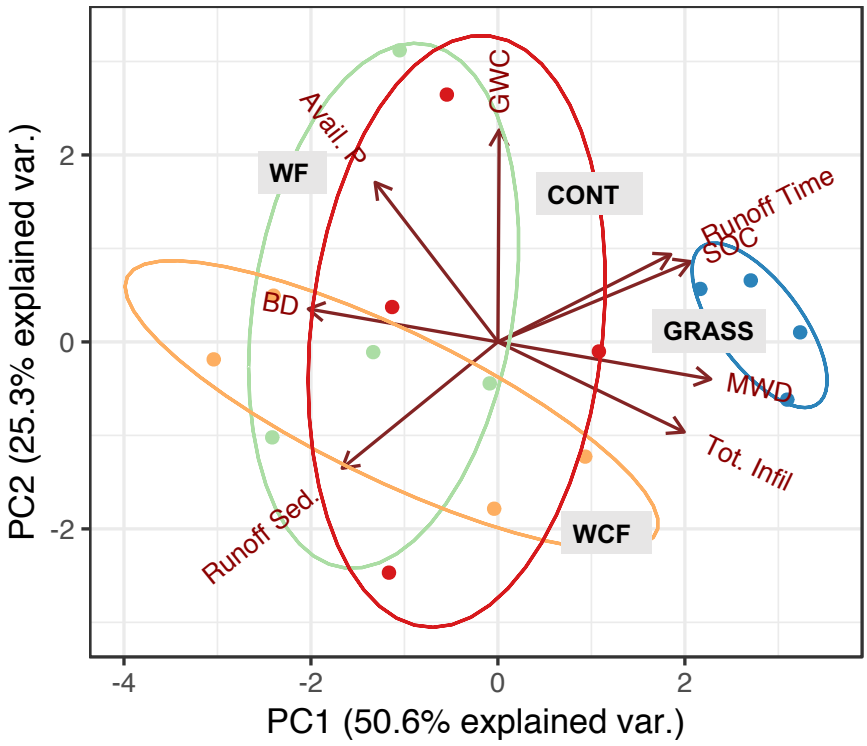

Figure 2.3. Principal component analysis (PCA) ordination of soil chemical and physical properties of four different management in a 34-year semi-arid, dryland, no-till experiment in eastern CO, USA. Permutational multivariate analysis of variance (PERMANOVA) analysis of treatment groups indicates that there is a significant ($p <$ 0.001) effect of rotation treatment. GWC, gravimetric water content; Avail. P, Olson phosphate; SOC, soil organic carbon; MWD, mean weight diameter; Tot. Infil, total water infiltrated; Runoff Time, time to runoff; Runoff Sed., concentration of sediment in runoff water; BD, bulk density.

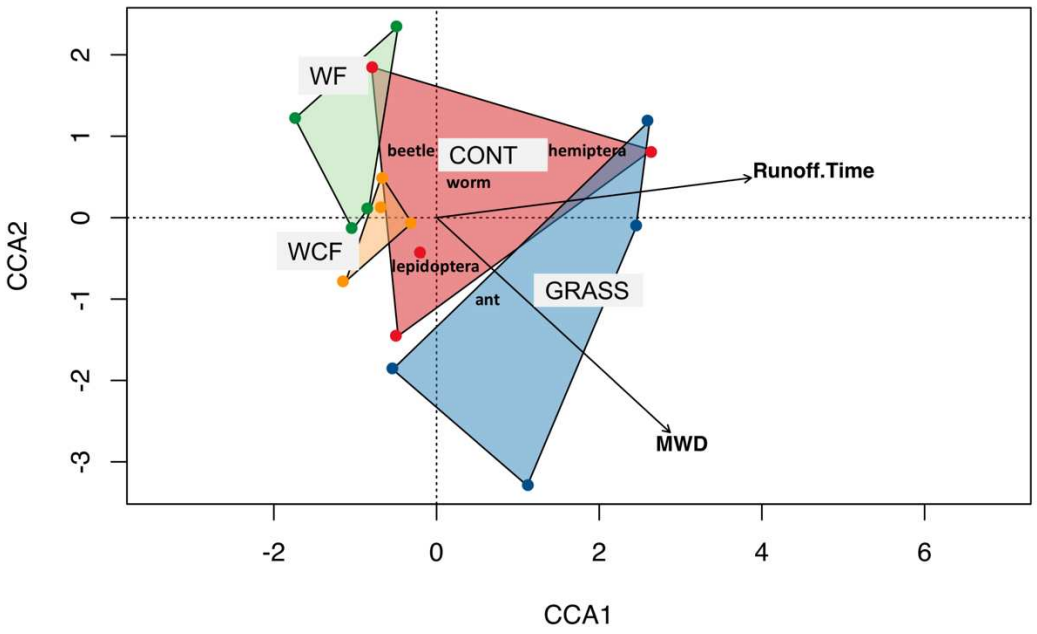

Figure 2.4. Canonical correspondence analysis (CCA) relating soil macrofauna communities to two soil parameters important for water infiltration: aggregate stability (mean weight diameter, MWD) and initial water infiltration (Time to Runoff). Eigenvalues of constrained axes 1 and 2 are 0.22 and 0.14, respectively, cumulatively explaining 20% of total inertia. Key taxa are overlaid on the ordination. Each point represents the macrofauna community, grouped to order, of a specific plot. Shaded polygons connect all sample points from the same rotation treatment. Runoff time is positively correlated with axis 1 and MWD is positively correlated with axis 1 and negatively with axis 2. ANOVA-like permutation test (Oksanen et al., 2018) with 999 permutations indicates the model (macrofauna community composition ~ MWD + Time to Runoff) is marginally significant ($p = 0.07$).

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CHAPTER 3: DRYLAND COVER CROP SOIL HEALTH BENEFITS ARE MAINTAINED WITH GRAZING IN THE U.S. HIGH AND CENTRAL PLAINS[2]

3.1 Introduction

Cover crops can play an important role in soil conservation and improving the sustainability of agricultural systems. Cover crops have been shown to increase soil organic matter, augment soil fertility, improve soil tilth, break pest cycles, and increase plant diversity and pollinator resources (Blanco-Canqui et al., 2013; Poeplau and Don, 2015; Tonitto et al., 2006). While in many systems cover crops can provide these multiple benefits to soil health as well as pest and weed control, competition for soil moisture between cover crops and the following cash crop can make cover cropping economically risky, especially in water-limited environments (Barker et al., 2018; Blanco-Canqui et al., 2013; Holman et al., 2018; Nielsen et al., 2016; Unger and Vigil, 1998).

In semi-arid dryland cropping systems of the High and Central Great Plains of the United States, winter wheat (*Triticum aestivum*) is the dominant crop and is traditionally grown with alternating years of bare fallow (Haas et al., 1974; Nielsen and Calderón, 2011). These fallows are maintained largely free of growing vegetation for 14 months to store soil moisture and stabilize wheat yields in the face of low and highly variable annual precipitation. However, on average only about 20-35% of precipitation remains in the soil at the end of the fallow period, with the highest storage efficiencies under no-till systems (Farahani et al., 1998; Hansen et al., 2012; Nielsen and Vigil, 2010). No-till management leaves crop residue on the field during fallow period and mitigates some of the detrimental effects of tillage on soil health, including soil organic matter loss and erosion (Merrill et al., 1999). With the adoption of no-till practices,

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cropping systems have intensified across the region by adding new crops into the rotation and reducing overall fallow frequency, though wheat-fallow rotations are still a common practice (Peterson and Westfall, 1996; Rosenzweig and Schipanski, 2019). The fallow period is increasingly costly to maintain due to the emergence of herbicide resistant weeds and low commodity prices. At the same time, greater frequency of bare fallow contributes to reduced soil health in dryland systems (Nielsen and Calderón, 2011; Rosenzweig et al., 2018a). The typical 14-month fallow period before wheat planting offers a long window for potential cover crop growth. However, water usage by crops that replace all or part of the typical fallow before wheat is of great concern, and these crops need to offer a level of profitability that can offset the reduction in wheat yields commonly seen in continuously cropped systems (Halvorson et al., 2004; Holman et al., 2018; Nielsen et al., 2002).

Across the Great Plains there is renewed interest in livestock integration to diversify, and potentially increase, whole-farm economics (Krall and Schuman, 1996; Martens and Entz, 2011; Russelle et al., 2007). Forage crops can be more profitable and lower risk than grain crops in the High and Central Plains because they are less susceptible to crop failure due to drought and heat stress (Holman et al., 2018; Nielsen et al., 2017). Grazing cattle directly on cropland eliminates the need for baling and transporting feed and may increase available soil nutrients through the return of animal waste (Cicek et al., 2014; Martens and Entz, 2011). Grazing may increase soil compaction in high traffic areas (Liebig et al., 2012). However, a study of the potential effects of livestock integration on crop yield found that severe declines in soil health and extensive field residue removal were required for yields to decline (Bell et al., 2011). Integration of annual legume pastures for grazing by sheep in Australia demonstrated dramatic increases in soil health, grain yields and economic returns (Puckridge and French, 1983), though adoption of this

integrated crop-livestock system has fallen off due to labor shortages and increasing farm sizes (Bell et al., 2014). A lack of established markets is often cited as a major barrier to farm diversification (Roesch-Mcnally et al., 2018). However, animal integration may be particularly feasible in the semi-arid croplands of the High and Central Plains because of relative proximity to native rangelands and market infrastructure already in place.

Allowing for limited grazing of cover crops integrated into crop rotations may provide the economic balance required for making cover crops viable in dryland rotations of the High and Central Plains (Gardner and Faulkner, 1991). Historic definitions of cover crops precluded grazing or haying management, though recent updates to NRCS cover crop guidelines have allowed for grazing, on the condition that conservation goals are not compromised (NRCS, 2014). However, crop insurance policy is generally more restrictive and grazing cover crops is not allowed if crops are insured in the summer fallow program, which precludes many areas in the High Plains and western Central Plains, where continuous cropping is not insurable (NRCS, 2019). As crop insurance policy is county-specific and largely driven by local agronomic outcomes, more data is needed to help inform the financial viability of grazing cover crops in semi-arid zones. Several studies integrating forages for grazing or haying in dryland regions of the Great Plains have shown their potential to boost farm income (Holman et al., 2018; Manske and Nelson, 1995; Nielsen et al., 2016; Twerdoff et al., 1999), but little work has been done to quantify the balance between soil health benefits of cover crops and the potential negative impacts of cattle grazing.

Through grazing management and plant species selection, integrating grazed cover crops into a crop rotation has the potential to balance soil health goals with farm profitability. Most plant species used as cover crops in dryland systems have good regrowth potential (Vipan et al.,

2020), and therefore careful grazing of cover crops can leave adequate residue to protect the soil to achieve soil health goals while providing good forage. Specific management may include rotational grazing that starts early in the season to allow for regrowth and maintaining a relatively low stocking rate. Rotational grazing with low stocking rates can reduce the risk of surface soil compaction (Twerdoff et al., 1999). Adequate residue on the field is essential for realizing many of the benefits of cover crops, including organic matter return to the soil, regulation of soil surface temperature, snow capture, and reduced erosion . Careful harvest of cover crops may still maintain these benefits, as biomass removal through haying of cover crops was shown to maintain key soil benefits in a dryland system over five years (Blanco-Canqui et al., 2013). While generally more expensive than a typical monoculture forage crop planting, diverse species mixtures commonly used as cover crops may offer greater benefits to soil and ecosystem health (Blanco-Canqui et al., 2015; Finney and Kaye, 2017), while also balancing forage quality and quantity to optimize livestock performance (Farney et al., 2018; Sanderson et al., 2018). For example, cool and warm season grasses can provide substantial biomass for grazing across the spring-summer growing season, while legumes add nitrogen (N) and, along with brassicas and other forbs, provide pollinator resources. However, for other management goals, such as water use and biomass production, differences between cover crop mixtures and monocultures may be negligible (Nielsen et al., 2015). Field-scale data on the potential benefits and challenges of grazing cover crops in the semi-arid cropping region of the High and Central Plains are needed to help inform producers looking to incorporate grazed covers into their systems.

The aim of this study was to evaluate short-term implications for key soil health metrics and winter wheat yield of integrating grazed, spring-planted cover crop mixtures in dryland

cropping systems. We hypothesized that cover crops would deplete soil water compared to summer fallow and this would lead to decreased grain yield in the following winter wheat crop. Cover crops were hypothesized to increase active pools of carbon (C) and N, but total C and N stocks were not expected to change during the short timeframe (i.e., a single season) of this study. We also hypothesized that cover crops would improve soil aggregate stability compared to fallow, but grazing would lead to a decrease in aggregation and increased compaction by livestock. We tested these hypotheses using a participatory, on-farm approach with replicated field trials within each farm to document changes in key soil health metrics and crop production at a commercial scale across a range of dryland production conditions. As summer fallow is still a common practice in the High and Central Plains, our objective was to compare water storage, soil properties and crop yields among three different treatments: 1) summer fallow, 2) un-grazed (traditional) cover crops, and 3) grazed cover crops in dryland no-till systems.

3.2. Methods

3.2.1 Field Sites Selection and Experimental Design

Five field sites in 2016 and another five in 2017 were established in collaboration with producers on dryland production fields located across eastern Colorado, western Kansas and southwestern Nebraska, USA (Fig. 3.1). All fields were under no-till management, and almost all (except Site 6) had been using cover crops before the onset of the experiment. Climate and soil type varied across field sites with average annual precipitation ranging from 464-657 mm and soils typically classified as Argiustolls (Mollisols with ustic moisture regime) with silt or clay loam texture (Table 3.1).

A common cover crop seed mixture was designed by collaborating producers each year and consisted of a mixture of cool season grasses, legumes and forbs which varied slightly between the two years: eight species were included in 2016 and nine in 2017, each having a similar cost of $45 USD ha^{-1} (Table 3.2). We are choosing to use "cover crops" to refer to these treatments because these mixtures were chosen by producers mainly for their purported soil health benefits, though forage production and palatability remained important objectives. The cover crop mixture was planted between March and May, depending on site, at a rate of 50 kg ha^{-1} in 2016 and 46 kg ha^{-1} in 2017 (Table 3.3). Fields of at least 16 hectares were divided into four replicate blocks of roughly equal area, with blocks arranged to account for field variability to the extent possible (See Fig. S3.1 for example field layout). Soil type was generally uniform across fields, and soil characteristics of each block are reported in Supplementary Table S3.1. Within each replicate, three treatments were established: 1) grazed cover crop, 2) un-grazed cover crop and 3) fallow. For the un-grazed cover crop treatment, grazing exclosures were established (approximately 25 x 120 m) using electric wire fencing within each replicate block. For the fallow treatment, a sub-plot (5 x 5 m) was created within the un-grazed exclosure, by applying the chemical herbicides glyphosate (Roundup) and fluroxypyr 1-methylheptyl (Starane), shortly after cover crop emergence (<5 cm tall), with additional applications as needed. Exclosures were generally randomized within each replicate block while avoiding field edges, with the exception of sites 1 and 9, where the exclosures were distributed across the edges of the field.

Grazing commenced between May and July depending on site, and each field was grazed for an average of 28 days. Cattle type, density, and grazing days varied by field and grazing was managed by the producer collaborators based on their needs. Cattle were weighed individually

prior to grazing and stocking rate ranged from 307 – 1052 kg liveweight (LW) ha^{-1}, with an average of 626 kg LW ha^{-1}. Most producers were concerned with leaving sufficient residue on the soil surface; as a result, stocking rates were generally lower than recommended to optimize forage utilization and not always well-matched to biomass production, especially in 2016, which was a warm and wet year. To account for the different types and body weights of cattle used on each field and the different lengths of grazing time, stocking rate-days (product of stocking rate in kg LW ha^{-1} and days grazed) was used as an indicator of relative cattle pressure on the field. All producers practiced rotational or strip grazing, allowing the cattle to graze part of the field and moving the herd in order to leave an adequate amount of standing crop residue, according to their individual management goals.

After the completion of grazing, the cover crop was terminated chemically and a short (2-3 month) late summer fallow (LSF) period was implemented before wheat planting in mid-September through mid-October. Exact planting time of the cover crop and winter wheat, timing of grazing, and type, density and timing of cattle movement was controlled by each producer (See Table 3.2 for detailed description of management at each field site). Five different fields were planted each year, for a total of ten fields over the course of the two-year experiment. Portable on-site weather stations (Onset Computer Corp, Bourne, MA) provided temperature and precipitation data when a nearby Colorado State (CoAgMet) or Kansas State (Kansas Mesonet) weather station was not available.

2.2 Soil sample collection

Soils were collected from each field at three different times throughout the duration of the experiment: baseline before treatment establishment in the spring, two weeks after cover crop

termination in mid- to late summer, and at the time of wheat planting in early autumn. For baseline sampling and all soil moisture measurements, samples collected to 180 cm (where possible) with a truck-mounted hydraulic probe (Giddings, Windsor, CO), and care was taken to obtain a representative sample, avoiding burrows, manure and wheel tracks. Baseline soil samples were collected across each of the four replicate blocks to measure field-level variability in soil texture, moisture and soil fertility parameters. Three soil cores (2 cm diameter) were collected from a center transect spanning each block, and divided into 30 cm increments pooled by depth. The composite soil samples for each depth were sealed in plastic bags and transported to the lab in a cooler. Air-dried samples from the top 60 cm were sent to a commercial lab (Ward Laboratories; Kearney, NE) for analysis of basic nutrients and texture. Soil moisture was determined gravimetrically for each depth increment and, for estimation of total profile available water, converted to volumetric water content using estimates of bulk density by depth from the NRCS Web Soil Survey (https://websoilsurvey.sc.egov.usda.gov/)

For evaluation of cover crop and grazing effects, soils were sampled within two weeks of chemical termination of the cover crop following grazing. Three cores were collected in each treatment plot and pooled into one composite sample for each depth increment. Each core was approximately 10-20 m apart in a transect along the length of the treatment plot except from the fallow treatment due to the small size of the treatment area, where cores were taken 3 m apart. To minimize variability within each block, grazed soil samples were collected in the area immediately adjacent to the un-grazed treatment plot (See Fig. S3.1). Cores were divided by depth as described above, except for the top 30 cm, which was further divided into 0-15 and 15-30 cm increments. Three surface soil samples (0-5 cm) were taken at a similar location for determination of surface bulk density and aggregate stability. Samples were collected by

inserting a sharpened metal cylinder (7 cm diameter) vertically into the soil by hand and then excavating. The timing of this sampling differed between sites due to variation in timing of grazing and chemical termination but was approximately early July – early August.

Adjacent to each soil core, surface residue cover was assessed by visually estimating the percent of covered soil in a 0.5 m^2 quadrat. These estimates were combined for an average cover estimate for each treatment plot.

Approximately 1 week before wheat planting in the fall (generally Sept - Oct), deep soil cores (down to 180 cm and divided into 30 cm increments) were again taken for evaluation of soil moisture storage. This occurred in the same manner as described above with three cores taken per treatment plot.

3.2.3 Soil physical properties

Bulk density was determined on the 0-5 cm cores by weighing each core, drying a subsample at 105 °C to determine the gravimetric water content, and an averaging the three cores to obtain plot-level estimates. The remaining soil from the surface cores was pooled, gently passed through an 8 mm sieve, and air dried in preparation for analysis of wet aggregate stability. Following Elliott (1986), a 40 g sample was spread on a 2 mm sieve and submerged in deionized water for 5 minutes of slaking. The sieved soil was then lifted and repeatedly submerged (total 50 cycles) over a 2 min. period. Soil remaining on the sieve surface was collected and dried at 60 °C. Soil passing through the sieve was transferred to a 250 μm sieve, and the submersion and transfer process repeated. The final round of submersion was completed on a 53 μm sieve and the smallest silt and clay fraction (<53 μm) estimated by difference. Mean weigh diameter

(MWD) was calculated as an index of aggregate stability by calculating the fraction of soil in each size class, multiplied by the average diameter of the size class (van Bavel, 1950).

3.2.4 Soil chemical analysis

Soil chemical analysis was performed on 2 mm sieved, air-dried soil from the top 15 cm. Total C and N were measured using a combustion analyzer (LECO Tru-SPEC, St. Joseph, MI). Organic C was determined by subtracting inorganic C, which was assessed using a modified pressure-calcimeter method (Sherrod et al., 2002). Permanganate oxidizable C (POXC) was determined following the method of Weil et al. (2003) to measure a somewhat labile (or more active) soil C fraction. Available P was measured using the Olson P method in a 0.5 M sodium bicarbonate extracting solution (Murphy and Riley, 1962). Potentially mineralizable N (PMN) was measured by comparing extractable ammonium concentrations in an immediate soil extraction (2M KCl) to a paired sample that had undergone a 7-day anaerobic incubation (Drinkwater et al., 1996). Ammonium concentrations in the baseline and incubated soil extracts were determined colorimetrically, as nitrification is assumed not to occur under these anaerobic conditions (Kandeler and Gerber, 1988). Buffered pH using a 1:1 modified Woodruff buffer solution (Brown and Cisco, 1984) and soil texture determined by hydrometer (Gee and Bauder, 1986).

3.2.5 Wheat harvest

Before commercial wheat harvest, a 1 m long row was hand harvested by cutting the plants at the soil surface. Samples were dried at 55 °C, weighed for biomass, and then threshed for grain yield. Two samples were taken from each grazed and un-grazed treatment, and one sample was taken from each fallow treatment. In addition, collaborating producers provided combine yield monitor data for the grazed and un-grazed treatments for a subset of fields. We

similarly did not find differences between these treatments based on yield monitor data and yield monitor estimates were similar to hand harvest yield estimates (1-12% difference in mean values, data not shown). We chose to only include hand-harvest data in order to include the fallow treatment in our analysis. Harvest index was calculated as the ratio of grain to total biomass weight. Not all fields had wheat harvest data because some crops were lost to hail, replaced with corn or harvested before samples could be taken (Table S3.2 Notes). Thus, wheat grain yield data was obtained from four fields in 2016 and three fields in 2017.

3.2.6 Statistical analysis

Soil properties, water storage, cover crop and wheat yields were compared among cover crop treatments using ANOVA. A mixed effects model with treatment and site-year as fixed effects and block nested within site as a random effect was used for all ANOVA and pairwise comparisons across farms. Inclusion of site-year accounts for differences in environmental and management effects of each site. Different soil depths were treated as separate units for moisture analysis using the same model to compare treatment effects. Treatment comparisons within a site considered cover crop treatment as a fixed effect and block as a random effect. All pairwise comparisons were made using a Tukey pairwise adjustment using modeled mean, though simple means of sample data are presented in all tables.

In addition to the treatment comparisons described above, overall relationships between wheat yield and soil, environmental and management factors were explored using multiple linear regression without explicitly considering site. A model to explain yield variability was generated by first selecting relevant explanatory variables, next removing variables with high collinearity (Variance Inflation Factor > 5) using the *olsrr* package (Hebbalia, 2020) and then using AIC

selection to choose the best model using the "step" function. Proportion of variance explained for each predictor variable was estimated by dividing the parameter sum of squares by the total sum of squares for the regression. Interaction terms were tested and ultimately excluded based on lack of significance.

For all analyses, the assumptions of ANOVA were verified; data transformation were not required. Treatment differences and parameters were considered significant if $p < 0.05$. All statistical analyses were conducted in R (R Core Team, 2020). Mixed effects models were created using the *lme4* package (Bates et al., 2015) and ANOVA and pairwise Tukey comparison tested using *lmerTest* (Kuznetsova et al., 2017) and *emmeans* (Lenth, 2018).

3.3. Results

3.3.1 Soil moisture and cover crop treatments

Soil moisture was depleted in cover cropped treatments at the time of termination, while grazing did not appear to affect soil moisture compared to the un-grazed treatment (Fig. 3.2a). The fallow plots had more water below 15 cm at cover crop termination, but recharge in the surface layers during the late summer fallow period removed cover crop effects above 60 cm by wheat planting (Fig 3.2b). The greatest treatment differences were seen below 30 cm depth, where fallow plots showed 12-20% greater gravimetric water content (GWC) than the cover cropped plots. Immediately following cover crop termination, surface soil showed the smallest change in soil moisture (4-10%) while the 90-120 depth increment showed the largest depletion, with a 22% decrease in GWC with cover crops. Fallow plots had on average 53 mm more water than grazed cover crop plots and 59 mm more water than un-grazed cover crop plots at cover crop termination, though this decreased to 32 and 36 mm more water, respectively, by wheat

planting. Soil moisture content was not different between grazed and un-grazed cover crop treatments for all depths and at both sampling time points. While not statistically significant, soil profile recharge during the short fallow after cover crop termination (late summer fallow, LSF) was on average 23 and 21 mm higher for grazed and un-grazed cover crop treatments, respectively, than for fallow ($p = 0.11$; Table 3.4).

3.3.2 Treatment effects on soil health metrics and soil cover

Cover crops improved aggregate stability in the surface layer, while cattle grazing showed no increase in soil compaction compared to fallow. Surface bulk density did not increase with grazed cover crops compared to fallow but was lowest with un-grazed covers (Fig. 3.3a). The un-grazed cover crop treatment had a 4% lower bulk density compared to the fallow and grazed treatments. Surface bulk density in the grazed plots ranged from 0.99 – 1.42 g cm^{-3}, with an average of 1.18 g cm^3 across all sites. Aggregate stability as measured by mean weight diameter (MWD) was higher with cover crops, and increased by 47% and 33% in the grazed and un-grazed treatment, respectively, relative to fallow (Fig. 3.3b; Table S3.2). Total soil organic C and N, permanganate-oxidizable C (POXC) and potentially mineralizable nitrogen (PMN) did not differ across treatments (Table 3.3).

Cover crop treatments increased soil cover compared to summer fallow. Residue cover on the soil surface primarily consisted of the previous years' crop residue and, to a lesser extent, the more recent cover crop residue. There was no difference in residue cover between grazed (72%) and un-grazed (78%) cover crop treatments, but fallow plots had less cover (64%) than both cover crop treatments (Table 3.3).

3.3 Treatment effects on wheat yields

Cover crops had a variable, but overall negative effect on wheat yields. There was no difference in the effects of grazed and un-grazed cover crops on wheat yield. Average wheat yield with fallow was 3,654 kg ha^{-1}, with un-grazed cover crop plots yielding 22% and grazed plots 19% less than fallow (average yields 2,980 and 2809 kg ha^{-1}, respectively; Fig. 3.4). Aboveground wheat biomass showed similar reductions in the cover crop treatments vs. fallow, with average un-grazed biomass of 7,370 kg ha^{-1} and grazed biomass of 7,720 kg ha^{-1}, respectively. This corresponds to a 16-20% reduction in wheat biomass compared to fallow (9230 kg ha^{-1}). Harvest index did not differ between treatments, with an average value of 0.34 across all farms and cover crop treatments.

3.4 Yield responses to weather and soil factors

Seasonal weather factors were the most important variables for explaining winter wheat grain yield. Field sites spanned a large gradient in local climate and, combined with variability in the timing of field operations, growing degree days (GDD) ranged from 3,191 to 5,626 degree-days across sites and years (Table 3.2). Winter wheat yield across all farms was best explained by wheat growing degree days, followed by available soil water at cover crop termination and precipitation during the late summer fallow (cumulatively 64% of model variability explained, $p < 0.01$; Table 3.5). On average, each mm of water stored in the soil profile at cover crop termination increased yield by 7 kg ha^{-1} and each mm of moisture received between cover crop termination and wheat planting (during LSF) resulted in an increase in wheat grain yield of 10 kg ha^{-1}. Precipitation received during the wheat growing season did not explain yield. Soil factors did not improve the model fit and were ultimately excluded.

3.4. Discussion

3.4.1 Grazed cover crops and soil health metrics

The aim of this study was to evaluate the potential for integrating grazed cover crops within dryland winter wheat rotations, with a specific focus on understanding livestock impacts on important metrics of soil health. Our results demonstrate that cover crops can rapidly increase soil aggregate stability compared to fallow in semi-arid, dryland cropping systems, and that cover crops can be grazed with minimal consequences for soil compaction or SOC loss in the short term.

Cover crops resulted in an appreciable and consistent increase in soil aggregation in the surface layer and residue cover compared to fallow after only about 90 days of growth (Fig. 3.3b; Table 3.4). Aggregate stability is an important indicator of soil structure and susceptibility to erosion, as well as infiltration potential (Barthès and Roose, 2002). Actively growing plants can stimulate soil aggregation through a variety of mechanisms, including the direct addition of organic material, stimulation of microbial communities, binding of soil particles by roots or associated fungal hyphae, and exacerbation of wet-dry cycles due to evapotranspiration (Czarnes et al., 2000; Denef et al., 2002). Residue cover is also important for protecting the soil surface from rainfall impact that can cause aggregate rupture and lead to water runoff and erosion (Ranaivoson et al., 2017). Standing residue also mitigates wind speeds, reducing loss of susceptible soil particles through wind erosion (Nielsen and Aiken, 1998).

Other studies generally show that a longer treatment time is necessary to see a soil structural response with cover crops (Blanco-Canqui et al., 2012; Franzluebbers and Stuedemann, 2008). We note that our results also point to the importance of living roots in these

cropping systems, as the short-term effects of cover crops reported in this study may also be interpreted as the rapid loss of soil surface aggregation under fallow. Improved aggregation seen under cover crops were no longer detectable nine months after cover crop termination in Garden City, KS (Blanco-Canqui et al., 2013). Also, dryland crop rotations with more fallow periods tend to have lower aggregation (Rosenzweig et al., 2018a), indicating that exposed soils in this region are highly susceptible to aggregate disruption.

Cattle grazing did not appear to disrupt soil aggregation; contrary to our hypothesis, grazed covers showed slightly, though not significantly, higher aggregation than un-grazed treatments (Fig. 3.4). We note that stocking rates in this study (average of 626 kg LW ha^{-1}) were generally lower than recommended (Ogle and Brazee, 2009), as cover crop residue retention was a primary management goal.

Our results demonstrate that cover crops can decrease surface bulk density compared to fallow, but only when they are not grazed. Therefore, grazing can diminish the bulk density improvements seen with cover crops. However, grazed cover crops did not have a higher bulk density than the summer fallow in this study. Variability in stocking rates was unintentionally achieved based on difference in site management, and though not an explicit question in the study, observation of the data shows no clear pattern between stocking rates and site-level bulk density responses to treatment (Table 3.2, Table S3.2). Some site-level increases in surface bulk density were observed with grazing (Table S3.2), but all fields were below the threshold bulk density of 1.5 or 1.6 g cm^{-3} for concerns of negative impacts on root growth (Reeves et al., 1984). It is important to note that grazing on wet soil can significantly deteriorate soil quality and lead to compaction, which can impede root and water penetration. Soils with high clay content are particularly susceptible to this effect. We note that this study area is a dry

environment (640-250 mm average precipitation) and grazing during this study generally occurred on dry soils. We did not repeat bulk density measurements after the winter freeze thaw, which may ameliorate compaction (Abdel-Magid et al., 1987).

Concerns about compaction are reported as being a major barrier to integrating grazing into crop rotations globally, despite evidence that compaction typically does not occur with proper management (de Faccio Carvalho et al., 2010). While the small effect we found of livestock on bulk density may be attributed to the relatively short time the cattle were on the field, longer-term studies of integrated crop-livestock systems have reported that grazing did not result in an increase in bulk density (Franzluebbers and Stuedemann, 2008; Liebig et al., 2012; Stavi et al., 2015). In Canada, Twerdoff et al. (1999) found that increased grazing intensity in annual forage systems increased surface bulk density in a curvilinear fashion, though plant growth was not influenced, and concluded that intensive rotational grazing could be a suitable management strategy. In the southern High Plains, Baumhart et al. (2011) documented increased penetration resistance in a no-till wheat-sorghum-fallow rotation when cattle grazed fall winter wheat growth and sorghum stubble, though wheat yields did not decrease despite fall grazing. In addition, grazing directly on the field may generally be a more suitable option to maximize some elements of soil health compared to harvesting forages with heavy equipment; in a study across fields in Nebraska, baling and removing crop residues reduced soil cover and increased soil erodibility compared to grazing (Rakkar et al., 2019). In the relatively flat, windy conditions of the Central Plains, standing residues are critical for soil conservation, and the producers in the present study intentionally managed cattle to maximize residue retention. Increase residue cover has potential to reduce susceptibility of soils to wind erosion.

Carbon and N pools remained unchanged across all treatments, which was not surprising due to the short timeframe of this study. A recent meta-analysis across tropical and temperate regions estimates the potential C sequestration rate from cover crops at 0.32 Mg ha^{-1} yr^{-1} (Poeplau and Don, 2015), which would be very difficult to detect after only one year and may be difficult to achieve in these dry and relatively low-productivity systems. However, noticeable increases in SOC and soil structure can be realized in this climate after just five years of cover crop implementation, with the most effective species contributing an average of 0.48-0.56 Mg C ha^{-1} yr^{-1} to SOC pools (Blanco-Canqui et al., 2013). Cover crops in this region may have a larger impact on soil organic C relative other systems because starting SOC levels are low and cover crops are typically grown during the warmer spring and summer growing windows with greater biomass production potential, as opposed to the late fall and early spring typical in more humid environments. However, it should be noted that increased residue return from cover crops may result in a commensurate decrease in cash crop biomass and residue, decreasing or potentially eliminating the net C gains from cover crops. While studies of long-term continuous cropping systems show greater SOC pools even with reduced average wheat yields relative to wheat-fallow rotation systems (Rosenzweig et al., 2018b), this is a question that merits further investigation in this region.

Soil health benefits of cover crops are largely attributed to increased biomass return to the soil. This is one of the reasons why grazing cover crops is an area of evolving policy for the Natural Resources Conservation Service (NRCS) in working to balance soil conservation practice effectiveness with economic feasibility (NRCS, 2019, 2014, 2013). Harvest of cover crop biomass reduces residue return to the soil, and as a result, high rates of utilization can lead to decreases in soil C compared to unharvested cover crops. Blanco-Canqui et al. (2013)

observed a noticeable, but non-significant decrease in SOC with cover crop removal, highlighting the importance of residue management for successful soil-building with grazed or harvested cover crops. Wheat stubble grazing by sheep in dryland systems of Australia did not detect a reduction in soil C with moderate sheep grazing (Stavi et al., 2015), reinforcing the idea that careful livestock use of plant residues can maintain soil conservation goals. Importantly, animal waste returns labile nutrients to the soil that may stimulate growth and promote crop yield (Gardner and Faulkner, 1991; Martens and Entz, 2011), and may also contribute to soil organic matter formation in the long term (Miles and Brown, 2011).

3.4.2 Cover crops, water and yield

Grazing cover crops did not change the subsequent wheat yield compared to un-grazed cover crops. This may be partially attributed to the small difference observed in standing residue biomass between grazed and un-grazed treatments (18% lower in grazed), which occurred due to conservative livestock management and cover crop regrowth (Table S3.3). Plant residue biomass and soil cover has been shown to positively affect soil water infiltration and reduce soil evaporation (Ranaivoson et al., 2017), which can translate to increased yields in water limited systems. Although not statistically significant, we did observe that the cover cropped treatments tended to have greater soil water recharge after termination compared to fallow.

Fallow plots, on average, had higher levels of stored soil moisture at wheat planting and greater wheat yields compared to plots following cover crops. While this was expected due to reduced transpiration without growing vegetation, it should be noted that nine out of the ten fields used in this study had cover crops integrated in the rotation for several years before our treatments were applied; therefore, our small fallow treatments within fields with a history of

cover crops are unlikely to be representative of the yield and soil health outcomes from a longer-term summer fallow-based rotation, where regular fallowing is shown to decrease soil health metrics (Nielsen and Calderón, 2011). For example, the fallow treatments in our study did not show deceased soil carbon levels, though fallow-based rotations tend to have reduced soil carbon stocks relative to continuously cropped systems (Rosenzweig et al., 2018a). Thus, our experimental design possibly overestimates yield and soil health measurements under frequent fallow, and only represent the short-term effects of fallow.

In water-limited agroecosystems, it is well documented that yield variability is highly related to annual precipitation (Sherrod et al., 2014), with fall precipitation shown to be most important for wheat yields (Holman et al., 2011). Our multiple regression results reinforce this, as soil moisture was strongly related to wheat grain yield, particularly precipitation during the later summer and fall before wheat planting (Table 3.5). This is in agreement with other studies demonstrating that soil water at wheat planting is a strong predictor of yield in these systems (Holman et al., 2018; Nielsen et al., 2016; Nielsen et al., 2002).

Soil health metrics were not retained in the model as important predictors of yield, suggesting that moisture was the overt driver of yield in these systems. We note that relatively large differences between sites, the short-term nature of the study and potential interactions between soil aggregation and moisture make this relationship difficult to discern. Summer fallow is typically used to store water for wheat germination and early growth, and so replacing the fallow with a cover crop generally leads to reduced yields in the following wheat crop (Holman et al., 2018; Unger and Vigil, 1998). However, increased economic returns were found in western Canada when leguminous cover crops were grown before spring wheat due to improved nitrogen availability, as long as the cover crops were terminated in early- to mid-July before full

bloom, increasing N availability while allowing for a longer late summer fallow period to store moisture (Mean growing season precipitation 210 mm; Zentner et al., 2004). Nitrogen-fixing cover crops similarly increased cash crop yields in southcentral Kansas (average annual precipitation 873 mm), which was largely attributed to N provisioning and amelioration of compaction by the cover crop (Blanco-Canqui et al., 2012).The yield increase seen in these green manure systems suggest that including substantial legumes in cover crop mixtures and terminating cover crops early in the summer may provide greater profitability than is generally observed in the study region (Nielsen and Vigil, 2005; Schlegel and Havlin, 1997). Indeed, the negative response of wheat grain yield to cover crop growing degree day suggests that shorter cover crop growing seasons provide the best yield outcomes for the following wheat crop. However, when the cover crop is used for grazing that can produce an income, the trade-off between forage production and moisture consumption may extend this ideal termination date further into the typical fallow season. While this study was not designed specifically to assess the efficacy of cover crop planting and termination timing and there are other potential factors influencing the relationship between cover crop growing degree days and yield, it reinforces the importance of timing for this complex management system to be successful in the Central and High Plains. Wheat grain yield responses to cover crops were variable across fields, highlighting the inconsistent effects of replacing fallow on wheat yields (Nielsen et al., 2016) and suggesting that cover crops may not always lead to reduced yield in the Central Plains.

3.5. Conclusions

Cover crops are an important strategy to improve agroecosystem health, but tradeoffs with water use in dryland systems necessitates a creative approach to cover crop use and

management. Here we demonstrate that cover crops can improve some aspects of soil health in dryland systems in the short term (i.e. increasing aggregation, decreasing bulk density) and that grazing the cover crop does not appear to appreciably degrade any measured soil parameters compared to summer fallow.

While soil organic carbon is a widely-used benchmark for soil health across many agroecosystems, producers in dryland systems that generally produce low plant biomass may find building soil carbon to be a somewhat elusive or longer-term goal. As a result, other soil health metrics may be more suitable in the short-term for producers looking to improve their soil. In particular, we found that soil aggregation was consistently improved with covers after only two to three months of treatment. Improvements to water capture and retention are particularly important in dryland systems to improve resilience to climate variability, and both may be achieved through increased aggregation and potential to increase soil water recharge following cover crop termination. Additionally, soil erosion benefits from residue cover and aggregation are particularly important in windy regions. Therefore, despite short-term water use, cover crops may contribute to improving resilience to changing and more variable precipitation regimes. Our results show that carefully managed grazing of cover crops may offer a promising option for improving some metrics of soil health in moisture-limited environments, with the potential to diversify farm economics.

CHAPTER 3 TABLES AND FIGURES

Table 3.1. Site factors associated with a spring-planted, on-farm grazed cover crop trial in eastern CO, western KS, and western NE, USA. Cover Crop Precip. and late summer fallow (LSF) Precip. were the amount of precipitation recorded during the cover crop growth period, and between the time of cover crop termination and wheat planting, respectively, during the study year. Soil particle size distribution and pH in top 60 cm; SOC, soil organic carbon, in top 15 cm.

Year	Location	Site No.	Elevation (m)	Sand (g kg^{-1})	Clay (g kg^{-1})	pH	SOC (g kg^{-1})	Soil Classification*	Cover Crop GDD‡	Wheat GDD	Cover Crop Precip. (mm)	LSF Precip. (mm)
2016	37°35'17" N 99°42'18" W	1	733	280	200	6.8	13.5	Fine, smectitic, mesic Typic Argiustoll	2243	3191	258	18
	39°14'41" N 100°34'02" W	2	962	220	240	6.8	11.8	Fine-silty, mixed, superactive, mesic Aridic Argiustoll	2821	5217	276	163
	39°37'23" N 100°32'57" W	3	833	230	240	7	11.7	Fine-silty, mixed, superactive, mesic Typic Argiustoll	2123	5439	150	274
	39°58'7" N 99°41'23" W	4	656	220	210	6.7	13.9	Fine-silty, mixed, superactive, mesic Typic Argiustoll	4632	5626	662	265
	40°49'14" N 101°56'58" W	5	1096	470	160	7.1	9.3	Fine-silty, mixed, superactive, mesic Pachic Argiustoll	2338	-†	71	4
2017	40°30'14" N 103°54'06" W	6	1087	630	220	7.2	4.8	Fine-loamy, mixed, superactive, mesic Aridic Argiustoll	3011	-	200	59
	37°35'16" N 99°43'32" W	7	733	130	360	6.8	11.3	Fine, smectitic, mesic Typic Argiustoll	2992	5144	153	65
	39°09'34" N 100°31'48" W	8	962	130	360	7.2	10.0	Fine-silty, mixed, superactive, mesic Aridic Argiustoll	2454	4046	36	231
	39°12'41" N 102°52'48" W	9	1436	230	360	7.2	12.7	Fine-loamy, mixed, superactive, calcareous, mesic Aridic Ustorthent	4726	4909	297	21
	39°58'14" N 99°41'04" W	10	656	160	350	6.9	9.6	Fine-silty, mixed, superactive, mesic Typic Typic Argiustoll	2827	-	39	181

*Taxonomic classification based on major soil type as listed in the NRCS Web Soil Survey. ‡GDD, growing degree day, is calculated for each site based on daily mean temperature, crop, and growing window. † No wheat yields.

Table 3.2. Cover crop seed mixtures for a spring-planted, on-farm grazed cover crop trial in eastern CO, western KS, and western NE, USA. Cost is actual seed price from seed supplier used for the experiment (Green Cover Seed, Bladen, NE)

2016		2017	
Species	**Seeding rate (kg ha^{-1})**	**Species**	**Seeding rate (kg ha^{-1})**
Spring Peas	5.6	Spring peas	5.6
Oats	16.8	Oats	11.2
Forage Barley	16.8	Forage Barley	11.2
Hay millet	5.6	Triticale	11.2
Rapeseed	2.2	Rapeseed	2.2
Flax	1.1	Flax	1.1
Safflower	1.1	Safflower	1.1
Sunflower	1.1	Sunflower	1.1
		Purple top turnip	1.1
Total	50.4		45.9
Total Cost (USD ha $^{-1}$)	$45		$45

Table 3.3. Management of grazed cover crop by site for a spring-planted, on-farm grazed cover crop trial in eastern CO, western KS, and western NE, USA.

Year	Site No.	CC[a] planting date	Grazing duration (days)	CC termin. date	CC growing days	LSF duration (days)	Wheat planting date	Stocking rate (kg ha^{-1})	Field Area (ha)	Grazed area (ha)
2016	1	3/1	36	7/15	136	75	9/28	315	16.8	15.5
	2	3/17	29	6/21	96	93	9/22	1022	36.6	35.0
	3	3/21	29	6/27	98	87	9/22	388	34.3	33.0
	4	4/11	28	8/9	120	43	9/21	533	17.3	16.1
	5	5/15	28	8/3	80	63	10/5	1221	20.6	20.0
2017	6	3/23	25	8/11	141	55	10/5	474	17.2	15.7
	7	3/20	31	7/19	121	86	10/13	426	20.6	19.2
	8	3/16	28	7/11	117	105	10/24	595	39.0	37.3
	9	3/14	22	8/10	149	37	9/19	607	40.5	38.9
	10	3/27	27	7/19	114	97	10/24	401	19.9	18.6

[a]CC, cover crop; termin., termination; LSF, late summer fallow, period between cover crop termination and wheat planting

Table 3.4. Key soil health metrics and moisture mean values and analysis of variance significance test results for treatment, site-year, and their interaction within three cover crop treatments: grazed cover crop, un-grazed cover crop, and fallow. Soil chemical data in top 0-15 cm, available water (AW) and recharge summed down to 180 cm. Data collected from a spring-planted, on-farm grazed cover crop trial in eastern CO, western KS, and western NE, USA. Arithmetic mean values across all sites and field replicates (n=40) are presented. Standard error (SE) values are italicized below means. Values followed by different letters within a row indicate treatment difference.

	Treatment			ANOVA P		
Soil Parameter[§]	Fallow*	Grazed	Un-grazed	Treatment	Site-Year	Trt x Site-Year
Org. C (g kg^{-1})	10.82	10.78	10.63	0.849	0.001	0.723
	(0.466)	*(0.431)*	*(0.412)*			
Total N (g kg^{-1})	1.22	1.21	1.20	0.779	0.001	0.865
	(0.049)	*(0.044)*	*(0.045)*			
POX C (mg kg^{-1})	309	316	309	0.849	<0.001	0.988
	(16.7)	*(17.1)*	*(15.6)*			
PMN (mg kg^{-1} wk^{-1})	13.5	14.9	14.3	0.569	<0.001	0.997
	(1.33)	*(1.50)*	*(1.28)*			
Olsen P (mg kg^{-1})	60.6	62.7	62.0	0.911	<0.001	0.778
	(7.23)	*(7.38)*	*(7.46)*			
Soil Cover (%)	64 a	72 b	78 b	<0.001	0.002	0.805
	(3.1)	*(2.4)*	*(1.9)*			
AW at CC term. (mm)	384 a	331 b	325 b	<0.001	<0.001	0.014
	(15.6)	*(12.1)*	*(13.9)*			
AW at wheat plant (mm)	411 a	379 b	375 b	0.002	< 0.001	0.017
	(20.0)	*(13.9)*	*(14.1)*			
LSF recharge (mm)	27	48	50	0.11	<0.001	0.261
	(10.0)	*(10.3)*	*(10.6)*			

[§]Org. C Organic carbon; POX C, permanganate oxidizable C; PMN, potentially mineralizable N; AW, available water; CC term, cover crop termination; LSF, late summer fallow. *Fallow plots have a variable history of cover cropping prior to experiment implementation

Table 3.5. Results from Akaike information criterion (AIC) model selection explaining winter wheat yield from a spring-planted, on-farm grazed cover crop trial in eastern CO, western KS, and western NE, USA. Only a subset of farms in the study produced wheat yield data. Operation timing and climate was variable across sites. Treatment and soil health variables were not retained as significant predictors.

Coefficient	Estimate	Df	F value	Pr(>F)	Variance Expl. (%)
LSF[§] Precip (mm)	10.6	1	24.6	<0.001	11.2
Wheat GDD	1.3	1	99.8	<0.001	45.4
ASW at CC Term (mm)	7.1	1	15.4	<0.001	7.0
Residuals		80	NA	NA	36.4
R^2	0.62				

[§]LSF, late summer fallow; GDD, growing degree day; ASW, available soil water; CC Term, cover crop termination

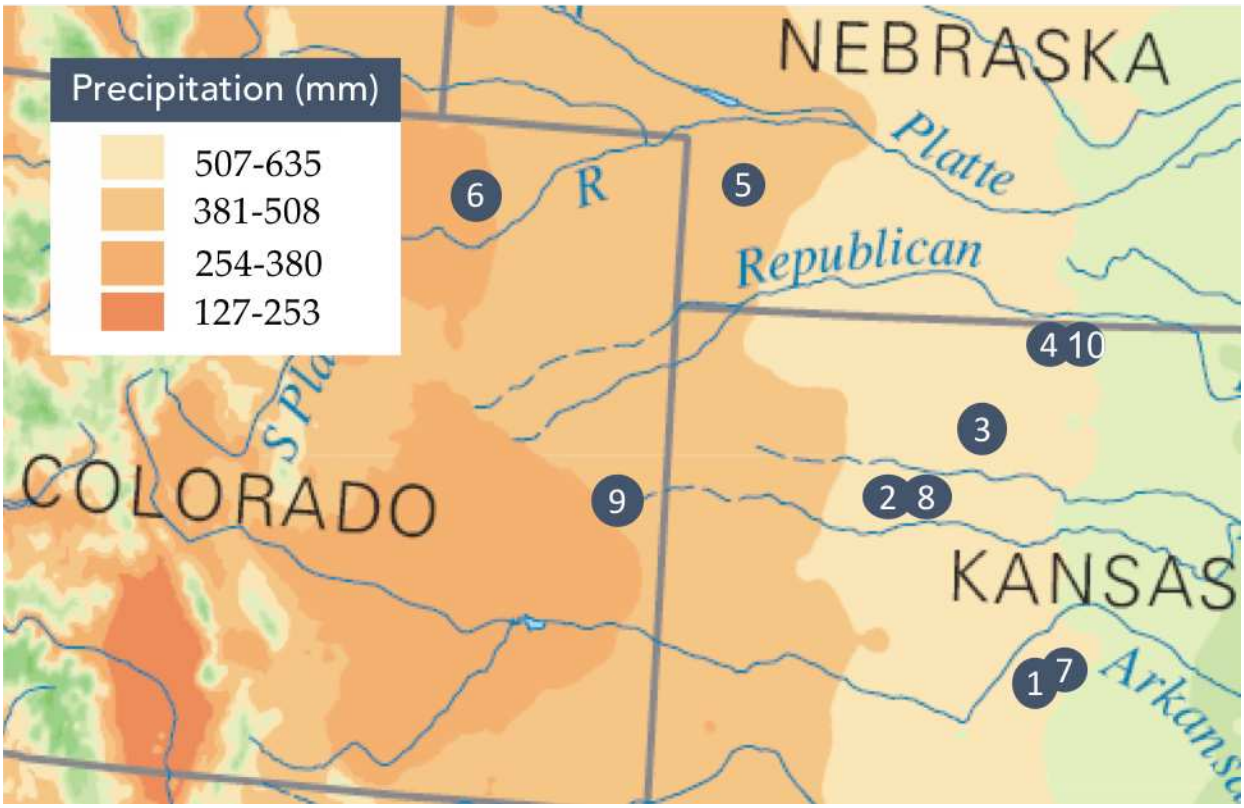

Figure 3.1. Location of experimental field sites for grazed cover crop study. Locations 1-5 were part of the study in 2016 and locations 6-10 in 2017. Colors correspond to precipitation averages 1961-1990. Map image is public domain downloaded from Wikimedia Commons (https://en.wikipedia.org/wiki/United_States_rainfall_climatology#/media/File:Average_precipitation_in_the_lower_48_states_of_the_USA.png)

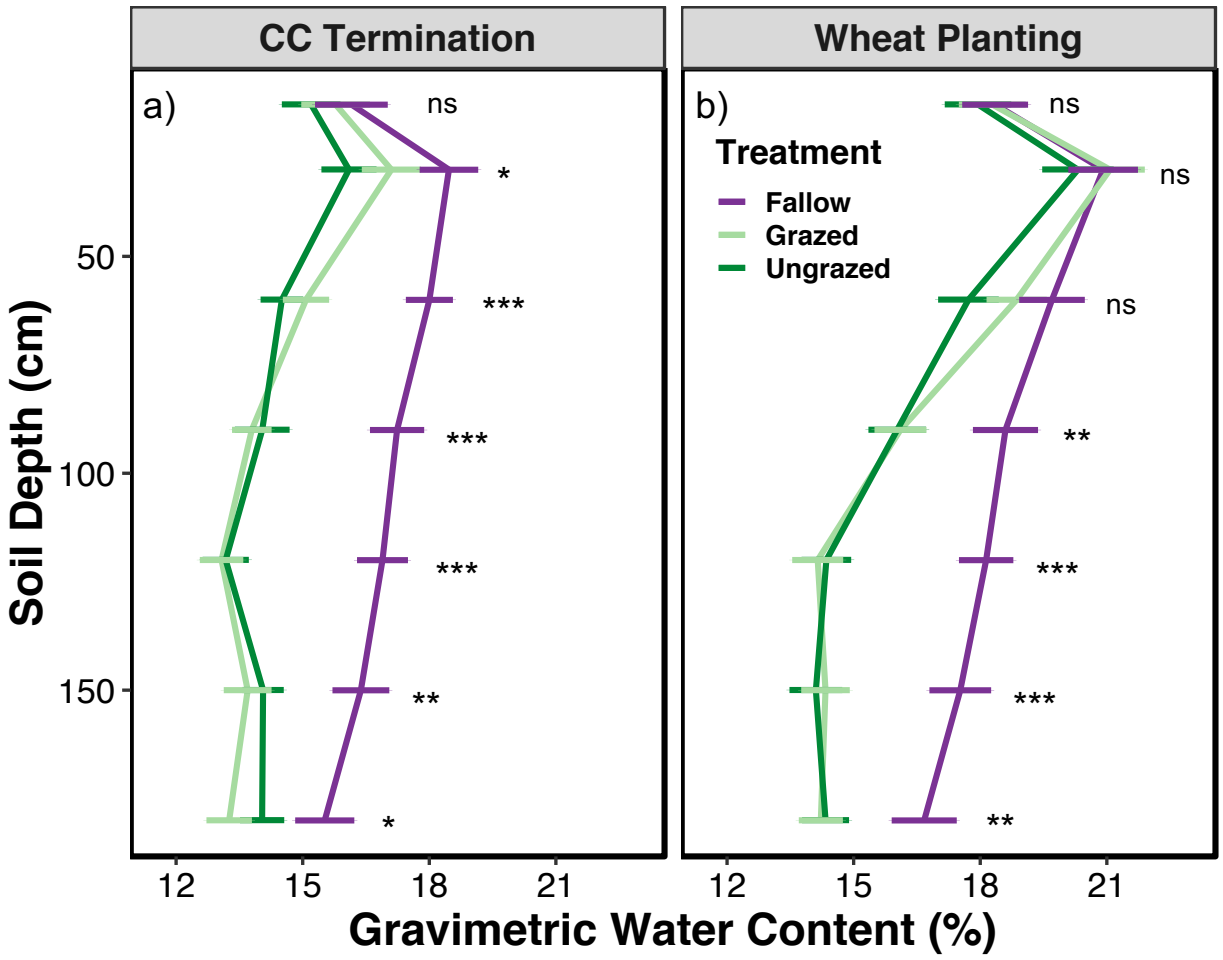

Figure 3.2. Soil moisture response of a spring-planted, on-farm grazed cover crop trial in eastern CO, western KS, and western NE, USA. Values are means ± SE at a) CC (cover crop) Termination (July-August) and b) Wheat Planting across all sites and both years. Differences by depth are indicated (ns=p>0.05; *= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$) and derived from a mixed-effects model using site-year and block nested with site-year as random factors. Error bars represent one standard error of the mean across all sites and blocks ($n = 20$).

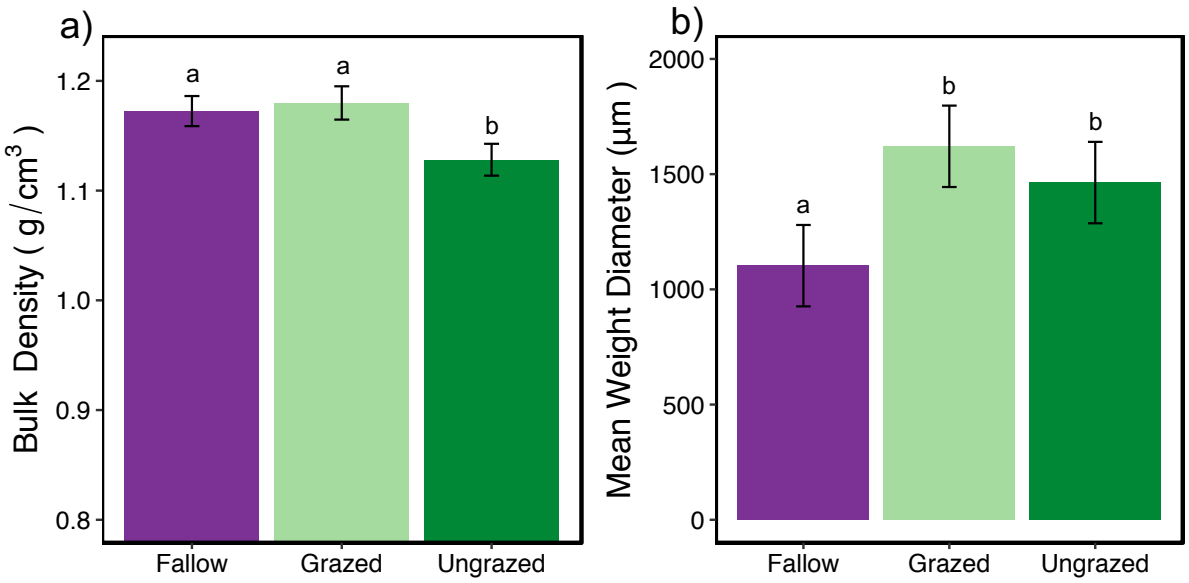

Figure 3.3. Soil physical properties in response a spring-planted, on-farm grazed cover crop trial in eastern CO, western KS, and western NE, USA: a) surface bulk density and b) aggregate mean weight diameter in surface (0-5 cm) soil after completion of grazing and termination of the cover crop. Values are means across all sites and replicates, and error bars are one standard error (n = 40). Different letters indicate treatment differences derived from a mixed-effects model using site-year and block nested with site-year as random factors. (Tukey post hoc $p < 0.05$).

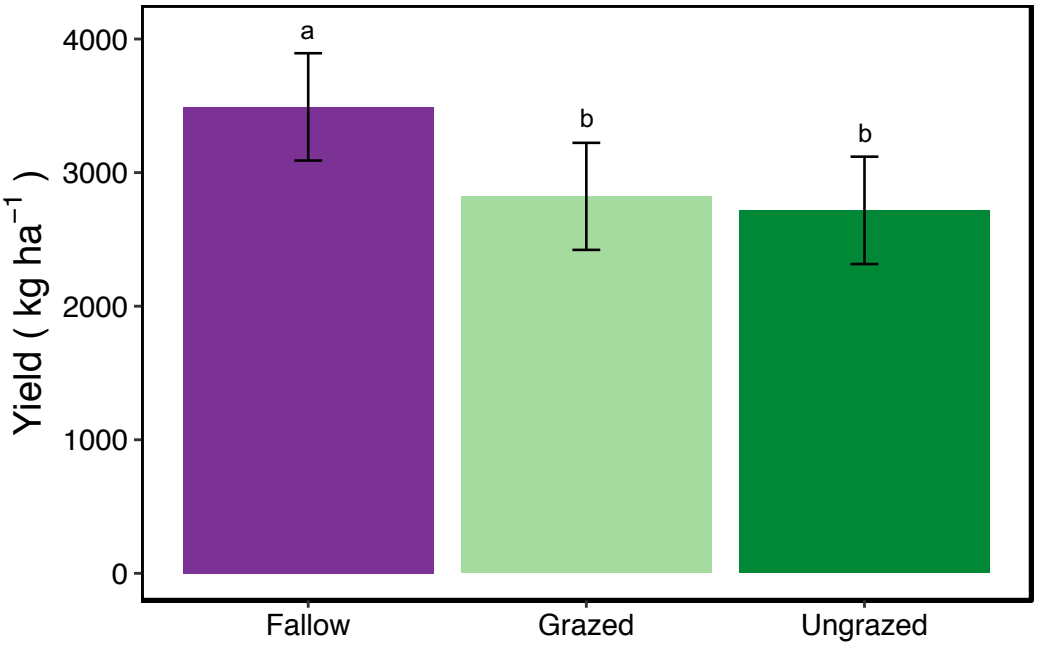

Figure 3.4. Mean wheat grain yields for spring-planted, on-farm grazed cover crop trial in eastern CO, western KS, and western NE, USA. Error bars ± SE. Different letters represent significantly different values ($p < 0.05$) derived from a mixed-effects model using site-year and block nested within site-year as random factors. Not all sites were harvested due to crop failures ($n = 28$).

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CHAPTER 4: DIVERGENT BELOWGROUND CARBON PATTERNS OF WINTER WHEAT SHAPE RHIZOSPHERE MICROBIAL COMMUNITIES AND NITROGEN CYCLING ACTIVITIES[3]

4.1. Introduction

Wheat (*Triticum aestivum* L.) is a globally important crop grown around the world that has been under selection by humans for millennia (Peng et al., 2011). Modern breeding efforts began around the 19th century, with wheat breeding being extensively developed in the mid-20th century to select for improvements in aboveground traits (Borlaug, 1968; Lupton, 1987; Waines and Ehdaie, 2007). However, there is concern that focus on aboveground traits during breeding inadvertently disrupted belowground traits and processes related to root morphology, carbon (C) sequestration and microbial associations important for sustainable production (Iannucci et al., 2017; Pérez-Jaramillo et al., 2016; Porter and Sachs, 2020; Waines and Ehdaie, 2007; Wissuwa et al., 2009).

Wheat genotypes vary in their belowground allocation of C (Aljazairi et al., 2015; de Graaff et al., 2009; Iannucci et al., 2017). Differences in both the quantity and quality of low molecular weight exudates can affect the structure and function of microbial communities in the rhizosphere (Badri and Vivanco, 2009; Chaparro et al., 2012). Rhizosphere microorganisms play a role in disease suppression and the production of plant-growth-promoting hormones and compounds, which directly impact plant health (Berg and Smalla, 2009; Mendes et al., 2013).

[3] In review at *Soil Biology and Biochemistry*. Authors: Courtland Kelly, Michelle Haddix, Patrick Byrne, M. Francesca Cotrufo, Meagan Schipansko, Cynthia Kallenbach, Matthew Wallenstein, and Steven J. Fonte

They are also the key drivers in controlling the mineralization and availability of nutrients including nitrogen (N). Reliance on organic N sources (e.g. such as cover crops and soil organic matter), which first have to be mineralized by the rhizosphere microbial community, is becoming more common to reduce reliance on highly mobile mineral N fertilizers and ameliorate the environmental impacts of agriculture (Drinkwater et al., 2017). Even in agricultural systems managed with synthetic N fertilizers, upwards of half of crop N uptake originates from mineralized organic N sources (Gardner and Drinkwater, 2009; Yan et al., 2020). Therefore, it is critical that modern crop varieties successfully support microbial communities that help mineralize N from organic sources and facilitate crop N uptake across diverse agricultural systems (Drinkwater and Snapp, 2007).

Soil microbes produce and excrete extracellular enzymes, which depolymerize larger organic residues, the rate-limiting step in the mineralization of soil organic matter (SOM) and organic residues to plant-available N (Burns et al., 2013). Plant exudates can stimulate N mineralization through stimulation of the microbial biomass, fostering plant N uptake and growth (Hamilton and Frank, 2001; Henneron et al., 2020a; Meier et al., 2017). This effect suggests that fresh and accessible C compounds in exudates provide the energy needed to produce enzymes that catalyze N mineralization from SOM or organic residues to alleviate microbial N limitation, which in turn can increase in plant available N (Allison and Vitousek, 2005; Chen et al., 2014; Cui et al., 2020; Zhu et al., 2014). However, the process of N release from microbial enzyme activity is complex, as the balance between immobilization in microbial biomass and mineralization will depend on the C:N ratio of added organic material and physiological characteristics of the microbial biomass, e.g. utilization efficiencies, biomass stoichiometry (Hall et al., 2011; Janzen and Kucey, 1988; Kallenbach et al., 2019).

As there is likely a strong connection between root C allocation traits, microbial activity, and N mineralization, there is a need to understand the role of crop breeding and genotype on C allocation patterns and the subsequent impact on organic N turnover in agroecosystems. Recent research efforts have explored the influence of crop genotype and exudate composition on microbial community structure, but there has been little done to explicitly link genotypic variation in belowground root C and microbial community change with rhizosphere function, particularly related to N nutrition (Brisson et al., 2019; Iannucci et al., 2021, 2017; Mahoney et al., 2017; Ndour et al., 2021; Schmidt et al., 2020, 2016) Therefore, the objectives of this study were to: 1) assess the impact of breeding and genotype variation on belowground C allocation patterns; 2) understand the effect of belowground C allocations patterns on plant residue N cycling; and 3) examine the role of microbial communities in mediating rhizosphere N cycling.

To address these objectives, we evaluated root architecture, rhizodeposit C, microbial community structure and activity, and subsequent cover crop residue-derived N acquisition by the growing plant across a range of winter wheat genotypes in a greenhouse experiment using stable isotope tracing. We hypothesized that different genotypes would show variation in belowground C allocation related to breeding history, which would affect residue N mineralization and accessibility. Additionally, we hypothesized that differences in microbial community structure and activity associated with genotypic variation would help explain these differences by driving N mineralization activities. Through whole-system tracing of C and N flows, this study allowed us to directly relate changes in root traits and belowground C allocation to rhizosphere community structure and to key C and N cycling functions in a plant-soil system.

4.2. Methods

4.2.1 Experimental design

This study was conducted in the Colorado State University Plant Growth Facility. We selected a group of eleven hexaploid cultivars and one diploid relative of winter wheat (Table 4.1) based on divergent pedigrees, relevance to current wheat production, year of release, and success in a preliminary greenhouse trial (data not shown). The wheat relative (*Triticum monococcum* L.) is a primitive domesticated diploid einkorn wheat having the A genome, whereas the hexaploid cultivars have the A, B, and D genomes. The Wheat Genetics Resource Center at Kansas State University and other sources provided the original seed, which we then grew in irrigated plots at the Agricultural Research, Development & Education Center (ARDEC) near Fort Collins, Colorado to produce seed sourced from a common environment.

We collected surface soil (0-30 cm) for the pot experiment from unfertilized dryland wheat plots located at the Central Great Plains Research Station near Akron, Colorado, USA (40°09'N, 103° 09'W, altitude 1384 m). The soil is classified as a Weld silt loam (fine, smectic, mesic Aridic Arguistoll) with 18.1 g kg^{-1} organic C, pH of 6.5 and a loam texture. No carbonates were detected in the soil, and inorganic N was low (NH_4-N: 4.0 g kg^{-1}, NO_3-N <1.0 g kg^{-1}). Collected soil was air-dried, passed through a 2 mm sieve, and combined with 40-grit clean quartz sand in a 2:1 sand:soil ratio by mass. We added 2.2 kg of the prepared soil mixture to cylindrical PVC pots (10 cm dia. 25 cm length) lined with plastic sleeves to facilitate soil and root harvest. Triple-super phosphate was added at a rate equivalent to 34 kg ha^{-1} (28.5 mg pot^{-1}) and incorporated into the top 15 cm of the pot to avoid issues of phosphorus limitation. We added hairy vetch (*Vicia villosa* Roth) shoot residues labelled with ^{15}N (see details below) to trace residue N mineralization and residue-derived plant N uptake. Residue was mixed into the

top 15 cm of the sand-soil mixture of all pots at a rate of 1.5 g dry mass pot^{-1}. This rate was equivalent to a field rate of 3.9 Mg ha^{-1} and a total N addition of 109 kg N ha^{-1}, which is within the typical range of aboveground cover crop residue production for dryland systems in the region (Kelly et al., 2021). Packed pots were watered from the bottom to water holding capacity and incubated in the greenhouse for 60 hr before transplanting wheat seedlings.

We germinated wheat seeds from each selected genotype on blotting paper inside plastic bags for 5 days before transplanting into prepared pots. Four seedlings were transplanted to each pot, placed in a greenhouse, and thinned to two seedlings 2 days after establishment. Five days after transplanting, all pots were transferred into a ^{13}C labelling chamber within the same greenhouse (details below). Five replicates of each genotype plus five unplanted controls with cover crop added were randomly arranged in the chamber for a total of 65 experimental units.

4.2.2 Plant growth and isotopic labelling

The experiment was carried out in a continuous ^{13}C labelling chamber (Soong et al., 2014), modified for the purpose of this experiment by adding independent valve controls for the flow of enriched and ambient CO_2 gas. The chamber atmosphere was maintained at a target 4.5 atm% ^{13}C-CO_2 by adjusting relative inflow rates of natural abundance CO_2 and 10 atm% ^{13}C-CO_2 (Cambridge Isotope Laboratories, Andover, Massachusetts) to accommodate the addition of unenriched CO_2 from soil respiration. We analyzed chamber gas samples regularly to maintain the ^{13}C-CO_2 enrichment at the target level of 4.5 atm% using a Delta V Advantage IRMS, coupled to a GC-isolink unit, with a Trace GC Ultra and PreCon (Thermo Scientific). Additionally, to minimize ^{13}C dilution and CO_2 fertilization from decomposing SOM and cover crop residues, we grew field corn (*Zea mays* L.) in pure sand at one extreme of the chamber. We

applied water using a reservoir and pump to the pots bi-weekly based on visual assessments of soil moisture and plant stress using a common drip line so that all pots, including the unplanted controls, received the same amount of water. Temperature and humidity were controlled by an air conditioning unit and dehumidifier inside the chamber, and air flow maintained with oscillating fans. Average daily temperature was approximately 27 ºC, relative humidity ranged between 40-60%, and natural light was supplemented with fluorescent grow lights to provide 16 hr of light day^{-1}.

We produced ^{15}N-labelled hairy vetch, a commonly used leguminous field cover crop, to incorporate into the experimental pots as the source of legume residue-derived N to simulate a cover crop residue addition. The vetch was grown in pure quartz sand and labelled with ^{15}N provided at 9 atm% as $K^{15}NO_3^-$ (Cambridge Isotope Laboratories, Andover, Massachusetts) in Hoagland's solution (Hoagland and Arnon, 1950). Vetch shoots were harvested at flowering, oven-dried at 60 ºC, and chopped with scissors to pass through a 4 mm sieve. The harvested vetch shoot contained 2.8% N, with a ^{15}N label of 8.98 atm%.

4.2.3 Harvest of experimental pots

We harvested wheat plants at 46 days after transplanting at the 5-6 leaf stage, after a total of 39 days in the labelling chamber. Aboveground plant biomass was collected by cutting the wheat plants at the soil surface and oven-drying at 60 ºC. Rhizosphere soil was collected by removing the whole soil column intact, cutting the plastic sleeves on top of a sterile tray and gently breaking apart the root-filled soil column to extract the root ball. Loose soil was shaken off the root mass and soil still clinging to the roots was considered rhizosphere soil. We transferred the root mass with tightly-adhered soil to a clean plastic bag and shook vigorously to

dislodge remaining rhizosphere soil. Rhizosphere soil was passed through a 2 mm sieve and then we subsampled for DNA extraction (~0.3 g), enzyme analysis (1 g), chloroform fumigation (10 g each for control and fumigated), and soil moisture determination (~40 g). The subsample for DNA analysis was transferred to a BeadBashing™ tube, vortexed with 700 mL BeadBashing™ buffer (Zymo Research Corporation, Irvine, CA), and kept frozen at -20 ºC until analysis. Pre-weighed soil for enzyme assays and chloroform fumigation was kept at 4 ºC until analysis (1-2 days). The remaining bulk soil was kept separate, sieved for roots, and air-dried in preparation for elemental analysis.

In preparation for root scanning and isotopic analysis, all recovered roots were thoroughly rinsed over a 4 mm mesh screen to remove any remaining soil and sand particles. A representative subsample of roots (~ 0.5 g) was collected for isotopic analysis by clipping small sections from different representative parts of the root system and oven drying at 60 ºC. We submerged the remaining roots in a solution of 25 % reagent alcohol and 0.05 % neutral red dye for initial staining and preservation of roots in preparation for root scanning (Junaidi et al., 2018). At the completion of imaging, all root and root crown tissue was oven-dried at 60 ºC for determination of total root biomass. Final biomass calculations included aboveground biomass and root crown together as shoot material, with all other roots considered as root biomass.

4.2.4 Root imaging and analysis

Wheat root systems were dyed, scanned, and measured with image-processing software to analyze difference in root architecture. Preserved root systems (excluding the root crown) were re-dyed to increase contrast by submerging in a 1% Neutral Red Dye solution for 1 minute. We then rinsed and detangled the roots in several dishes of water, and then arranged the roots in

shallow acrylic trays with water, illuminated from behind to remove shadows, and imaged using a ScanMaker 9800XL (Microtek International Inc., Santa Fe Springs, CA USA) in 16-bit greyscale mode with 600 dpi resolution, and then converted to 8-bit TIFF for software compatibility. Each root system was separated into 3-6 scans depending on the size of the root system to minimize root overlap and ensure distinct imaging of roots. Root length in diameter size classes and average root diameter were determined using the skeletonization method in WinRhizo (Version 2009; Regent Instruments, Quebec, Canada). Roots were divided into eight size classes between 0.05 mm diameter and 0.35 mm diameter in 0.05 mm increments and the total root length in each size class calculated. Total root length, average root diameter and total root volume were also calculated. Size class fractions were calculated by dividing the root length in a size class by the total root length for each sample pot. Fine roots were determined as those with diameter < 0.2 mm.

4.2.5 Plant and soil analysis

Wheat plant and whole soil samples were ground in preparation for elemental and isotopic (^{13}C and ^{15}N) analysis. We ground dried wheat shoots using a small Wiley mill (Thomas Scientific), and roots using a mortar and pestle with added liquid N to make the roots brittle. Rhizosphere and bulk soil were 2-mm sieved and ground using a mortar and pestle and passed through a 200 um sieve to pulverize the quartz sand. Total ammonium and nitrate in rhizosphere soil were measured in unfumigated K_2SO_4 extracts (described below) to obtain soil inorganic N measurements using a Alpkem Flow Solution Automated wet chemistry system (O.I. Analytical, College Station, TX). Due to low N concentrations, isotopic signal in the inorganic N pools were not measured.

4.2.6 Soil microbial biomass and activity

We assessed microbial activity in the rhizosphere through estimation of microbial biomass C and N, and fluorometric enzyme activity assays. Total microbial biomass C and N in the rhizosphere was estimated as using chloroform fumigation-extractions (Vance et al., 1987). Briefly, each sample was divided into "control" and "fumigated" samples; control soils were immediately extracted with 0.05 M K_2SO_4 by adding 50 mL to 10 g fresh soil and shaken on a rotary shaker at 200 rpm for 2 hr Extracts were filtered through a Whatman 40 filter (8 um). Fumigated samples were enclosed in a vacuum chamber with 30 mL of chloroform, brought to negative pressure to boil the chloroform and then left to fumigate for 24 hr. After fumigation, samples were evacuated for 4 hr and then extracted with the same protocol as the control soils. All extracts were frozen at -20 ºC and then lyophilized (freeze-dried) and the resulting salts collected for isotopic analysis. As the same soil was used across all samples, extraction efficiency was assumed to be constant, and no extraction efficiency factor was applied. Therefore, microbial biomass C (MBC) and N (MBN) throughout refer to the difference in C or N between control (unfumigated) values from fumigated values. The total extracted C or N after chloroform fumigation (DOC + MBC) is combined as the total extractable C or N as an indicator of net accessible C and N.

Soil enzyme activity was determined fluorometrically on rhizosphere soil samples to assess N cycling activity via decomposition of organic N [β-1,4-glucosaminidase (NAG), leucine aminopeptidase (LAP)], labile C (β-Glucosidase), and cellulose (β-D-cellobiosidase; DeForest, 2009). Activities of BG, CB, and NAG were measured using substrate with methylumbelliferone as the fluorescing agent and LAP activity was measured with 7-amino-4-

methylcoumarin as the fluorescing agent. Activity was measured on a microplate fluorometer with 365 nm excitation and 450 nm emission filters.

4.2.7 Molecular soil microbial community analysis

We used qPCR of microbial protease genes and amplicon sequencing to assess the functional potential and community structure of the microbial community in rhizosphere soil. Genomic DNA was extracted from 0.3 g of fresh rhizosphere soil using the Quick-DNA Fecal/Soil Microbe kit (Zymo Research Corporation, Irvine, CA) following manufacturer's instructions and quantified using the Qubit dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA).

We used qPCR to quantify microbial marker genes (16S and ITS2) and protease genes in gDNA extracted from rhizosphere soil. The universal primer sets EUB 338/EUB 518 and ITS1F/5.8s were used for quantification of all bacteria and fungi, respectively, at an annealing temperature of 53 ºC ((Fierer et al., 2005). Protein degrading genes encoded alkaline metallopeptidases (*apr*), neutral metallopeptidases (*npr*), and serine peptidases (*sub*) using the primers reported by Bach et al. (2001) and were run using an annealing temperature of 50 ºC. All reactions were run using a Bio-Rad CFX96 Real-Time System coupled to a C1000 Touch Thermal Cycler with the 2X SensiFAST SYBR No-ROX Mix (Bioline) in 10uL reactions.

Amplicon libraries were prepared for the 16S rRNA region using the 515/806 Earth Microbiome Project standard primer pair (Caporaso et al., 2011), and the V3-V4 region of the ITS gene (ITS-2; White 1990). Libraries were pooled and paired-end reads were sequenced on an Illumina MiSeq at the Colorado State University Sequencing Core. Sequence data generated for this project is available in the NCBI SRA database under the project ID PRJNA735275.

Demultiplexed 16S and ITS-2 sequences were processed using QIIME2 (version 2019.10; (Caporaso et al., 2010). Paired-end 16S reads were denoised using DADA2 to identify unique amplicon sequence variants (ASVs) that is most similar to species, and avoids the 97% similarity threshold used to group and identify operational taxonomic units (OTUs; (Callahan et al., 2016). Only the forward reads were used for analysis of ITS gene sequences to improve ASV clustering, as significant read loss was observed when merging and read quality was highest in forward reads, likely due to variability in region length. ITS forward reads were trimmed to 200 bp before denoising, and read-through primers were not specifically removed due to the short read length.

ASVs were assigned taxonomy using the Native Bayes feature classifiers trained on the study-specific primer pairs through QIIME2 (Bokulich et al., 2018) using the 99% similarity SILVA data base for 16S sequences (Quast et al., 2013)and the UNITE reference database version 8.2 (Abarenkov et al., 2020)for aligning fungal ITS sequences. Unidentified, chloroplast, and mitochondrial sequences were removed from the 16S dataset after taxonomic classification.

4.2.8 Microbial diversity and relation to plant and soil parameters

For the calculation of diversity metrics, prokaryotic 16S samples were rarefied to 19233 reads and fungal ITS rarefied to 653, which excluded 1 and 4 samples from each analysis, respectively. Total number of ASVs (richness), Pielou's evenness (Smith and Wilson, 1996) and Shannon diversity (Spellerberg and Fedor, 2003) were calculated using rarefied data as measures of within-sample alpha diversity. Read subsampling and diversity metrics calculations were completed in QIIME2 using the Core Metrics function (Bolyen et al., 2019).

In preparation for additional downstream statistical analysis, prokaryotic and fungal datasets were normalized using cumulative sum of squares (Paulson 2013) followed by a log transformation. This normalization method helps account for different read numbers in each sample that is unrelated to ecological differences, while minimizing data loss, as occurs with rarefaction to even sampling depth (McMurdie and Holmes, 2014; Weiss et al., 2017). Samples with fewer than 1000 (16S) and 300 (ITS) reads were excluded and taxa with an overall relative abundance below 0.01% were removed.

4.2.9 Isotopic analysis and calculations

All soil, plant, and K_2SO_4 extract samples were analyzed for total C, ^{13}C, total N and ^{15}N at the UC Davis Stable Isotope Facility using a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Ltd., Cheshire, UK). These readings for the unfumigated K_2SO_4 extracts were used to determine salt-extractable C and N as a proxy for dissolved organic C (DOC) and total dissolved N (TDN). Total extractable C is considered as the total C concentration from the fumigated samples (i.e. sum of DOC and chloroform-extractable C). We considered C derived from rhizodeposits as the wheat-derived ^{13}C recovered in the rhizosphere and bulk soil samples, which includes ^{13}C assimilated into microbial biomass. We calculated total rhizodeposit C by multiplying the concentration of soil C by the *f*-value (calculation below) using the measured ^{13}C value, and then multiplying this by the mass of soil.

The relative contribution of isotopically labelled source material (^{13}C in growing wheat plants or ^{15}N in legume residue) to various C and N pools were calculated using atm% values in a mixing model equation:

$$f_{label} = \frac{(atm\%_{sample} - atm\%_{control})}{(atm\%_{label} - atm\%_{control})}$$

Where f_{label} is the relative contribution of the labeled source to the sample, $atm\%_{sample}$ is the atom% of the sampled material, $atm\%_{control}$ is the atom% of the natural abundance control, and $atm\%_{label}$ is the atom% of the labelled source material (either the wheat plant (^{13}C) or the legume residue (^{15}N)).

To accurately calculate the root-C and residue-N contribution to MBC and MBN pools, respectively, the isotopic signatures of the end member pools needed to be calculated separately, as the chloroform fumigated extracts represented the mixed signal from the microbial biomass and background salt-extractable soil C and N pools. Therefore, one additional calculation was made for these mixed samples to first determine the isotopic signature of the isolated microbial biomass pool (subtracting the unfumigated soil contribution), using the known fraction of microbial biomass C or N in the fumigated sample, and rearranging the above mixing model equation:

$$atm\%_{MB} = \frac{atm\%_{fum} - (f_{no\text{-}fum} \times atm\%_{no\text{-}fum})}{f_{MB}}$$

Where $atm\%_{MB}$ is the calculated isotopic enrichment value of the microbial biomass (chloroform-extractable fraction), $atm\%_{fum}$ is the measured isotopic value of the fumigation soil extracts, $f_{no\text{-}fum}$ is the fraction of C or N present in the fumigated sample attributed to the non-fumigated soil (*non-fumigated / fumigated*), $atm\%_{no\text{-}fum}$ is the isotopic value of the unfumigated control sample and f_{MB} is the fraction of C or N in the fumigated sample from the microbial biomass [*(fumigated – control) / fumigated*.]

4.2.10 Statistical analysis of plant and soil parameters

We performed one-way ANOVA on all measured variables to assess the influence of genotype on plant, soil chemical and microbial parameters. We also implemented Tukey pairwise comparisons to identify differences between individual genotypes; an alpha level of $p = 0.05$ was determined to be significant. Spearman correlations between plant, soil and microbial variables were used to investigate relationships between different aspects of the plant-soil-microbe system and to inform further analyses. Pearson correlations were also performed for some plant and enzyme parameters were assumptions we met. Net rhizodeposit C was calculated by summing the contribution of wheat C to rhizosphere and bulk soil, and then multiplying by the total C in each compartment on a per pot basis. Net belowground C (NBC) was calculated as the sum of net rhizodeposit C and root tissue C.

A structural equation model (SEM) was developed to simultaneously assess the relationship between different elements of our hypothesized network of interactions, utilizing data collected from rhizosphere soil samples (Grace, 2006). The initial network was informed by ANOVA results and hypothesized linkages based on the literature. Potential linkages were assessed by reviewing model fit parameters, and competing models were compared using the corrected Akaike Information Criterion (AICc), adjusted for small sample size (< 250). Overall goodness of fit for the model was assess using the Chi-square test (χ^2; the model has acceptable fit where $0.01 < p < 0.05$ and good fit when $0.05 < p < 1.0$), the root mean square error of approximation (RMSEA; the model has acceptable fit when RMSEA < 0.1 and good fit when RMSEA < 0.8) and the comparative fit index (CFI; the model has good fit when CFI > 0.9). All input parameters were scaled to have similar variance for use in the model. The model was specific using the *sem* function in the *lavaan* R package (Rosseel, 2012), utilizing the default

maximum likelihood method of estimation. Linear regression assumptions were verified for each model component. All statistical analyses were completed using R version 4.0.3 (R Core Team, 2020).

4.2.12 Multivariate analysis of microbial communities

Normalized taxon abundances were tested for relationships with rhizodeposition and N uptake parameters using ordination methods and Spearman correlations. Multivariate statistical analyses were completed using the *vegan* package for R (Oksanen et al., 2020). Microbial community composition was compared between winter wheat genotypes using PERMANOVA of Bray-Curtis dissimilarities. Distance-based redundancy analysis (db-RDA), a constrained ordination method that utilizes distance-based dissimilarity matrices to describe communities, was used to assess the relationships between microbial community composition and plant and soil variables (also called Canonical Analysis of Principal Components (CAP); (Buttigieg and Ramette, 2014; Legendre and Anderson, 1999) db-RDA was implemented using the *capscale* function in *vegan*, and stepwise backwards model selection with *ordistep* used to select the most important parameters. Overall model fit was assessed with a model fit of $R^2 > 0.15$, or 15% of inertia explained, determined to be acceptable. We computed Spearman correlations between normalized taxa abundances and measured plant and soil parameters for taxa present in > 50% of samples.

Network analysis helped to visualize co-occurrence of taxa and relationships to other plant and soil variables. Co-occurrence networks were constructed for bacterial and fungal taxa separately using the 500 most abundant taxa by first generating Spearman correlations between taxa. Correlation results were then converted into dissimilarities and plotted in two dimensions

using Principal Coordinate Analysis (PCoA) so that taxa that tended to be in the same samples were plotted close together. Edges, or lines, between nodes indicate significant ($p < 0.05$) positive correlations with Spearman's $\rho > 0.25$, and node size corresponds to relative taxa abundance. Nodes were then colored based on the strength of the positive correlation between the taxon and the plotted plant or soil parameter. Different associated variables were explored and the most informative presented. Network analysis and plotting was completed using Calypso (version 8.84) using default parameters (Zakrzewski et al., 2017).

4.3. Results

4.3.1 Wheat morphology

Wheat genotypes varied in above- and below-ground biomass. Average aboveground biomass was 2.7 g pot^{-1}, with Scout 66 having significantly higher biomass (3.2 g) than *T. monococcum* (2.2 g; Table 4.2). Average root biomass across all genotypes was 1.2 g pot^{-1}, where again Scout 66 (ca. 1967) had the highest value (1.5 g) and *T. monococcum* the lowest (0.9 g) biomass. Conversely, *T. monococcum* had the highest fraction of biomass allocated to roots (31.9%), while Scout 66 had the lowest (24.1%; Table 4.2).

Root architecture metrics varied between genotypes and highlighted contrasting root system characteristics. Average root length across all genotypes was 17.9 m (Table 4.2). Snowmass and Sandy had the shortest root system (14.3 and 15.7 m, respectively), while Byrd and Scout 66 roots were the longest (21.4 and 22.7 m, respectively). Overall, Snowmass (ca. 2009) differed from other varieties as having shorter, coarser roots: the average diameter of Snowmass roots was 14% larger than Ripper (ca. 2006), which had the finest roots, on average (Table 4.2). Ripper, Wichita, and Byrd had the largest proportion of fine roots (< 0.2 mm) at

73%, 73%, and 72%, respectively, which was greater than Snowmass (65%) and Sandy (65%). Specific root length (SRL), a relative measure of root investment to nutrient and water acquisition - analogous to specific leaf area (Ostonen et al., 2007), was lowest for Snowmass (125 m g^{-1}) and greatest for the landrace Turkey Red (178 m g^{-1}). The effect of breeding history was not apparent, except for the high SRL of Turkey Red (ca. 1874), and the wheat relative, *T. monococcum*, was overall smaller than its domesticated counterparts.

4.3.2 Belowground C allocation

Wheat plants allocated 41% on average of net recovered C to belowground pools (root + rhizodeposition), of which an average of 32% was recovered as net rhizodeposit C, defined as the total wheat-derived C found in soil. Average plant tissue enrichment with ^{13}C-CO_2 was 4.35 atm% for both aboveground and belowground plant tissue. Average enrichment of the soil was 1.14 atm% ^{13}C in the rhizosphere and 1.13 atm% ^{13}C in the bulk soil (standard error = 0.002 for both), while the isotopic signature of the unplanted control soil used in the mixing model was 1.08 atm% ^{13}C. Total recovered rhizodeposit C in rhizosphere and bulk soil amounted to an average of 258 mg C pot^{-1}, ranging from 81-597 mg C pot^{-1}, and did not differ among genotypes (Table 4.3). This amounted to 1.8-18.5% (mean: 6%) of the recovered C in all plant and soil pools. Genotype differences were not observed for rhizodeposit C, net belowground C (NBC), or their relative proportion of rhizodeposit C in NBC (Table 4.3). Wheat-derived extractable C (sum of DOC and MBC) displayed a marginally significant genotype difference ($p = 0.09$), with Snowmass (ca. 2009) having the highest wheat-dervied extractable C (23 mg C kg^{-1} soil) and Cheyenne (ca. 1933) the lowest (7.8 mg C kg^{-1} soil; Table 4.3). Wheat-derived C comprised 10%

(6-23% range) of the total extractable soil C pool, which included DOC and MBC as an indicator of total accessible C.

4.3.2 Nitrogen cycling and uptake

On average, 6% of the N added as legume residue was recovered in total wheat tissue, ranging from 3 to 14% with no effect of genotype. Wheat shoot N concentration were 13.3 – 17.5 mg N g^{-1}, while root N concentration ranged from 12.3 – 15.3 mg g^{-1} (Table 4.4). Residue-derived N accounted for an average of 17% of shoot N and 15.5% of root N (Table 4.4), with no significant genotype effect. Total residue-derived N uptake by the wheat plants was highly related to overall plant biomass (Pearson's $r = 0.83$, data not shown) and was not different among genotypes (Table 4.4).

Soil N pools were overall highly variable, though some genotype effects were detected. For instance, the relative contribution of residue-derived N in the MBN pool had a significant genotype effect ($p = 0.03$; Table S4.1); Turkey Red and *T. monococcum* pots had the highest relative contribution of residue N to the MBN pool (43-45%), while Scout 66, Ripper and Cheyenne only had about 16% of the MBN derived from the residue. Total MBN and residue-derived MBN did not differ between genotypes, and we did not detect an effect of genotype on nitrate and ammonium concentration. On average, ammonium levels were 73% higher than nitrate (Table S4.1). Soil moisture at harvest was negatively correlated to root volume ($\rho = -0.54$, $p < 0.001$), but not related to enzyme activity or N availability.

4.3.4 Enzyme activity andiIndicators

Regardless of the enzyme, potential enzyme activities did not vary by genotype. LAP and NAG activities were correlated with each other (Pearson's $r = 0.67, p < 0.001$) and added together to compute an "N-cycling enzyme activity" composite variable, which was positively related to residue-derived dissolved N ($\rho = 0.35, p < 0.01$), wheat-derived DOC ($\rho = 0.45, p < 0.01$), wheat-derived MBC ($\rho = 0.47, p < 0.01$) and average root diameter ($\rho = 0.42, p < 0.001$; Table 4.5). Inorganic N content in the rhizosphere soil was positively correlated with the N-cycling enzyme NAG ($\rho = 0.53, p < 0.001$), and the C-cycling enzyme CB ($\rho = 0.30, p < 0.05$), while correlations with LAP and BG were positive but non-significant (Table 4.6). Abundance of peptidase genes in the rhizosphere soil showed some notable patterns with genotype and N-cycling indicators. Specifically, abundance of the *npr* gene was elevated in the landrace Turkey Red (Table S2), and positively related to wheat-derived DOC, but not with N-cycling enzyme activity or measure N pools (Table 4.5).

4.3.5 Structural equation modeling

SEM analysis explained 74% of the variation in total residue-derived N uptake in wheat plants, and identified direct and indirect pathways between wheat root traits, C allocation, microbial N-cycling enzyme activity, and residue N dynamics (Fig. 4.1). The model provided a good fit according to χ^2, RMSEA and CFI (Grace, 2006; Lefcheck, 2019). Standardized coefficients (β) are reported to allow for comparison of relative importance of linkages across the model. Total uptake of residue-derived N by wheat was best explained by the proportion of residue N in wheat biomass ($\beta = 0.69, p < 0.001$) followed by total root length ($\beta = 0.51, p < 0.001$), and microbial biomass C (MBC) from wheat ($\beta = 0.27, p < 0.001$). Average root

diameter was the best root morphology variable to explain wheat C patterns in the rhizosphere, while total root length was not important in the SEM model and only weakly related to rhizodeposition using Spearman correlation (Table 4.5). N-cycling enzyme activity, expressed as the sum of LAP and NAG activity, was highly influenced by wheat-derived MBC ($\beta = 0.52$, $p < 0.001$), which was in turn controlled by wheat C rhizodeposition ($\beta = 0.40$, $p < 0.001$) and average wheat root diameter ($\beta = 0.28$, $p = 0.018$). The relative proportion of N in wheat tissue derived from added legume residue (% residue N in wheat) was not well predicted by either of the model predictor variables, nor other measured variables tested; we retained two predictor variables in the model based on our conceptual model of system function (N-cycling enzyme activity and wheat-derived MBC), but the linkages were non-significant ($p = 0.24$ and $p = 0.09$, respectively).

4.3.6 Microbial community composition

In both the prokaryotic and fungal datasets, ordination analysis did not reveal sample clustering based on wheat genotype, and PERMANOVA analysis confirmed that wheat genotype did not significantly explain microbial community composition (Fig. 4.2). Both prokaryotic and fungal communities produced significant db-RDA models by backwards selection of phyla relative abundances, and root length and root-derived soil C had the strongest effect on both prokaryotic and fungal community ordination structures (Fig. 4.2).

Alpha diversity indices did not differ among genotypes in terms of overall richness, evenness, or Shannon index for prokaryotic or fungal taxa (Table S4.3). Prokaryotic taxa richness ranged from 899 to 3823 amplicon sequence variants (ASVs) per sample, with an average of 2535 ASVs per sample and a total of 1781 unique ASV's detected. Prokaryotic

samples were dominated by bacteria, comprising 90-95% of relative abundance; the most abundant bacterial phyla were Proteobacteria (27%), Actinobacteria (16%) and Acidobacteria (11%; Fig. S4.1). No prokaryotic phyla differed in abundance among genotypes (Bonferroni-adjusted Wilcoxon rank-test $p > 0.05$; data not shown). Fungal communities were dominated by Ascomycota, which comprised 50-79% of all identified sequences (Fig. S4.2), and similarly did not differ among wheat genotypes.

4.3.8 Relationships between microbial taxa and C and N parameters

Network analysis revealed that a subset of bacterial genera was highly associated with wheat-derived soil C, but that these taxa are distinct from those associated with the fraction of residue-derived N assimilation by the wheat plant (Fig. 4.3a). Extractable wheat-derived C, which was calculated as the sum of wheat-derived DOC and chloroform-extractable C, was used in the analysis to represent the more microbially accessible C. Genera associated with the relative proportion of legume residue-derived N in wheat biomass (blue) are largely non-overlapping with the extractable wheat C-associated genera (red) in the network. Repeating the analysis with fungal ASV abundance reveals a somewhat similar but much less distinct pattern (Fig. 4.3b), and the lower node density indicates overall weaker correlations between fungal taxa.

Specific bivariate relationships analyzed with Spearman correlations revealed both root C pools and N cycling/uptake variables were associated with a variety of bacterial genera. The Actinobacteria *IMCC2614*, the Alphaproteobacterial *Iamia*, the Gammaproteobacteria *Luteolibacter*, and the Gemmatimonadetes *Glycomyces* had the strongest association with total wheat N uptake.

Fungal phyla demonstrated the strongest relationships with wheat N uptake and availability. Ascomycota, the most abundant phyla, was negatively correlated with relative legume residue N content in wheat tissue (Table S4.4). Basidiomycota was positively correlated to both total root length and total N uptake by wheat. Glomeromycota, the group containing mycorrhizal fungi, was also positively related to total N uptake, and negatively associated with TDN. Olpidomycota was positively related to both total N uptake and legume residue N uptake, as well as N-cycling enzyme activity (Table S4.4). No fungal groups correlated with total rhizodeposition or extractable wheat C, though db-RDA identified extractable wheat C as important for explaining fungal community composition (Fig. 4.2b).

4.4. Discussion

This research sought to elucidate how variation in wheat genotypes, specifically in belowground traits, can potentially shape rhizosphere microbial communities and associated implications for crop N nutrition. Our approach using ^{13}C provided unique insight on belowground C allocation that is often ignored in studies of how plants shape their rhizosphere communities. Additionally, we sought to evaluate the functional role of rhizosphere communities, specifically regarding N acquisition from organic N pools (e.g., leguminous cover crops residue) that are likely to become more important with increasing reliance on organic nutrient sources in the future.

4.4.1 Tradeoffs in C allocation

Our results suggest that there are divergent nutrient acquisition strategies within winter wheat, such that shorter and coarser roots with less foraging area appear to rely more on

microbial activation through rhizodeposition or mycorrhizal colonization to produce enzymes and mineralize organic soil N pools. Specifically, average root diameter was positively related to dissolved wheat C pools (Fig. 4.1; Table 4.5). While the quantity of rhizodeposits is thought to be a function of growing roots, as mucilage is produced by the growing root cap (Farrar et al., 2003), we found that root thickness was most predictive of root-derived C deposition. Iannucci et al. studied the relationships between root morphology, exudates, and microbial communities in eight cultivars of durum wheat [*Triticum turgidum ssp. durum* (Desf.)] and found evidence of a similar tradeoff. They suggested the presence of two different resource-use strategies: a conservative strategy with large root systems but low exudation, and an explorative/competitive strategy that relies on high exudation and microbial activity to access nutrients (Iannucci et al., 2021). We observed a similar effect in our study, where short, coarse root systems were associated with the highest amount of rhizodeposit C. Net belowground C was similar across all plants, indicating that the total amount of C allocated belowground was relatively constant, but the form (i.e. exudation vs. root structures) was variable, leading to a trade-off in belowground C allocation. Root thickening in wheat has been shown to be stimulated by some hormone-secreting microbes in the rhizosphere, suggesting that the microbial community may play a role in shaping in root architecture (Narula et al., 2006). This relationship may hold across other crop species; Wen et al. found root thickness to be an important predictor exudation amounts across several different annual crops (Wen et al., 2019). In a study of switchgrass ecotypes, thicker roots were associated with greater assimilation of root-derived C by Gram-negative PLFAs, suggesting that thicker roots can lead to higher microbial assimilation of rhizosphere C compounds, at least in some grass species (Roosendaal et al., 2016).

Coarse roots have been shown to have higher mycorrhizal dependency across wheat and other annual crops (Hetrick, 1991; Wen et al., 2019). Mycorrhizae can greatly increase effective root surface area and acquisition of limiting nutrients, which is especially important for smaller root systems (Marschener, 1998). Though we did not find a positive relationship between root thickness and Glomeromycota sequence abundance (Table S4.4), our detection methods were not optimized for detecting mycorrhizal association, which would be improved though 18S sequencing or microscopy methods (Saito et al., 2004; Shi et al., 2012).

We found partial support of our hypothesis that genotype would influence root architecture and C allocation, though the effect of breeding history was not straightforward. The landrace Turkey Red (ca. 1874) had the highest specific root length (SRL), which may indicate an acquisition strategy adapted for conditions of low nutrient availability (Table 4.2). Snowmass (ca. 2009) was notable for its shorter, coarse root system (Table 4.2), coupled with high extractable C pools in the rhizosphere (Table 4.3). The contrasting responses of these two genotypes represent opposite extremes of the root C resource-use spectrum, which may reflect responses to selection. Notably, while SRL is generally associated with increased uptake of mineral N, a study of wheat N uptake across a breeding gradient demonstrated a negative effect of crop selection on root length and density, but an overriding increase in N uptake efficiency led to increased N uptake despite smaller root systems of modern wheat (Aziz et al., 2017).

Our experimental approach included a wide range of genotypes, which provided within-species variation that allowed us to evaluate the relationships between plant traits and C allocation, and microbial processes linked to N cycling relevant for breeding objectives. Including more landrace genotypes would have allowed us to group genotypes into age groups, as genotype-specific differences were difficult to distinguish, particularly in our isotopically-

determined pools. Genotype differences may become more pronounced under different soil health and nutrient management regimes, as has been observed in spring wheat, where plants with higher root:shoot ratios exhibited greater plasticity and responded more favorably to compost addition (Junaidi et al., 2018).

4.4.2 Linkages between rhizodeposition, microbial activity and N dynamics

We observed a strong effect of microbial biomass stimulation, estimated as the amount of wheat-derived C recovered in microbial biomass C (MBC), on both N-cycling activity and wheat uptake of residue-derived N (Fig. 4.1). We found evidence of both direct and indirect pathways of residue-derived N uptake. Total root length was directly, positively associated with residue N uptake, which may have been related to higher foraging area, but also related to overall plant size. Microbial biomass indirectly mediated the effect of different root C allocation patterns on N availability; specifically, overall rhizodeposit C and average root diameter had a positive effect on residue-derived N mediated through the microbial biomass (Fig. 4.1).

The other linkages in the model connecting N-cycling enzyme activity to wheat N uptake were not as clear. This may be attributed to the dual role of soil microbes in both mineralizing, but also competing for, available N, as other labelling experiments have struggled to directly link rhizodeposition with N uptake (Allard et al., 2006; de Graaff et al., 2009). Mwafulirwa et al. (2017) found a relationship between barley rhizodeposition and residue N uptake, but genotype differences were only apparent between C pools and not for N uptake. Stronger linkages between belowground C and N dynamics have been measured in natural systems, where increased root exudation has been linked to elevated N turnover following simulated grazing or elevated CO_2 conditions (Hamilton and Frank, 2001; Phillips et al., 2011).

Enzyme activity was positively related to MBC assimilated from wheat, demonstrating a clear link between microbial community growth in response to wheat root C and resulting N-cycling enzyme production (Fig. 4.1; Table 4.6). As is expected, elevated microbial biomass would provide a larger potential source of hydrolytic enzymes, and this has been seen in field and greenhouse studies (Bowles et al., 2014; Chander et al., 1997; Kabiri et al., 2016). While we were not able to detect a direct positive response of rhizosphere enzyme activity on plant growth, a previous study examining the effects of putative plant-growth-promoting (PGP) *Streptomyces* inoculation of wheat suggested that microbial enzyme activity was responsible for increased plant growth, though enzyme activity was only measured in the isolate incubations and not under growing plants (Jog et al., 2012).

4.4.3 Recruitment and functioning of microbial taxa

Ecological grouping frameworks originally developed for plants (Grime, 1977) have been applied to soil microbes to help simplify and interpret the overwhelming diversity of soil microbial communities (Fierer, 2017; Fierer et al., 2012, 2007). Our network analysis revealed a distinction between bacterial taxa associated with extractable root C, and those associated with mineralization and plant uptake of residue-derived N in wheat plants (Fig. 4.2). The differences in chemical composition between exudates and the added legume residue, both in terms of stoichiometry and lability, likely stimulated taxa from distinct ecological niches, with perhaps different nutrient demands or life history strategies (Fierer, 2017). Other chemicals, like phytohormones, may have also led to distinct community selection in plants with high exudation (Carvalhais et al., 2015).

Further analysis revealed that specific bacterial genera appeared to be selectively stimulated by wheat rhizodeposition, while other genera showed a relationship with different N pools (Table 4.7). We observed contrasting relationships to C and N variables within a phylum, suggesting that broad phyla-level generalizations about ecological niches and life history strategies may not hold true (Martiny et al., 2015). For example, within the Bacteroidetes, which has been previously described as a copiotrophic phylum (Fierer et al., 2007), *Devosia* and *Flavobacterium* were positively associated with rhizodeposition, while *Gemmatimonas* showed a negative relationship (Table 4.7). *Flavobacterium* was found to dominate wheat root surface only under phosphorus fertilization in Japan, suggesting sensitivity to nutrient availability (Sato and Jiang, 1996). We found that *Massilia*, a gram-negative Betaproteobacteria in the Oxalobacteraceae family, was positively related to rhizodeposition, wheat N uptake and N-cycling enzyme activity. This taxon was highly abundant across all samples, accounting for 1.5% of all sequences. Ecological investigation of *Massilia* growing in association with cucumber found this genus to behave as a copiotroph and aggressively colonize young root tissue and mycorrhizal hyphae, and some isolates have exhibited plant growth promoting attributes, though a mechanism was not suggested (Ofek et al., 2012).

While a few taxa, including *Massilia*, were associated both with root C and N uptake, the majority of taxa were associated with only one of these responses, as demonstrated via network analysis (Fig. 4.3). Thus, our results suggest that N mineralization responses seen with exudation (Meier et al., 2017; Tian et al., 2019) may integrate the activities of several distinct microbial groups, requiring many members to generate the functional responses that may be observed at the broad phylogenetic level. Indeed, it is generally understood that complex ecological interactions between microbial communities give rise to emergent ecosystem properties, though

the relatively sparse colonization of soil surfaces questions the importance of inter-species interactions in the soil (Young and Crawford, 2004).

Fungal taxa observed in our samples may have played a role in N uptake through increased foraging capabilities, and likely not through direct mineralization of residue N. The Glomeromycota phylum, which contains arbuscular mycorrhizal species, was positively correlated with total wheat N uptake and low dissolved N in the soil, suggesting that mycorrhizal associations with wheat may have improved N acquisition (Table S4.4). The mycorrhizal group was only associated with total N uptake, and not specifically residue-derived N, which could be explained by the ability of mycorrhizal associations to access, but not mineralize, soil N (Govindarajulu et al., 2005).

Microbial communities did not appear to respond specifically to the wheat genetic variation present within our study; we did not detect differences in diversity, overall community composition, or abundance of specific taxa between wheat genotypes (Fig 4.2, Table S4.4). Other studies have found that group identity based on genome duplication and domestication status, rather than individual genotypes, resulted in differences in microbial communities and metabolite profiles in wheat (Iannucci et al., 2017; Rossmann et al., 2020; Tkacz et al., 2020), suggesting that including additional landrace genotypes may have revealed more distinct patterns due to age. Additionally, we note that wheat is generally planted in dry, marginal lands, and thus has been bred for stress tolerance under relatively low N application rates compared to other irrigated crops where breeding efforts have focused on yield potential. Thus, the effect of modern nutrient regimes in the selection pressure for wheat may have been lower than for crops like corn (*Zea mays* L.), where directed selection has resulted in large changes in root structure and microbial communities (Brisson et al., 2019; Gaudin et al., 2011; Schmidt et al., 2020).

4.5. Conclusions

Plant rhizodeposition significantly influences the rhizosphere community, potentially altering functions important to plant success, such as nutrient acquisition. Our work demonstrates that winter wheat rhizodeposition can influence N availability through stimulation of total microbial biomass, associated enzyme activity, and the selection of specific microbial taxa that directly and indirectly influence N mineralization. Our results also suggest that distinct microbial taxa are responsible for different aspects of the N mineralization effect due to exudation. Wheat genotype had a noticeable influence on some rhizosphere characteristics, but genotype alone did not explain differences in microbial community structure or N acquisition. While we did not observe strong genotype differences in this study, the variability in traits that we captured allowed us to analyze relationships between plant C allocation patterns and microbially mediated soil N processes.

Greater focus on belowground traits in crop breeding programs could be an important avenue to increase crop fitness in agricultural systems that rely on complex organic nutrient sources, as our results indicated a linkage between rhizodeposition on N cycling and wheat N uptake. Further work exploring these observed relationships across different agricultural management systems and the specific activity of important microbial taxa may lead to improvements in agronomic and environmental metrics. Considering the growing focus by farmers, agribusiness and consumers on soil health and environmental quality, organic nutrient sources are becoming increasingly important for meeting crop nutrient demand, necessitating efficient promotion of biological activity to drive nutrient availability and related crop success.

Plant traits, microbial communities and biogeochemical cycles are intrinsically connected, and improved understanding of their interplay may help the sustainability of agricultural systems.

CHAPTER 4 TABLES AND FIGURES

Table 4.1. Information on eleven winter wheat (*Triticum aestivum*) genotypes and one related species (*Triticum monococcum*) used in a greenhouse study investigating the influence of root and rhizosphere C inputs on microbial communities and N cycling.

Genotype	Acc. No.[1]	Origin	Release/Collection Date	Dwarfing
Byrd	PI 664257	Colorado State Univ.	2011	Semi-dwarf[2]
Snowmass	PI 658597	Colorado State Univ.	2009	Semi-dwarf
Ripper	PI 644222	Colorado State Univ.	2006	Semi-dwarf
Ron L	PI 648020	Kansas State Univ.	2006	Semi-dwarf
Prowers	PI 605389	Colorado State Univ.	1997	Tall
TAM 107	PI 631352	Texas A & M Univ.	1984	Semi-dwarf
Sandy	CItr 17857	Colorado State Univ.	1981	Semi-dwarf
Scout 66	CItr 13996	Univ. Nebraska	1967	Tall
Wichita	CItr 11952	Kansas State Univ.	1944	Tall
Cheyenne	CItr 8885	Univ. Nebraska	1933	Tall
Turkey Red	PI 565343	E. Europe	1874	Tall
T. monococcum[3]	TA142	Turkey	1972	Tall

[1] Accession number in the USDA-ARS GRIN database (http://www.ars-grin.gov/) for wheat cultivars and the Wheat Genetic Resources Center for *T. monococcum*.
[2] Semi-dwarf genotypes possess either allele *Rht-B1b* or *Rht-D1b*, and Tall genotypes lack both those alleles.
[3] Wheat relative with non-shattering morphology

Table 4.2. Summary of plant growth metrics and root architecture for genotypes of winter wheat (*T. aestivum*) grown in a greenhouse, in order of descending release date. Overall ANOVA *p*-value for the effect of genotype is presented below. Values are mean ± standard error ($n = 5$); different letters indicate variety difference using Tukey's test ($p < 0.05$). No letters present indicate no pairwise difference.

Variety	Shoot Biomass (g pot $^{-1}$)	Root Biomass (g pot $^{-1}$)	Root Mass Frac.‡ (%)	Total Root Len. (m pot $^{-1}$)	Surface Area (cm^2 pot $^{-1}$)	Avg. Root Diam. (μm)	Fine Roots (%)	SRL (m g^{-1})
Byrd	2.6 ± 0.2ab	1.4 ± 0.2ab	34 ± 3	21.4 ± 1.8a	1,393 ± 396ab	224 ± 7ac	72 ± 1 a	155 ± 7 ab
Snowmass	2.6 ± 0.2ab	1.2 ± 0.2ab	31 ± 1	14.3 ± 1.5b	1,080 ± 253b	244 ± 16a	65 ± 3c	125 ± 13 b
Ripper	2.4 ± 0.1ab	1.1 ± 0.1ab	32 ± 1	17.8 ± 0.7ab	1,190 ± 87ab	214 ± 6c	73 ± 1a	160 ± 12 ab
Ron L	2.5 ± 0.0ab	1.2 ± 0.1ab	32 ± 1	18.2 ± 1.2ab	1,284 ± 171ab	227 ± 9ac	71 ± 2a	151 ± 10 ab
Prowers	2.8 ± 0.1ab	1.1 ± 0.1ab	29 ± 1	16.0 ± 1.5ab	977 ± 326b	222 ± 12ac	70 ± 2ab	141 ± 8 ab
TAM 107	2.6 ± 0.3ab	1.2 ± 0.2ab	32 ± 2	18.1 ± 2.3ab	1,220 ± 344ab	220 ± 9bc	72 ± 2a	151 ± 10 ab
Sandy	2.9 ± 0.1ab	1.2 ± 0.1ab	29 ± 1	15.7 ± 0.9b	1,176 ± 153ab	241 ± 12ab	65 ± 2bc	134 ± 3 ab
Scout 66	3.2 ± 0.1a	1.5 ± 0.1a	32 ± 1	22.7 ± 1.2a	1,660 ± 147a	236 ± 14ac	68 ± 3ac	149 ± 4 ab
Wichita	2.7 ± 0.2ab	1.2 ± 0.1ab	31 ± 1	18.6 ± 1.9ab	1,180 ± 266ab	217 ± 13bc	73 ± 3a	152 ± 9 ab
Cheyenne	2.8 ± 0.1ab	1.3 ± 0.1ab	32 ± 1	18.8 ± 1.1ab	1,332 ± 147ab	226 ± 11ac	69 ± 3ac	143 ± 10ab
Turkey Red	2.8 ± 0.2ab	1.1 ± 0.1ab	29 ± 1	19.9 ± 0.8ab	1,392 ± 137ab	225 ± 12ac	69 ± 3ac	178 ± 7a
T. monococcum	2.2 ± 0.3b	0.9 ± 0.1b	28 ± 1	13.5 ± 2.4b	904 ± 342b	215 ± 5c	71 ± 0.9a	151 ± 14 ab
Mean	2.7	1.2	31	17.9	1,232	226	69	137.2
CV	0.15	0.23	10	0.23	0.24	0.06	0.06	0.15
ANOVA	*p*-values							
Genotype	**0.024**	0.076	**0.018**	**0.003**	**0.002**	**<0.001**	**<0.001**	0.081

‡ Root mass fraction (root biomass/ total plant biomass); Fine roots are < 0.2mm and percentage calculated on a per length basis; SRL, specific root length; CV, coefficient of variation of all samples (standard deviation divided by mean)

Table 4.3. Wheat-derived soil carbon (C) pools for 12 genotypes determined by using ^{13}C labelling of growing wheat plants. Values are mean ± standard error (n = 5); different letters indicate variety difference using Tukey's test ($p < 0.05$). The C concentrations are mg kg^{-1} rhizosphere soil.

Variety	Rhizodeposition (mg C pot^{-1})	Net Belowground C[a] (mg C pot^{-1})	DOC from wheat (mg C kg^{-1} soil)[b]	MBC from wheat (mg C kg^{-1} soil)[b]	Extractable C from wheat (mg C kg^{-1} soil)
Byrd	212.5 ± 52.17	765.1 ± 57.90	2.48 ± 0.57	11.3 ± 2.42	13.7 ± 2.86 ab
Snowmass	232.0 ± 73.28	722.3 ± 92.70	4.52 ± 1.73	18.5 ± 5.23	23.0 ± 6.90 a
Ripper	177.1 ± 74.65	641.4 ± 60.41	2.23 ± 0.62	8.6 ± 2.34	10.7 ± 2.94 ab
Ron L	271.7 ± 100.23	768.7 ± 91.71	3.44 ± 0.84	15.1 ± 4.29	18.5 ± 5.08 ab
Prowers	249.2 ± 40.87	713.5 ± 40.70	1.72 ± 0.20	8.8 ± 1.23	10.5 ± 1.33 ab
TAM 107	189.0 ± 80.01	688.9 ± 63.94	1.92 ± 0.23	7.3 ± 1.10	9.2 ± 1.20 ab
Sandy	367.2 ± 84.14	849.7 ± 65.89	1.84 ± 0.41	10.3 ± 2.78	12.1 ± 2.63 ab
Scout 66	279.6 ± 73.02	903.9 ± 80.11	2.30 ± 0.39	11.8 ± 3.88	14.1 ± 4.07 ab
Wichita	384.9 ± 76.60	880.8 ± 96.38	1.73 ± 0.22	7.6 ± 0.88	9.3 ± 1.08 ab
Cheyenne	241.5 ± 68.89	784.6 ± 73.04	1.67 ± 0.08	7.3 ± 2.69	7.8 ± 2.52 b
Turkey Red	199.4 ± 40.33	667.0 ± 32.94	2.73 ± 0.53	11.4 ± 2.02	14.1 ± 2.52 ab
T. monococcum	295.1 ± 91.02	653.6 ± 113.30	1.91 ± 0.33	7.6 ± 1.47	9.4 ± 1.59 ab
Mean	258.3	753.3	2.38	10.5	12.7
CV	0.62	0.23	0.66	0.61	0.63
ANOVA	*p*-values				
Genotype	0.63	0.22	0.14	0.19	0.09

[a]Net belowground C, estimated as sum of root biomass C and rhizodeposition C; DOC, dissolved organic carbon; MBC, microbial biomass C, estimated as chloroform-extractable C, calculated as fumigated C minus DOC; Extractable C, total fumigated and non-fumigated extractable C; CV, coefficient of variation of all samples (standard deviation divided by mean)
[b]Data transformed to meet assumptions of ANOVA.

Table 4.4. Total and residue-derived N pools (mean ± standard error, n = 5) in wheat plants for 12 winter wheat genotypes used in a greenhouse study with application of ^{15}N-labelled cover crop (*Vicia villosa*) residues to the soil. No genotype differences were detected using Tukey's test ($p > 0.05$).

Variety	Leaf N (mg g^{-1})	Root N (mg g^{-1})	Residue N in leaf N pool (% of total leaf N)	Residue N in root N pool (% of total root N)	N uptake (mg pot^{-1})	Residue N uptake (mg pot^{-1})
Byrd	16.1 ± 0.97	13.1 ± 0.42	17.2 ± 1.10	15.7 ± 1.42	59.2 ± 6.6	9.87 ± 1.16
Snowmass	15.7 ± 1.53	12.9 ± 1.04	16.2 ± 0.97	13.5 ± 0.78	54.4 ± 1.5	8.42 ± 0.62
Ripper	16.2 ± 0.73	15.3 ± 0.71	17.6 ± 0.36	16.7 ± 0.91	55.8 ± 3.5	9.70 ± 0.75
Ron L	16.1 ± 1.34	13.1 ± 0.75	15.4 ± 1.41	13.1 ± 1.25	56.6 ± 4.8	8.54 ± 1.27
Prowers	13.9 ± 0.31	13.8 ± 0.33	18.2 ± 0.91	17.4 ± 0.90	54.2 ± 3.4	9.65 ± 0.28
TAM 107	15.0 ± 0.85	13.0 ± 0.62	17.4 ± 1.08	16.9 ± 1.06	53.2 ± 4.8	9.06 ± 0.69
Sandy	13.3 ± 0.49	12.5 ± 0.52	16.9 ± 0.58	16.4 ± 0.87	53.3 ± 1.8	8.90 ± 0.43
Scout 66	14.8 ± 0.81	12.7 ± 0.48	16.4 ± 1.01	16.2 ± 2.37	66.3 ± 3.9	10.88 ± 1.27
Wichita	13.6 ± 0.95	13.4 ± 0.56	17.5 ± 1.05	14.9 ± 1.87	53.1 ± 4.7	8.80 ± 0.86
Cheyenne	15.1 ± 0.60	12.3 ± 0.62	18.5 ± 0.54	16.4 ± 0.65	58.1 ± 2.9	10.38 ± 0.59
Turkey Red	14.4 ± 1.06	13.2 ± 0.55	16.6 ± 0.49	13.9 ± 0.71	55.8 ± 5.6	8.82 ± 0.86
T. monococcum	17.5 ± 1.25	14.3 ± 0.94	15.6 ± 0.59	15.3 ± 1.13	50.6 ± 5.3	7.71 ± 0.52
Mean	15.2	13.3	16.9	15.5	55.9	9.2
CV[a]	0.15	0.12	0.12	0.19	0.17	0.21
ANOVA	*P*-values					
Genotype	0.12	0.14	0.35	0.19	0.57	0.35

[a]CV, coefficient of variation of all samples (standard deviation divided by mean)

Table 4.5. Spearman correlation coefficients (ρ) relating wheat root carbon (C), cover crop residue nitrogen (N), and rhizosphere microbial community metrics from a greenhouse study investigating twelve genotypes of winter wheat. Winter wheat was labelled with ^{13}C-CO_2, and cover crop residue with ^{15}N for tracing purposes. Values in bold are significant ($p < 0.05$), n=57-60 due to some missing observations.

	Root Length	Average Root Diam.	Wheat DOC	Wheat MBC	Residue N uptake	% Res. N wheat	Res. TDN	N-cycling enz.	*npr* gene copies
Root Length									
Avg. Root Diam.	-0.21								
Wheat DOC	0.19	0.19							
Wheat MBC	0.17	**0.31**	**0.61**						
Res. N uptake	**0.40**	0.15	0.24	0.19					
% Res. N wheat	-0.22	0.11	-0.17	**-0.28**	**0.49**				
Residue TDN	-0.07	-0.03	**0.48**	**0.35**	-0.03	-0.09			
N-cycling enz.	-0.17	**0.42**	**0.45**	**0.47**	0.06	0.04	**0.35**		
npr gene copies	0.08	-0.13	**0.27**	0.00	-0.08	-0.08	0.22	0.13	
Inorg. N	-0.21	0.09	-0.10	0.04	-0.06	0.09	**0.40**	**0.40**	0.07

[a]Avg. Root Diam., average root diameter; Rhizodep. C, concentration of wheat-derived C in the rhizosphere soil; Wheat DOC, wheat-derived C in K_2SO_4 extractable fraction of rhizosphere soil; Wheat MBN, wheat-derived C in chloroform-extractable fraction of rhizosphere soil; Res. TDN, cover crop residue-derived total dissolved N; N-cycling enz., sum of LAP and NAG enzyme activity in rhizosphere soil; *npr*, neutral metallopeptidase gene copy abundance; Inorg. N, sum of nitrate and ammonium.

Table 4.6. Mean enzyme activities in rhizosphere soil for twelve winter wheat genotypes used in a greenhouse study (n=5). ANOVA *p*-values for genotype effect are below, followed by relevant Spearman correlations, with significance: ns ($p > 0.05$); * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$)

	LAP[†]	NAG	BG	CB
Variety	----- nmol h^{-1} g^{-1} -----			
Byrd	35.0 ± 0.9	28.9 ± 1.7	70.4 ± 3.3	14.7 ± 0.6
Snowmass	40.0 ± 3.4	31.3 ± 3.8	81.3 ± 9.6	19.7 ± 2.5
Ripper	38.4 ± 3.5	25.2 ± 1.7	74.7 ± 7.3	18.2 ± 1.9
Ron L	38.8 ± 3.2	31.0 ± 2.9	77.2 ± 5.5	17.5 ± 0.9
Prowers	35.6 ± 2.2	28.1 ± 1.8	62.0 ± 4.5	17.1 ± 1.6
TAM 107	34.7 ± 2.4	22.8 ± 3.4	64.0 ± 4.1	16.4 ± 1.7
Sandy	34.1 ± 3.5	27.8 ± 1.7	70.4 ± 4.8	17.3 ± 1.3
Scout 66	37.0 ± 2.6	28.3 ± 2.4	79.2 ± 6.7	19.9 ± 1.5
Wichita	36.4 ± 2.9	31.0 ± 2.0	75.1 ± 6.1	19.9 ± 1.1
Cheyenne	36.2 ± 3.1	29.0 ± 2.5	78.5 ± 4.1	19.6 ± 2.0
Turkey Red	44.4 ± 4.8 a	34.0 ± 3.7	79.2 ± 5.3	19.8 ± 2.2
T. monococcum	34.3 ± 2.7	27.3 ± 3.1	71.0 ± 5.3	18.9 ± 2.1
Mean	37.1	28.7	73.6	18.3
CV	0.18	0.21	0.18	0.21
ANOVA	*p*-values			
Genotype	0.51	0.29	0.36	0.5
Select Spearman Correlations (ρ)				
Extractable wheat C	0.55***	0.36**	0.35**	0.37**
Inorganic N	0.22 ns	0.43***	0.24 ns	0.30 *

[†]LAP, Leucine aminopeptidase; NAG, N-Acetyl-glucosaminidase; BG, β-Glucosidase; CB, β-D-cellobiosidase; CV, coefficient of variation; Extractable wheat C, fumigated and unfumigated extractable C derived from wheat; DOC/DON, total DOC/DON in rhizosphere soil; wheat DOC/DON, wheat-derived DOC/DON in rhizosphere soil

Table 4.7. Spearman correlation coefficients (ρ) between rhizosphere prokaryotic genera abundance (cumulative sum square + log normalization) and environmental variables measured in a greenhouse study of winter wheat genotypes. Wheat contributions to soil carbon (C) and cover crop (CC) derived nitrogen (N) uptake were determined using stable isotope tracing. Significance of correlation coefficients is indicated with the following symbols: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Phylum[a]	Genus	Rhizodep.[b]	Extractable Wheat C	Root Length	N uptake	CC-N Uptake	TDN	N-cycl. enzyme	Normalized Avg. Abundance
Acidobacteria	*Rhizobacter*				0.33**	0.29*			5.37
Actinobacteria	*Aeromicrobium*	0.38**	0.37**	0.38**	0.48***				6.06
Actinobacteria	*IMCC26134*		0.27*	0.57***	0.61***	0.51***	0.27*		4.01
Actinobacteria	*Pseudomonas*	0.33*							7.25
Actinobacteria	*Saccharum_hybrid*	0.38**	0.48***	0.35**	0.47***		0.29*		3.02
Actinobacteria	*Sulfurifustis*	-0.45***	-0.42***		-0.27*				2.58
Alphaproteobacteria	*Asticcacaulis*				0.45***	0.35**	0.31*		2.67
Alphaproteobacteria	*Dyadobacter*	0.49***						0.29*	3.61
Alphaproteobacteria	*Iamia*	0.40**	0.40**	0.37**	0.59***	0.40**		0.26*	4.57
Alphaproteobacteria	*Polycyclovorans*				0.38**	0.33*			5.52
Alphaproteobacteria	*Steroidobacter*				-0.26*	-0.26*			7.47
Bacteroidetes	*Devosia*	0.26*	0.31*				0.44***		7.35
Bacteroidetes	*Flavobacterium*	0.34**	0.29*		0.36**	0.34**		0.36**	6.66
Bacteroidetes	*Gemmatimonas*	-0.32*	-0.39**		-0.29*				6.78
Chloroflexi	*Hirschia*	-0.32*						-0.37**	4.33
Deltaproteobacteria	*Anaeromyxobacter*		-0.27*	-0.42***	-0.42***	-0.29*			2.21
Deltaproteobacteria	*Bdellovibrio*	-0.33**			-0.44***	-0.28*			5.71
Gammaproteobacteria	*Acidibacter*				-0.27*				7.37
Gammaproteobacteria	*Arenimonas*	0.34**			0.32*				5.32
Gammaproteobacteria	*Cellvibrio*							0.28*	7.76
Gammaproteobacteria	*Luteolibacter*	0.27*	0.27*	0.38**	0.61***	0.46***	0.37**		4.43
Gammaproteobacteria	*MND1*	-0.46***	-0.50***	-0.26*	-0.58***	-0.35**		-0.28*	7.18

Gammaproteobacteria	*Pedobacter*	0.36**	0.37**	0.29*			0.27*		5.11
Gammaproteobacteria	*Promicromonospora*	0.48***	0.47***	0.37**	0.54***	0.30*	0.31*	0.34**	3.11
Gammaproteobacteria	*RB41*				0.29*				9.65
Gammaproteobacteria	*Rhizobium*		0.26*				0.33*		6.43
Gammaproteobacteria	*Streptomyces*	0.50***	0.37**	0.33*	0.36**		0.44***		7.5
Gammaproteobacteria	*SWB02*		-0.31*	-0.29*	-0.38**			-0.28*	3.03
Gemmatimonadetes	*Glycomyces*	0.44***	0.50***	0.38**	0.58***	0.29*	0.31*		3.86
Nitrospirae	*Panacagrimonas*		-0.33*	-0.43***	-0.28*				2.45
Omnitrophicaeota	*Candidatus_Omnitrophus*	-0.61***	-0.37**		-0.42***			-0.28*	6.03
Patescibacteria	*Skermanella*	-0.36**	-0.30*		-0.48***	-0.46***			6.26
Planctomycetes	*Cytophaga*	-0.32*							3.18
Verrucomicrobia	*Legionella*			-0.30*	-0.37**				2.96
Verrucomicrobia	*Massilia*	0.45***	0.43***		0.34**	0.30*		0.33*	8.38

[a]Except Proteobacteria, which is defined to Class

[b]Rhizodep. Concentration of wheat C in the rhizosphere; Extractable wheat C, sum of dissolved organic C and chloroform-extractable C; N uptake, total N uptake in mg pot^{-1}; CC-N uptake, total cover crop-derived N recovered in wheat tissue; TDN, total dissolved N in rhizosphere soil;; N-enzyme, sum of all LAP and NAG enzyme activity; Normalized avg. abundance; overall average abundance of each genus across all samples, normalized by cumulative sum of squares followed by log transformation

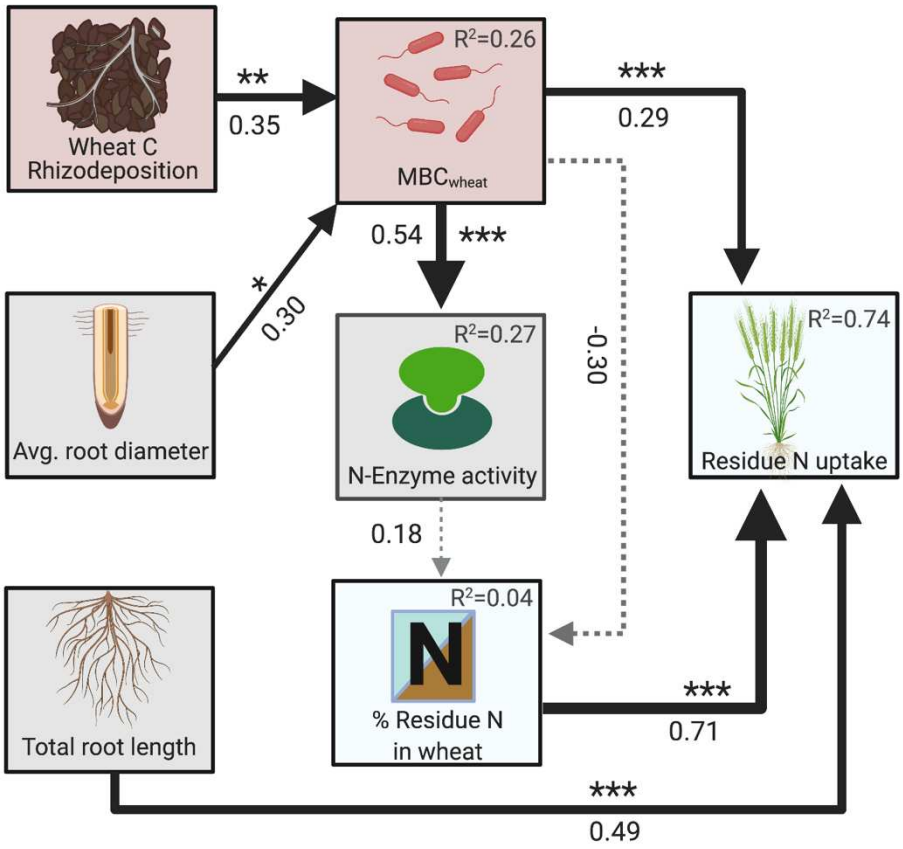

Figure 4.1. Structural equation model (SEM) relating wheat root traits, belowground carbon (C) allocation, nitrogen (N)-cycling enzyme activity and added cover crop residue N uptake in the growing wheat plants. Wheat and CC derived C and N pools were calculated using stable isotope tracing. Arrow thickness and color represents the strength and direction of relationship between the variables, labelled with the standardized parameter value and significance symbol. Black arrows are positive and red arrows are negative standardized parameter values. Significance labels: * ($p < 0.05$); ***($p < 0.001$). Dashed arrows indicate nonsignificant ($p > 0.05$) linkages that were retained in the model. R^2 values for each endogenous variable are included in the parameter tile. Rhizodeposition, concentration of wheat-derived C in rhizosphere soil; CC-N uptake, total cover crop-derived N recovered in wheat tissue; % Residue N in wheat, relative proportion of cover crop-derived N in wheat tissue N pool. Figure created with BioRender.com.

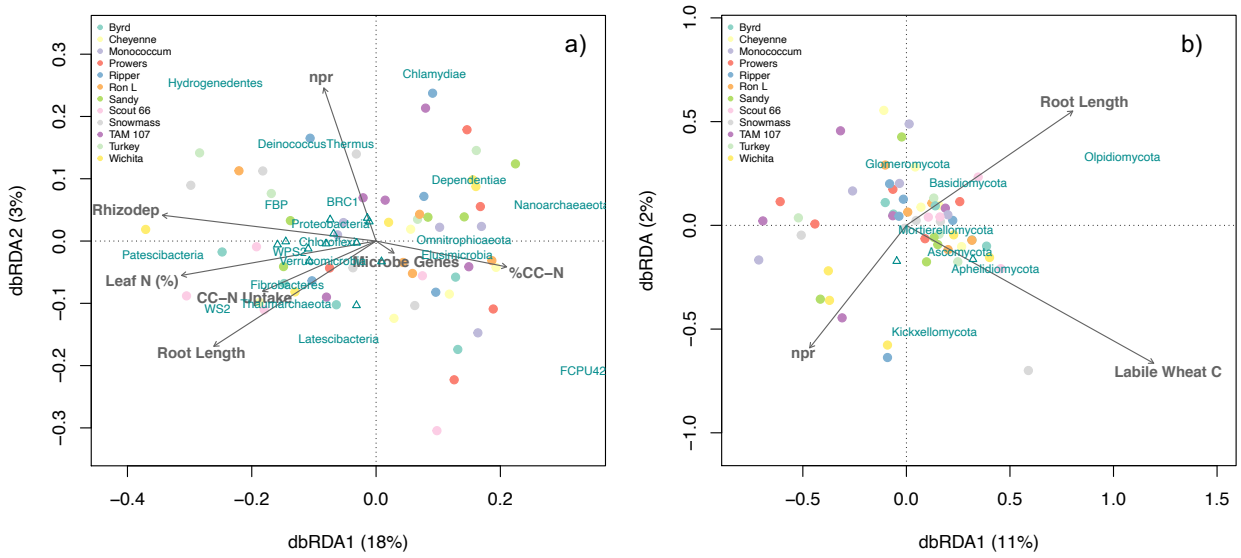

Figure 4.2. Distance-based Redundancy Analysis (db-RDA) relating **a)** bacterial family composition and **b)** fungal family composition to wheat plant and rhizosphere variables. Analysis was conducted using Bray-Curtis dissimilarities based on 16S and ITS fingerprinting and backwards selection to select most important variables. **Bacterial model results**: $R^2 = 0.31$, adj. $R^2 = 0.21$; Axis 1, $p = 0.001$; Axis 2, $p = 0.53$. Label abbreviations and ANOVA p-values for model parameters: Root Length, total wheat root length (p=0.07); %CC-N, relative proportion of leaf N from labelled cover crop ($p = 0.018$); CC-N uptake, total cover crop-derived N in wheat tissue (p=0.012); Rhizodep, wheat-derived C in rhizosphere soil ($p = 0.028$); Microbe Genes, sum of 16S and ITS gene abundance ($p = 0.07$); npr, neutral metallopeptidase gene abundance in rhizosphere (p=0.047)**. Fungal model results**: $R^2 = 0.17$; adj. $R^2 = 0.13$. Overall model $p = 0.001$; Axis 1, $p = 0.001$; Axis 2, $p = 0.67$. Variable explanation and significance: Root Length ($p = 0.033$); Labile Wheat C, sum of dissolved organic C and microbial biomass C ($p = 0.001$); npr, abundance of neutral metallopeptidase gene ($p = 0.095$).

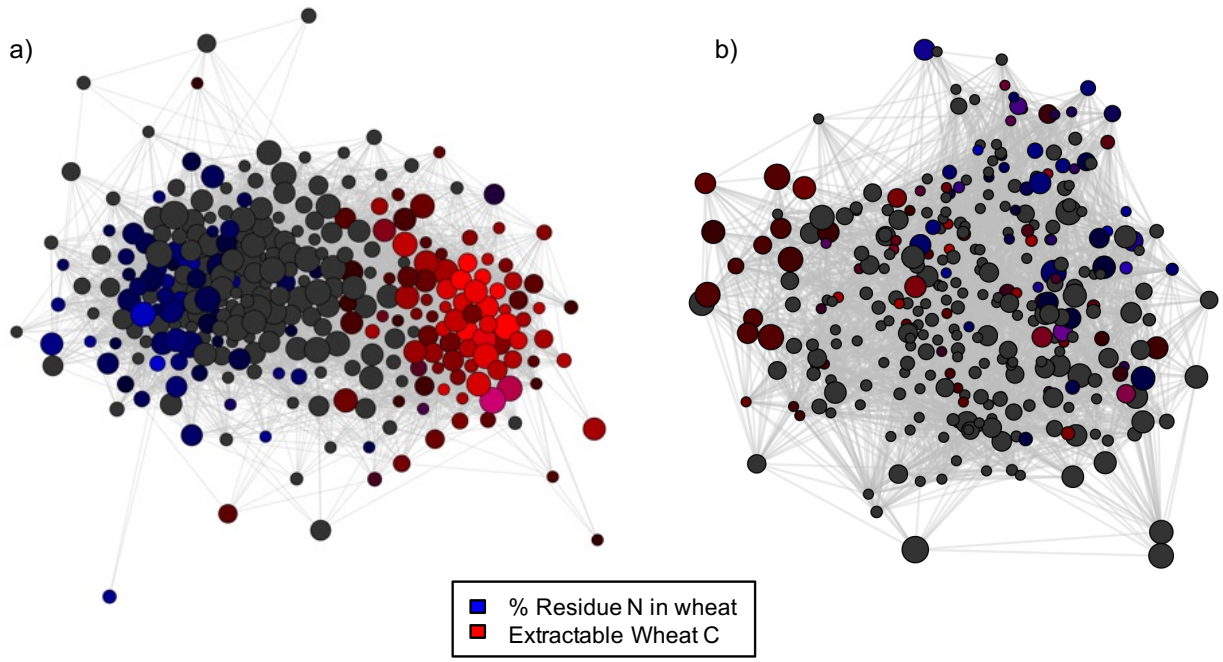

Figure 4.3. Correlation network analysis of (a) bacterial genera and (b) fungal ASVs placed by co-occurrence and colored by correlation with plant and soil variables. Degree of color saturation is proportional to positive Spearman correlation with Labile Wheat C (red) or proportion of cover crop N in wheat tissue (blue). The size of each node (circle) is proportional to the relativized abundance of taxa that it represents, and node placement is by dissimilarity matrix distance. Edges connect positively correlated nodes with Spearman $\rho > 0.25$. Extractable Wheat C, sum of dissolved organic C and microbial biomass C from wheat; % Residue N in wheat, the relative fraction of wheat N derived from added legume residue.

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CHAPTER 5: WHEAT GENOTYPES CHANGE BELOWGROUND C ALLOCATION, RHIZOSPHERE RECRUITMENT, AND N ACQUISITION WITH DIFFERENT SOIL NUTRIENT LEGACY

5.1. Introduction

There is a growing understanding that leveraging biologically-mediated nutrient cycling in agroecosystems can increase many aspects of soil and environmental health while meeting crop nutrient demands (Drinkwater et al., 2017). Greater reliance on nitrogen (N)-fixing cover crops, compost, and other organic amendments as crop nutrient sources can increase soil organic matter (SOM) and multiple aspects of ecosystem health while helping to avoid deleterious nutrient losses like leaching, erosion, and N_2O emissions (Abbott and Manning, 2015; Kumar et al., 2018). This approach to soil management relies on soil organisms to process organic amendments and release plant-available forms of limiting nutrients, such as N, through the process of enzyme production, microbial assimilation, and biomass turnover, and on their N-fixation abilities (Kuypers et al., 2018; Kuzyakov and Xu, 2013).

Plants allocate C belowground into physical root structures and through rhizodeposition pathways like exudation. Increased root length and surface area directly enhance the foraging area of roots, while exudation improves nutrient availability indirectly by stimulating microbial activity and mineralization (Barber and Silberbush, 2015; Zhu et al., 2014). Root exudates are comprised of primary (carbohydrates, organic acids) and secondary metabolites (flavonoids, glucosinolates, etc.) and are estimated to constitute 11-40% of fixed C (Vives-Peris et al., 2020; Badri and Vivanco, 2009). The relative allocation of belowground C to rhizodeposits has been

proposed as a key root trait, or plant economic strategy, that regulates nutrient cycling and availability in the rhizosphere (Henneron et al., 2020).

There is growing evidence that, via exudate release patterns and composition, plants can cultivate distinct rhizosphere communities that may impart benefits to plant health (Chaparro et al., 2012). In addition to the clear mutualistic relationship between root-modulating plants (e.g. legumes) and N-fixing rhizobia, there is evidence that plants also recruit taxa that stimulate productivity through disease suppression, nutrient mobilization, hormone interaction, and other signaling pathways (Chaparro et al., 2012; Zhou et al., 2016). Rhizosphere communities are influenced by plant species and plant growth stage, and recent evidence suggests differences in rhizosphere communities between genotypes or cultivars within a species (Berendsen et al., 2012; Tkacz et al., 2015; Brisson et al. 2019).

Breeding efforts have led to substantial changes in agronomically-important plant traits, but the effects of selection in reshaping plant relationships with belowground communities remains poorly understood. For example, distinct rhizosphere recruitment has been observed across wild relatives, inbred lines, and modern cultivars of maize, and among different cultivars of durum wheat (Brisson et al., 2019; Iannucci et al. 2021). In some cases, changes in rhizosphere recruitment has been linked to specific benefits for plant health, as has been found for fungal resistance in common bean (*Phaseolus vulgaris* L.) which may be mediated by anti-fungal properties of the rhizosphere microbial community (Mendes et al., 2018).

The native soil environment also exerts a strong influence on the composition and activity of the rhizosphere microbiome, and when coupled with interactive effects of soil on plant growth, leads to complex relationships between plants, soils, and the microbial community. The native bulk soil supplies the initial pool of potential community members from which plant

traits may enrich or deplete (de Vries and Wallenstein, 2017; Weinert et al., 2011). Importantly, soil nutrient status has a strong regulating effect on the activity of the background microbial community, as a field study of different nutrient management in tomato fields found that enzyme activities were highly dependent on available C and inorganic N (Bowles et al., 2014). At the same time, soil amendments and nutrients status influences plant root and rhizodeposition patterns, and likely in a non-linear fashion (Bowsher et al., 2018).

As a globally important staple crop in semi-arid agricultural regions, wheat supplies about 21% of global food production (www.fao.org). Wheat has a complex domestication history as the result of three genome duplication events, which has resulted in relatively little genetic diversity within breeding populations (Peng et al., 2011). However, despite wheat's evolutionary bottleneck, modern breeding efforts have documented substantial diversity in the morphology and trait diversity within wheat lines under different environmental contexts (Awad et al., 2017; Becker et al., 2016; Latshaw et al., 2016). As crop breeding efforts work to improve yields under various environmental conditions, it is important to consider the soil nutrient management context and how different root traits and rhizosphere communities may respond. This issue is particularly relevant as more farmers become interested in managing for soil health, including organic nutrient strategies that can alter the timing and availability of crop nutrients. To best support farmers in this transition, there is a need to understand whether crop traits differ among genotypes in ways that influence their performance under organic nutrient management strategies. We sought to explore this question using winter wheat and assess the relative plasticity of resource acquisition strategies under soils with different nutrient management legacies.

The objective of this study was to understand the relationship between belowground C allocation patterns in wheat, rhizosphere microbial community structure and activity, and the acquisition of N from an organic nutrient source (i.e., cover crops) in soil with contrasting nutrient management legacies. Using dual isotopic labelling (^{13}C and ^{15}N) within a greenhouse setting, we examined the interactive effect of wheat genotype and long-term compost amendment on belowground C allocation patterns, which we further linked to rhizosphere microbial communities and N cycling activities. We hypothesized that wheat genotypes modulate root growth and exudation patterns in response to SOM and nutrient availability, leading to differences in belowground C allocation between soils. We predicted that high-SOM soils, with higher biological activity and mineralization rates, would elicit lower levels of C allocation. We also hypothesized that genotypes vary in their phenotypic plasticity of belowground C allocation in response to different soil conditions. Specifically, we predicted that a high-exudation strategy would change the most between soils because it is microbially-motivated and may be more responsive to native microbial conditions. We also hypothesized that rhizodeposition selects for specific microbial taxa, and so predicted that higher exudation would result in recruitment of specific microbial taxa. Finally, we hypothesized that through stimulation of microbial activity like extracellular enzymes, rhizodeposition stimulates the mineralization of residue-derived N, increasing plant availability and uptake of residue N. We predicted that a high-exudation strategy would be more successful at acquiring fresh residue N in a high organic C context, as the higher background nutrient content would prevent short-term immobilization by the microbial community in response to wheat C.

5.2. Materials and methods

5.2.1 Experimental design and plant material

Soils for the greenhouse experiment were collected from a dryland field trial at the USDA-ARS Central Great Plains Research Station in Washington County, Colorado, USA (40°09'22.4"N 103°08'26.1"W, altitude 1,384 m) and are classified as a Weld silt loam (fine, smectitic, mesic Aridic Argiustoll). The plots had been under a wheat-fallow rotation for 10 years with biennial applications of beef feedlot compost at a rate of 0 (control) or 108.7 Mg ha^{-1}, which corresponds to five times the expected crop N demand (Calderón et al., 2018). The plots were managed without synthetic herbicides or fertilizers, and weeds were controlled with shallow sweep tillage (ca. 8 cm depth) twice each summer. Topsoil was collected from 0-10 cm depth from multiple locations within the two treatment plots, sieved to 2 mm, and air-dried. Initial soil characteristics were evaluated (Table 5.1) and include: total soil C and N (measured by combustion using an elemental analyzer (LECO Corp, MI, USA)); ammonium and nitrate (extracted with 2M KCl and analyzed colorimetrically); buffered pH (using a 1:1 modified Woodruff buffer solution; (Brown and Cisco, 1984) and soil texture (determined by hydrometer; (Gee and Bauder, 1986). The air-dried soils were mixed with clean 40-grit quartz sand in a 2:1 ratio (2 parts soil:1 part sand by weight). Roughly 4.5 kg of the soil-sand mixture from the two soil treatments was added to cylindrical mesocosms (50 cm height, 10 cm diameter). Each mesocosm was capped at the bottom using plastic sleeve with slits cut for drainage. The two soil treatments were ultimately created for this design: soils receiving 108.7 Mg ha^{-1} compost biennially (5x) and control soil with no recent history of compost application (0x).

Isotopically-labelled plant residue material was generated by growing a mixture of hairy vetch (*Vicia villosa*) and Triticale (*xTriticoscale*) grown in pure quartz sand with N-free

Hoagland's solution (Hoagland and Arnon, 1950) and N supplied as 9 atm% ^{15}N-KNO_3 (Cambridge Isotopes, MA, USA). The cover crop residue was harvested at vetch flowering after ~ 2 months of growth, dried in paper bags at 55 ºC, and chopped with scissors into ~2cm pieces. We incorporated the ^{15}N labelled cover crop residue into the top 15 cm of soil at a rate of 0.58 g oven-dry biomass mesocosm $^{-1}$ (equivalent to 1500 kg ha^{-1}), which is within the typical range for dryland cover crop production in the semi-arid Great Plains (Kelly et al., 2021). The cover crop residue had an average N content of 21.2 mg N kg^{-1} with an enrichment of 6.4 atm% ^{15}N.

We selected two genotypes of winter wheat with distinct rooting and belowground C allocation characteristics determined previously ((Kelly et al., *in review*.)). The Byrd cultivar is drought-tolerant cultivar released in 2011 with high root biomass, while The Snowmass cultivar is a more drought susceptible cultivar released in 2009 with high rhizodeposition and a relatively short, coarse root system (Haley et al., 2012, 2011). These genotypes therefore exhibit different belowground C allocation strategies that may alter the role of microbial activity in nutrient acquisition. The Wheat Genetics Resource Center at Kansas State University provided the original seed for each genotype, which we then grew out in a common environment at the Agricultural Research, Development & Education Center (ARDEC) near Fort Collins, Colorado to produce seed for the experiment. We vernalized wheat seeds by sprouting surface sterilized seed on moistened blotting paper inside plastic bags for 3 days, and then storing the sprouted seed in the dark at 4 ºC for 8 weeks. Prior to transplanting, the prepared mesocosms were wetted to water holding capacity and incubated in the greenhouse for 7 days. Three wheat plants were transplanted to each pot and left to establish for 6 days before thinning to two plants per pot and placing the pots into ^{13}C-CO_2 labelling chamber. Six replicates of each genotype × soil treatment

as well as unplanted controls (with residue incorporated) were arranged in a randomized design within the labelling chamber, resulting in a total of 24 experimental pots and 12 control pots.

5.2.2 Isotopic labeling

The wheat plants were grown in a large, transparent plexiglass chamber located in the Plant Growth Facilities at Colorado State University using continuous flow ^{13}C-CO_2 labelling (Soong et al., 2014), and modified to allow independent injections of ambient and 10 atm% ^{13}C CO_2 (Cambridge Isotope Laboratories, Inc., MA, USA). Enrichment of the chamber was kept at approximately 4.5 atm% ^{13}C-CO_2 and maintained between 360-400 ppm during the day when drawdown was occurring, with higher levels reached overnight due to respiration. We measured chamber enrichment regularly on a Delta V AdvantageIRMS, coupled to a Gas Bench II (Thermo Fisher Scientific). Field corn (*Zea mays* L.) grown in quartz sand acted as an additional C sink on the opposite end of the chamber to help control for ^{13}C-CO_2 dilution by respiration from the soil and cover crop residues. Pots were watered 1-2 times per week with tap water using a drip irrigation system based on visual assessments of soil wetness and plant stress. Unplanted control pots were watered less frequently using a separate line, due to the absence of transpiration. An air conditioning unit and dehumidifier were used to maintain temperature at an average 23 ºC and relative humidity between 40-60%. Fluorescent grow lights were used to supplement natural light and provide 16 hrs of light per day.

5.2.3 Plant and soil harvest

We harvested wheat plants at the heading/flowing stage, 54 days post-transplant. We made detailed notes on plant survival, tillering, number of reproductive heads visible and the

height of the tallest leaf. We then clipped plants at the soil surface and transferred this aboveground material to paper bags for drying. Intact soils and roots were removed from the mesocosms by gently sliding out the plastic sleeve and then roots were harvested by carefully loosening the soil column to minimize root damage. Roots and attached soil were transferred to a zip-top plastic bag and kept at 4 ºC. Roots were subsequently removed and cleaned (detailed below), thus leaving rhizosphere soil for further analysis. Fresh rhizosphere soil was stored at 4 ºC for ~ 1 week before subsequent microbial biomass and enzyme analyses (described below) or freezing in lysis buffer and storing at -20 ºC in preparation for DNA extraction. The non-rhizosphere bulk soil was collected separately, sieved for roots, and air-dried for storage and subsequent analysis.

We cleaned the roots with tap water over a 1 mm sieve, removing attached soil-derived organic fragments with forceps. Clean roots were stored in 35% reagent alcohol mixed with 0.03% Neutral Red Dye at 4 ºC until image analysis (see details below). The aboveground plant material and the roots (after imaging) were oven-dried at 60 ºC for determination of dry biomass and isotopic analyses (described below). Root mass fraction was calculated as the ratio of root biomass to that of the whole plant. Soil and plant material was ground in preparation for elemental and isotopic analysis.

5.2.4 Root scanning and image analysis

To stain roots more deeply in preparation for scanning, we submerged each root system in a 1% Neutral Red Dye solution for 1 min. before rinsing and arranging in scanning trays. Root scans were analyzed using WinRHIZO v. 2009 (Regent Instruments, Quebec, Canada). Root systems were divided into eight diameter size classes (0-50 μm, 50-100 μm, 100-150 μm, 150-

200 μm, 200-250 μm, 250-300 μm, 300-350 μm, and >350 μm), which were determined to be appropriate for our samples in order to obtain a relatively even distribution in each size class. Additional measurements generated from the root scanning include total root length, average root diameter, surface area, and root volume. We calculated size class fractions by dividing the total root length in each size class by the total root length of the system.

5.2.5 Soil microbial biomass and enzyme activity

Chloroform-extractable C and N and enzyme activity were measured on fresh rhizosphere soil to obtain estimates of microbial utilization of C from wheat rhizodeposits and residue-derived N, as well as the hydrolytic enzyme capacity within the rhizosphere soil. We used the chloroform fumigation-extraction method following Vance et al. (1987). Briefly, each fresh rhizosphere soil sample was divided into two 10-g subsamples, the first being immediately extracted, and the second subjected to 24 hr of fumigation with vaporized chloroform in a sealed vacuum chamber. Both control and fumigated samples were extracted with 50 mL 0.05M K_2SO_4 on a rotary shaker at 200 rpm for 2 hr and then gravity filtered through a Whatman 40 filter (8 um). A subsample of the control extracts were analyzed for nitrate and ammonium using a Alpkem Flow Solution Automated wet chemistry system (O.I. Analytical, College Station, TX). We froze all extracts at -20 ºC and then freeze-dried the samples at -80 ºC to collect the resulting salts for isotopic and elemental analysis. The unfumigated control samples were considered an estimation of dissolved organic C (DOC) and total dissolved N. Microbial biomass C (MBC) and N (MBN) concentrations were estimated by subtracting the unfumigated control values from those of the paired fumigated samples; because the experimental soils were all collected from the same location, we assumed an extraction efficiency of 1.

We evaluated soil enzyme activity to assess the *in-situ* hydrolytic capacity of the rhizosphere soil and its response to plant growth. Carbohydrate-degrading, protein-degrading, and phosphatase enzymes were assessed. We determined enzyme activity fluorometrically using substrates bound to fluorescing agents as described by DeForest (2009) for the following enzymes and their respective element cycling activities: *L*-leucine aminopeptidase (LAP), *L*-Tyrosine aminopeptidase (TAP), and N-Acetyl-β-D-glycosaminidase (NAG) indicate N-cycling activities; β-1,4-glucosidase (BG) and β-D-cellobiosidase (CB) indicate C cycling activities; phosphatase (PHOS) indicates P-cycling activities. Briefly, we combined 1 g rhizosphere soil with 120 mL 50 mM sodium acetate buffer in a blender for 1 min to create slurries, which were incubated with substrates in 250 μL reactions with 16 replicates for 4 hours. Quenching was estimated by reacting the un-bound 4-methylumbelliferone or methylcoumarin fluorescing agent with the soil slurry, and substrate mixed with buffer was used to estimate background fluorescence.

5.2.6 Isotope calculation and mixing model

We used mixing models to determine the relative contribution of labelled source material (^{13}C for wheat C, ^{15}N for residue N) to our measured C and N pools of interest. All soil, plant, and dried salt extracts were analyzed for total C, ^{13}C, total N and ^{15}N at the UC Davis Stable Isotope Facility using a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Ltd., Cheshire, UK). The relative contribution of isotopically-labelled source material (^{13}C in growing wheat plants or ^{15}N in legume residue) to various C and N pools were calculated using atm% values in a mixing model equation:

$$f_{label} = \frac{(atm\%_{sample} - atm\%_{control})}{(atm\%_{label} - atm\%_{control})}$$

where f_{label} is the relative contribution of the labeled source to the sample, $atm\%_{sample}$ is the atom% of the sampled material, $atm\%_{control}$ is the atom% of the natural abundance control, and $atm\%_{label}$ is the atom% of the labelled source material (either the wheat plant (^{13}C) or residue (^{15}N)). The natural abundance control values for ^{13}C calculations were the unplanted control pot values from the same soil treatment to account for any non-plant C-fixation. Natural abundance ^{15}N values for the 5x soil was different from the control (0x) soil, and thus a different control value was used for these calculations (Table 5.1). The contribution of enriched end-members (wheat or residue) to different C or N pools was calculated by multiplying the f-value by the total concentration of C or N. Wheat-derived MBC was calculated by subtracting the total wheat-derived C in unfumigated control extracts from wheat-derived C in chloroform-fumigated extracts.

5.2.7 Statistical analysis of plant and soil parameters

We used two-way ANOVA to test for the effect of wheat genotype, soil treatment, and their interaction on our plant, soil, and biological parameters. Tukey's test was implemented to determine pairwise differences between treatments. An alpha value of $p < 0.1$ was used to determine statistical significance. Log transformations were applied as needed to meet the assumptions of ANOVA. All statistical analyses were performed in R version 4.0.3 (R Core Team, 2020), and plots were constructed using ggplot2 (Wickham et al., 2018).

5.2.8 Microbial amplicon sequencing and analysis

Genomic DNA was extracted from rhizosphere soil using the Quick-DNA Fecal/Soil Microbe kit (Zymo Research Corporation, Irvine, CA) following manufacturer's instructions.

Amplicon libraries were prepared for the 16S rRNA region using the 515/806 Earth Microbiome Project standard primer pair (Caporaso et al., 2011), and the V3-V4 region of the ITS gene (ITS-2; White 1990). Extracted DNA was quantified using the Qubit ds DNA High Sensitivity quantification system (Invitrogen). The Colorado State University Sequencing Core constructed the libraries and sequenced paired-end reads (250 bp) using an Illumina MiSeq. Sequence data is available in the NCBI SRA database under project ID PRJNA735275.

We processed the demultiplexed sequences using QIIME2 version 2019.10 (Caporaso et al., 2010). Amplicon sequence variants (ASVs) were determined using DADA2 (Callahan et al., 2016) using paired-end reads trimmed to 206 bp (forward) and 180 bp (reverse) for 16s, and using only forwards reads trimmed to 200 bp to improve alignment. We assigned taxonomy to ASVs using a Native Bayes classifier trained on the study-specific primer pairs (Bokulich et al., 2018). Classifiers used the 99% similarity SILVA databased for 16s sequences and the UNITE reference database version 8.2 for fungal ITS sequences. Unidentified sequences were removed from both datasets, and mitochondrial and chloroplast sequences were removed from the 16S dataset through filtering.

Diversity metrics were calculated using datasets rarefied to an even sampling depth; the 16S dataset was rarefied to 2027 reads per sample, and the ITS to 13,709 to maximize sequences while minimizing the samples with read counts that fall below the threshold and thus must be excluded. Total taxa richness, Shannon diversity, and Pielou's evenness were computed for both datasets (Pielou, 1966; Shannon and Weaver, 1949), and Faith's phylogenetic diversity for the 16S dataset only, as the ITS marker gene is considered too variable for tree construction (Faith, 1992; Simmons and Freudenstein, 2003). We determined wheat genotype and soil legacy effects on diversity metrics using two-way ANOVA.

We performed data filtering and normalization on relative read counts to prepare microbial sequence data for statistical analysis. Samples with less than 1000 reads were removed, as were taxa with less than 0.01% relative abundance to remove likely errors. Filtered datasets were normalized using cumulative-sum scaling followed by a $\log_2$ transformation to account for count data distribution (Paulson et al., 2013).

Differences in prokaryotic and fungal community composition were analyzed using Bray-Curtis dissimilarities calculated from normalized abundances, a metric appropriate for community data that computes distances between samples (Sorensen, 1948). We used principal coordinate analysis (PCoA) to plot sample dissimilarities in space, and PERMANOVA to statistically test the effect of our experimental factors on overall bacterial and fungal community composition. Data normalizing and PCoA plotting was performed using Calypso (version 8.84) using default parameters (Zakrzewski et al., 2017). PERMANOVA was completed using the *adonis* function in the *vegan* package in R (Oksanen et al., 2020).

5.2.9 Indicator species analysis

Indicator taxa analysis was used to identify microbial families most associated with a wheat genotype and soil management treatment, as well as each genotype × soil treatment combination. Indicator taxa are those that are found more frequently and in higher abundance in certain conditions, and thus may be used to indicate those same conditions (Dufréne and Legendre, 1997). Species occurring in less than three samples were removed from the analysis to avoid bias due to rare taxa. The Indicator Value Index was calculated for each bacterial or fungal family within each environmental group (i.e. wheat genotype, soil, or the treatment combination), which integrates abundance and frequency to create a value of specificity to that

environmental group using the *multipatt* function in the *indicspecies* package (Cáceres and Legendre, 2009). A level of $\alpha = 0.05$ was used to determine significance using 999 permutations.

5.3. Results

5.3.1 Wheat morphology

Total wheat root and shoot biomass did not vary across treatments, but we did observe a significant effect for the root mass fraction, or relative proportion of root biomass compared to the whole plant (Fig. 5.1a; Table 5.2). Specifically, root mass fraction showed a genotype, soil treatment, and interaction effect, such that the Snowmass cultivar had a greater proportion of biomass as roots and had a greater response to soil treatment (Fig.1a). The Snowmass cultivar grown in amendment-free (0x) soil had the greatest proportion of biomass as roots (7.8 ± 0.8%), 42-81% more than other treatments (Fig. 5.1a). Total root length varied by genotype, with the Snowmass cultivar having 62% greater total root length than the Byrd cultivar, while no soil or interaction effect was observed (Table 5.2). We saw no effect of genotype or soil legacy on total root surface areas or specific root length. Average root diameter across both genotypes was 4.1% greater in the 5x soils (Table 5.2).

When plant roots were divided into different size classes, the cumulative root length in each size class followed a similar trend to total root length, with Snowmass having greater root length in all except the three largest size classes (i.e., >250 μm), with no effect of soil treatment (Table S5.1). However, unlike total length, the fraction of root length in each size class demonstrated soil effects for all size classes below 300 μm, with a greater percentage of fine roots (0-150 um) in the 0x soils, and a greater proportion of medium-coarse roots (150-300 μm) in the 5x soils (Table 5.3). A genotype effect on proportion of roots in each root length fractions

was present only in the smallest and largest size classes, where Snowmass had a greater proportion of the finest roots (0-50 um), and Byrd a greater proportion of the coarsest roots (>350 μm; Table 5.3). There were no significant interactive effects between genotype and soil on total root length or the relative fraction for any of the size classes.

5.3.2 Wheat-derived C in soil pools

We did not observe genotype or soil treatment effect on total wheat-derived C in rhizosphere or bulk soil (Table 5.4). At the same time, dissolved organic C (DOC) derived from wheat exhibited both a genotype and soil treatment effect. Overall, wheat-derived DOC was 3x higher in the 0x soil treatment and 2 times greater with the Snowmass cultivar (Fig. 5.1b). The genotype effect on DOC was generally more pronounced in the 0x soils. When we calculated the wheat-derived DOC relativized per gram of total wheat biomass, we found a significant effect of soil treatment and a genotype x soil interaction (Fig. 5.1c). Snowmass had over 2x higher relative DOC than Byrd in the 0x soil, while conversely in the 5x soil, Byrd had 26% greater relative DOC Snowmass. Wheat-derived microbial biomass C (MBC) did not change with wheat genotype, though was almost 3-fold greater in 0x microcosms compared to 5x (Table 5.4). Total rhizosphere C and DOC was both about 1.5-fold greater in the 5x soils compared to the 0x. Average isotopic enrichment of the wheat plant biomass was 3.54 (standard deviation 0.083) atm% ^{13}C.

5.3.3 Nitrogen dynamics

Total wheat N concentration and uptake did not differ by genotype or soil treatment, with an overall average wheat tissue concentration of 26.9 g kg^{-1} N, and average total N uptake per

mesocosm of 64.8 mg (Table 5.5). However, residue-derived N in the wheat plants (per mesocosm) showed an interactive effect of soil and genotype, such that the Byrd cultivar took up 1.45 times more residue-derived N than Snowmass in the 0x treatment, but the relationship flipped in the 5x treatment, where Snowmass took up 1.8 times more residue N than Byrd (Fig. 2a). Residue-derived N as a fraction of total aboveground plant N was 63% greater in 0x soil than 5x soils across both genotypes, but was low overall, with residue-derived N averaging less than 1% of the total wheat N uptake (Table 5.5).

Residue N in the rhizosphere soil was different between genotypes and showed a genotype × soil interaction (Table 5.5), such that Byrd rhizosphere soil had more residue-derived N than Snowmass and this difference was most pronounced in the 0x soils. Dissolved soil N pools did not demonstrate an effect of genotype, and the only difference based on soil treatment was in total dissolved N (TDN) in rhizosphere soil (Table S5.2).

5.3.4 Enzyme analysis

Enzyme activities for LAP, NAG, BG, CB, and PHOS were elevated in the 5x soil for most tested enzymes, with increases between 24 and 59% compared to 0x (Table 5.6; Fig. 5.2b). LAP activity also demonstrated a genotype x soil management interaction, such that Snowmass had generally higher LAP activity in the 0x treatment, with genotype differences less apparent under 5x (Fig. 5.2b). TAP activity did not differ with soil treatment or genotype.

5.3.5 Microbial community diversity and composition

Rhizosphere microbial diversity responded to soil treatment but not wheat genotype, demonstrating reduced bacterial/archaeal diversity and increased fungal evenness in 5x soils

(Table 5.7). Rhizosphere soil from the 0x treatment exhibited roughly twice as many bacterial/archaeal ASVs as the 5x compost treatment soil, and a 50% increase in Faith's phylogenetic diversity (Table 5.7).

Overall microbial species composition was highly affected by soil treatment, whereby both bacterial and fungal communities were different according to soil legacy (PERMANOVA $p < 0.01$; Fig. 3). Fungal communities in the 5x soil were particularly distinct from the 0x, as displayed by clear separation in principal coordinate analysis (PCoA; Fig. 5.3c). Similar to the diversity metrics, there was no effect of wheat genotype on overall species composition (PERMANOVA $p > 0.1$).

While overall species composition did not respond to genotype, indicator species analysis reveals that several bacterial and fungal families were closely associated with a specific wheat genotype, soil treatment, or a combination of genotype and soil treatment (Fig. 5.4, Fig. 5.5). A total of nine bacterial families were indicative of Snowmass, while Byrd had only one bacterial indicator taxa (Fig. 5.4). In line with the results from the PERMANOVA, there were many fungal families associated with specific soil treatments, and relatively few with wheat genotypes (Fig. 5.5). The Snowmass cultivar had seven fungal families as indicator taxa across all treatments (four highly significant, shown in Fig. 5.5), while Byrd had four indicator taxa only in combination with the 0x soil, and only one highly significant (Fig. 5.5; Table S5.4). Due to the high number of fungal indicator taxa identified, only the most significant ($p < 0.01$) taxa are reported within different soil treatments in Fig. 5.5; the complete list of fungal indicator taxa is reported in Table S5.4.

5.4. Discussion

The aim of this study was to evaluate the relative success of two contrasting belowground C allocation strategies employed by different winter wheat genotypes and the ability to access residue-derived N in high and low SOM contexts. We combined stable isotope techniques with assessment of microbial community structure and function to provide novel insight into plant-soil-microbe interactions in two contrasting native soil environments. These interactions are important for driving nutrient availability and may be especially important in changing soil health paradigms.

5.4.1 Genotype controls on rhizosphere strategy

The two genotypes of winter wheat used in this study exhibited clear differences in belowground C allocation and in plasticity of responses to soil management legacy. Specifically, we found that the Snowmass cultivar had a greater proportion of biomass as roots and greater wheat-derived DOC than the Byrd cultivar (Fig. 5.1). We interpreted wheat-derived dissolved organic C (DOC) as an indicator of root exudate C, recognizing that our experimental design does not allow us to quantify total exudate C due to decomposition of rhizodeposits (including exudates) over the course of the experiment and associated losses through respiration.

The two genotypes evaluated in this study showed different levels of belowground C allocation (Fig. 5.1), suggesting that each genotype has different mechanisms for interacting with the rhizosphere microbial community. This difference suggests contrasting resource-use strategies in terms of belowground C allocation patterns. Resource-use and plant economic theory identifies a tradeoff between acquisition and conservation of resources, which creates feedbacks in nutrient availability (Grime, 2006). Plant economic theory can be extended to root exudation, whereby high exudation is a resource-intensive acquisition strategy to increase

nutrient availability by stimulating microbial activity (Guyonnet et al., 2018; Henneron et al., 2020). Using grassland plant species from various functional groups, Henneron et al. (2020) found evidence of two distinct resource acquisition strategies between different plant functional groups, as well as evidence that the plants with high-exudation increased turnover of the microbial N pool, contributing to increased N uptake. Iannucci et al. (2021) explored this idea further using within-genotype variability across eight cultivars of durum wheat [*Triticum turgidum ssp. durum* (Desf.)]. The authors found differences in exudation amount between durum wheat cultivars resulting in high- and low-exudation groups, and a positive relationship between exudation amount and bacterial abundance. Our results corroborate the idea that distinct nutrient-acquisition strategies are based on differing levels of exudation and microbial community stimulation (Fig 5.2a; Fig. 5.4,5.5). Using this framework, we propose that the high-exudate genotype, Snowmass, falls into the "acquisitive" category with a more facilitative or mutualistic relationship with the rhizosphere microbiome, while the low-exudate genotype Byrd may be considered "conservative" with respect to root exudation and microbial partnerships, perhaps leading to a more competitive relationship with the microbial biomass.

4.2 Belowground C allocation plasticity and responses to soil legacy

In this study, we observed striking differences in phenotypic plasticity depending on genotype. The high exudation genotype, Snowmass, showed dramatic changes in root mass fraction and exudation metrics with soil type, compared to the Byrd cultivar (Fig. 5.1). Snowmass similarly showed high rates of exudation in a related study, while root architecture was different than that observed here, suggesting that the high exudation capacity is conserved across environments (Kelly et al., *in review*). Therefore, it appears that phenotypic plasticity is an

important component of this genotype's nutrient acquisition strategy, though it is unclear how plasticity in root structure and rhizodeposition are related. While we found higher plasticity in root mass fraction and relative exudation in our high-exudation genotype (Fig. 5.1), we only evaluated a subset of genotypes within a single, high-cultivated species. Therefore, further research is needed to understand the link between phenotypic plasticity and plant economic strategies related to nutrient acquisition.

Our results supported our hypothesis that root exudation responds negatively to soil nutrient amount. High rates of available N and N mineralization in soils with high SOM likely precludes the need for root stimulation of mineralization through exudation. Additionally, high nutrient availability decreases the need for expansive root systems for foraging and may also reduce root system size. Previous work has found soil nutrient limitation to lead to release of specific exudate compounds that may increase nutrient availability, while reducing release of limiting elements (Carvalhais et al., 2011). Our results support the deployment of root exudation to help mineralize N, particularly in the more nutrient-limited 0x soils, in line with other reports of a positive rhizosphere priming effect (Zhu et al., 2014).

However, the relationship between N availability and plant belowground C allocation is not straightforward: higher nutrient availability can lead to larger plants, which tend to have larger root systems, while high nutrient availability tends to reduce root growth. A systematic review of rhizodeposition responses to soil N availability found a negative relationship between soil N and rhizodeposition as a fraction of fixed C; however, there was positive relationship with soil N when considering rhizodeposition per plant, suggesting rhizodeposition amount is interactively influenced by plant size and N availability (Bowsher et al., 2018). This phenomenon may explain apparently conflicting reports of increased root growth with moderate

nutrient addition: for example, small amounts of compost addition to N-depleted soils have been shown to stimulate root growth for some spring wheat genotypes, and N fertilization of perennial ryegrass was associated with higher root production (Dodd and Mackay, 2011; Junaidi et al., 2018).

In addition to changes in the total amount of C allocated belowground, root morphology also shifted in response to soil nutrient management legacy. The 0x soils had a greater proportion of fine roots, whereas medium-coarse roots made up a greater fraction of root length in the 5x soils (Table 5.3). Studies from forest ecosystems often report negative relationships between fine root biomass and soil nutrient availability, and studies of *Arabidopsis* responses to soil nitrate show a negative relationship with lateral root growth (Hendricks et al., 1993; Linkohr et al., 2002).

5.4.3 Rhizodeposition C effects on soil microbial communities and function

The Snowmass cultivar showed higher specificity for distinct rhizosphere taxa, indicating recruitment in line with greater exudation reported above. Indicator taxa analysis identified nine bacterial families to be associated with Snowmass overall, compared to only one bacterial family associated with Byrd (Fig. 5.4). The higher exudation observed in Snowmass may explain this result, since the complex mixture of sugars, organic acids, and hormones contained in exudates are known to stimulate specific microbial communities across species, and thus are more likely to recruit specific taxa than other forms of rhizodeposition, like root cell sloughing and senescence (Sasse et al., 2018; Tkacz et al., 2015).

We also examined potential N-cycling activity by assessing extracellular enzyme activity. LAP also showed a genotype × soil treatment interaction, where the Snowmass cultivar had

elevated LAP activity compared to the Byrd cultivar in the 0x soils but not the 5x treatment (Fig. 5.2b). We suspect that increased exudation by the Snowmass cultivar in the 0x soil stimulated LAP activity, though this did not result in higher total N or residue-derived N uptake by the Snowmass cultivar. Increased enzyme cycling does not necessarily lead to an increase in available N for plants, as microbes often outcompete plants for N, and plants rely on microbial turnover for N release (Kuzyakov and Xu, 2013). While we did not observe evidence of residue N immobilization by the microbial biomass in any treatments (Table S5.3), our results were highly variable and previous work has demonstrated that root exudation can lead to temporary microbial immobilization of N (Fisk et al., 2015; Marschner et al., 2012).

4.4 Nitrogen uptake dynamics

The wheat genotypes studies here showed evidence of differing abilities to access residue-N depending on the soil legacy. While overall leaf N concentration and N uptake by wheat did not differ between genotypes, total uptake of residue-derived ^{15}N exhibited a clear genotype × soil treatment interaction (Fig. 5.3a). In the 5x treatment, the Snowmass cultivar took up, on average, 1.8 times greater residue-derived N than the Byrd cultivar (Fig. 5.2a), though the relationship was flipped in the 0x soils. The shift in N uptake patterns for the two genotypes across the different soils suggests that in the more biologically-active soil (5x), the high-exudation genotype (Snowmass) was relatively better at accessing residue-derived N.

Interestingly, the pattern of residue N uptake was contrary to the patterns of belowground wheat C allocation observed (Fig. 5.1). This result contrasts with our hypothesis that increased belowground wheat C allocation would lead to increased mineralization and uptake of residue N. Our results may indicate that high exudation resulted in the mineralization of SOM in the 0x

soils, diluting the labelled residue N. Previous work has suggested that microbial taxa associated with higher wheat exudation were distinct from taxa related to residue-N uptake (Kelly et al., *in review*). Root exudate C may therefore be most important for stimulating the mineralization of SOM, but less important in catalyzing the decomposition and mineralization of residue N for rapid plant uptake.

4.6 Soil legacy impacts on soil biological activity and diversity

We observed elevated rhizosphere enzyme activities of the compost amended treatments for most enzymes (38%-55% increase), which was consistent with previously measured enzyme activity and aggregate C from this site (Calderón et al., 2018; Liu et al., 2021). Compost amendment significantly increased SOM, a major food source for soil microbes, which generally correlates well with enzyme activity (Dick, 1994). A common method to account for differences in SOM or overall increases in microbial biomass is to normalize enzyme activity values by MBC; applying this normalization to our data completely accounts for amendment effects, indicating that changes in enzyme activity could be well-explained by increases in the microbial biomass (Table 5.4).

Microbial community structure, particularly of fungal communities, showed strong responses to soil nutrient management legacy (Fig. 5.3). Long-term compost addition has been shown previously to alter soil microbial communities, which is likely due to drastic changes in SOM, nutrient availability and soil structure (Liu et al., 2021). We also observed a decrease in bacterial diversity in the 5x treatment, which is likely due to the copiotrophic soil conditions which favor fast-growing "weedy" taxa and reduce community diversity (Fierer and Lennon, 2011). Fungal communities did not show a change in diversity with compost addition, despite the

strong difference in community composition, suggesting replacement of fungal taxa, and highlighting basic measurements of diversity are often not adequate for understanding complex community responses to environmental conditions (de Vries and Wallenstein, 2017; Shade, 2017).

5.5. Conclusion

Developing sustainable agroecosystems will rely on choosing crops that can perform optimally under changing soil conditions, including a shift to more organic nutrient sources in changes to soil health. Our data indicate clear differences in how distinct wheat genotypes respond to changing soil conditions. Specifically, we saw that the Snowmass cultivar responded to conditions of low nutrient availability and biological activity by increasing belowground allocation to roots and exudates (i.e. wheat-derived DOC). This strategy may have increased mineralization of native SOM but did not contribute to higher levels of residue-N uptake. However, despite relatively small differences in belowground investment under that 5x soil, we note that the Snowmass cultivar was more effective at recruiting microbial taxa in this environment and appears to be relatively more effective than the Byrd cultivar at accessing organic (residue-derived) N in this environment. While root exudation likely plays an important role in recruiting and stimulating microbial activity, changing soil health contexts and nutrient availability likely alters the relative importance of these relationships. Therefore, plants with high plasticity may prove most adaptable and able to respond to changing nutrient regimes.

As agricultural systems move towards an increased reliance on soil health promoting practices to support a range of ecological benefits, adequately managing new and biologically-controlled nutrient regimes will be a challenge for many producers. These systems will require

improved understanding of the relationships between crops and soil organisms to better regulate nutrient cycling and improve resilience and ecosystem health, including reducing reliance on external fertilizer inputs. Such knowledge will facilitate breeding and selection of crop varieties that are best adapted to using organic nutrients like cover crops and compost, and the increased SOM and biological activity that comes along with these practices. While our knowledge of plant-soil-organism interactions is still in its infancy, this study and others like it, suggest great promise for the development of more sustainable and resilient agroecosystems by elucidating how plant interact with the soil microbiome.

CHAPTER 5 TABLES AND FIGURES

Table 5.1. Initial soil characteristics of experimental field plot soils used in the greenhouse experiment. Field soils were amended biennially (every two years) with beef feedlot compost at a rate of 0 t ha^{-1} (0x) or 108.7 t ha^{-1} (5x), calculated as five times the expected N demand for the established alternating wheat-fallow rotation.

Soil Legacy	SOC (g kg^{-1})	$\delta^{13}C$	Total N (g kg^{-1})	$\delta^{15}N$	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	Total P (mg kg^{-1})	1:1 pH	% clay
0x	1.8	-18.55	2.2	14.66	21.8	4.5	1.0	7.3	17
5x	3.1	-18.24	4.0	26.63	30.7	6.8	2.0	7.2	20

Table 5.2. Plant biomass and root architecture metrics for two genotypes of winter wheat (*Triticum aestivum* L.) grown under greenhouse conditions. Plants were vernalized for 6 weeks before transplanting to experimental mesocosms. Values are mean ± standard error. ANOVA *p*-values in bold indicate significant ($p < 0.1$). Failed plant survival resulted in $n < 6$ for some treatments.

Soil Legacy	Genotype	n	Shoot Biomass (g mesocosm^{-1})	Root Biomass (g mesocosm^{-1})	Root Length (m)	Root surface area (cm^2)	SRL[a] (m g^{-1})	Avg. Root Diam (μm)
0x	Byrd	5	2.4 ± 0.3	0.15 ± 0.04	27.1 ± 9.40	180.0 ± 61.1	232.9 ± 8.6	214.3 ± 3.6
	Snowmass	5	2.2 ± 0.2	0.19 ± 0.03	45.8 ± 6.12	254.6 ± 60.6	239.9 ± 8.0	222.5 ± 13.6
5x	Byrd	3	1.8 ± 0.4	0.09 ± 0.04	21.4 ± 7.12	122.6 ± 55.0	267.9 ± 29.4	230.5 ± 8.6
	Snowmass	6	2.4 ± 0.5	0.14 ± 0.03	32.3 ± 8.76	222.5 ± 56.7	237.2 ± 13.6	224.1 ± 10.5
ANOVA	***p*-values**							
Genotype			0.62	0.23	**0.10**	0.12	0.53	0.65
Soil Legacy			0.79	0.16	0.37	0.50	0.43	**0.07**
Genotype x Soil Legacy			0.32	0.97	0.66	0.63	0.23	0.78

[a]SRL, specific root length

Table 5.3. Winter wheat (*Triticum aestivum* L.) genotype and soil treatment effects on root fraction in different diameter size classes. Values are mean percent ± standard error of root in different size classes by length. ANOVA *p*-values are below, with significant values ($p < 0.05$) in bold. Different letters following values within a column indicate pairwise difference by Tukey's test ($p < 0.05$); a column without letters has no pairwise differences between treatment combinations.

		Percent (%) of total root length in each diameter size class							
Soil Legacy	Genotype	0 – 50 μm	50-100 μm	100-150 μm	150-200 μm	200-250 μm	250-30 μm	300-350 μm	> 350 μm
0x	Byrd	11 ± 1.0	19 ± 1.1	19 ± 0.3	19 ± 0.9	12 ± 0.4 b	7.1 ± 0.4	3.0 ± 0.2	9.8 ± 0.7
	Snowmass	123 ± 0.8	20 ± 0.8	18 ± 0.4	20 ± 1.0	13 ± 0.4 b	6.6 ± 0.3	2.8 ± 0.1	7.9 ± 0.6
5x	Byrd	10 ± 1.4	17 ± 1.9	15 ± 1.6	21 ± 1.6	15 ± 0.7 a	8.0 ± 0.8	3.1 ± 0.5	11.7 ± 1.2
	Snowmass	11 ± 0.7	17 ± 1.5	17 ± 1.4	21 ± 1.1	14 ± 0.7 a	7.7 ± 0.6	3.3 ± 0.4	8.9 ± 1.3
ANOVA	***p*-values**								
Genotype		**0.057**	0.35	0.84	0.92	0.69	0.35	0.56	**0.049**
Soil Legacy		**0.033**	**0.034**	**0.064**	**0.062**	**< 0.01**	**0.091**	0.16	0.34
Genotype x Soil Legacy		0.47	0.38	0.59	0.33	0.25	0.91	0.97	0.65

Table 5.4. Total and wheat-derived soil C in whole soil and extractable rhizosphere soil pools in a ^{13}C-labeled greenhouse experiment comparing two wheat genotypes and three soil organic matter/fertility treatments. Treatment combination values are means ± standard error. ANOVA *p*-values for factor and interactive effects follow.

Soil Legacy	Genotype	Rhizosphere total C concentration (g kg^{-1})	Wheat-derived C in rhizosphere (mg kg^{-1}) †	Wheat-derived C in bulk soil (mg kg^{-1})	DOC (mg kg^{-1})	MBC (mg kg^{-1})	Wheat-derived MBC (mg kg^{-1})
0x	Byrd	12.1 ± 0.7	93 ± 31.9	9.7 ± 3.3	129 ± 9.9	54 ± 14.7	9.4 ± 3.8
	Snowmass	13.4 ± 0.5	155 ± 46.0	4.2 ± 2.1	127 ± 5.4	50 ± 8.9	16.1 ± 3.8
5x	Byrd	20.4 ± 1.3	82 ± 22.5	7.9 ± 1.2	190 ± 12.6	63 ± 17.2	4.2 ± 2.0
	Snowmass	19.8 ± 0.6	65 ± 11.4	8.0 ± 3.2	193 ± 7.7	77 ± 21.1	4.4 ± 1.5
ANOVA	***p*-values**						
Genotype		0.93	0.42	0.82	0.65	0.61	0.24
Soil Legacy		**<0.001**	0.11	0.43	**< 0.001**	0.35	**0.015**
Genotype x Soil Legacy		0.08	0.25	0.34	0.99	0.44	0.32

† Wheat rhizodeposition, concentration of wheat-derived C in rhizosphere soil; DOC, dissolved organic C, estimated as salt-extractable DOC; Wheat derived DOC, salt-extractable C derived from wheat (same data presented in Fig 5.1a); MBC, microbial biomass C, estimated as difference between chloroform-fumigated, salt-extractable C and unfumigated salt-extractable C

Table 5.5. Total and cover crop residue-derived N in wheat tissue and soil pools in an isotopically-labelled greenhouse experiment comparing two winter wheat genotypes and two soil treatments. ANOVA *p*-values for factor and interactive effects below.

Soil Legacy	Genotype	Wheat tissue N conc. (g kg^{-1})	Total wheat N uptake (mg $mesocosm^{-1}$)	Fraction of shoot N from residue (%)	‡Rhiz. N conc. (g kg^{-1})	Residue N in Rhiz. soil (mg kg^{-1})
0x	Byrd	24.5 ± 2.0	68.7 ± 8.6	0.84 ± 0.1	1.5 ± 0.1	5.9 ± 1.3
	Snowmass	25.9 ± 1.0	64.9 ± 6.4	0.56 ± 0.1	1.6 ± 0.1	1.9 ± 0.6
5x	Byrd	31.4 ± 1.3	55.9 ± 12.5	0.27 ± 0.1	2.6 ± 0.1	6.5 ± 2.9
	Snowmass	27.2 ± 2.2	66.7 ± 13.4	0.41 ± 0.1	2.5 ± 0.1	3.6 ± 1.1
ANOVA	*p*-values					
Genotype		0.70	0.55	0.33	0.81	**0.056**
Soil Legacy		0.12	0.96	**0.003**	**< 0.001**	0.97
Genotype x Soil Legacy		0.20	0.78	**0.05**	0.46	**0.089**

‡Rhiz. N conc, rhizosphere soil nitrogen concentration

Table 5.6. Additional enzyme activities in rhizosphere soil of two varieties of winter wheat (*Triticum aestivum* L.) grown in greenhouse conditions with soil from unamended (0 t ha^{-1}) or high rate of beef feedlot compost (108.7 t ha^{-1}) field treatments. Values are means ± standard error in units of nmol h^{-1} g^{-1} soil.

			TAP[†]	**NAG**	**BG**	**CB**	**PHOS**
Soil Legacy	**Genotype**	**n**	----------nmol h^{-1} g^{-1} soil----------				
0x	Byrd	5	52 ± 27	27 ± 1.9	92 ± 5.8	16. ± 1.4	39 ± 5.8
	Snowmass	6	30 ± 6.6	34 ± 3.4	109 ± 8.9	19 ± 2.1	47 ± 5.9
5x	Byrd	4	34 ± 3.2	54 ± 4.2	141 ± 13.6	29 ± 4.1	67 ± 6.5
	Snowmass	6	35 ± 9.0	44 ± 6.6	137 ± 14.4	29 ± 2.8	54 ± 8.3
ANOVA	***p*-values**						
Genotype			0.49	0.89	0.54	0.48	0.70
Soil Legacy			0.93	**0.005**	**< 0.01**	**<0.001**	**0.03**
Genotype x Soil Legacy			0.94	0.14	0.38	0.52	0.39

[†]*L*-Tyrosine aminopeptidase (TAP), and N-Acetyl-β-D-glycosaminidase (NAG) indicate N-cycling activities; β-1,4-glucosidase (BG) and β-D-cellobiosidase (CB) indicate C cycling activities; phosphatase (PHOS) indicates P-cycling activities

Table 5.7. Rhizosphere microbial diversity based prokaryotic and fungal DNA fingerprinting in rhizosphere soil of two winter wheat genotypes and three soil treatments. Values are mean ± standard error based on amplicon sequence variant (ASV)-level taxonomic data. Soil treatment means showing pairwise differences are presented below, followed by overall parameter means and coefficient of variation (CV) for comparison. ANOVA p-values are presented in the bottom of the section, with significant values ($p < 0.05$) in bold.

		Prokaryotic (16S)				Fungal (ITS)		
Soil Legacy	Genotype	Richness (ASV sample^{-1})	Diversity (Shannon)	Diversity (Faith)	Evenness (Pielou)	Richness (ASV sample^{-1})	Diversity (Shannon)	Evenness (Pielou)
0x	Byrd	192 ± 46	7.0 ± 0.4	14.9 ± 2.3	0.94 ± 0.01	250 ± 24	5.3 ± 0.1	0.67 ± 0.01
	Snowmass	209 ± 30	7.2 ± 0.2	16.6 ± 1.4	0.94 ± 0.01	274 ± 31	5.4 ± 0.1	0.67 ± 0.01
5x	Byrd	109 ± 22	6.4 ± 0.2	11.3 ± 1.4	0.95 ± 0.01	217 ± 28	5.5 ± 0.1	0.71 ± 0.02
	Snowmass	69 ± 11	5.7 ± 0.2	8.8 ± 1.2	0.96 ± 0.00	214 ± 30	5.5 ± 0.2	0.71 ± 0.01
ANOVA	*p*-values							
Genotype		0.77	0.47	0.90	0.28	0.68	0.77	0.94
Soil Legacy		**0.0017**	**0.0013**	**0.0023**	0.16	0.13	0.31	**<0.001**
Genotype x Soil Legacy		0.35	0.17	0.23	0.40	0.66	0.66	0.72

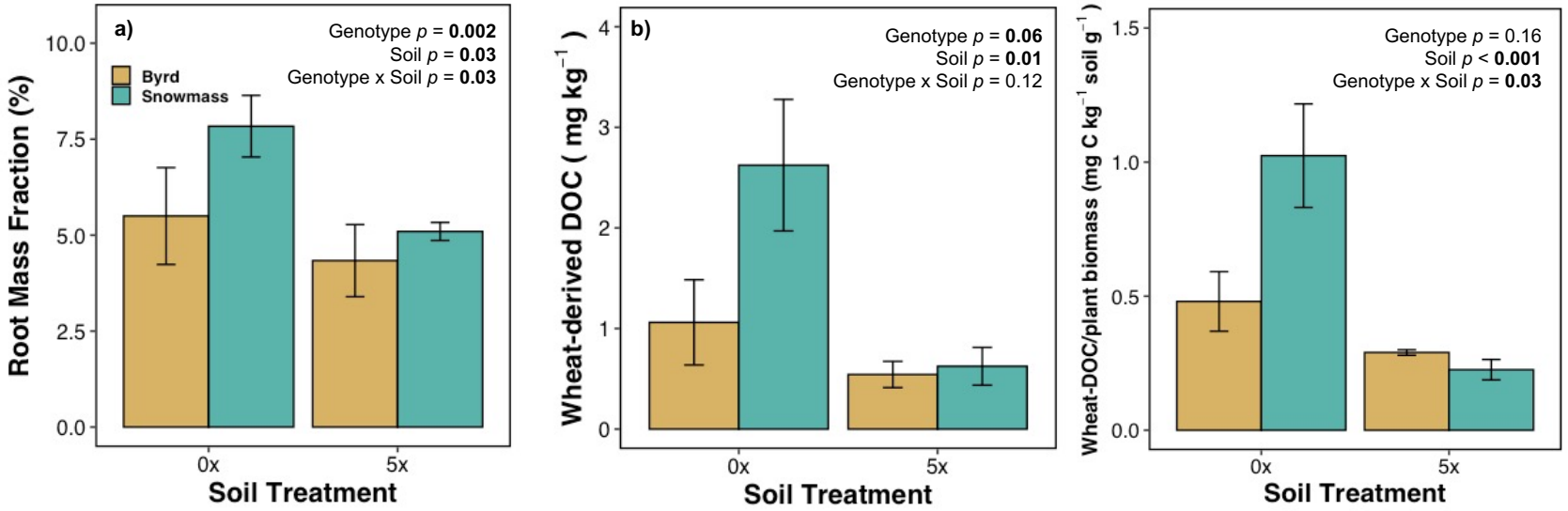

Figure 5.1. Belowground carbon allocation metrics of two different winter wheat (*Triticum aestivum* L.) genotypes (colors) grown in two different soil treatments (x-axis). Bars are mean ± standard error for a) root mass fraction; b) concentration of wheat-derived DOC in rhizosphere soil using ^{13}C labeling; and c) the relative ratio of root-derived DOC concentration to total wheat plant biomass. Soil legacy treatments are no amendment (0x) and long-term beef feedlot compost addition (5x). Two-factor ANOVA *p*-values for genotype, soil treatment, and their interaction is shown for each variable.

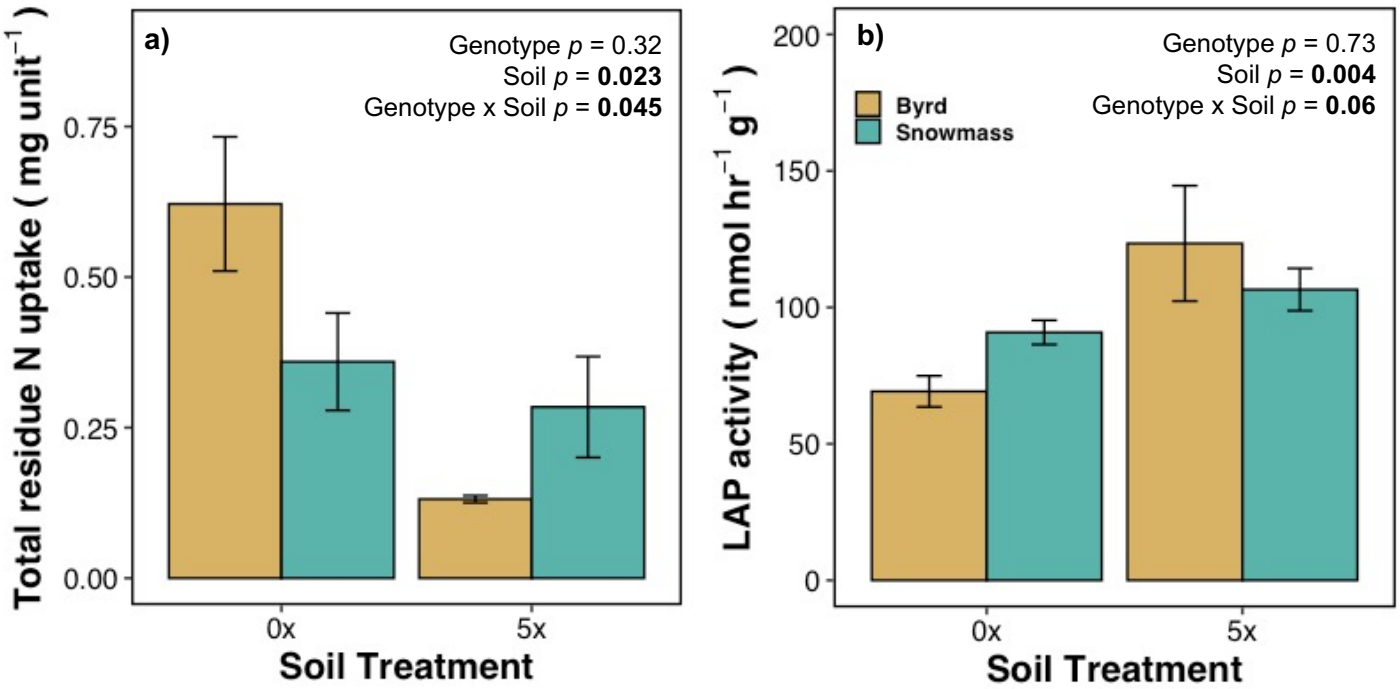

Figure 5.2. Nitrogen functional responses to residue addition of two different winter wheat (*Triticum aestivum* L.) genotypes (colors) grown in two different soil treatments (x-axis). Bars are mean ± standard error for a) fraction of wheat N derived from added residue using ^{15}N labeling and b) activity of leucine amino-peptidase (LAP), an enzyme that cycles nitrogen. Soil treatments are no amendment (0x) and long-term beef feedlot compost addition (5x). Two-factor ANOVA *p*-values for genotype, soil treatment, and their interaction is shown for each variable.

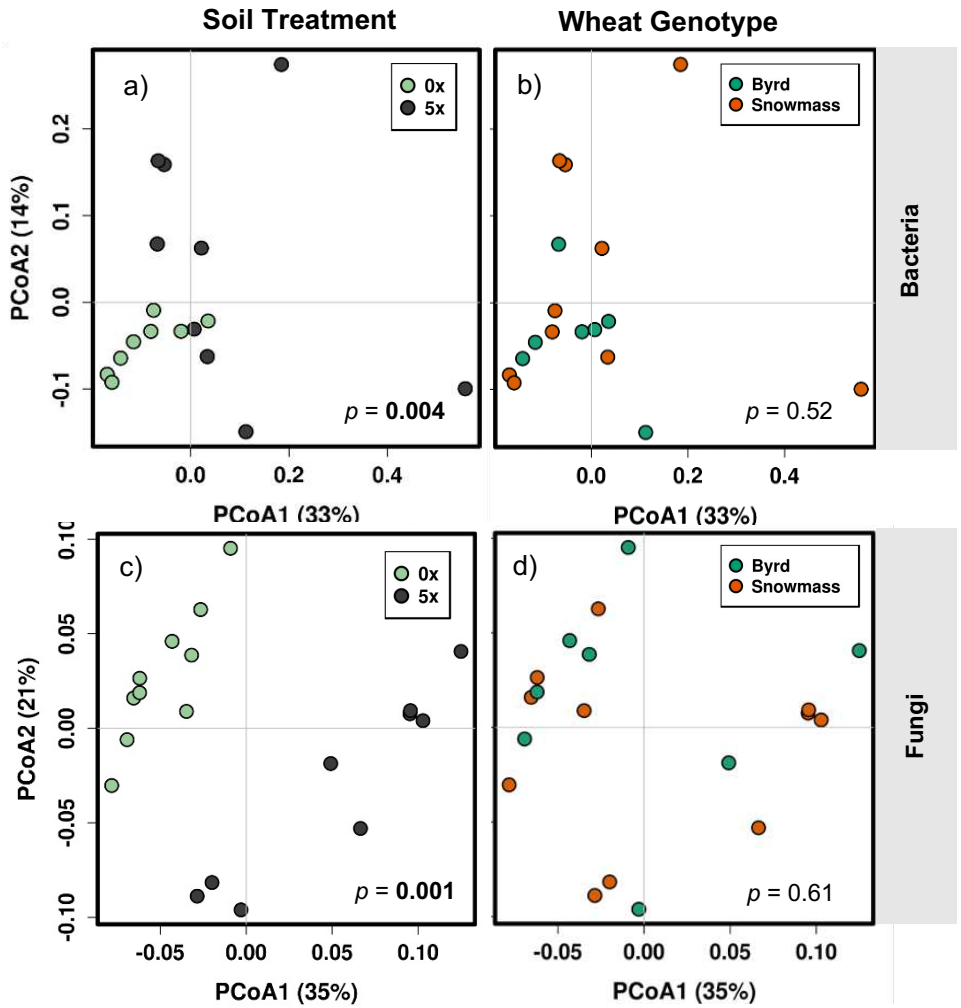

Figure 5.3. Principle coordinate analysis (PCoA) ordination plots of bacterial (16S; a, b) and fungal (ITS; c, d) marker-based community profiles at the taxonomic resolution of family. Points are placed based on Bray-Curtis dissimilarities and colored based on soil treatment (left column; a, c) or wheat genotype (right column; b, d). PERMANOVA *p*-values are shown in the bottom-right corner of each plot based on the factor used to color the points. Soil × genotype interaction was non-significant for all analyses.

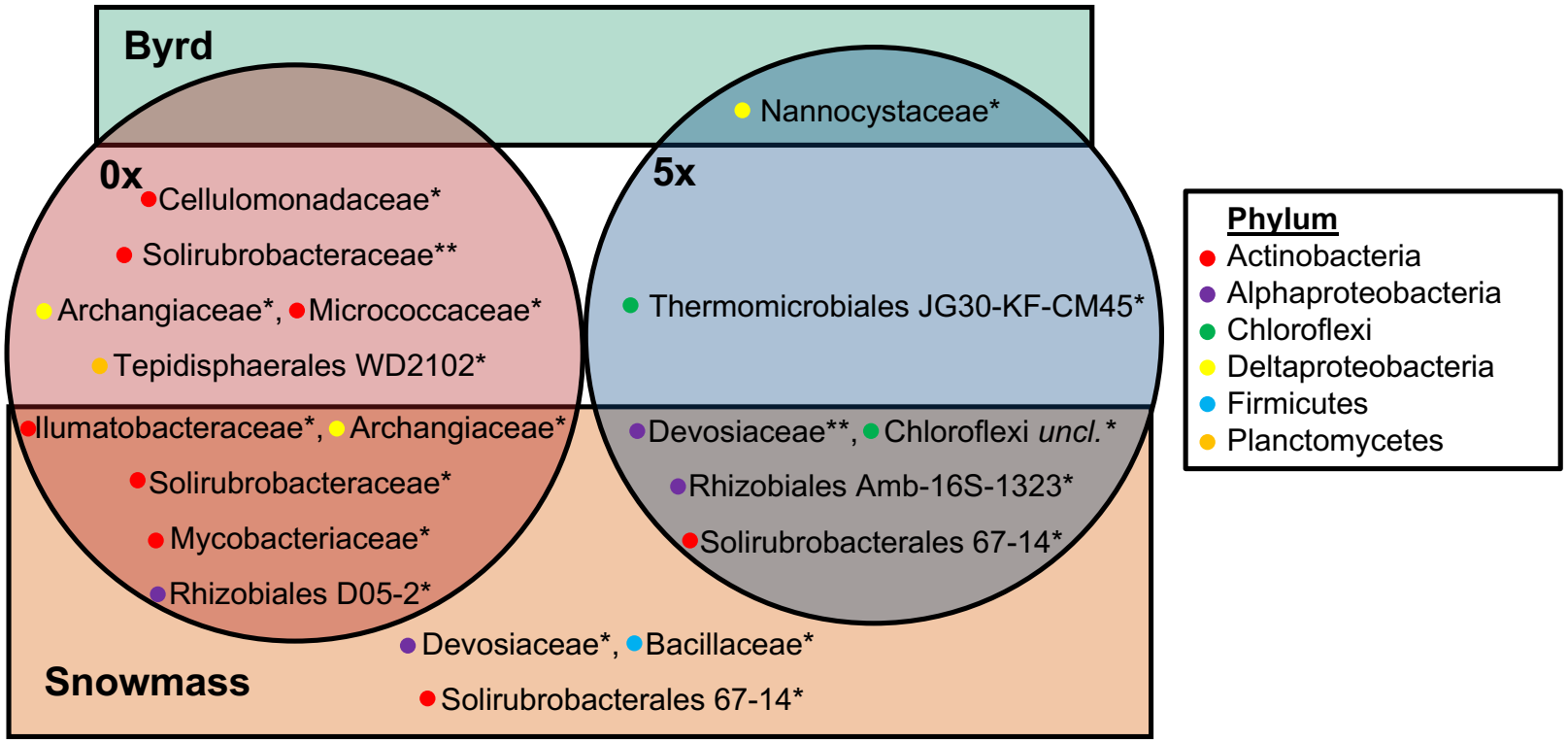

Figure 5.4. Bacterial indicator taxa (family level) in rhizosphere communities for different experimental factors: wheat genotype (rectangles), soil treatment (circles), and the combined factors (overlapping area). The phyla containing each indicator family is denoted with colored points preceding the name, and significance of group identity via permutation by * $p < 0.05$; ** $p < 0.01$.

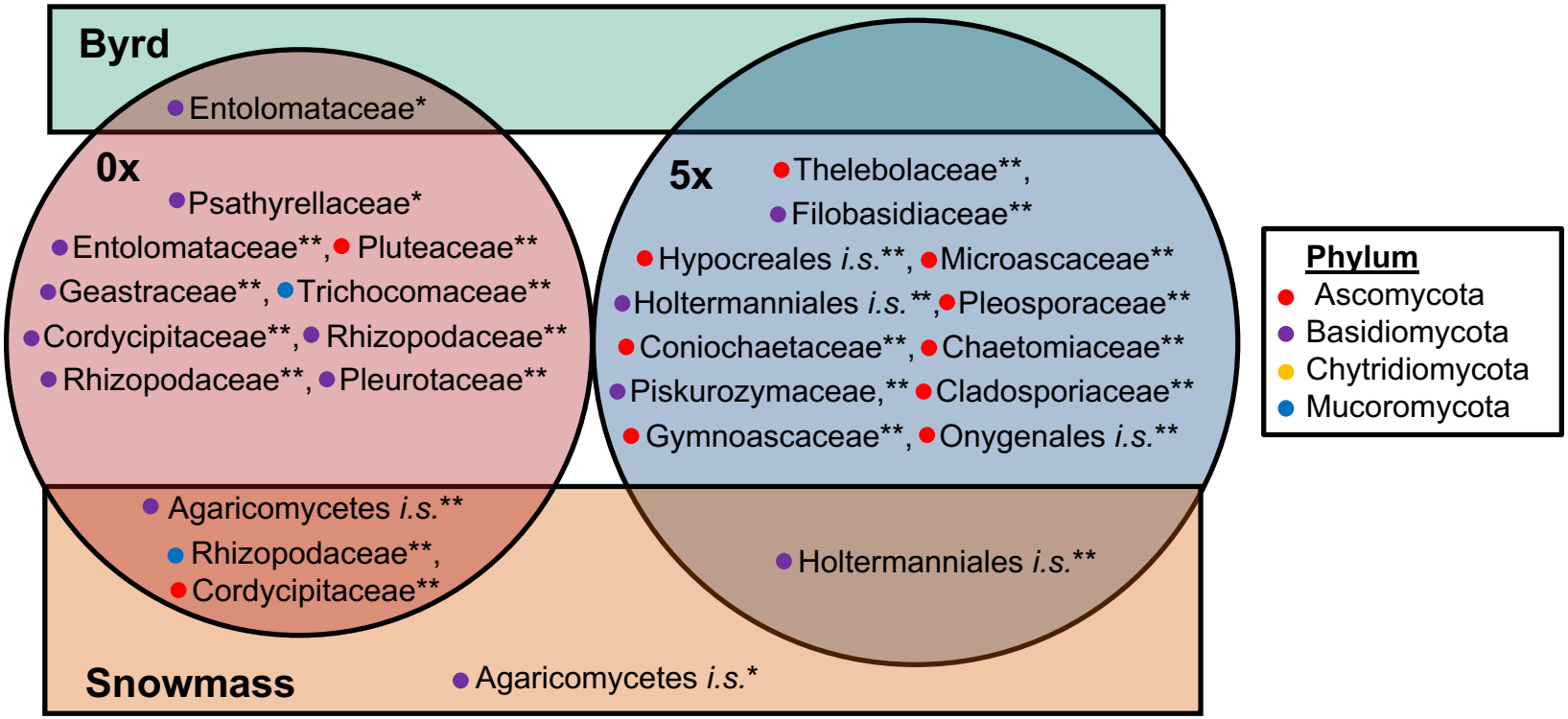

Figure 5.5. Fungal indicator taxa (family level) in rhizosphere communities for different experimental factors: wheat genotype (rectangles), soil treatment (circles), and the combined factors (overlapping area). The phyla containing each indicator family is denoted with colored points preceding the name, and significance of group identity via permutation by * $p < 0.05$; ** $p < 0.01$. Taxa with uncertain family grouping are labeled *inserte sedis* (*i.s.*) and the taxonomic order is given. For clarity, only highly-significant taxa ($p < 0.01$) are reported here for most categories (those including soil effects); a full list of all selected indicator taxa is reported in Table S5.3.

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APPENDIX: SUPPLEMENTARY TABLES AND FIGURES

Table S2.1: Density (individuals m^{-2}) distribution of macrofauna in different cropping systems in two sites of a 32-year dryland cropping rotation experiment in eastern Colorado, USA. Values are means (SE) of two experimental plots. WF, wheat fallow; WCF, wheat-corn-fallow; CONT, continuous cropping; GRASS, native perennial grass.

		Sterling				Stratton			
		WF	WCF	CONT	GRASS	WF	WCF	CONT	GRASS
Coleoptera	Staphylinidae	4 (4.0)	0 (0.0)	0 (0.0)	4 (4.0)	8 (8.0)	4 (4.0)	4 (4.0)	4 (4.0)
	Latridiidae	36 (28.0)	4 (4.0)	8 (8.0)	8 (8.0)	4 (4.0)	28 (4.0)	4 (4.0)	0 (0.0)
	Carabidae	16 (0.0)	20 (20.0)	4 (4.0)	4 (4.0)	8 (0.0)	8 (8.0)	4 (4.0)	4 (4.0)
	Scarabaeidae larvae	0 (0.0)	0 (0.0)	12 (12.0)	0 (0.0)	0 (0.0)	12 (12.0)	4 (4.0)	4 (4.0)
	Elateridae larva	0 (0.0)	0 (0.0)	8 (0.0)	12 (12.0)	4 (4.0)	8 (0.0)	4 (4.0)	0 (0.0)
	Carabidae larvae	0 (0.0)	4 (4.0)	4 (4.0)	4 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Ptinidae larva	0 (0.0)	0 (0.0)	0 (0.0)	12 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hymenoptera	Myrmicinae	28 (28.0)	4 (4.0)	56 (56.0)	12 (12.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Solenopsis sp.	4 (4.0)	8 (8.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Ponera sp.	24 (8.0)	12 (12.0)	24 (8.0)	248 (72.0)	0 (0.0)	12 (12.0)	96 (96.0)	20 (20.0)
	Tapinoma sp.	0 (0.0)	16 (16.0)	0 (0.0)	24 (16.0)	0 (0.0)	0 (0.0)	4 (4.0)	0 (0.0)
	Lasius sp.	0 (0.0)	0 (0.0)	0 (0.0)	312 (312.0)	0 (0.0)	0 (0.0)	0 (0.0)	276 (196.0)
	Crematogaster sp.	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	28 (28.0)	0 (0.0)	120 (48.0)
Hemiptera	Lygaeidae	0 (0.0)	0 (0.0)	4 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	12 (4.0)
Arachnida	Araneae	8 (8.0)	8 (0.0)	4 (4.0)	4 (4.0)	0 (0.0)	8 (8.0)	4 (4.0)	4 (4.0)
Orthoptera	Orthoptera eggs	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	20 (20.0)	4 (4.0)	4 (4.0)	0 (0.0)
Other	Other	8 (0.0)	4 (4.0)	0 (0.0)	16 (8.0)	4 (4.0)	12 (4.0)	4 (4.0)	24 (16.0)

Table S3.1. Detailed soil characteristics across each experimental field in the short-term grazed cover crop study. Particle size distribution reported for surface 30 cm.

					Gravimetric Water Content (g kg^{-1})					
Site	Block	Sand (g kg^{-1})	Clay (g kg^{-1})	Texture class	0-30 cm	30-60 cm	60-90cm	90-120 cm	120-150 cm	150-180 cm
1	1	270	180	Silt Loam	216	237	193	127	116	112
	2	400	200	Loam	208	225	188	120	109	114
	3	240	200	Silt Loam	226	224	213	174	140	147
	4	210	200	Silt Loam	228	236	223	183	146	145
2	1	180	300	Silty Clay Loam	217	163	140	152	161	168
	2	240	230	Silt Loam	230	170	118	113	113	109
	3	230	220	Silt Loam	239	198	153	119	111	108
	4	230	220	Silt Loam	228	166	120	113	114	112
3	1	210	220	Silt Loam	234	203	125	114	126	140
	2	290	240	Loam	221	189	142	115	119	132
	3	220	240	Silt Loam	251	214	183	135	129	125
	4	210	240	Silt Loam	232	209	127	113	118	134
4	1	210	200	Silt Loam	242	230	212	173	138	152
	2	200	200	Silt Loam	234	229	212	171	131	140
	3	230	200	Silt Loam	221	218	198	182	143	141
	4	250	220	Silt Loam	229	219	193	138	149	150
5	1	45	16	Loam	198	190	147	68	-$^{\phi}$	-
	2	45	18	Loam	198	184	117	79	-	-
	3	46	16	Loam	223	193	191	163	114	-
	4	51	15	Loam	236	230	150	-	-	-
6	1	65	22	Sandy Clay Loam	104	106	132	120	125	103

	2	55	32	Sandy Clay Loam	153	165	67	75	88	129
	3	73	22	Sandy Clay Loam	109	81	78	94	60	143
	4	59	26	Sandy Clay Loam	112	140	88	63	63	56
7	1	13	32	Silty Clay Loam	221	195	134	139	138	146
	2	11	34	Silty Clay Loam	227	225	164	144	140	150
	3	13	38	Silty Clay Loam	222	198	161	154	153	162
	4	15	38	Silty Clay Loam	229	200	138	131	137	134
8	1	14	37	Silty Clay Loam	169	163	128	119	130	134
	2	14	34	Silty Clay Loam	147	122	120	138	140	131
	3	12	34	Silty Clay Loam	170	124	113	124	151	148
	4	13	38	Silty Clay Loam	177	141	135	131	155	154
9	1	21	36	Clay Loam	196	164	133	130	172	108
	2	31	36	Clay Loam	161	142	103	110	105	109
	3	21	34	Clay Loam	179	167	153	159	151	164
	4	19	36	Silty Clay Loam	174	170	151	138	140	129
10	1	11	34	Silty Clay Loam	235	191	153	162	158	159
	2	13	36	Silty Clay Loam	238	181	134	143	157	157
	3	12	35	Silty Clay Loam	237	190	127	120	134	138
	4	12	33	Silty Clay Loam	247	186	123	131	142	153

$^{\phi}$ reached maximum soil depth

Table S3.2. Site level mean ± SE soil parameter values at each farm site in a spring-planted, on-farm grazed cover crop trial in eastern CO, western KS, and western NE, USA. Different letters within a column section (grouped by site) indicate cover crop treatment differences within a site. All soil chemical properties 0-15 except MWD and BD (0-5 cm). ASW summed to 180cm depth.

Year	Site	Treatment	MWD μm	BD g cm^3	Olson P g kg^{-1}	Total N g kg^{-1}	Org. C g kg^{-1}	POX C mg kg^{-1}	PMN mg kg^{-1}	ASW CC term. (mm)	ASW wheat plant. (mm)	Wheat Yield kg ha^{-1}
2016	1	Fallow	664± 176.4a	1.24± 0.02a	52± 5.5a	1.4± 0.12a	13.5± 1.17a	309±18.8a	12.8± 2.51a	502± 12.1a	518± 10.8a	5,330± 715a
		Grazed	1,622± 131.1b	1.31± 0.01b	53± 2.9a	1.3± 0.05a	10.7± 0.50a	297±18.5a	9.1± 0.48a	421± 15.5b	461± 12.4b	5,050± 136a
		Un-grazed	1,242± 118.1b	1.21± 0.02a	56± 8.8a	1.4± 0.03a	12.1± 0.99a	315 ± 3.1a	14.8± 3.64a	416± 10.6b	445± 23.6b	4,690± 704a
	2	Fallow	939 ± 143.2a	1.10± 0.05a	109± 17.8a	1.4± 0.03a	11.8± 0.45a	415± 27.8a	9.9± 1.22a	370± 10.7a	402± 19.7a	4,440± 875a
		Grazed	1,645 ± 158.6a	1.15± 0.04a	104± 16.5a	1.5± 0.10a	13.1± 1.01a	421± 21.8a	10.6± 1.78a	256± 6.3b	339± 14.1a	2,630± 53a
		Un-grazed	1,295 ± 321.2a	1.06± 0.04a	99 ± 7.3a	1.4± 0.07a	11.7± 0.65a	392± 27.0a	13.0± 4.43a	283± 16.3b	352± 32.7a	2,660± 241a
	3	Fallow	1,022± 129.4a	1.13± 0.03a	55 ± 5.5a	1.3± 0.07a	11.7± 0.76a	401± 18.1a	8.9± 0.76b	425± 16.8a	495± 35.2a	4,970± 854a
		Grazed	1,184± 181.9a	1.09± 0.04a	62±13.2a	1.3± 0.02a	11.8± 0.47a	408± 30.7a	12.8± 1.40a	366± 19.3b	431± 8.6a	4,970± 299a
		Un-grazed	1,210± 199.2a	1.07± 0.04a	50 ± 1.3a	1.3± 0.05a	11.7± 0.42a	410± 11.3a	9.0± 1.39b	325± 4.0c	455± 10.7a	4,460± 278a
	4	Fallow	2,138± 249.8a	1.09± 0.01a	161 ± 9.5a	1.6± 0.06a	13.9± 0.83a	415± 34.8a	15.9± 1.93a	383± 11.5a	441± 29.1a	4,250± 570a
		Grazed	2,008± 249.2a	1.09± 0.04a	161 ± 5.3a	1.6± 0.15a	14.1± 1.79a	409± 41.3a	12.5± 1.43a	330± 14.6b	403± 4.5ab	3,200± 581a
		Un-grazed	2,373± 112.0a	1.05± 0.05a	184±14.1a	1.5± 0.14a	12.9± 1.64a	395± 37.8a	13.7± 2.78a	301± 14.9c	364± 15.0b	3,100± 230a
	5	Fallow	566 ± 99.5a	1.23± 0.02a	49± 7.1a	1.2± 0.09a	9.3± 0.81a	286± 29.3a	6.2± 0.32a	177± 17.7a	183± 23.1a	-*
		Grazed	1,384± 431.5a	1.24± 0.03a	48± 6.7a	1.1± 0.06a	9.5± 0.49a	274± 17.3a	7.1± 1.14a	175± 17.5a	243± 19.0a	-
		Un-grazed	823 ±144.4a	1.15± 0.05a	45± 2.9a	1.1± 0.07a	9.0± 0.64a	258± 28.4a	8.1± 0.96a	124± 16.0a	219± 13.8a	-
2017	6	Fallow	732± 112.5a	1.32± 0.05a	32 ± 4.2a	0.6± 0.09a	4.8± 0.70a	79± 30.7a	12.1± 2.60a	282± 45.3a	211± 17.0a	-¥
		Grazed	1,201± 218.1a	1.28± 0.05a	63±33.7a	0.7± 0.09a	5.3± 0.93a	91± 36.8a	14.3± 5.19a	297± 17.3a	305±35.4b	-
		Un-grazed	862 ± 47.7a	1.28± 0.04a	39 ± 4.6a	0.6± 0.04a	5.1± 0.35a	88± 24.4a	12.0± 0.50a	285± 21.1a	276± 20.8ab	-
	7	Fallow	1,506± 50.5a	1.19± 0.01a	56 ± 2.2a	1.1± 0.03a	11.2± 0.60a	329± 8.9a	9.5± 2.85a	457± 15.2a	509± 9.3a	2,190± 158a§
		Grazed	2,465± 71.6b	1.14± 0.03a	38 ± 8.1a	1.1± 0.04a	10.8± 0.82a	319± 44.1a	13.9± 7.42a	390± 10.3b	471± 12.5a	1,600± 143a
		Un-grazed	2,300±179.1b	1.15± 0.01a	54±10.4a	1.1± 0.02a	10.7± 0.48a	314± 13.5a	8.4± 0.39a	384± 7.0b	471± 17.4a	1,690± 136a
	8	Fallow	831± 194.4a	1.12± 0.02a	31± 7.2a	1.0± 0.06a	9.1± 0.69a	252± 26.9a	15.3± 2.10a	447± 33.8a	548± 17.0a	3,500± 315a
		Grazed	856 ± 64.0a	1.17± 0.02a	26± 6.6a	1.0± 0.07a	9.9± 0.48a	278± 25.1a	17.6± 2.78a	405± 8.8a	439± 35.6b	2,450± 522b
		Un-grazed	1,086± 244.6a	1.11± 0.02a	28± 2.5a	1.1± 0.02a	10.2± 0.17a	266± 13.7a	18.3± 2.30a	398± 18.5a	463± 21.2ab	3,100± 307ab
	9	Fallow	1,594± 283.4a	1.13± 0.02a	12 ± 1.5a	1.5± 0.06a	13.5± 0.63a	271± 25.9a	34.3± 3.29a	382± 27.5a	368± 18.6a	900± 104a
		Grazed	2,268± 454.7a	1.20± 0.03a	38±16.2a	1.3± 0.05a	11.9± 0.50a	288± 29.5a	33.4± 5.84a	330± 25.8a	319± 64.7a	960± 130a
		Un-grazed	2,303± 195.9a	1.11± 0.05a	26 ± 6.2a	1.4± 0.11a	12.9± 0.94a	330± 36.1a	33.1± 1.14a	405± 18.8a	325± 14.0a	460± 17b†,€

10	Fallow	1,038± 128.7a	1.19± 0.01a	47±22.7a	1.1± 0.05a	9.6± 0.54a	333± 16.8a	10.4± 1.22b	410± 13.0a	432± 15.2a	-‡
	Grazed	1,576± 126.7b	1.14± 0.03a	34 ± 7.4a	1.2± 0.02a	10.7± 0.30a	372± 24.5a	17.7± 0.71a	341± 7.2b	384± 16.0a	-
	Un-grazed	1,142± 66.2ab	1.15± 0.01a	38 ± 2.2a	1.1± 0.05a	10.1± 0.33a	322 ± 9.4a	12.9± 1.14b	328± 19.5b	381± 23.8a	-

*Wheat was harvest before samples could be taken. ¥Wheat crop lost due to hail. §Harvested early (dough stage). †Un-grazed exclosures and fallows were located on the perimeter of the field €Heirloom wheat variety planted ‡Corn planted instead of wheat. ASW, available soil water; MWD, mean weight diameter; POX C, permanganate oxidizable C; PMN, potentially mineralizable N; CC term, cover crop termination

Table S4.1. Total and cover crop (CC)-derived N in rhizosphere soil pools for twelve winter wheat genotypes used in a greenhouse study with application of ^{15}N-labelled cover crop residue (*Vicia villosa*) residues to the soil. Values are mean ± standard error (n = 5). ANOVA *p*-values for genotype effect on each variable are reported below means. No pairwise differences between genotypes were detected. Pearson correlations (*r*) with specific root length (SRL) are reported; significant values are in bold.

Variety	TDN‡ mg kg^{-1} soil	TDN from Residue (mg kg^{-1})[a]	DON (mg kg^{-1})	MBN (mg kg^{-1})	MBN from Residue (mg kg^{-1})	Proportion Residue N in MBN (%)[a]	Nitrate (mg kg^{-1}) [a]	Ammonium (mg kg^{-1})
Byrd	6.1 ± 0.6	0.37 ± 0.05	4.5 ± 0.6	6.4 ± 1.4	1.2 ± 0.14	20 ± 3	0.51 ± 0.19	1.0 ± 0.1
Snowmass	8.0 ± 1.4	0.39 ± 0.06	6.9 ± 1.4	6.0 ± 1.2	1.2 ± 0.18	20. ± 7	0.32 ± 0.08	0.9 ± 0.1
Ripper	7.9 ± 1.9	0.44 ± 0.11	6.3 ± 1.9	7.3 ± 0.5	1.2 ± 0.41	16 ± 5	0.55 ± 0.06	1.00 ± 0.1
Ron L	9.0 ± 1.6	0.49 ± 0.05	7.7 ± 1.5	4.7 ± 1.6	1.5 ± 0.19	39 ± 9	0.39 ± 0.13	0.9 ± 0.1
Prowers	6.5 ± 0.6	0.44 ± 0.07	4.7 ± 0.7	8.0 ± 1.0	2.0 ± 0.36	25 ± 2	0.61 ± 0.14	1.1 ± 0.1
TAM 107	7.3 ± 1.2	0.37 ± 0.07	6.1 ± 1.3	8.1 ± 2.3	1.7 ± 0.39	31 ± 13	0.33 ± 0.08	0.9 ± 0.1
Sandy	5.2 ± 0.4	0.40 ± 0.03	3.6 ± 0.3	9.7 ± 1.4	1.7 ± 0.18	18 ± 2	0.81 ± 0.33	0.8 ± 0.1
Scout 66	5.9 ± 0.7	0.30 ± 0.04	4.8 ± 0.7	5.5 ± 0.6	0.8 ± 0.16	16 ± 4	0.28 ± 0.07	0.8 ± 0.1
Wichita	7.1 ± 0.6	0.49 ± 0.05	5.2 ± 0.6	8.6 ± 1.7	1.6 ± 0.21	21 ± 5	0.78 ± 0.09	1.1 ± 0.1
Cheyenne	5.0 ± 0.4	0.28 ± 0.04	3.8 ± 0.3	6.6 ± 1.1	1.1 ± 0.22	16 ± 3	0.32 ± 0.07	0.9 ± 0.1
Turkey Red	8.9 ± 0.5	0.69 ± 0.23	6.6 ± 0.9	4.2 ± 1.0	1.5 ± 0.31	45 ± 11	1.13 ± 0.74	1.1 ± 0.2
T. Monococcum	7.8 ± 1.0	0.36 ± 0.07	6.3 ± 1.1	5.5 ± 2.0	1.3 ± 0.32	44 ± 14	0.56 ± 0.16	0.9 ± 0.1
Mean	7.05	0.42	5.56	6.62	1.32	25.4	0.55	0.95
CV	0.35	0.50	0.44	0.49	0.51	0.73	1.03	0.25
ANOVA	*P*-values							
Genotype	0.11	0.35	0.17	0.18	0.15	**0.03**	0.25	0.27

[a]log transformation for ANOVA; ‡TDN, total dissolved N; DON, dissolved organic N; MBN, microbial biomass N; CC, cover crop; SRL, specific root length or wheat; CV, coefficient of variation of all samples (standard deviation divided by mean)
[b]Only 2 samples were included in analysis due to negative values

Table S4.2. Average abundance and standard error of microbial marker (16S and ITS) and peptidase-encoding genes in rhizosphere soil for twelve winter wheat genotypes used in an isotopically labelled greenhouse study (n=5). Values within a column followed by a different letter indicate a pairwise difference between genotypes (Tukey's p<0.05). *apr*, alkaline metallopeptidase; *npr*, neutral metallopeptidase; *sub*, subtilisin-like serine peptidase

	16S			**ITS**			***apr***			***npr***			***sub***			
Variety	---------- ng^{-1} gDNA ----------															
Byrd	$3.0 \cdot 10^6$	±	$2.7 \cdot 10^5$	$4.6 \cdot 10^7$	±	$2.3 \cdot 10^7$	$10.8 \cdot 10^6$	±	$3.6 \cdot 10^6$	$1.7 \cdot 10^3$	±	$0.6 \cdot 10^3$	ab	$20.3 \cdot 10^1$	±	$9.7 \cdot 10^1$	ab
Snowmass	$3.0 \cdot 10^6$	±	$1.6 \cdot 10^5$	$7.0 \cdot 10^7$	±	$4.1 \cdot 10^7$	$6.3 \cdot 10^6$	±	$1.8 \cdot 10^6$	$3.4 \cdot 10^3$	±	$1.0 \cdot 10^3$	ab	$5.6 \cdot 10^1$	±	$2.0 \cdot 10^1$	b
Ripper	$3.3 \cdot 10^6$	±	$3.1 \cdot 10^5$	$19.2 \cdot 10^7$	±	$7.1 \cdot 10^7$	$8.0 \cdot 10^6$	±	$1.9 \cdot 10^6$	$3.2 \cdot 10^3$	±	$1.0 \cdot 10^3$	ab	$18.8 \cdot 10^1$	±	$8.4 \cdot 10^1$	ab
Ron L	$3.1 \cdot 10^6$	±	$3.7 \cdot 10^5$	$10.6 \cdot 10^7$	±	$4.6 \cdot 10^7$	$7.7 \cdot 10^6$	±	$2.5 \cdot 10^6$	$2.9 \cdot 10^3$	±	$1.1 \cdot 10^3$	ab	$6.3 \cdot 10^1$	±	$4.3 \cdot 10^1$	ab
Prowers	$3.0 \cdot 10^6$	±	$4.3 \cdot 10^5$	$6.1 \cdot 10^7$	±	$5.4 \cdot 10^7$	$14.7 \cdot 10^6$	±	$1.6 \cdot 10^6$	$1.5 \cdot 10^3$	±	$0.7 \cdot 10^3$	ab	$48.9 \cdot 10^1$	±	$15.2 \cdot 10^1$	a
TAM 107	$2.3 \cdot 10^6$	±	$1.5 \cdot 10^5$	$11.9 \cdot 10^7$	±	$1.4 \cdot 10^7$	$6.5 \cdot 10^6$	±	$1.5 \cdot 10^6$	$1.9 \cdot 10^3$	±	$1.0 \cdot 10^3$	ab	$8.2 \cdot 10^1$	±	$1.9 \cdot 10^1$	ab
Sandy	$3.3 \cdot 10^6$	±	$2.6 \cdot 10^5$	$2.6 \cdot 10^7$	±	$1.5 \cdot 10^7$	$8.6 \cdot 10^6$	±	$2.5 \cdot 10^6$	$1.5 \cdot 10^3$	±	$0.8 \cdot 10^3$	ab	$16.7 \cdot 10^1$	±	$10.4 \cdot 10^1$	ab
Scout 66	$3.0 \cdot 10^6$	±	$4.6 \cdot 10^5$	$12.3 \cdot 10^7$	±	$8.8 \cdot 10^7$	$4.6 \cdot 10^6$	±	$2.2 \cdot 10^6$	$0.7 \cdot 10^3$	±	$0.4 \cdot 10^3$	b	$27.6 \cdot 10^1$	±	$9.4 \cdot 10^1$	ab
Wichita	$2.4 \cdot 10^6$	±	$2.6 \cdot 10^5$	$13.0 \cdot 10^7$	±	$5.3 \cdot 10^7$	$4.0 \cdot 10^6$	±	$0.6 \cdot 10^6$	$4.1 \cdot 10^3$	±	$1.0 \cdot 10^3$	ab	$4.0 \cdot 10^1$	±	$0.8 \cdot 10^1$	b
Cheyenne	$2.8 \cdot 10^6$	±	$2.6 \cdot 10^5$	$14.4 \cdot 10^7$	±	$6.6 \cdot 10^7$	$8.9 \cdot 10^6$	±	$2.1 \cdot 10^6$	$1.4 \cdot 10^3$	±	$0.8 \cdot 10^3$	ab	$12.4 \cdot 10^1$	±	$5.9 \cdot 10^1$	ab
Turkey Red	$3.5 \cdot 10^6$	±	$3.7 \cdot 10^5$	$7.5 \cdot 10^7$	±	$1.0 \cdot 10^7$	$10.5 \cdot 10^6$	±	$2.4 \cdot 10^6$	$6.6 \cdot 10^3$	±	$0.4 \cdot 10^3$	a	$18.7 \cdot 10^1$	±	$7.2 \cdot 10^1$	ab
T. monococcum	$3.1 \cdot 10^6$	±	$4.3 \cdot 10^5$	$10.5 \cdot 10^7$	±	$5.7 \cdot 10^7$	$9.8 \cdot 10^6$	±	$3.7 \cdot 10^6$	$1.6 \cdot 10^3$	±	$0.8 \cdot 10^3$	ab	$20.1 \cdot 10^1$	±	$12.5 \cdot 10^1$	ab
Mean	$3.1 \cdot 10^6$			$12.6 \cdot 10^7$			$9.1 \cdot 10^6$			$2.5 \cdot 10^3$			$23.4 \cdot 10^1$			
CV	0.26			1.2			0.66			0.98			1.5			
ANOVA	*P*-values															
Genotype	0.56			0.74			0.4			**0.027**			**0.034**			

Table S4.3. Alpha diversity indices for prokaryotic (16S) and fungal (ITS) communities in rhizosphere soil for twelve winter wheat genotypes used in an isotopically labelled greenhouse study (n = 5).

	Prokaryotic (16S)			Fungi (ITS)		
Variety	**Richness** (ASV pot^{-1})	**Evenness**	**Shannon Index**	**Richness** (ASV pot^{-1})	**Evenness**	**Shannon Index**
Byrd	2,160 ± 927	0.91 ± 0.01	10.0 ± 0.6	100 ± 25	0.80 ± 0.02	5.2 ± 0.3
Snowmass	2,340 ± 956	0.90 ± 0.01	10.0 ± 0.5	80 ± 20	0.76 ± 0.03	4.8 ± 0.4
Ripper	2,300 ± 1,017	0.91 ± 0.02	10.0 ± 0.7	100 ± 15	0.77 ± 0.04	5.1 ± 0.4
Ron L	2,720 ± 338	0.91 ± 0.00	10.4 ± 0.2	90 ± 18	0.77 ± 0.03	5.0 ± 0.4
Prowers	2,640 ± 318	0.91 ± 0.00	10.3 ± 0.1	100 ± 24	0.80 ± 0.01	5.3 ± 0.3
TAM 107	2,420 ± 887	0.91 ± 0.01	10.2 ± 0.5	70 ± 30	0.79 ± 0.04	4.8 ± 0.7
Sandy	2,170 ± 916	0.92 ± 0.01	10.0 ± 0.5	80 ± 23	0.78 ± 0.04	4.9 ± 0.4
Scout 66	2,380 ± 832	0.91 ± 0.01	10.2 ± 0.5	100 ± 18	0.79 ± 0.02	5.2 ± 0.2
Wichita	3,010 ± 550	0.91 ± 0.01	10.5 ± 0.3	80 ± 17	0.76 ± 0.04	4.9 ± 0.3
Cheyenne	2,760 ± 968	0.91 ± 0.00	10.3 ± 0.5	80 ± 13	0.77 ± 0.02	4.8 ± 0.3
Turkey Red	2,340 ± 785	0.90 ± 0.01	10.1 ± 0.4	80 ± 15	0.78 ± 0.01	4.9 ± 0.2
T. Monococcum	2,550 ± 937	0.91 ± 0.00	10.2 ± 0.6	90 ± 23	0.76 ± 0.02	4.9 ± 0.4
Mean	2486	0.91	10.2	86.4	0.78	5.0
CV	0.31	0.01	0.04	0.24	0.04	0.08
ANOVA	*P*-values					
Genotype	0.74	0.87	0.59	0.61	0.44	0.50

Table S4.4. Spearman correlation (ρ) coefficients between rhizosphere fungal genera and phyla (normalized abundance) and environmental variables measured in a greenhouse study of winter wheat genotypes. Wheat contributions to soil carbon (C) and cover crop (CC) derived nitrogen (N) uptake were determined using stable isotope tracing. Significance of correlation coefficients is indicated with the following symbols and refer to Bonferroni corrected P-values for multiple testing: *p < 0.05; **p < 0.01; ***p < 0.001.

Phylum	Genus	Root Length	N uptake	CC-N Uptake	%CC-N	TDN	LAP	N-enzyme	Normalized Avg. abundance
Ascomycota	NA				-0.39*				
Basidiomycota	NA	0.42*	0.48***						10.34
Glomeromycota	NA		0.72***			-0.55***			7.69
Glomeromycota	*Claroideoglomus*					-0.44*			5.74
Glomeromycota	*Funneliformis*					-0.44*			6.22
Mucoromycota	NA				-0.52***				10.53
Olpidiomycota	NA		0.53***	0.39*			0.39*	0.41**	4.97

[b]Rhizodeposition in the rhizosphere; Labile wheat C, sum of dissolved organic C and microbial biomass C; N uptake, total N uptake in mg pot^{-1}; CC-N uptake, total cover crop-derived N recovered in wheat tissue; TDN, total dissolved N in rhizosphere soil; LAP, leucine amino peptidase activity; N-enzyme, sum of all N-cycling enzyme activity; Normalized avg. abundance; overall average abundance of each genus across all samples, normalized by cumulative sum of squares followed by log transformation

Table S5.1. Length of winter wheat roots (cm) in eight different root diameter size classes grown in a stable isotope labelling chamber inside a greenhouse in Colorado, USA. Plants were vernalized for 6 weeks before transplanting into cylindrical pots with 2 parts agricultural soil to 1-part clean quartz sand. ANOVA p-values are reported below for reference comparing two genotypes and 3 soil treatments: 0x, no amendments for 10 years; 5x, beef feedlot compost added biennially based on 5x estimated wheat nitrogen requirements; Fert, 0x field soil amended with KNO_3 and triple-super-phosphate all alleviate possible nutrient limitation. No treatment differences were detected.

		-- Root length (cm) in diameter size class --							
Soil Legacy	Genotype	0 – 50μm	50-100μm	100-150μm	150-200μm	200-250μm	250-30μm	300-350μm	> 350μm
0x	Byrd	337 ± 143.3	562 ± 208.9	497 ± 167.2	479 ± 157.3	312 ± 99.3	180 ± 55.8	82 ± 26.5	262 ± 91.1
	Snowmass	580 ± 100.5	906 ± 139.8	831 ± 101.7	889 ± 92.7	569 ± 69.2	301 ± 39.5	131 ± 18.1	373 ± 72.8
5x	Byrd	138 ± 53.1	239 ± 85.2	259 ± 110.8	373 ± 167.9	256 ± 111.0	134 ± 58.4	57 ± 27.0	199 ± 95.9
	Snowmass	350 ± 104.0	585 ± 178.0	577 ± 188.2	687 ± 190.8	444 ± 106.3	229 ± 52.5	97 ± 20.1	256 ± 49.8
ANOVA	***p*-values**								
Genotype		**0.06**	**0.10**	**0.10**	**0.08**	**0.08**	0.11	0.14	0.45
Soil Treatment		0.48	0.19	0.32	0.70	0.74	0.58	0.48	0.44
Genotype x Soil Treatment		0.59	0.86	0.78	0.55	0.49	0.56	0.58	0.48

Table S5.2. Total and residue-derived N in dissolved soil pools in rhizosphere soil of greenhouse study comparing two winter wheat genotypes and two soils from different management legacy. Values are treatments means ± standard error. ANOVA *p*-values for genotype and soil management effects are below.

Variety	Soil	Total Dissolved N (mg kg^{-1} soil)	Residue-derived TDN (ug kg^{-1})	MBN (mg kg^{-1})	Residue-derived MBN (ug kg^{-1})	MBC/MBN
Byrd	0x	54.4 ± 9.59	872.3 ± 278.78	10.0 ± 4.26	255.6 ± 107.4	12.3 ± 6.76
	5x	98.5 ± 27.09	606.4 ± 406.34	16.9 ± 7.31	148.6 ± 12.2	2.12 ± 0.12
Snowmass	0x	51.4 ± 4.11	443.5 ± 116.41	12.7 ± 3.56	211.8 ± 71.4	7.7 ± 3.59
	5x	99.1 ± 12.29	816.8 ± 471.86	19.2 ± 1.22	60.4 ± 22.8	5.3 ± 0.17
ANOVA	*p*-values					
Variety		0.61	0.11	0.82	0.80	0.83
Soil Treatment		**0.002**	0.46	0.34	0.53	0.35
Variety x Soil Treatment		0.89	0.70	0.54	0.84	0.53

Table S5.3. Complete indicator analysis results for fungi at the family level in rhizosphere soil of two winter wheat genotypes grown in two soils with contrasting nutrient management legacies. Note: Incertae sedis refers to taxa with uncertain family placement in the taxonomy; in these cases, the order is reported.

	Phylum	Taxa (family)	Statistic	*p* -value for group membership	sig
Byrd		None			
Snowmass	Basidiomycota	Agaricomycetes_fam_Incertae_sedis	0.739	0.04	*
0x	Basidiomycota	Pluteaceae	0.949	0.005	**
	Basidiomycota	Psathyrellaceae	0.93	0.005	**
	Ascomycota	Helotiales_fam_Incertae_sedis	0.89	0.005	**
	Basidiomycota	Geastraceae	0.876	0.005	**
	Ascomycota	Trichocomaceae	0.841	0.005	**
	Ascomycota	Bionectriaceae	0.836	0.01	**
	Basidiomycota	Pleurotaceae	0.823	0.005	**
	Basidiomycota	Entolomataceae	0.814	0.005	**
	Olpidiomycota	Olpidiaceae	0.793	0.045	*
	Ascomycota	Cordycipitaceae	0.792	0.005	**
	Ascomycota	Pleosporales_fam_Incertae_sedis	0.782	0.02	*
	Basidiomycota	Bolbitiaceae	0.766	0.045	*
	Mucoromycota	Rhizopodaceae	0.751	0.005	**
	Basidiomycota	Rhynchogastremataceae	0.747	0.015	*
	Ascomycota	Didymosphaeriaceae	0.742	0.04	*
	Chytridiomycota	Powellomycetaceae	0.738	0.04	*
	Ascomycota	Hypocreaceae	0.731	0.03	*
5x	Basidiomycota	Holtermanniales_fam_Incertae_sedis	0.776	0.005	**
	Ascomycota	Thelebolaceae	0.77	0.01	**
	Basidiomycota	Piskurozymaceae	0.75	0.005	**
	Ascomycota	Plectosphaerellaceae	0.74	0.005	**
	Ascomycota	Microascaceae	0.731	0.005	**
	Basidiomycota	Filobasidiaceae	0.731	0.01	**
	Ascomycota	Gymnoascaceae	0.729	0.005	**
	Ascomycota	Onygenales_fam_Incertae_sedis	0.729	0.005	**
	Ascomycota	Pleosporaceae	0.727	0.005	**
	Ascomycota	Coniochaetaceae	0.726	0.005	**
	Ascomycota	Chaetomiaceae	0.721	0.005	**
Byrd-0x	Ascomycota	Teratosphaeriaceae	0.684	0.015	*
	Ascomycota	Bionectriaceae	0.661	0.045	*
	Basidiomycota	Geastraceae	0.634	0.015	*
	Basidiomycota	Entolomataceae	0.595	0.01	**

Snowmass 0x	Basidiomycota	Agaricomycetes_fam_Incertae_sedis	0.783	0.01	**
	Basidiomycota	Pluteaceae	0.753	0.02	*
	Ascomycota	Diatrypaceae	0.707	0.035	*
	Ascomycota	Helotiales_fam_Incertae_sedis	0.633	0.02	*
	Ascomycota	Cordycipitaceae	0.581	0.01	**
	Mucoromycota	Rhizopodaceae	0.536	0.01	**
Snowmass 5x	Basidiomycota	Holtermanniales_fam_Incertae_sedis	0.556	0.03	*

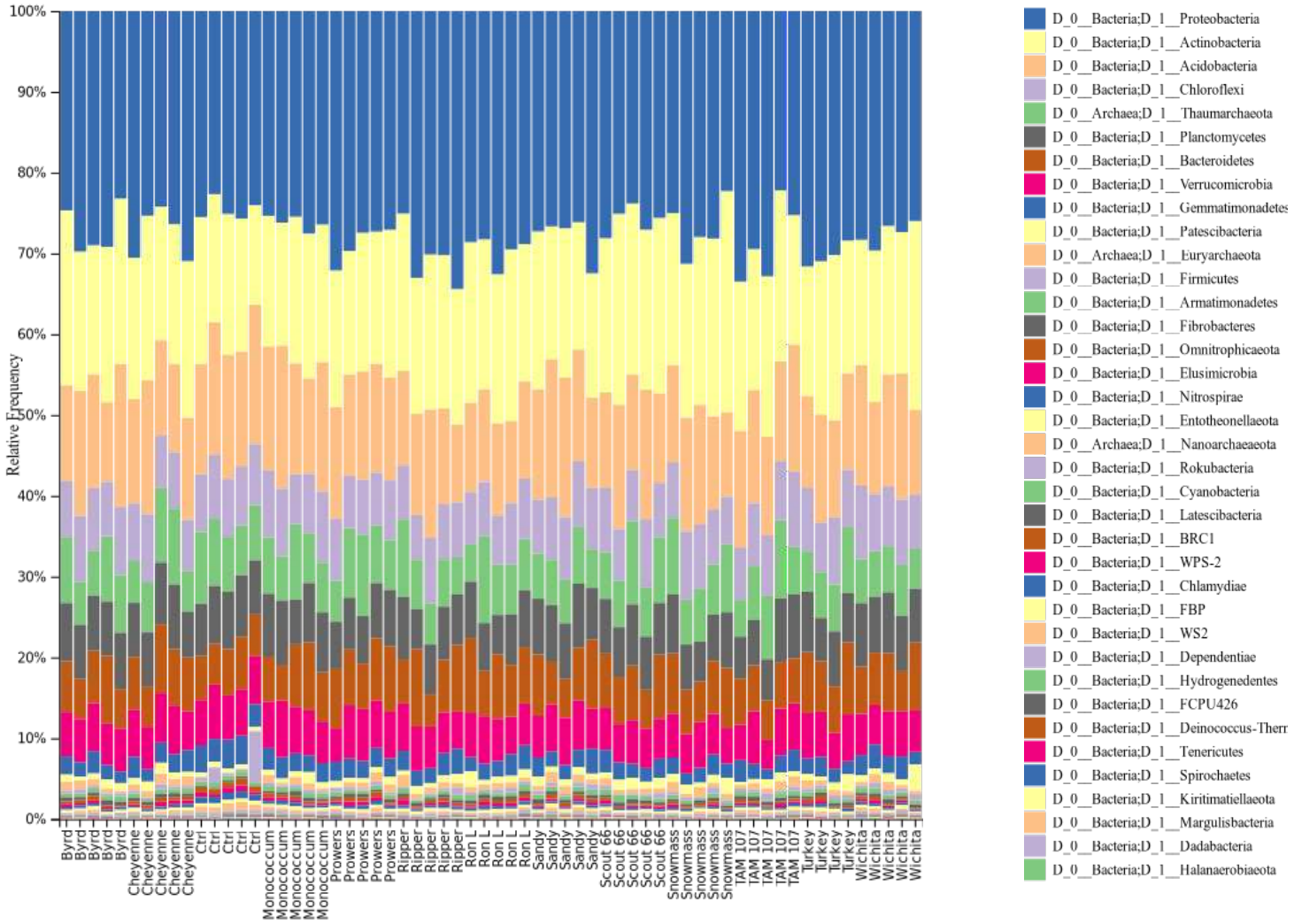

Figure S4.1. Relative abundance of prokaryotic phyla found in rhizosphere soil samples of winter wheat genotypes grown in a greenhouse study.

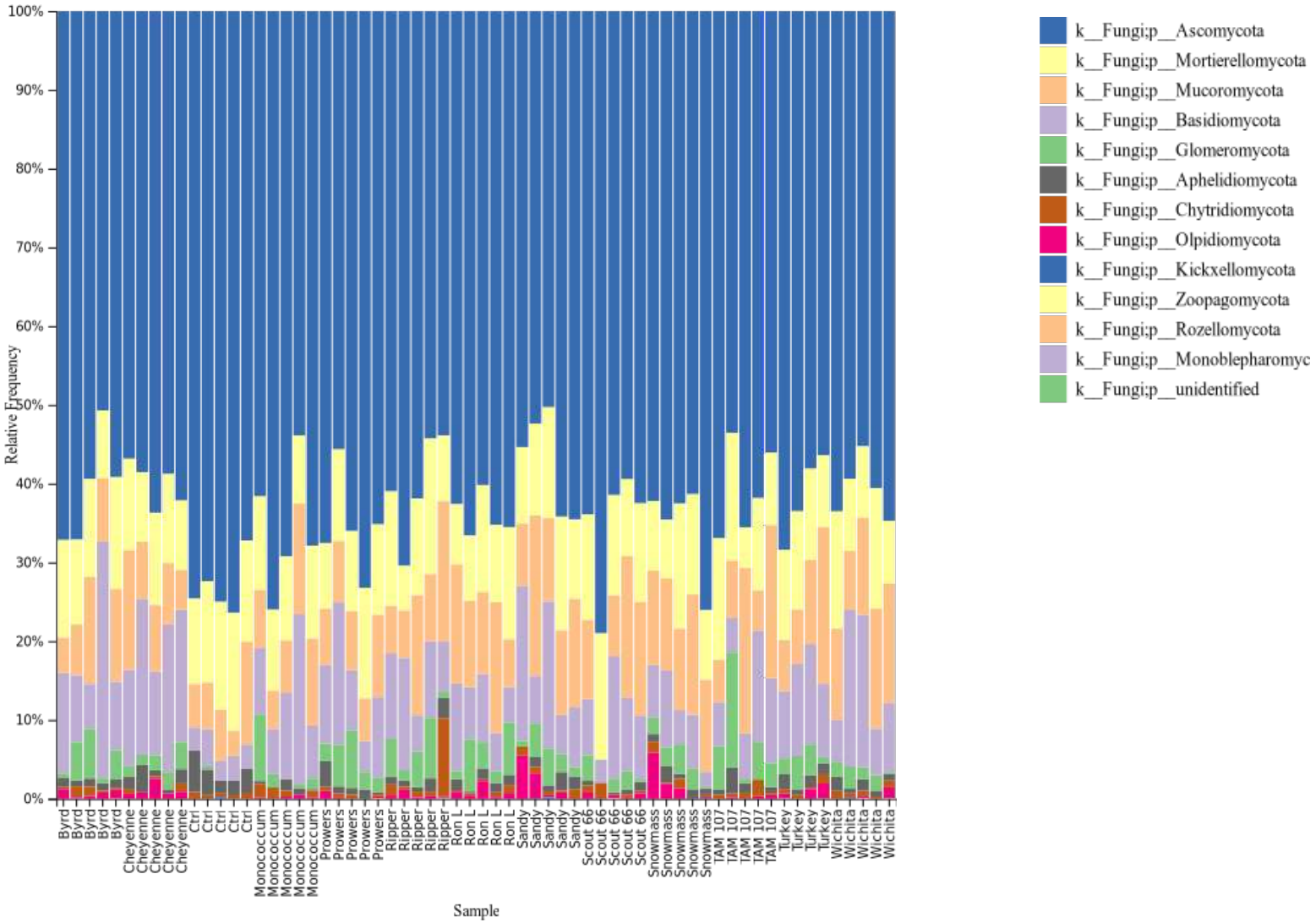

Figure S4.2. Relative abundance of fungal phyla found in rhizosphere samples of winter wheat genotypes grown in a greenhouses study