LONG TERM MULTISPECIES COVER CROPS IN SEMI-ARID MONTANA: SOIL RESPONSE AND ABOVEGROUND BIOMASS

by

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

of

Master of Science

in

Land Resources and Environmental Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

November 2020

ACKNOWLEDGEMENTS

I was lucky enough to be co-advised by two amazing scientists Dr. Catherine Zabinski and Dr. Perry Miller both of whom put immense work into this research. Dr. Zabinski provided endless amounts of support, knowledge, encouragement, and expertise during every step of the project, while also prioritizing happiness and belonging which I am so grateful for. Dr. Miller's knowledge of Montana agriculture made this research directly applicable to producers in the area which is all I hoped for out of this masters degree, while simultaneously answering all my questions and teaching me everything I know about Montana agriculture. I would like to extend my gratitude to my committee member Clain Jones whose wealth of soil chemistry knowledge and attention to detail made me a better scientist, and to Maryse Bourgault for answering all questions and helping with all aspects of the termination study. Additional thanks to Jeff Holmes for teaching me the ropes, executing field work with precision/accuracy, and for never running out of stories; Rosie Wallander for assisting in countless analyses and for her incredible ability to troubleshoot any problem; Christine Gobrogge for assisting in sample analysis; Terry Rick for her help with soil analyses and so much more; and Bailey Servais and Isaack Aasgaard for countless hours of hard work. Thank you to my phenomenal undergrad technician and great friend Zane Ashford, who put in long days in the lab/field and longer nights processing endless samples while maintaining a positive optimistic outlook. Thanks for taking a personal interest in this research and following it every step of the way, long after your time at Montana State came to an end; you made even the longest most grueling days fun. I would also like to thank my parents for their never-ending encouragement and support to follow my passions no matter the direction; for showing through example how important education/adventure can be and lastly to my brother and parents for always coming to Montana to experience this adventure with me.

FUNDING ACKNOWLEDGEMENTS

This project was funded by USDA's Western Sustainable Agriculture Research and Education program, Montana Fertilizer Advisory Committee, and the Montana Wheat and Barley Committee.

Additional thanks to Stephanie Adams and Jamie Dillon for your friendship over the years and your weekend distractions that made the whole process worth it.

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

Table Page

LIST OF TABLES CONTINUED

Table Page

LIST OF FIGURES

ABSTRACT

Low and variable annual precipitation (250-350 mm) make management strategies that conserve soil moisture imperative for wheat producers in semi-arid Montana. A wheat-fallow rotation was historically the most common dryland cropping system in semi-arid Montana, due to its ability to conserve soil water; however, summer fallow has negative environmental impacts (Campbell et al., 1991). There is interest to incorporate cover crops into a rotation as a partial replacement for summer fallow to enhance soil quality.

An eight-yr study explored the effect of cover crops on biological soil properties through aboveground biomass inputs of four plant functional groups: brassica (BC), fibrous root (FR), tap root (TR), and nitrogen fixers (NF) grown as two-species mixes, six-species mixes (three functional groups), a full eight-species mix, and two controls—chemical fallow and sole pea. Cover crops grew for about 60 days, were terminated with glyphosate, then soil samples were taken nine months after termination at wheat seeding.

The only difference in biological parameters based on functional group was that mycorrhizal colonization in wheat was higher following FR than BC at one site. Potentially mineralizable nitrogen (PMN) was 1.6-1.7 times higher and microbial biomass was 1.4 times higher in soils from cover crop treatments relative to fallow at one of two sites. PMN was 1.2-1.3 times higher in soils from six-species mixes than two-species mixes at both sites, and six-species mixes produced 1.4 times more biomass at one site. Nitrogen fixers had the lowest C:N ratio of the functional groups at both sites, while FR had the highest at one site.

In a second study of cover crop termination, cover crops were grown about 90 days and terminated with one of three strategies: chemically, grazing, or haying. Soils were sampled nine months after termination at the time of wheat seeding. Few enzyme differences and no PMN differences or meaningful patterns were discovered among termination strategies. Minimal differences in biological parameters, even when shoot biomass was removed, may mean grazing or haying could improve net revenue without detracting from soil health. In semi-arid annual systems, water limitations may be the main concern with growing cover crops.

CHAPTER ONE

INTRODUCTION TO THE THESIS

Sustainable Agriculture and Summer Fallow Concerns

With a growing population and global diet changes associated with economic growth, great stresses will be put on land and producers to intensify existing crop and livestock systems, and unprecedented demand will be put on our agricultural systems as a whole (Godfray et al., 2010; Alexander et al., 2015). The need to further develop sustainable management systems comes after seeing the negative effects that agricultural practices have generally had on ecological systems. This includes a decline of soil quality, released water pollutants, increased water scarcity, and interruption of natural animal and pest cycles (Gold, 2016). For this reason, management practices centered around sustainable agriculture are frequently being studied, used, and adopted.

Dryland farming is the dominant agricultural practice in the northern Great Plains (NGP) region, including Montana (Tanaka et al., 2010). Summer fallow is a common farming technique used in semi-arid regions with limited and erratic annual precipitation, to conserve available soil water and nitrogen for crops. In Montana, summer fallow remains a common practice in the largest wheat growing region of north central Montana, where annual precipitation averages 250 to 350 mm yr^{-1}, occupying annually 40% or slightly more of the total dryland cropping area (USDA NASS). This management technique involves leaving a field plant-free every other year in rotation with a cash crop, typically a small grain (Janzen, 2011). This summer fallow practice dates back more than a century in this region and was shown to be three times more productive than continuous wheat in the NGP (Smika, 1970). However, this productivity difference may be much smaller in the presence of modern farming techniques, as evidenced by a massive conversion of summer fallow area to annual cropping throughout the northern Great Plains since the early 1970s (Tanaka et al., 2010). Although summer fallow may conserve valuable soil moisture, it is thought to be an unsustainable practice since it degrades soil (Tanaka et al., 2010). Downfalls of the summer fallow technique include increased likelihood for nitrate leaching (Campbell et al., 1991; John et al., 2017) and soil erosion (Campbell et al., 1991). Additional downfalls of summer fallow consist of decreased rates of soil organic matter accumulation (Campbell et al., 2000), and a decline in biological activity in the soil (Acosta-Martinez et al., 2007).

Summer fallow area in the NGP decreased from a peak of 17 million ha in 1971 to 4 million ha by 2007 (Tanaka et al., 2010), and has continued to decline (USDA-NASS, 2019), and is coincident with increased no-tillage management, which increases soil water use efficiency (Baccara et al., 2003; Tanaka et al., 2010), decreasing the need for fallow. No-till management can increase crop residue in annual cropping systems which increases the soil's water holding capacity and soil organic matter accumulation (Campbell et al., 2000; Shrestha et al., 2013; Engel et al., 2017). With summer fallow area steadily declining, more research is required to determine best management practices to conserve moisture and increase soil quality in areas like north central Montana where producers continue the practice of fallow (pers. comm., P. Miller). One potential summer fallow replacement strategy is cover crops and cover crop mixes. Cover crops grown during what used to be the fallow period may have the ability to enhance certain soil properties and be more sustainable than the traditional wheat-fallow system. For this study cover crops are thought to be a crop that is grown for a reason other than income, such as soil health practices.

Cover Crop Possibilities and Challenges

A survey of producers in Montana found that soil water usage was one of the top reasons producers weren't planting cover crops (Jones et al., 2015). Cover crops can deplete soil water and in turn reduce cash crop yields in the short term, greatly increasing short term financial risks for producers (O'Dea et al., 2013; Tallman, 2014; Miller, 2015). In some environmental contexts, soil water use was minimized by strategic timing of the cover crop termination while still gaining soil benefits (Zentner et al., 2004; Miller et al., 2011).

Early cover crop work in the Montana has focused on single species cover crops, specifically nitrogen fixers (Burgess et al., 2014; Miller et al., 2015; Miller et al., 2018). Miller et al. (2006) found promising results for a sole pea cover crop, where pea used equal or less water while providing equal or more nitrogen to the soil, and having equal or greater effect on the following cash crop, than other single species cover crops. Other cover crop species may provide specific benefits such as nutrient scavenging abilities or rapid ground cover, farmers in the region have shown interest in planting more diverse species mixes, as it is thought that diversity could lead to a greater benefits to the soil. Cover crops can enhance soil quality by increasing soil organic matter, soil structure, and enhance the cycling of nutrients (Zentner et al., 2004; Lu et al., 2000); while decreasing the potential for erosion (Tanaka et al., 1997), and suppressing the growth of weeds (Fiksel et al., 2003). Increased cover crop diversity and intensification increased biological activity in some studies in semiarid Texas, which under certain conditions, had a positive correlation with improved crop yields (Acosta-Martinez et al., 2011).

Many benefits seen from growing cover crops, such as weed suppression or nitrogen retention, have had positive correlations with cover crop biomass production regardless of species or mix in subhumid Pennsylvania (Finney et al., 2016). Cover crop mixes with higher species richness are thought to produce a higher quality and quantity of biomass with annual plants in subhumid Nebraska (Wortman et al., 2012). This is because species that have variable physiologies, phenology, and resource requirements can coexist and if complementary, increase diversity according to ecological niche theories (Bulleri et al., 2016). Finney et al. (2017) found that while growing multispecies cover crop mixes provides some soil benefits, there are negative tradeoffs towards other soil parameters. Species within cover crop mixes should be carefully selected to best support a producer's goal to increase desired benefits while avoiding species that may cause a disservice like reduced nitrogen supply or weed suppression.

The species composition of cover crop mixes can affect the ecosystem services the mixture provides (Finney et al., 2017). This thesis looks at two cover crops studies; one with mixes designed based on seed availability to local producers (termination study, Chapter 4), and the other designed by functional group inclusion. Although there are many functional groups, the four functional groups that will be examined in this study are nitrogen fixers, brassica, fibrous roots, and tap roots. Legumes (nitrogen fixers) are commonly chosen for their ability to fix nitrogen through a symbiotic relationship with rhizobia bacteria. Spring pea and lentil are two common legumes grown in Montana, that thrive during the region's optimal precipitation period (Tanaka et al., 2010). Brassicaceae species are chosen as cover crops for their ability to produce rapid ground cover (Lawley et al., 2011) and their potential to increase soil biological activity (Larkin and Griffin, 2007). Fibrous rooted species are chosen for their rooting structure and high carbon additions to soil. Tap rooted species are chosen to reduce the potential for nitrate leaching (Dunbabin et al., 2003) and for their ability to reduce and break up soil compaction (Chen and Weil, 2010).

Termination

The initial cost associated with planting cover crops with no economic return is an additional barrier preventing the adoption of cover crops (Jones et al., 2015). One of the cover crop studies in this thesis specifically compares termination strategies. Using an alternative termination strategy to chemical fallow, such as haying or grazing may provide an economic benefit to the producer to offset the cost of seed, equipment use, and time spent managing the cover crops. This strategy would also present an immediate economic benefit that could potentially offset the initial cash crop yield loss, until long-term soil benefits can be achieved.

Research Objectives

The research goal of this thesis is to provide a knowledge base on the effects of long-term cover crops, that will allow for the creation of cover crop mixes based on how the chosen mix will affect biological, chemical, and physical soil parameters. Designing cover crop mixes based on functional groups in lieu of specific species allows for the results of this study to be transferable to other areas where different species within the same functional groups may thrive.

Chapter Two of this research investigates the long-term effects of spray-terminated multispecies cover crops (after four cycles) on biological soil parameters. Potentially mineralizable nitrogen (PMN), five soil enzymes, mycorrhizal colonization, and microbial biomass were monitored and compared between ten cover crop treatments and a summer fallow control. Four functional groups were compared, with two species in each functional group, along with a full mix plot containing all four functional groups (eight cover crop species). Four additional treatments containing the full mix minus one functional group (containing six cover crop species) was investigated to determine whether the presence or absence of a functional group affected soil quality. A sole-species legume cover crop and chemical summer fallow were used as controls due to the commonality of these practices in semi-arid Montana. The data collected in this chapter allowed me to address fundamental questions about soil change driven by different plant functional groups.

Chapter Three of this thesis examines the effects of varying cover crop mixes on aboveground cover crop biomass quantity and carbon to nitrogen ratios (C:N). Ecological theories based on natural ecosystems predict that cover crop mixes with higher functional richness will produce more biomass (Trenbath, 1999; Wortman et al., 2012). Therefore, this chapter examines differences in aboveground biomass for the 11 treatments from

Chapter Two, exploring functional group differences as well as differences by functional group richness. Since wheat stubble is an important biomass contributor, the total biomass added over two years—cover crop plus wheat aboveground biomass— was examined. The C:N of cover crop biomass inputs can influence the development of soil organic matter (SOM), therefore differences were investigated between the four functional groups.

Since cover cropping in areas semi-arid environments in Montana that receive little rainfall is inherently a financially risky practice, some producers are interested in selecting termination strategies that add financial benefit to growing cover crops, without substantial negative soil impacts. Therefore, in Chapter Four of this research, I compared three different cover crop termination strategies—spraying, grazing or haying—and how they affect biological soil properties. In Chapter Two, I quantified differences in soil parameters due to functional groups, whereas in Chapter 4, I investigated how forage removal, in the form of haying or grazing, may alter those differences. Two cool season cover crop mixes with many of the same species used in the study described in Chapter Two were selected from a larger study as well as a barley monoculture and summer fallow control. The main goal of the work summarized in Chapter 4 was to determine whether forage removal altered the measured soil parameters.

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CHAPTER TWO

SOIL BIOLOGICAL EFFECTS OF HERBICIDE-TERMINATED MULTI-SPECIES COVER CROP MIXES, IN SEMI-ARID MONTANA

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

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Contributions: Secured funding, provided assistance with study execution, advised on statistical analysis and reviewed manuscript.

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Contributions: Secured funding, provided assistance with study execution, advised on all agronomic practices and reviewed manuscript.

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Manuscript Information

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[TBD]

Status of Manuscript:
_X_ Prepared for submission to a peer-reviewed journal
____ Officially submitted to a peer-reviewed journal
____ Accepted by a peer-reviewed journal
____ Published in a peer-reviewed journal

1. Introduction

Growing crops in semi-arid Montana poses a unique set of issues when trying to transition to more sustainable agriculture practices. This area receives very little annual precipitation, mostly ranging from 250 mm – 350 mm, with dramatic annual variation and hot dry summer periods, making management strategies that conserve soil moisture imperative for producers. Summer fallow is a common practice in semi-arid Montana, where fields are left plant-free the growing season following a cash crop, allowing for crops to use the precipitation from more than one growing season (Le Roy et al., 2016). Although summer fallow helps recharge valuable soil water, in the process, it degrades soil (Tanaka et al., 2010). As we gain a better understanding of the biological processes that take place in the soil, shortfalls of the summer fallow management practices have become clear. Summer fallow increases the likelihood for soil erosion (Campbell et al., 1991), and decreases the rate of accumulation of soil organic matter (Campbell et al., 2000) and biological activity in the soil (Acosta-Martinez et al., 2007).

Cover crops can be used as a partial summer fallow replacement strategy, especially in regions with marginal precipitation to continuously crop. Cover crops can enhance soil quality by increasing soil organic matter and soil structure (Zentner et al., 2004), enhancing the cycling of nutrients (Lu et al., 2000), increasing weed suppression (Fiksel et al., 2003), and decreasing the potential for erosion (Tanaka et al., 1997). Cover cropping (specifically with legume species) over a longer period may reduce financial uncertainty for the producer by providing nitrogen at key times and increasing soil quality (Miller et al., 2015). Despite some advantages, cover crops can deplete soil water

(O'Dea et al., 2013; Tallman, 2014; Miller et al., 2017), and hence reduce crop yields, which is one of the top reasons why producers in the semi-arid Montana don't plant cover crops (Jones et al., 2015). In some environmental contexts, soil water use is minimized by strategic timing of the cover crop termination while still gaining soil benefits (Zentner et al., 2004; Miller et al., 2011).

Early cover crop work in Montana focused on single-species legume mixes, specifically pea (Miller et al., 2011; McCauley et al., 2012; Burgess et al., 2014; Miller et al., 2015). However, farmers in the region have shown interest in planting more diverse species mixes, as the diversity could lead to greater soil benefits (Tallman, 2012). Increased cover crop diversity and intensification increases biological activity in studies in semiarid Texas, which under certain conditions, has a positive correlation with improved crop yields (Acosta-Martinez et al., 2011). Increased functional diversity increases plant productivity and other ecosystem services in an ecological grassland study in Minnesota (Tilman et al., 1997). Tilman et al. (2006) found that when compared to monocultures, polycultures lead to more temporal stability and lower inter-annual variation of above ground perennial plant biomass. Finney et al. (2017) found that while growing multispecies cover crop mixes provides some soil benefits, there are negative trade off towards other soil parameters in a study in subhumid Pennsylvania. Species within cover crop mixes need to be carefully selected based on a producer's goal to increase desired benefits while avoiding species that may cause a disservice like reduced nitrogen supply or weed suppression (Finney et al. 2017).

The full range of cover crop benefits can be addressed by assessing changes in soil quality due to the application of cover crops. Soil quality indicators can be either static or dynamic. Static indicators are affected by geological history and climate, whereas dynamic indicators change over relatively short periods of time (Gianfreda & Rao, 2019). Ideally, a soil quality indicator should be sensitive to changing conditions, measure a function or multiple functions within the soil, provide reference or threshold values, be easily interpreted, and be easy and inexpensive to obtain (Dalal, 1998). However, these characteristics are harder to quantify than to define.

Extracellular soil enzymes are enzymes excreted from microbes or plants that live freely in the soil and are important for organic matter breakdown and nutrient cycling. Soil enzymes are ideal soil quality indicators because they respond more quickly to management changes than other indicators and because they have a direct relationship with sustaining biological diversity, activity, and productivity, as well as storing and cycling nutrients (Lehman et al., 2015). Due to enzymes' quick response to changes, they have the potential to predict outcomes of certain management techniques (Nannipieri et al., 2018).

Another soil quality indicator is microbial biomass, which accounts for only a small amount of soil carbon, but is the biologically active component of the soil. Microbial activity is limited by labile carbon (C) and available energy, which crop residues greatly affect (Schimel and Weintraub, 2003, Nair and Ngouajio, 2012). A meta-analysis on soil microbial biomass found that increased crop diversity by adding one or more crops to a monoculture resulted in a 21% increase in microbial biomass carbon and a 26% increase in microbial biomass nitrogen regardless of crop type in annual continuous cropping systems (McDaniel et al., 2014).

A common limiting nutrient in agricultural systems is nitrogen; therefore, parameters related to plant available nitrogen can be helpful soil quality indicators. Potentially mineralizable nitrogen (PMN) is an estimate of how much nitrogen will become available to the plant during the growing season due to microbial breakdown of organic compounds (Drinkwater et al., 1997). Cropping systems with shorter fallow periods, more diverse cropping systems, and limited tillage, have higher PMN values than conventional cropping systems (Liebig et al., 2006).

Within the soil biological realm, arbuscular mycorrhizal fungi (AMF) have been used as a soil quality indicator in natural and agricultural settings. These fungi form a mutualistic relationship with many plants where plant-released carbon is exchanged for nutrients taken up by the fungus (Pirozynski, 1975). Arbuscular mycorrhizal fungi can provide beneficial services to the cash crop (host crop), including improved phosphorus uptake (Jansa et al., 2006) and pathogen protection (Rillig, 2004). Chemical fertilization can reduce AMF colonization since plants can access key nutrients without establishing a symbiosis that requires providing carbon to the symbiont (Corkidi et al., 2002). Fallow periods have also been shown to reduce AMF populations, and the use of cover crops reduce that fallow period (Lehman et al., 2012). Certain families do not form mycorrhizal associations, such as plants in the Brassicaceae family.

In 2019, 2.2 million ha of wheat was grown in Montana (USDA-NASS, 2019). Summer fallow area was 1.1 million ha, with the largest dryland wheat growing region of north central Montana having the greatest proportion of fallow area, greater than 40%. For producers in areas possibly too dry for continuous cropping, but who want to improve soil health, cover crops could be a good fit, specifically with legume green manures (LGM), as a summer fallow replacement strategy. Although there has been research done on LGM in the semi-arid Montana this research is specifically focused on the effects of multispecies cover crop mixtures, asking the following questions: Does having any cover crop increase the activity of biological parameters when compared to summer fallow? What are the effects of cover crop plant functional groups on biological soil properties; does the presence of a specific functional group affect biological soil parameters? Does increased plant diversity increase activity of biological parameters?

This research investigated the long-term effects of multispecies cover crops on biological soil parameters after four rotations of cover crops in dryland wheat systems. In semi-arid regions, where annual fluctuations in weather can greatly impact soil parameters, long term studies are important. Long term studies are also important because it may take several years for a soil response to cover crops to be detectable from experimental noise. To assess soil quality change, PMN, mycorrhizal colonization, soil enzymes, and microbial biomass were monitored and compared among fallow, pea, and nine cover crop mixtures derived variably from four plant functional groups. Assessing multiple soil parameters can give us a better picture of soil quality, because different aspects of the soil can respond differently.

Because plants provide organic matter inputs to soil systems, we expected that the presence of cover crops would increase enzymatic activity, microbial biomass, and PMN, when compared to the summer fallow control. It was also expected that cover crop functional groups would affect soil biological parameters differently due to varying phenologies, biomass production, and nutrient requirements. With this study design, we could assess the effects of a cover crop versus summer fallow (SF) by comparing the PEA and FULL treatments to the SF. We compared SF (0-spp), PEA (1-spp), and FULL (8-spp) since SF and LGM are more common practices hence used as controls. To explore how increased plant diversity may affect biological parameters, we compared the single functional groups, which each contained two species, with four 6-spp mixes (the minus treatments). Pairwise comparisons between each functional group and the coinciding minus functional group were used to determine whether the presence of a specific functional group affected soil biological parameters. However, because cover crops in dryland wheat systems are only grown for about two months, and then the field is left fallow for nine months before planting spring wheat again, differences among treatments may be ephemeral and thus unimportant to the following cash crop. Thus, we focused on sampling soil coincident with spring wheat planting.

2. Materials and Methods:

2.1 Study sites

This study was conducted on two private farm fields located near Amsterdam (N 45.72°, W 111.37°) and Conrad (N 48.22°, W 111.48°), Montana. Both sites had no-till farming histories prior to this study and were managed primarily as commercial wheat farms. The Amsterdam site receives an average of 356 mm of precipitation annually and its soil is classified as a frigid, Aridic Calciustoll silt loam. In 2018, the Amsterdam site received 229 mm of precipipitation during the growing season (April-June). The Conrad site receives an average of 280 mm of precipitation annually and is classified as a frigid, Aridic Argiustoll clay loam (Table 2.1). In 2018, the Conrad site received 138 mm of precipipitation during the growing season (April-June).

2.2 Study Design

The study at each site consisted of four randomized complete blocks, each containing 11 treatments. Cover crop mixtures included four plant functional groups: brassicas (BC), fibrous rooted crops (FR), nitrogen fixers (NF), and tap-rooted crops (TR). Brassicas were selected for rapid establishment of ground cover and their unique phytochemistry; fibrous rooted plants were chosen for their expansive root system and hence C inputs into the soil; nitrogen fixers were picked for their capacity to add nitrogen to the system; and tap-rooted plants were chosen for the root structures to potentially reduce soil compaction. Two species of each functional group (Table 2.2) were grown in individual plots (8 x 12 m), along with a full mix (FULL) plot containing all four functional groups (8 cover crop species). Four additional treatments contained the full mix minus one functional group (containing 6 cover crop species; minus brassica, MBC; minus fibrous root, MFR; minus nitrogen fixers MNF; and minus tap root, MTR) to determine if the absence of a functional group affected soil quality. Two control treatments were a sole species legume cover crop (PEA) and chemical summer fallow (SF).

2.3 Plot Design

The cover crop plots were planted in early May at a depth of 1 to 2 cm and a consistent target density of 120 plants m^{-2} to eliminate biasing biomass in favor of plant species with higher seeding rates recommended for grain production. Following cover crop treatments (2012, 2014, 2016, 2018), spring wheat (Triticum aestivum L. cv. Duclair) was grown (2013, 2015, 2017, 2019) to assess the cash crop response to cover crop treatments. Wheat was seeded in rows perpendicular to the cover crop rows. Nitrogen (N) fertilizer was applied to the wheat crop at three rates, (0 kg N ha^{-1}, 67 kg N ha^{-1}, and 135 kg N ha^{-1}) in a split plot design with fertilizer rates as the subplot level. The study began in 2012 with the first round of cover crop treatments and has generally followed this rotation and fertilizer treatments since. Cover crop plots were terminated with glyphosate in early July after approximately 60 days of growth.

2.4 Soil Investigation

After four cover crop cycles, soil samples were collected in early April 2019 at the time of spring wheat planting (~9 months after cover crops were terminated), to measure soil conditions coincident with the start of spring wheat growth. Soils in the SF, PEA, and FULL treatment groups were sampled at all three N rates. Samples for the remaining treatments were only taken at the middle N fertilization rate (67 kg N ha^{-1}), unless otherwise stated. Soils were cored manually (2-cm diam.) to sample the top 10 cm of soil by compositing six randomly located cores to represent each subplot. The corer was flame-sterilized after rinsing with 97% ethanol between plots. Field-moist composited samples were passed through a 2-mm sieve and stored at 4 °C for a maximum of 30 days, until lab analyses were performed.

2.5 Potentially Mineralizable Nitrogen

Potentially mineralizable nitrogen (PMN) was analyzed as adapted from Keeney and Nelson (1982). Six flasks received 5 g of soil, three of which were immediately extracted for 30 minutes with 1 M KCl (mechanically shaken for 30 minutes, 310 rpm) in 25 mL KCl and 5 g soil and analyzed using a Lachat auto analyzer (Lachat Instruments, Loveland, CO), with the average value reported. The additional three flasks were pumped with N_2 gas for five seconds to create anoxic conditions and were kept in a dark incubator at 30 °C for 14 days. These samples were then analyzed using the same KCl extraction method and Lachat. PMN was calculated by subtracting the difference between plant available nitrogen (ammonium only) at the time of soil collection and after the 14-day incubation period.

2.6 Soil enzymes

Soil enzyme activity was analyzed for all treatments using the procedure outlined by Dick (1997; 2011) and Parham and Deng (2000). Duplicates of 1g of field moist soil for each sample and controls were incubated with the enzyme specific substrate for 1 hour at 37 °C, then filtered and analyzed spectrophotometrically. Controls were treated the same as samples but didn't get the substrate specific p nitrophenyl until right before filtration. Dry weight equivalent measurements were used to express enzyme concentrations per g of dry soil. Five enzymes were analyzed: β-1,4-glucosidase (EC 3.2.1.21, involved with Carbon cycling), β-1,4-N-acetyl glucosaminidase (EC 3.2.1.30, involved in carbon and nitrogen cycling), arylsulfatase (EC 3.1.6.1, involved with sulfur cycling), acid phosphatase and alkaline phosphatase (EC 3.1.3.1/2, involved in phosphorus cycling). Geometric means were calculated for each plot by taking the fifth root of the product of all enzymatic activity (Garcia-Ruiz et al., 2008). Enzyme analyses were performed on soils from the four functional groups, FULL, and both controls (SF, PEA) at the 67 kg N ha^{-1} fertilization rate. β-1,4-N-acetyl glucosaminidase was also analyzed at all three N fertilization rates for the SF, PEA, and FULL treatments to assess fertilizer effects on enzymes involved in nitrogen cycling.

2.7 Microbial Biomass

Microbial biomass was inferred for soil using substrate induced respiration (SIR; Fierer et al., 2003) from the four single-functional group plots, FULL, and both controls (SF, PEA) at the 67 kg N ha^{-1} fertilization rate. Five g of soil was weighed and put into a 50-mL falcon tube, then a yeast solution was added and tubes were put on a shaker table for 4 hr. The CO_2 concentration in the headspace of the tubes was measured at the time of yeast addition, as well as 2 and 4 hr after the yeast addition, via gas chromatography (Varian CP 3800 gas chromatograph). The rate of SIR is used to infer microbial biomass (Fierer et al., 2003). Mixed linear regression models were used to assess SIR-induced respiration rate as an index of microbial biomass at each site separately due to varying initial site conditions.

2.8 Mycorrhizal colonization

Mycorrhizal colonization of wheat roots was measured in plants following the four functional groups, FULL, both controls (SF, PEA), and the minus brassica treatment (due to lack of mycorrhizae on brassica species) in 2019 at the 67-kg N ha^{-1} fertilization rate. Single wheat plants were harvested at the time of wheat anthesis in duplicate. Roots were washed, cleared with KOH and stained with trypan blue (McGonigle et al., 1990). Mycorrhizal structures (arbuscules, vesicles, hyphae) were counted using the gridline intersection method on a compound microscope under 200x magnification.

2.9 Statistical Analysis

The R statistical package (R Core Team, 2017; Pinheiro et al., 1997; Fox, 2003; Fox and Weisberg, 2011; Hothorn et al., 2008; Wickham et al., 2017) was used for all statistical analyses for the objectives in this study. The ANOVA function was used to compare cover crop treatments, and Tukey's HSD with a 90% confidence interval was used for comparisons among treatments. Cook's distance (Cook, 1977) with a limit of four times the mean, was used to assess outliers. Residuals vs fitted value plots and Q-Q plots (Fox and Weisberg, 2011) were used to check the assumptions (normality and equal variance) for both ANOVAs and mixed models. If an assumption was not met, transformations were explored to normalize data. Several enzymes were log transformed (base 10) before being analyzed. Linear mixed effects models were used, where block was always a random effect variable, treatment was a fixed effect variable and the biological parameter was the response variable. Site locations were analyzed separately due to site differences and varying levels of precipitation. Mixed models (Pinheiro et al., 2017) and two-way ANOVAs were used to test for a treatment effect on PMN, single soil enzymes, enzyme geometric means, microbial biomass, and mycorrhizal colonization.

Interaction terms were considered when appropriate and top-down model refinement techniques were used. A correlation matrix was created using Pearson's coefficients (r) between all biological parameters and 2018 aboveground cover crop biomass with an alpha level of 0.10.

3. Results:

3.1 Potentially Mineralizable Nitrogen

At both sites, N fertilizer rate did not significantly affect PMN values and interaction terms were not significant in the models (Table 2.3). When PMN values were averaged across all three nitrogen rates, a cover crop treatment effect was present at both Conrad (F = 4.37, p = 0.02) and Amsterdam (F = 21.1, $p < 0.01$; Table 2.4). At Conrad, soils following PEA had 1.6 times higher PMN values than those following SF (p = 0.02). There were no differences between PEA and FULL or FULL and SF. At Amsterdam, PMN was 1.6 times higher in soils following PEA and FULL than soils following SF ($p < 0.01$). There were no differences in PMN between PEA and FULL at either site.

Potentially mineralizable nitrogen was also analyzed at just the medium nitrogen rate for all 11 treatments. At the Amsterdam site, medium N rate PMN values were 68% higher in the FULL plot than SF ($p < 0.01$) and PEA had 56% higher PMN values than SF ($p < 0.01$), while there were no differences among the FULL, PEA, and SF treatments at Conrad in the medium N rate. Pairwise comparisons between the presence vs absence of each functional group (functional group vs minus functional group) at the medium N rate (Figure 2.1) found there were no differences between any of the pairs at either site. A two-species (the single-functional group treatments) versus six-species (minus treatments) linear orthogonal contrast found that six-species mixes had 20% and 34% higher PMN values than soils following two-species mixes at Amsterdam ($p < 0.01$) and Conrad ($p = 0.02$) respectively (Table 2.5).

3.2 Soil Enzymes

No differences among treatments were found for any enzymes at the Conrad site (Table 2.6). The Conrad site had herbicide resistant weeds present, which grew substantial weed biomass (Chapter Four). This could explain the lack of enzyme concentration differences seen at this site. At the Amsterdam site, alkaline phosphatase activity was ~10% higher in SF and FULL soil than PEA soil ($p = 0.02$, $p = 0.01$), with no other differences in individual enzymes. Soil enzyme activity did not differ in soils among the single functional groups for any of the individual enzymes or the geometric means at either site (Table 2.7). Arylsulfatase data was extremely variable leading to large numerical variation within cover crop treatments without any significance at Conrad. This high variability was also seen for Arylsulfatase in 2015 at the Conrad field site (Housman et al., 2016). An earlier soil assessment after two rotations of cover crops, revealed enzyme geometric means were greater in PEA and FULL cover crop treatments than SF (Housman et al., 2016). However, after four rotations of cover crops (7 yr) distinct differences among treatments were not seen in any of the five enzymes or the geometric mean at either site.

3.3 Microbial Biomass

The microbial respiration rate was 1.4 times higher in soil following PEA than soil following SF at Conrad ($p = 0.04$) and 1.3 times higher at Amsterdam ($p = 0.06$). At the Conrad site, the FULL treatment had 1.4 times higher microbial biomass than the SF treatment ($p = 0.07$, Figure 2.2). No statistical differences were seen among functional groups at either field site.

3.4 Arbuscular Mycorrhizal Colonization

At both field sites, there were no differences in AMF colonization rates among the FULL, PEA, and SF treatments during the subsequent wheat phase in 2019 (Table 2.8). The four functional groups were analyzed to assess the role of specific functional groups on mycorrhizal colonization. At Conrad, AMF colonization of wheat was higher in the FR (26% colonization) than in BC treatment (16% colonization; $p < 0.01$). There were no differences in AMF colonization in wheat following any of the other functional groups. At the Amsterdam location, there were no differences in mycorrhizal colonization levels of wheat among any of the treatments. Importantly, at both field sites, there were no differences in AMF colonization of wheat between the BC and MBC.

3.5 Cover Crop Biomass Correlations with Biological Soil Parameters

All soil biological parameters were tested to see if they correlated with the 2018 aboveground cover crop biomass data. The five individual enzymes as well as the geometric mean did not correlate with the previous cover crop aboveground biomass at either site (Table 2.9). Microbial biomass and arbuscular mycorrhizal colonization also did not correlate with the aboveground biomass at either site. The only soil biological parameter that correlated with the 2018 cover crop aboveground biomass was PMN and only at one site (Amsterdam, r = 0.50, $p = 0.01$).

4. Discussion:

Sustainable management farming practices are encouraged and incentivized by federal soil conservation programs, and tried by an increasing number of producers. Although summer fallow helps recharge valuable soil water, it contributes to loss of soil quality (Tanaka et al., 2010). Soil systems are dynamic, and an adequate understanding of the abiotic and biotic processes taking place beneath the surface is still lacking. The experimental design of this project provided a unique opportunity to test a variety of cover crop mixes and plant functional groups, whereby 1) each functional group appeared separately as four functional group treatments ('presence'), 2) they appear together in a complete mixture, and 3) the complete mixture minus each functional group ('absence').

4.1 Fallow versus cover crop

To determine how cover crops affect soil biological parameters compared to fallow, mycorrhizal colonization, enzymes, microbial biomass, and PMN, were measured for the FULL, PEA, and SF treatments. Soil that had cover crops growing in it prior had no difference on AMF colonization, or on single enzymes and enzyme geometric mean when compared to SF. Many studies have shown that reducing or eliminating the fallow periods (plant-impoverished) with the use of cover crops increases AMF formation in the soil (Garcia-Gonzalez et al., 2016; De Vries and Bardgett, 2012; Schipanski et al., 2014).

However, none of these experiments took place in a semi-arid environment with a comparably limited water supply. Microbial biomass values were 1.4 times higher in both cover crop treatments than SF at the drier field site (Conrad) and 1.3 times higher for PEA than SF at Amsterdam. Potentially mineralizable nitrogen values for cover crop treatments were 1.6 times higher than SF at the Amsterdam site. This is likely the case because N returns are higher in the cover crop treatments due to cover crop biomass. Supporting this idea, no differences were seen between FULL or PEA because these treatments produced similar amounts of biomass (Chapter 4). This follows similar trends to the previous study years (2013 and 2015), where no PMN differences were seen at the Conrad site, while cover crop treatments had higher PMN values than SF at the Amsterdam site (Housman, 2016). Similarly, O' Dea et al. (2013) found that PMN was higher following a legume green manure than fallow at most sites in the same region as this study. It is important to note that PMN is dynamic and we measured only once, nine months after cover crop termination. However little N will be mineralized in the winter months. In summary, we measured only small increases in soil biological parameters nine months after cover crop termination compared to fallow.

4.2 Functional group effects

There were no differences in soil enzymes, PMN, or microbial biomass among cover crop plant functional groups in this study. However, because cover crops in dryland wheat systems are only grown for about two months, leaving the field fallow for nine months before planting spring wheat again, differences between treatments may disappear before the cash crop is grown. Arbuscular mycorrhizal fungi colonization was the only parameter that differed among functional groups in this study at one of the two sites. The AMF colonization of wheat was higher in plots following fibrous rooted cover crops than brassica cover crops, at Conrad. This is an expected result since brassicas are non-mycorrhizal, and plants in the Gramineae family are generally mycorrhizal. However, in contrast, AMF colonization of wheat did not differ in treatments following BC relative to MBC, at either site.

Wheat following BC had the lowest observed AMF colonization of any of the functional groups at one if the sites. This is likely due to species in the Brassicaceae family not forming mycorrhizal associations and mycorrhizal fungi may not survive well in soil over a two-year period. A meta-analysis on field and greenhouse studies using mostly annual crops, showed that using mycorrhizal crops instead of nonmycorrhizal crops leads to increased colonization (Lekberg and Koide, 2005). Another study, in Australia with similar in-season precipitation as our Amsterdam site, found that AMF colonization in wheat was higher following a legume (chickpea) than a brassica (canola; Bakhshandeh et al., 2017). The study highlighted the importance of selecting species that could provide multiple benefits to the rotation (reduce pathogens, fix nitrogen). Bowles et al. (2017) meta-analysis also highlighted that cover crop type plays an important role in AMF colonization, and cash crops following legumes specifically, saw increased AMF colonization mainly in maize systems.

Functional groups also had no measurable effect on PMN at either site. In semi-arid environments, where the system is most likely limited by biomass inputs, it is possible that the microbial community is decomposing residues of cover crop biomass quickly enough so that any soil biological changes that may have been present during cover crop growth and shortly after termination do not persist in the soil long enough for the changes to affect the cash crop.

Acosta-Martínez et al. (2018) found a strong positive correlation between soil organic carbon and soil enzyme activity in soils from semi-arid Texas and subhumid Minnesota. The soil carbon inputs measured in this study would be cover crop biomass as well as wheat stubble biomass. However, biomass C is only a small fraction of the much higher soil organic carbon (SOC). Soil organic carbon for this experiment is relatively similar among treatments so the large SOC present in all treatments may make it difficult to see smaller changes from biomass C (Jones unpub data). Leading to the conclusion that in semiarid regions, it is especially hard to influence soil quality due to low biomass returns.

4.3 Species richness or functional group richness

To explore how increased plant diversity may increase biological parameters, we compared the four functional groups (2-spp) versus the four minus functional groups (6-spp). Values for PMN were higher for 6-spp cover crop mixes than for 2-spp mixes. Chu et al. (2017) found that a 2-spp and a 5-spp cover crop mix both containing legumes, had higher PMN values than the fallow control but no differences between the two cover crop mixes. Although we talk about species richness, it should be noted that this could be functional group richness and not species driven. A two-year field study in Pennsylvania found a slight correlation between species richness of cover crop mixes and multifunctionality of the mix, however multifunctionality was more strongly predicted by functional diversity (Finney & Kaye, 2017).

Although observed differences in enzyme activity was not seen, Lehman et al. (2015) points out an important limitation on current soil enzyme interpretation. The processes that control soil enzymatic communities (structure and function) must first be understood in order to optimize ecosystem functions and services from soil enzymes. Enzyme production should increase when simple nutrients are scarce and complex nutrients are abundant, because the enzymes have the ability to break down these complex nutrients (Allison and Vitousek, 2005). Enzyme geometric means were calculated in this study because no single enzyme can represent the cycling of all nutrients in the soil. The total microbial activity serves much more complex functions within the soil which many biochemical reactions contribute to (Gil-Sotres, 2005). Acosta-Martinez et al. (2011), points out the importance of the data evaluation tools chosen to interpret the soil enzyme results and the cruciality of understanding the actual trends present. An avenue for future research that would make soil enzymatic activity data more reliable would be to determine reference levels within certain soil types and to determine a threshold at which values could be considered different and not associated with random variation and heterogeneity of the soil.

This research investigated the role of cover crops versus fallow ground, plant functional groups within cover crop mixtures, and diversity within mixtures on soil biological parameters, which all may allow for more strategic design of cover crop seed mixes for targeted soil improvement. However, our results overall did not detect many soil biological responses to cover crop mixes. Species selection for cover crop mixes should be based on adaptiveness to the environmental conditions and should likely include a N-fixer to optimize soil biological benefits related to nitrogen availability.

Table 2.1 Site conditions for the two field sites for a 0 – 15 cm depth at the start of the 8-yr study (2012).

Site	Amsterdam	Conrad
Elevation (ft)	4740	3410
Texture	Silt loam	Clay loam
pH	8.2	6.5
SOM (%)	2.4	2.4
NO_3-N (ppm)	6.0	8.5
Olsen P (ppm)	13	28
Extractable K (ppm)	359	498

Table 2.2 Plant species included in 10 cover crop treatments and a chemical fallow control.

Treatment		Plant Species
Fallow	SF	Incidental weeds
Pea	PEA	Forage Pea
Full Mix	FULL	Forage pea (*Pisum sativum* L. cv. Arvika) Black lentil (*Lens culinaris* Medik. cv. Indianhead) Oat (*Avena sativa* L.) Canaryseed (*Phalaris canariensis* L.) Turnip (*Brassica rapa* L.) Safflower (*Carthamus tinctorius* L.) Forage Radish (*Raphanus sativus* L. var. *longipinnatus)* Winter canola (*Brassica napus* L.)
Brassicas	BC	Forage radish, Winter canola
Minus Brassicas	MBC	All but canola, radish and turnip
Fibrous Roots	FR	Oat, Canaryseed
Minus Fibrous Roots	MFR	All but oat and canaryseed
Nitrogen Fixers	NF	Forage pea, Black lentil
Minus Nitrogen Fixers	MNF	All but pea and lentil
Taproot	TR	Turnip, Safflower
Minus Taproots	MTR	All but turnip and safflower

Table 2.3 p-values from ANOVAs testing the interaction term of fertilizer rate and cover crop treatment on potentially mineralizable nitrogen (PMN), then testing fertilizer after the interaction was removed on PMN at Amsterdam and Conrad, MT.

	Amsterdam	Conrad
Fert*Treatment	0.41	0.31
Fert	0.42	0.78

Table 2.4 PMN (mg-N kg^{-1}) for summer fallow, full 8-spp mix, and PEA treatments averaged across all three N rates, following four rotations of cover crops at two field sites.

	Amsterdam			Conrad		
	SF	FULL	PEA	SF	FULL	PEA
	25.4^{b}	40.9^{a}	42.5^{a}	18.2^{b}	22.5^{ab}	29.9^{a}
p-value	<0.01			0.02		
$F\text{-stat}_{2,28}$	21.99			4.37		

Letters signify significant differences over an average of all three nitrogen rates ($p = 0.1$).

Table 2.5 Potentially mineralizable nitrogen (mg-N kg^{-1}) for 2-spp mixes (functional groups) vs 6-spp mixes (minus treatments), following four rotations of cover crops at two field sites.

	Amsterdam	Conrad
2-spp	32.5^{b}	18.8^{b}
6-spp	39.3^{a}	25.3^{a}
p-value	<0.01	0.02

Letters signify significant differences over an average of all three nitrogen rates within a site ($p = 0.1$).

Table 2.6 Enzymatic activity (mg PNP g soil^{-1} hr^{-1}) of five soil enzymes (ß- glucosidase, ß -glucosaminidase, acid and alkaline phosphatases, and arylsulfatase) for 3 treatments at the medium N rate, following four rotations of cover crops at two field sites.

	Acid Phosphatase		Alkaline Phosphatase		Arylsulfatase		B-glucosidase		B-glucosaminidase		Geometric mean	
Treatment	A	C	A	C	A	C	A	C	A	C	A	C
SF	90.6	193	330[a]	73.4	327	48.5	84.4	81.5	12.5	17.7	99.7	60.4
FULL	88.1	218	336[a]	72.9	260	70.9	78.8	92.0	10.3	17.2	89.6	69.6
PEA	89.1	170	296[b]	70.7	251	82.6	76.3	87.9	10.4	19.2	85.6	64.5
p-value	0.94	0.27	**0.02**	0.99	0.45	0.51	0.61	0.63	0.42	0.77	0.34	0.42
F-stat $_{2,6}$	0.06	1.62	7.66	0.01	0.91	0.76	0.55	0.50	1.01	0.27	1.31	1.00

Different letters signify significant differences within a site (*p* = 0.1).

Table 2.7 Enzymatic activity (mg PNP g soil^{-1} hr^{-1}) of five soil enzymes (ß- glucosidase, ß -glucosaminidase, acid and alkaline phosphatases, and arylsulfatase) for 4 functional group treatments at the medium nitrogen rate, following four rotations of cover crops at two field sites.

	Acid Phosphatase		Alkaline Phosphatase		Arylsulfatase		B-glucosidase		B-glucosaminidase		Geometric mean	
Treatment	A	C	A	C	A	C	A	C	A	C	A	C
BC	92.8	169	304	79.5	318	84.4	85.3	75.5	11.1	17.2	95.8	62.7
FR	84.0	203	333	37.2	303	42.8	82.7	68.4	11.1	19.0	94.6	51.5
NF	95.3	199	348	54.0	303	51.5	84.6	86.1	11.0	21.6	98.0	62.5
TR	82.1	189	299	59.6	316	52.4	76.3	70.0	10.5	18.9	89.6	56.4
p-value	0.51	0.60	0.36	0.19	0.5	0.53	0.56	0.43	0.98	0.5	0.65	0.53
F-stat$_{3,9}$	0.82	0.66	1.21	1.93	0.85	0.79	0.73	1.02	0.06	0.85	0.56	0.79

Table 2.8 Arbuscular mycorrhizal fungi colonization (%) for eight treatments at the medium nitrogen rate, following four rotations of cover crops at two field sites.

Treatment	Amsterdam	Conrad
SF	14	19 [ab]
PEA	17	22[ab]
FULL	21	16[b]
BC	16	16[b]
MBC	20	18 [ab]
BC	16	16[b]
FR	19	26[a]
NF	22	20[ab]
TR	18	21[ab]
p-value	0.39	0.01
$F\text{-stat}_{7,21}$	1.11	3.37

Letters signify significant differences within a site ($p = 0.1$).

Table 2.9 Correlation matrices (r) of soil biological response with 2018 aboveground cover crop biomass for all 11 treatments (Mg ha^{-1}).

	Amsterdam	Conrad
Microbial biomass	0.07	0.08
β-glucosidase	-0.04	0.00
β-glucosaminidase	-0.19	0.30
Arylsulfatase	-0.28	-0.09
Acid Phosphatase	0.09	0.11
Alkaline Phosphatase	0.12	-0.30
Geometric Mean	-0.13	-0.10
PMN	**0.50***	-0.02
Mycorrhizal Colonization	0.27	0.17

* *p-value* < 0.01

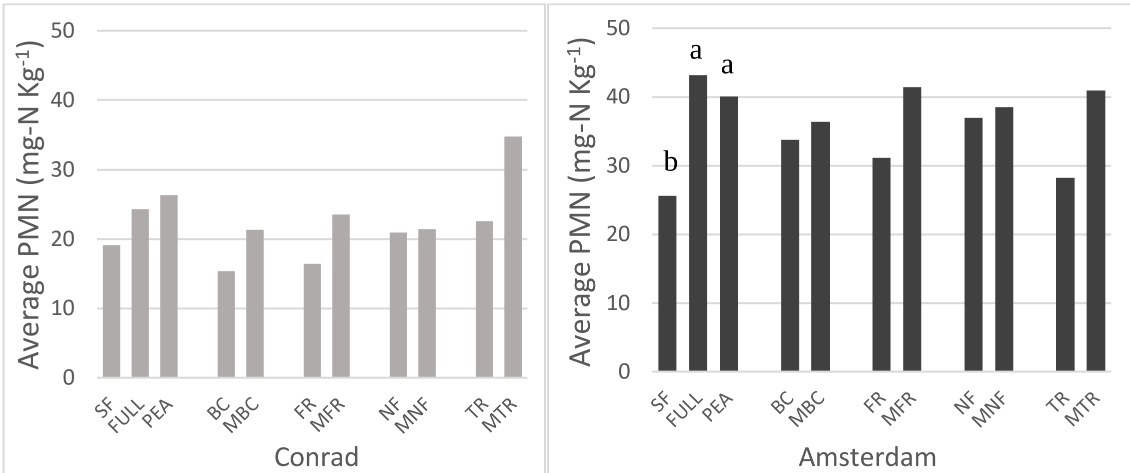

Figure 2.1 Potentially mineralizable nitrogen for 11 treatments at the medium nitrogen rate, following four rotations of cover crops at two field sites. Letters show significant differences ($p = 0.1$).

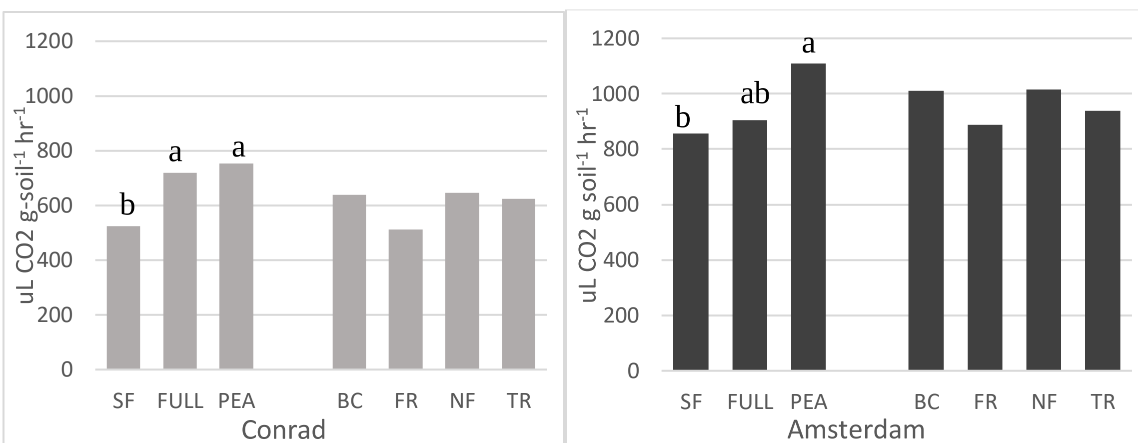

Figure 2.2 Microbial SIR-rate for 7 treatments at the medium nitrogen rate, following four rotations of cover crops at two field sites. Letters show significant differences ($p = 0.1$).

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CHAPTER THREE

ABOVEGROUND BIOMASS QUALITY AND QUANTITY OF LONG-TERM MULTISPECIES COVER CROP MIXES, IN THE SEMI-ARID MONTANA

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Manuscript Information

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[TBD]

Status of Manuscript:
_X_ Prepared for submission to a peer-reviewed journal
____ Officially submitted to a peer-reviewed journal
____ Accepted by a peer-reviewed journal
____ Published in a peer-reviewed journal

1. Introduction

In 2019, 2.2 million ha of wheat was grown in Montana (USDA-NASS, 2019). In the same year, 1.1 million ha were in Summer fallow, with the largest dryland wheat growing region of north central Montana having the greatest proportion of fallow area, greater than 40%. This region of semi-arid Montana receives only 250 to 350 mm of annual precipitation, resulting in the large percent still using summer fallow management techniques.

Although summer fallow, a common practice in the area, helps recharge valuable soil water, in the process it leaves soil vulnerable to increased erosion (Campbell et al., 1991), decreased soil organic matter accumulation (Campbell et al., 2000) and decreased soil biological activity (Acosta-Martinez et al., 2007). For producers in areas possibly too dry for continuous cropping who want to improve soil health, cover crops as a partial summer fallow replacement strategy can be a good fit, trading off some soil water use for plant biomass investment into the soil.

Previous cover crop work in Montana has focused on single-species legume mixes (Miller et al., 2011; McCauley et al., 2012; Burgess et al., 2014; Miller et al., 2015). However, farmers in the region have shown interest in switching to more diverse species mixes, as there is interest among producers in exploring the potential benefits of diversity in cover crops. Greater functional diversity increased plant productivity and other ecosystem services in an ecological perennial grassland study in subhumid Minnesota (Tilman et al., 1997). Cover crop mixes with higher species richness are thought to produce a higher quality and quantity of biomass than mixes with lower species richness (Wortman et al., 2012). This is because species that have variable physiology, phenology, and resource requirements can coexist and if complementary, increase diversity according to ecological niche theories developed with perennial ecosystems (Bulleri et al., 2016).

In natural systems, niche differences, such as varying rooting depths or most limiting nutrient, help to stabilize community diversity in annual serpentine plants (Levine and HilleRisLambers, 2009). Tilman et al. (2006) found that when compared to monocultures, polycultures lead to more temporal stability of aboveground perennial plant biomass. These ecological theories and experiments on the effects of species richness on ecosystem properties are all based on natural systems; annual agricultural systems, with harvesting and soil disturbance, may be affected by diversity differently. Khan and McVay (2019) highlight the benefit of resilience that comes with multi species cover crop mixes—a single species may perform better one season but perform poorly the next; therefore, the mixes on average stabilize biomass compared to monocultures. The reliance on research from predominantly natural or perennial systems is the result of scarcity of research conducted on diverse annual mixes. Within agricultural systems, research in different regions is expected to yield different results, depending on environmental constraints.

Considerations of diversity in agricultural systems also need to account for the importance and difficulty of selecting a cover crop mix that minimizes the negative impacts on the following cash crop via excessive soil water use (Nielsen et al., 2016). While growing multispecies cover crop mixes can provide soil benefits such as increased biomass inputs, nitrogen retention, and weed suppression in sub-humid systems, there are negative tradeoffs for other parameters (Finney et al., 2017). Species within cover crop mixes need to be carefully selected based on a producer’s goal to increase desired benefits while avoiding species that may cause a disservice, like reduced nitrogen supply or reduced weed suppression (Finney et al., 2017).

Cover crop species can provide different benefits and qualities of biomass inputs. Legumes, for example, fix atmospheric nitrogen and provide biomass with lower carbon to nitrogen ratios (C:N), tap-rooted species increase nutrient scavenging and decrease leaching at depth (Dunbabin et al., 2003), and fibrous rooted species provide biomass with higher C:N which may help to increase soil carbon (Housman, 2016). Cover crop biomass inputs with varying C:N can provide a variety of benefits to the soil. A model created by White et al. (2016), predicted that when plant residue has a C:N higher than 25, N immobilization is likely to occur, whereas if the residue has a C:N lower than 25, N mineralization is likely. It is important to note that this model was derived from corn-based systems in subhumid environments and this critical value may not apply in a semi-arid water constrained system.

One example of the advantage of cover crop mixtures over single species cover crops is with grass-legume bicultures, which produce equal or more biomass than the component monocultures since the grass species use the soil N while the legumes fix atmospheric N (Alonso-Ayuso et al., 2014; Hayden et al., 2014). Miller et al. (2018) found similar results when in a semiarid environment; a barley-pea mix significantly out-yielded sole pea and produced the same yield as sole barley but with better forage quality.

Although these are promising findings, more research is needed to explore the cover crop diversity and biomass quality and quantity relationship on more complex mixes.

The main objectives of this research are to examine the effects of varying cover crop mixes on aboveground cover crop biomass quantity and C:N. Specifically, I compared biomass quantity and C:N of four functional groups. I expected mixes composed solely of nitrogen fixers to produce the most biomass and have the lowest C:N when compared to the other functional groups. I further explored whether the presence of legumes or a specific functional group affected aboveground biomass by comparing it to a multispecies mix without the functional group present. I expect mixes containing legumes will produce more biomass than mixes without legumes because of the added nitrogen the legumes fix. Finally, I examine whether multi-functionality increases biomass quantity by comparing two-species functional group mixes to six-species multifunctional mixes. I expect more diverse cover crop mixtures to produce higher amounts of biomass. Cover crops in other agroecosystems have increased soil biological activity (Acosta-Martinez et al., 2011; Garcia-Gonzalez et al., 2016; De Vries and Bardgett, 2012).

2. Materials and Methods:

2.1 Study sites

This study was conducted on two private farm fields located near Amsterdam (N 45.72°, W 111.37°) and Conrad (N 48.22°, W 111.48°), Montana. Both sites had no-till farming histories prior to this study and were managed primarily as commercial wheat farms. The Amsterdam site receives an average of 356 mm of precipitation annually and its soil is classified as a frigid, Aridic Calciustoll silt loam. In 2018, the Amsterdam site received 229 mm of precipipitation during the growing season (April-June). The Conrad site receives an average of 280 mm of precipitation annually and is classified as a frigid, Aridic Argiustoll clay loam (Table 2.1). In 2018, the Conrad site received 138 mm of precipipitation during the growing season (April-June).

2.2 Study Design

The study at each site consisted of four randomized complete blocks, each containing 11 treatments. Cover crop mixtures included four plant functional groups: brassicas (BC), fibrous rooted crops (FR), nitrogen fixers (NF), and tap-rooted crops (TR). Brassicas were selected for rapid establishment of ground cover and their unique phytochemistry; fibrous rooted plants were chosen for their expansive root system and hence C inputs into the soil; nitrogen fixers were picked for their capacity to add nitrogen to the system; and tap-rooted plants were chosen for the root structures to potentially reduce soil compaction. Two species of each functional group (Table 3.2) were grown in individual plots (8 x 12 m), along with a full mix (FULL) plot containing all four functional groups (8 cover crop species). Four additional treatments contained the full mix minus one functional group (containing 6 cover crop species; minus brassica, MBC; minus fibrous root, MFR; minus nitrogen fixers MNF; and minus tap root, MTR) to determine if the absence of a functional group affected soil quality. Two control treatments were a sole species legume cover crop (PEA) and chemical summer fallow

(SF).

2.3 Plot Design

The cover crop plots were planted in early May at a depth of 1 to 2 cm and a consistent target density of 120 plants m^{-2} to eliminate biasing biomass in favor of plant species with higher seeding rates recommended for grain production. Following cover crop treatments (2012, 2014, 2016, 2018), spring wheat (Triticum aestivum L. cv. Duclair; cv. Vida in 2015 at Amsterdam) was grown in 2013, 2015, and 2019, while winter wheat (cv. Warhouse) was grown in 2017 to assess the cash crop response to cover crop treatments. Wheat was seeded in rows perpendicular to the cover crop rows. Nitrogen (N) fertilizer was applied to the wheat crop at three rates, (0 kg N ha^{-1}, 67 kg N ha^{-1}, and 135 kg N ha^{-1}) in a split plot design with fertilizer rates as the subplot level. The study began in 2012 with the first round of cover crop treatments and has generally followed this rotation and fertilizer treatments since. Cover crop plots were terminated with glyphosate in early July after approximately 60 days of growth.

2.4 Cover Crop Sampling

Aboveground cover crop biomass was sampled by hand immediately prior to glyphosate application in all 11 treatments with weeds included. Plants were cut at the soil surface (for turnip, the root bulb portion above soil surface is included), separated by functional group or weeds, dried for >72 hour at 50 °C and weighed directly out of the oven to represent dry matter. Each sample was then ground to pass a 2-mm sieve for analysis of C and N with a LECO combustion analyzer (LECO Corp., St. Joseph, Michigan).

2.5 Statistical Analysis

The R statistical package (R Core Team, 2017; Pinheiro et al., 1997; Fox, 2003; Fox and Weisberg, 2011; Hothorn et al., 2008; Wickham et al., 2017) was used for all statistical analyses. The ANOVA function was used to compare cover crop treatments, and Tukey's HSD with a 90% confidence interval was used for comparisons among treatments. Outliers were identified by applying Cook's distance (Cook, 1977) with a limit of four times the mean. To test for treatment effects on biomass quality and quantity, linear mixed effects models were applied, where block was always the random effect variable and treatment was the fixed effect variable. Residuals vs fitted value plots and Q-Q plots (Fox and Weisberg, 2011) were used to check the assumptions (normality and equal variance) for both ANOVAs and mixed models. Site locations were analyzed separately due to site differences and varying levels of precipitation. Mixed models (Lme, Pinheiro et al., 2017) and two-way ANOVAs were used to test for a treatment effect on aboveground biomass and C:N ratios.

This study design allowed me to explore specific questions of interest. I explored the effects of cover crop plant functional groups on above ground biomass quantity and C:N. Pairwise comparisons between each functional group and the coinciding minus functional group, as well as comparisons between the minus functional groups and the full mix were used to determine whether the presence of a specific functional group affects biomass quantity. To explore how the presence of legumes affect biomass quality and quantity when compared to cover crop mixes without legumes, I compared NF versus the three other functional groups. I also compared the single functional groups, which each contained two species, with four 6-spp mixes (the minus treatments) to explore how functional group richness affects the biomass quantity.

3. Results:

3.1 Cover Crop Biomass Quantity

Aboveground cover crop biomass quantity was measured at the medium N rate for all 10 cover crop mixes and fallow in 2018 (Table 3.3). First, we considered the four functional groups as a subset of treatments. At Amsterdam, NF produced the highest amount of biomass ($p < 0.1$). At Conrad, NF and FR produced the same amount of biomass, and more than BC and TR ($p < 0.1$), which produced less biomass, but similar to one another.

Each functional group was compared to the coinciding minus functional group to show variation in pairwise comparisons (Figure 3.1). At both sites, MBC had more biomass than BC ($p < 0.1$). The MTR treatment had more biomass than TR at both sites as well ($p < 0.1$). This may be partially due to significant turnip biomass being belowground and not sampled, unlike the other functional groups. Each minus treatment was then compared to the full mix to see if the lack of a specific functional group affected biomass quantity. At Amsterdam, FULL had 1.6 times more biomass than MNF, and had no differences with any of the other minus treatments. This is consistent with the NF effect stated below. MBC and MTR both produced more biomass than FULL at Conrad (1.5 times as much and 1.3 times as much respectively), while no differences were seen between FULL and MFR or MNF (Table 3.3).

To examine the effects of legumes when included in a mix on aboveground cover crop biomass, I contrasted biomass of NF to the other functional groups (BC, FR, TR). At Amsterdam, NF had 2.1 times as much biomass on average than the other functional groups ($p < 0.01$; Table 3.3). At Conrad, NF produced 1.4 times as much biomass on average than the other three functional groups ($p < 0.01$; Table 3.3). An additional approach to test for effects of legumes on cover crop biomass was to compare NF to MNF to confirm a legume effect was present. At both sites NF produced significantly more biomass than MNF. At Amsterdam, NF produced 2.3 times the biomass MNF and at Conrad, NF produced 1.6 times the biomass ($p < 0.01$, for both; Figure 3.1). MNF had significantly lower biomass production than any of the other minus treatments at both sites, providing support that nitrogen fixers were key in producing more biomass if cover crops aren't fertilized with N (Table 3.3).

To explore how functional group richness affects aboveground cover crop biomass production, I compared the four functional groups each containing two species (one functional group), with the four minus treatments each containing six species (three functional groups). At Conrad, six-species mixes produced 1.4 times the biomass compared to the two-species mixes at Conrad ($p = 0.01$), while no differences were seen at Amsterdam ($p = 0.33$; Table 3.3).

3.2 Biomass C:N

The C:N of the cover crop biomass of the four functional groups was compared at the medium N rate. As expected, NF had a lower C:N at both field sites than any of the other functional groups (Table 3.4). The largest difference was with FR, which had 1.9

times higher C:N than NF at both sites (Amsterdam: $p < 0.01$ Conrad: $p = 0.02$). At Conrad, no other differences among treatments were seen. At Amsterdam, BC and TR were both higher than NF and lower than FR but not different from eachother (Table 3.4).

4. Discussion:

4.1 Functional group effects

Biomass Yield

Biomass inputs into an agricultural system are linked to increased soil organic matter (Engel et al., 2017), so knowing relative biomass production between potential cover crop functional groups could improve the capacity of producers to consider soil carbon accrual. In previous years of this study, cover crop biomass results varied annually and were mainly related to differences in precipitation (Housman, 2016). In 2014, there were no differences in biomass among functional groups at either site. In 2018, TR and BC produced the least amount of biomass. When considering precipitation during the months of May-June (when cover crops were growing), 2018 was a wetter year with Amsterdam receiving 160 mm and Conrad receiving 119 mm, than 2014 when Amsterdam received 142 mm and Conrad received 97 mm. It should also be noted that safflower, one of the TR species was preferentially grazed by pronghorn at the Conrad field site, contributing to the low biomass in 2018. Brassica seedlings can be more temperature sensitive, and looked especially drought stressed at Conrad in 2018, also a possible explanation for BC having lower biomass.

When comparing each of the functional groups with their coinciding minus treatments, the low producing functional groups, BC and TR, had less biomass than the minus treatments. This is what one might expect since when you have a low producing functional group, the overall biomass without that functional group is being replaced with more productive species. Whereas when looking at NF, the highest performing functional group, it out yielded MNF.

C:N of Cover Crop Biomass

In some circumstances, for example in soil having relatively high SOM, cover crop biomass production may not be the main objective. Perhaps a producer is more concerned with the quality of biomass inputs than quantity, for example seeking an N contribution from the cover crop. Nitrogen fixers had the lowest C:N at both sites and FR had the highest C:N at one of the two sites. Similarly, Hunter et al. (2019) found that cover crop mixes with mainly grass species had the highest C:N ratios, a brassica monoculture and mixed functional group mixes were intermediate, and the legume monoculture had the lowest C:N ratio. Hunter et al (2019) points out the potential financial risk of planting cover crop mixes with high C:N ratios before cash crops like corn, which has a high N demand, but they did not see an effect on wheat yield in western Pennsylvania. High protein wheat that is grown in Montana has much higher N demands per unit yield than corn, so wheat protein could benefit from the lower C:N biomass inputs on a wet year. Given the challenges faced in semi-arid Montana however, water limitations can override the N benefits of low C:N biomass inputs, causing ‘haying off’ when N availability stimulates early season wheat growth without sufficient soil water to sustain that growth trajectory (Miller et al., 2011).

4.2 Functional group richness

Our research found that the three functional group (six species) cover crop mixes produced 1.4 times the biomass than the one functional group (two species) mixes on average at one of the two field sites. This coincides with much of the research that exists where on average greater diversity within a mix leads to more biomass. A study in Nebraska found that increased cover crop diversity (all mixes containing 50% legumes) increased biomass in two of the three study years (6 species produced 2.1 times the biomass 2-species did), and shows that having more diverse cover crop mixes helped to create a more resilient system by stabilizing cover crop biomass especially with weather disturbance (Wortman et al., 2012). Finney et al. (2016) found that in a two-year field study in subhumid eastern Pennsylvania, on average, increased species number increased cover crop biomass by 533 kg ha^{-1} for each additional crop species added. Their study also found some evidence that mixes containing species with varying nutrient scavenging techniques provided greater ecosystem services. This could relate back to increase soil quality and producer goals. In semi-arid systems the effects of diversity may be less pronounced. Khan and McVay (2019) measured 1.2 times more biomass in cover crop mixtures than single species in one year of their two-year study in semi-arid south central Montana. Although more diverse mixes have been shown to increase biomass on average, both Finney et al. (2016) and Khan and McVay (2019) did not have any diverse mixtures that outperformed the most productive monoculture, which was also true for our study with sole PEA Multi-functional diverse cover crop mixes may not out yield the most productive monoculture, but they offer increased resilience and stability, making them a valuable technique. Khan and McVay (2019) highlighted that increased resilience and stability when looking at the range variation between monocultures and mixes. The cover crop monocultures ranged a span of 2.6 Mg ha^{-1} while the cover crop mixes ranged 0.36 Mg ha^{-1}. The same trend was seen in our study, with a range of 1.73 Mg ha^{-1} for the two species mixes and a range of 1.38 Mg ha^{-1} for the six species cover crop mixes at Conrad and 1.53 Mg ha^{-1} for the range of two species mixes and 0.75 Mg ha^{-1} for the range of six species mixes at Amsterdam.

Cover crop mixes should be designed based on the specific goals of the producer. A mix could be designed to maximize biomass production to help build soil organic matter; or nitrogen addition may be the primary concern, so C:N of the biomass is more important. These goals can be best addressed using legume species, as seen in this study. In both cases, the soil properties the producer is concerned with are being affected by the cover crop biomass. Since these cover crops are being grown as a partial summer fallow replacement strategy, there are important precipitation use tradeoffs to consider with increasing cover crop biomass. More SOM building will occur, the more cover crop biomass is produced and left on the field. However, the more biomass that is produced the more soil water is used, frequently leading to decreased cash crop yields (Miller et al, 2006; 2018. In semi-arid annual systems such as the one studied here, water limitations is most often the main concern with growing cover crops. In years with above-average precipitation during the growing season, or after cover crop growth, may reduce or eliminate the water deficit for the cash crop. However, in dry years, yield loss in the following cash crop can be very significant (Miller et al., 2017). Without knowing in advance, the producer has to weigh the risk in planting cover crops.

Table 3.1 Site conditions for the two field sites for a 0 – 15cm depth at the start of the 8-yr study (2012).

Site	Amsterdam	Conrad
Elevation (m)	1445	1039
Texture	Silt loam	Clay loam
pH	8.2	6.5
SOM (%)	2.4	2.4
NO_3-N (ppm)	6.0	8.5
Olsen P (ppm)	13	28
Extractable K (ppm)	359	498

Table 3.2 Plant species included in 10 cover crop treatments and a chemical fallow control for Amsterdam and Conrad, MT.

Treatment		Plant Species
Fallow	SF	Incidental weeds
Pea	PEA	Forage Pea
Full Mix	FULL	Forage pea (*Pisum sativum* L. cv. Arvika) Black lentil (*Lens culinaris* Medik. cv. Indianhead) Oat (*Avena sativa* L.) Canaryseed (*Phalaris canariensis* L.) Turnip (*Brassica rapa* L.) Safflower (*Carthamus tinctorius* L.) Forage Radish (*Raphanus sativus* L. var. *longipinnatus)* Winter canola (*Brassica napus* L.)
Brassicas	BC	Forage radish, Winter canola
Minus Brassicas	MBC	All but canola, radish and turnip
Fibrous Roots	FR	Oat, Canaryseed
Minus Fibrous Roots	MFR	All but oat and canaryseed
Nitrogen Fixers	NF	Forage pea, Black lentil
Minus Nitrogen Fixers	MNF	All but pea and lentil
Taproot	TR	Turnip, Safflower
Minus Taproots	MTR	All but turnip and safflower

Table 3.3 2018 cover crop biomass (dry Mg ha^{-1}) at Amsterdam and Conrad, MT.

	Amsterdam	Conrad
ANOVA	CC	CC[y]
	---------- *P*-value ----------	
Cover crops	<0.01	<0.01
	---------- Mg ha^{-1} ----------	
SF	0.31	1.06
PEA	2.45	3.28
FULL	1.58	2.22
BC	0.80	1.76
FR	1.56	3.19
NF	2.33	3.08
TR	0.90	1.46[z]
MBC[w]	1.70	3.30
MFR	1.43	2.60
MNF	0.99	1.92
MTR	1.74	2.99
$LSD_{0.1}$	*0.24*	*0.59*
	Orthogonal linear contrasts	
2 vs 6 species	$P = 0.33$	$P = 0.01$
2	1.40	2.32
6	1.47	2.70
N-fix vs Non	$P < 0.01$	$P < 0.01$
NF	2.33	3.08
Non-N[x]	1.08	2.14

w – The minus Brassica mix had only 5 species since one Tap Root species was also a Brassica spp.
x – ‘Non-N’ was the average of all 2-species treatments except N fixers.
y – In 2018, glyphosate-tolerant kochia (*Bassia scoparia* (L.) A. J. Scott) infested the Conrad site averaging 9% of the total cover crop treatment biomass for the Medium N rates.
z – Safflower was preferentially grazed by antelope (*Antilocapra americana* Ord.) greatly reducing the biomass of the Tap Root treatment.

Table 3.4 C:N ratios of cover crop biomass for the four functional groups at the medium N rate at Amsterdam and Conrad, MT, 2018.

Treatment	Amsterdam	Conrad
BC	25.0[b]	23.0[a]
FR	34.9[a]	29.2[a]
NF	18.1[c]	15.4[b]
TR	25.9[b]	23.2[a]
p-value	<0.01	0.02
$F\text{-stat}_{3, 9}$	29.61	5.23

*Letters show significant differences (*p* = 0.1) within a site.

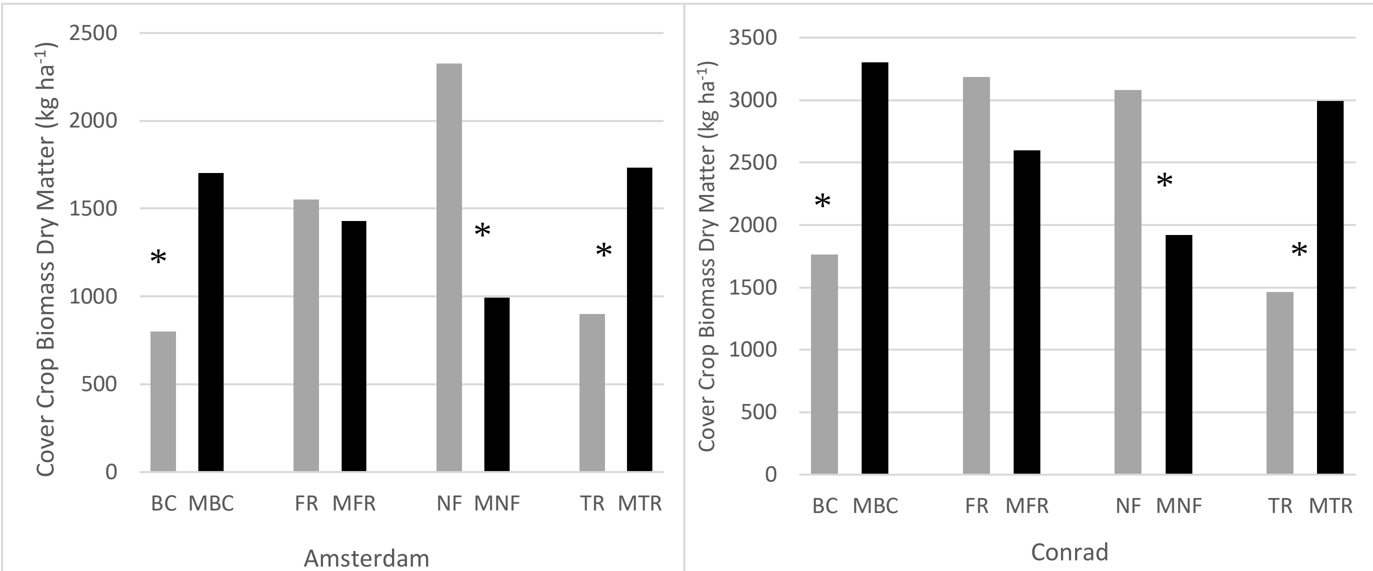

Figure 3.1 Aboveground cover crop biomass for the four functional groups and the corresponding minus treatments, at the medium N rate in 2018. Asterisks show significant differences between pairs (p = 0.1).

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CHAPTER FOUR

SOIL BIOLOGICAL RESPONSE TO SPRAYING, GRAZING, OR HAYING OF LONG-TERM MULTISPECIES COVER CROPS IN SEMI-ARID MONTANA

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Manuscript in Chapter 4

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[TBD]

Status of Manuscript:
_X_ Prepared for submission to a peer-reviewed journal
____ Officially submitted to a peer-reviewed journal
____ Accepted by a peer-reviewed journal
____ Published in a peer-reviewed journal

1. Introduction:

Summer fallow is a common practice in semi-arid Montana, where fields are left plant-free for a growing season, allowing crops to use the precipitation from more than one growing season (Le Roy et al., 2016). Because of this ability to save water, a wheat – fallow rotation is one of the most common dryland cropping system in semi-arid Montana, where continuous cropping is not possible. Although summer fallow helps recharge valuable soil water, in the process, it increases the likelihood for soil erosion (Campbell et al., 1991), and decreases soil organic matter (Campbell et al., 2000) and biological activity in the soil (Acosta-Martinez et al., 2007; Tanaka et al., 2010).

In 2019, 2.2 million ha of wheat was grown in Montana (USDA-NASS, 2019). Summer fallow area was 1.1 million ha, with the largest dryland wheat growing region of north central Montana having the greatest proportion of fallow area, greater than 40%. For producers in areas possibly too dry for continuous cropping, but who want to improve soil health, cover crops could be a good fit.

Cover crops can be incorporated into a rotation as a partial summer fallow replacement strategy to enhance soil quality by increasing soil organic matter and soil structure (Zentner et al., 2004), decreasing the potential for erosion (Tanaka et al., 1997), enhancing the cycling of nutrients (Lu et al., 2000), and suppressing weed growth (Fiksel et al., 2003). Cover cropping (specifically with legume species) over a longer period may reduce financial uncertainty for the producer by providing nitrogen at key times and increasing soil quality (Miller et al., 2015, O'Dea et al., 2013).

Growing crops in semi-arid Montana is especially difficult due to the low amount of annual precipitation this area receives, along with dramatic annual variation and prolonged hot dry summer periods (Padbury et al., 2002). Soil water usage is one of the top reasons why producers in Montana don't plant cover crops (Jones et al., 2015). Cover crops can deplete soil water and in turn reduce cash crop yields in the short term (O'Dea et al., 2013; Tallman, 2014; Miller et al., 2017). However, soil water use has been minimized in some soil-climatic contexts through precise timing of cover crop termination while still gaining soil benefits (Zentner et al., 2004, Miller et al., 2011).

The initial cost associated with planting cover crops with no economic return from harvest is an additional barrier preventing the adoption of cover crops (Jones et al., 2015). Using an alternative termination strategy such as haying or grazing may provide an economic benefit to the producer to offset the cost of seed, equipment use, and time spent managing the cover crops. This strategy would also present an immediate economic benefit that could potentially offset the initial cash crop yield loss, until long-term soil benefits can be achieved. In semi-arid Montana, biomass quality and quantity produced by cool season cover crop mixes was similar to other common types of commercial forage in the area (Walker, 2017). In that short-term study, grazing tended to reduce some soil quality aspects, such as potentially mineralizable nitrogen (PMN), when compared with spraying and leaving the cover crop biomass in place, and there was higher soil phosphatase enzyme activity in grazed plots as well, when measured the following spring, nine months after cover crop termination.

A farming system that manages both crops and livestock to benefit one another is called an integrated crop-livestock system (ICLS; Franzluebbers and Stuedemann, 2014). Historically, crop and livestock production went hand in hand; however, as countries became more developed, the production decoupled and became two unique specialized agricultural systems within our current industrial model for agriculture (Franzluebbers and Stuedemann, 2006; Martin et al., 2016). These more intensified, specialized systems can lead to loss of beneficial ecological interactions and loss of resilience to changing climate (Regan et al., 2017). Grazing cover crops is one way to incorporate crops and livestock into the same agricultural system.

Benefits of recoupling crop and livestock production can include improved soil productivity, reduced fertilizer/herbicide inputs, and better utilization of resources (Sulc and Franzluebbers, 2014). Livestock waste supplies soil with nutrients in different forms through excrements, organic carbon, and cation exchange capacity, all which can benefit future crops and improve or sustain soil fertility in warm wet environments (Franzluebbers and Stuedemann, 2014; Magdoff et al., 1997). High quality litter can accelerate decompositon; specifically, when an animal excretes nutrient-rich biomass, these nutrients can be more easily incorporated into belowground foodwebs because the excrements are very labile (Seastedt, 1984; Wardle et al., 2002; Ruess and Ferris, 2004). This grazing component can also lead to increased available phosphorus (Costa et al., 2014) and available nitrogen (Tracy and Zhang, 2008) due to increased speed of organic matter decomposition. With this being said, there is not much literature available on the effects on soil of grazing annual plants, especially for semi-arid Montana. When cover crops are grazed, crop residue is removed and only a portion of the nutrients are returned through manure (Hatfield et al., 2000).

With any biomass removal technique (graze or hay), carbon and mineral nutrients leave the system and no longer are returned to the soil. This carbon is imperative for the formation of soil organic matter (Blanco-Canqui and Lal, 2007). Chemically terminating cover crops allows for all the crop residue to remain on the field, and has been shown to increase soil organic matter (Wagger et al., 1998).

In semi-arid regions, where annual fluctuations in weather can greatly impact soil parameters, long term studies investigating the same rotation multiple times are important, yet rare. My research investigates the long-term effects of cover crop treatments and termination strategies on several soil parameters after three rotations of cover crop – wheat in a dryland system near Havre, MT. Soil enzymes and PMN were the chosen soil parameters to explore in this study. Since nitrogen is often limiting in agricultural systems, soil parameters related to nitrogen such as PMN can be helpful in quantifying soil quality and estimating potential benefits to subsequent crops. Measures of PMN are an estimate of how much nitrogen will become available to the plant during the growing season due to microbial breakdown of organic compounds (Drinkwater et al., 1997).

Cropping systems with shorter fallow periods, more diverse rotations, and limited tillage, have been shown to have higher PMN values than conventional cropping systems (Liebig et al., 2006). Soil enzymes are ideal soil quality indicators because they respond more quickly to management changes than other indicators and because they have a direct relationship with sustaining biological diversity, activity, and productivity, as well as storing and cycling nutrients (Lehman et al., 2015). Five soil enzymes were selected to address specific nutrient cycling within the soil (phosphorus, nitrogen, carbon, and sulfur). For these reasons, PMN and soil enzymes were monitored and compared among chemically sprayed, grazed, and hayed termination treatments to address the following questions: 1) Does biomass removal (haying or grazing) affect the soil parameters differently than chemical termination? 2) Do cover crops increase PMN or soil enzyme activity when compared to summer fallow? I expect biomass removal will have a negative effect on soil parameters since the carbon and nitrogen inputs from the cover crops will not be fully returned to the soil. I hypothesized that cover crops will increase PMN and soil enzymes when compared to fallow because cover crops provided living roots for about 90 days and biomass inputs into the system while fallow does not.

2. Materials and Methods:

2.1 Study site

This study took place at the Northern Agricultural Research Center (NARC) of Montana State University (48°29’N, -109°48’W), near Havre, MT. The field site receives an average of 305 mm of annual precipitation and is classified as Telstad-Joplin clay loam (Table 4.1). The field site had an extensive no-till management history prior to the start of this study.

2.2 Study design

This study was established in 2012 and consisted of 16 randomized treatments. In addition to a chemical fallow control, there were 15 cover crop treatments as well as a non-randomized barley buffer strip that can be compared as a single species control. Each treatment was established in a 7 x 37 m strip (259 m^2). Each block of plots was replicated three times and each replication was terminated (perpendicular to cover crop mixtures) using three strategies: herbicide spraying, grazing, or haying. The cover crop mixtures were made up of cool season species and planted on April 20th and terminated in early July. Cover crop treatments were fertilized with 22 kg N ha $^{-1}$, 10 kg P ha $^{-1}$, and 18 kg K ha $^{-1}$ to promote growth at the time of planting. To more easily manage grazing with cattle, the grazing termination section was confined to the central part of each plot, and spraying and haying were also not randomly assigned to each block to facilitate machinery operations (Figure 4.1). Each plot was divided in half and winter wheat and spring wheat were planted separately in each half (in alternating perpendicular strips across all plots) the following autumn or spring, respectively.

The cover crop treatments consisted of a variety of warm season, cool season, and a combination of warm and cool season cover crop mixes. A subset of the mixes in the larger study was analyzed for this study, including two multispecies cover crop mixes, sole barley, and a chemical fallow plot (Table 4.2). The two mixes selected for this study were both cool season mixes and included a five species mix (5-spp) and a seven species mix (7-spp) that were designed with species that would be readily available to farmers in the area. The 7-spp mix contained all the species in the 5-spp mix plus spineless safflower and lentil.

2.3 Termination Procedure

Three termination strategies were used to terminate the cover crop mixes shortly after the cereals started to head. For the haying treatment, cover crop trial plots were hayed using a swather that leaves approximately 5 cm of residual stubble from the ground. For the grazing treatment, 12 bulls spent about a week in the grazed strip. Animals were fenced into the entire grazing strip at one time and removed when similar amounts of biomass were taken as the haying treatment. This meant termination strategies could not be randomized; however, a spatial analysis was done with soil nutrient data at the beginning of the experiment as well as on yield data for the six years prior to sampling and no consistent spatial effects were observed in the east-west direction that would confound termination effects (Bourgault pers. comm). For the spraying treatment, 69 oz RT3 ha^{-1} (48.8 % glyphosate, N-(phosphonomethyl)glycine, in the form of its potassium salt) and 30 oz RIFLE (48.2% dimethylamine salt of dicamba) was used to terminate the cover crop treatments. In years where regrowth occurred in both the hayed and grazed sections, plots were sprayed with glyphosate after the chemically terminated plots were first sprayed.

2.4 Soil Sampling

Soil samples were taken in mid-April 2018 to assess the soil conditions at the time of spring wheat planting. For biological parameters, six manual cores (2-cm dia), were collected in the top 10 cm of soil in each treatment plot. The corer was flame-sterilized between plots. Field moist composited samples were put through a 2-mm sieve, stones and residue larger than 2-mm were discarded, and remaining sample was stored at 4 °C until lab analyses were performed.

2.5 Biological Parameters

Potentially mineralizable nitrogen was analyzed as adapted from Keeney and Nelson (1982), by measuring the plant available nitrogen (ammonium only) at the time of soil collection and after a 14-day incubation period, with a cadmium reduction on a Lachat auto analyzer (Lachat Instruments, Loveland, CO). Six flasks received 5 g of soil, three of which were immediately extracted for 30 minutes with 25 mL of 1 M KCl (mechanically shaken for 30 minutes, 310 rpm) and analyzed using a Lachat auto analyzer. The additional three flasks were pumped with N_2 gas for 5 seconds to create anoxic conditions and were kept in a dark incubator at 30 °C for 14 days. These samples were then analyzed using the above method. The difference between the initial value and the incubated value was calculated to get the PMN value for each sample.

Soil enzyme activity was analyzed using the procedure outlined by Dick (1996; 2020) and Parham and Deng (2000). Duplicates of 1 g of field moist soil for each sample and controls were incubated with the enzyme specific substrate for 1 h at 37 °C, then filtered and analyzed spectrophotometrically. Five enzymes were analyzed: β-1,4-glucosidase (Enzyme Commission; EC 3.2.1.21, related to carbon cycling), β-1,4-N-acetyl glucosaminidase (EC 3.2.1.30, related to carbon and nitrogen cycling), arylsulfatase (EC 3.1.6.1, related to sulfur cycling), acid phosphatase and alkaline phosphatase (EC 3.1.3.1/2, related to phosphorus cycling). In addition to individual enzyme analyses, geometric means were calculated for each treatment by taking the fifth root of the product of the activity level of the five enzymes (Garcia-Ruiz et al., 2008).

2.7 Statistical Analysis

The R statistical package (R Core Team, 2017; Pinheiro et al., 1997; Fox, 2003; Fox and Weisberg, 2011; Hothorn et al., 2008; Wickham et al., 2017) was used for all statistical analyses. The ANOVA function was used to compare cover crop treatments, and Tukey's HSD with a 90% confidence interval was used for comparisons among treatments (Alpha level = 0.10). Cook's distance (Cook, 1977) with a value of 4 times the mean, was used to determine outliers. Residuals vs fitted value plots and Q-Q plots (Fox and Weisberg, 2011) were used to check the assumptions (normality and equal variance) for both ANOVAs and mixed models, and transformations were used to normalize data when necessary. Linear mixed effects models (Pinheiro et al., 2017) were used, where block was a random effect variable, treatment and termination were fixed effect variables and the soil parameter was the response variable. Mixed models and two way ANOVAs were used to test for a cover crop treatment effect and termination strategy effect on PMN, single soil enzymes, and enzyme geometric means. An interaction term between treatment and termination strategy was considered but was never significant and the term was dropped from the model.

3. Results:

3.1 Potentially Mineralizable Nitrogen

I predicted that termination strategies that removed biomass (haying and grazing) would negatively affect PMN since less of the N in the cover crop biomass would not be returned to the soil when compared to chemical termination. However, there were no differences in PMN levels among termination strategies after accounting for variation due to cover crop treatment (F = 0.46, p = 0.71, Table 4.3), nor were there cover crop treatment effects (F = 0.38, p = 0.69, Table 4.3). Values were exceptionally low with high variation within the three blocks, with barley having the largest range of values.

3.2 Soil Enzymes

Five soil enzymes were analyzed individually across the three termination strategies after accounting for variation due to treatment. A termination effect was seen for alkaline phosphatase (p = 0.07). Alkaline phosphatase enzyme activity was 2.5 times higher in the grazing treatment soils than in the chemically sprayed soils (p = 0.06), and was 1.8 times higher in the hayed plots than in the sprayed plots (p = 0.04; Table 4.4). The enzyme concentrations for the grazed and hayed plots did not differ. No differences were seen among termination strategies for any of the additional four enzymes. The geometric mean of the five enzymes was analyzed across termination strategies and cover crop treatments. There were no differences among termination strategies after accounting for variations due to cover crop treatments.

The same five soil enzymes were analyzed across the cover crop treatments after accounting for variation due to termination. Alkaline phosphatase activity was higher in soils following summer fallow and 7-spp mix than following 5-spp mix, and intermediate in soils following the barley treatment ($p < 0.01$ for both; Table 4.5). *B*-glucosidase activity was higher in soils following 7-spp mix than soils following summer fallow and 5-spp mix, and intermediate following the barley treatment ($p = 0.05$; Table 4.5). *B*-glucosaminidase activity was the lowest in soils following the 7-spp mix than in the other treatments ($p = 0.10$; Table 4.5). There were no differences among treatments for acid phosphatase or arylsulfatase. Among cover crop treatments, the geometric mean of soil enzyme activity was higher in soils following 7-spp than fallow or 5-spp ($p = 0.04$; Table 4.5). Soils following the barley treatment had higher geometric means than following 5-spp.

4. Discussion:

Farm management practices and adoption of new techniques (or lack thereof) are dictated by economic sustainability. Haying or grazing may add an economic benefit to the producer while inclusion of cover crops can simultaneously change soil health parameters. In Montana, 93% of cover crops were used as forage from 2014 to 2018 (MASS data, as summarized by P. Miller), highlighting the importance of understanding their fit within livestock systems. Another potential reason for the majority of cover crops being associated with forage in this area could be producers with livestock who are searching for an alternative forage source. This study assessed whether forage-oriented termination strategies limit or alter how soil properties change compared with a non-forage option – spraying.

4.1 Termination Strategies

No differences were seen in PMN among termination strategies. A previous study in this same region (Walker, 2017) found that PMN was 60% higher in soils from plots that had chemically terminated cover crops than grazed cover crops, and the grazed plots had 22% higher PMN values in soils with hayed cover crops in one of two site-years. The field site was very windy and much of the residue aboveground seemed to blow away, whereas all three termination strategies left belowground biomass in the form of roots. This could explain why PMN differences among termination strategies were not seen. In our study, soils from the grazing and haying methods both had higher alkaline phosphatase activity than those where cover crops were chemically terminated. Similarly, Walker (2017) measured higher acid phosphatase levels in soil from grazed cover crop treatments when compared to soil from chemically terminated cover crop treatments. Phosphatase enzyme activity (both acid and alkaline) is inversely related to inorganic phosphorus availability (Allison et al., 2007; Marklein and Houlton, 2012) and adding phosphorus to the soil has been correlated with lower phosphatase activity (Marklein and Houlton, 2012). Thus, the lower phosphatase activity in the grazed and hayed termination plots could be from the removal of cover crop residue containing phosphorus.

In addition to consideration of effects of cover crop termination approach on soil quality, possible economic returns related to termination will influence producer adoption rates. There are few published studies that look at residue removal (grazing or haying) of cover crops in semi-arid environments such as Montana. One study on dryland wheat in the NGP documented the negative effects on economic return of removing crop residue both by grazing and haying versus leaving the residue on the field (Archer et al., 2020). Although haying and grazing offered additional income, there were costs associated with those termination strategies, such as equipment, fencing, etc. For a wheat – pea/cover crop rotation, the grazing costs were greater than the income associated with the grazing (Archer et al., 2020). The grain in this rotation, when crop residue was grazed, also had a lower associated income than the same rotation not grazed (Archer et al., 2020). This research highlights the difficulties in making cover crop rotations economically profitable in low rainfall semi-arid environments. It is important to note that the Archer et al. (2020) experiment took place in a slightly wetter environment receiving on average 406 mm of annual precipitation.

4.2 Cover Crops

To determine whether cover crops affect soil parameters differently than summer fallow, PMN, and enzymes were measured for three cover crop treatments and compared to summer fallow. There were no differences for PMN or enzyme geometric mean. Similarly, Housman (2016) found at the field site near Conrad, MT (see Chapters Two and Three), with similar annual precipitation to this site, that PMN did not differ between fallow and cover crop treatments. Results from Chapter 2 also found no differences in PMN values at the drier site location (Conrad); however, at the wetter site (Amsterdam) differences in PMN values were seen. Several other studies have measured higher PMN values following a cover crop than summer fallow in areas that receive more annual precipitation on average (Housman, 2016; O'Dea et al., 2013). The research site for this study was located on a hill with initial soil conditions varying spatially within the blocks in the north-south direction, possibly confounding the cover crop treatment effects on both PMN and enzymes.

To determine whether the barley control affects soil parameters differently from the multispecies cover crop mixes, we compared a single species cover crop treatment (barley) and two multispecies cover crop mixes (7-spp and 5-spp). No differences were seen between single species and multispecies mixes for PMN or enzymes. Similarly, other studies that have shown differences in PMN values between cover crops and summer fallow, also found no differences between single or two species cover crop mixes and more diverse cover crop mixes (Chu et al., 2017; Chapter 2). This could be explained by the single and multispecies mixes producing similar amounts of biomass, given that PMN sometimes correlates with the previous season's cover crop biomass (Chapter Two).

The cover crop treatments were randomly assigned to plots within each of the three blocks and a blocking variable was accounted for, so a causal statement can be made. In contrast, the termination strategies were not randomly assigned due to the need for containment of the grazing animals during the study. Future research is needed to further explore how grazing and haying cover crops affect soil parameters differently than chemically terminating cover crops. Additional economic analyses of the cost of growing cover crops versus the potential economic value the cover crop forage (haying or grazing) could provide for multiple moisture regimes in the NGP, specifically semi-arid

Montana, would be beneficial for producers looking to adopt these risky management practices.

Table 4.1 Site conditions for the field site near Havre, MT, for 0 – 15 cm depth, at the start of the 8-yr study (2012).

Elevation (ft)	2684
Texture	Clay loam
pH	7.1
SOM (%)	1.4
NO_3-N (ppm)	6.3
Olsen P (ppm)	24
Extractable K (ppm)	270

Table 4.2 Plant species included in 3 cover crop mixes and a summer fallow control.

Treatment	**Plant Species**
Fallow	Incidental weeds
Barley	Barley (Hordeum vulgare L.)
Multi species cover crop mix 1 (CCM1, 7-spp)	Turnip (Brassica rapa L.) Radish (Raphanus sativus L.) Forage pea (Pisum sativum L. cv. Arvika) Hairy Vetch (Vicia villosa L.) Oat (Avena sativa L.) Spineless Safflower (Carthamus tinctorius L. cv. Baldy) Lentil (Lens culinaris Medik. cv. Indianhead)
Multi species cover crop mix 2 (CCM2, 5-spp)	Turnip (Brassica rapa L.) Radish (Raphanus sativus L.) Forage Pea (Pisum sativum L. cv. Arvika) Hairy Vetch (Vicia villosa L.) Oat (Avena sativa L.)

Table 4.3 Potentially mineralizable nitrogen (mg-N kg^{-1}) for 3 crop termination strategies and 4 treatments, following three rotations of cover crops in 2017, near Havre, MT.

Termination Strategy		Cover Crop Treatment	
CHEM	1.50	Fallow	1.15
GRZ	1.62	Barley	2.37
HAY	1.87	CCM1 7-spp	1.92
		CCM2 5-spp	1.20
p-value	0.69	p-value	0.71
F-stat $_{2,28}$	0.38	F-stat $_{3,28}$	0.46

Table 4.4 Enzymatic activity (mg PNP g soil^{-1} hr^{-1}) of five soil enzymes (ß- glucosidase, ß -glucosaminidase, acid and alkaline phosphatases, and arylsulfatase) for three crop termination strategies, following three rotations of cover crops in 2017, near Havre, MT.

	Acid Phosphatase	Alkaline Phosphatase	Arylsulfatase	B-glucosidase	B-glucosaminidase	Geometric mean
CHEM	91.3	2.51^{b}	11.9	14.9	15.8	12.7
GRZ	91.2	6.18^{a}	11.3	13.7	13.8	13.5
HAY	100.5	4.59^{a}	10.3	11.1	14.7	13.3
p-value	0.63	**0.07**	0.33	0.39	0.36	0.72
F-stat $_{2,28}$	0.47	2.92	1.17	0.96	1.05	0.34

Letters show significant differences (*p* = 0.1).

Table 4.5 Enzymatic activity (mg PNP g soil^{-1} hr^{-1}) of five soil enzymes (ß- glucosidase, ß -glucosaminidase, acid and alkaline phosphatases, and arylsulfatase) for four treatments, following three rotations of cover crops in 2017, near Havre, MT.

	Acid Phosphatase	Alkaline Phosphatase	Arylsulfatase	B-glucosidase	B-glucosaminidase	Geometric mean
Fallow	85.2	5.17^{a}	11.0	9.53^{b}	16.3^{a}	12.7^{bc}
Barley	101	3.53^{ab}	12.3	14.4^{ab}	15.1^{a}	13.6^{ab}
CCM 7-spp	98.9	6.73^{a}	11.4	18.2^{a}	12.3^{b}	15.1^{a}
CCM 5-spp	91.9	2.27^{b}	10.1	10.8^{b}	15.5^{a}	11.3^{c}
p-value	0.59	**0.01**	0.37	**0.05**	**0.10**	**0.04**
F-stat $_{3,28}$	0.64	4.43	1.09	3.03	2.33	3.18

Letters show significant differences (*p*= 0.1).

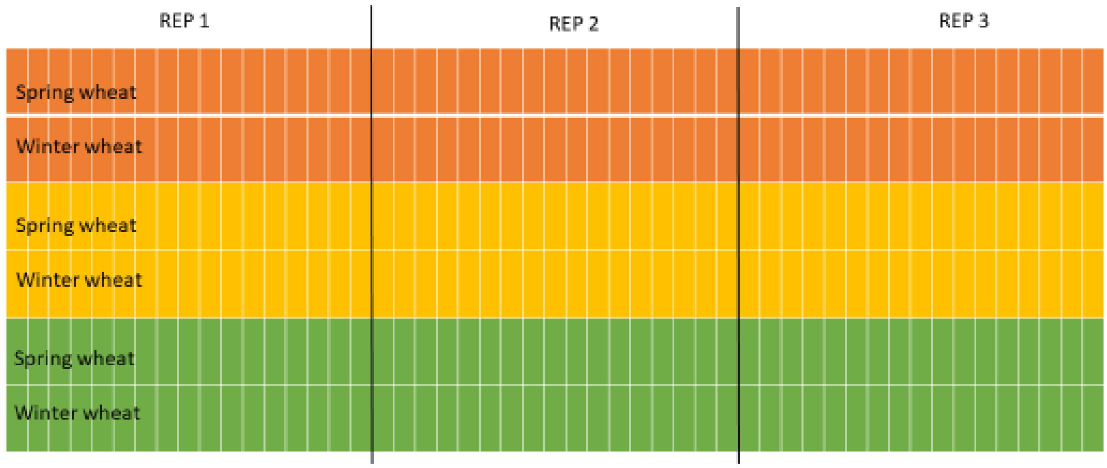

Figure 4.1. Randomized three block design where columns represent cover crop mixes (17 total, 4 used for this study) and row represents perpendicular wheat seeding as well as three termination strategies (chemically spraying in orange, grazing in yellow, and haying in green) for the Northern Agricultural research center near Havre, MT.

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CHAPTER FIVE

CONCLUSION

The main goal of this research was to explore how multispecies cover crop mixes affect soil parameters and how biomass compares among mixes. In the semi-arid Montana where a wheat – fallow rotation is a common agricultural practice, environmental concerns with leaving a field fallow hinders the sustainability potential of the system. This research explored the use of cover crops as a partial summer fallow replacement strategy to increase sustainability through possible increased ground cover, biological activity, and nutrient cycling. However, the problem is that low precipitation limits crop production, so economics is an important part of the sustainability equation.

The objectives of this research were: to investigate the long term effects of cover crop mixes with different number of functional groups on biological soil parameters, to examine the effects of varying cover crop mixes on aboveground cover crop biomass quantity and C:N, and to investigate the effects of termination strategies used to manage cover crop mixes on biological soil parameters.

Chapter 2 revealed that under certain conditions species composition might matter for affecting soil quality, but more research is needed to determine this as this thesis focused on biological soil parameters. If species do not matter, a mix may still make sense for the added resilience to the system that a mix brings. Not many differences were seen in the biological parameters analyzed for this study. The data had very high variation within cover crop treatments, especially for enzymes, with some average values being more than two times other treatments without showing significance. This shows the difficulty in picking up differences within treatments when variation is so high. Possibly, if other explanatory soil parameters were accounted for in a mixed model, a stronger enzyme signal could be seen. For example, if chemical parameters that related to nitrogen were included in the β-1,4-N-acetyl glucosaminidase model, an enzyme trend may be seen, but that would defeat the purpose of using enzymes as an early indicator for management change.

Chapter 2 and 3 were part of a larger study that examined how the 11 cover crop treatments affected biological, chemical, and physical soil parameters and the associated wheat yield and protein. Further analysis is required to correlate cover crop biomass to other soil parameters as well as biological parameters and biomass to wheat yield and protein. Biological soil parameters are only one piece in a much larger picture of soil health. Once all the soil parameters are analyzed and correlated, conclusions about how cover crops affect soil health can be made.

If maximizing cover crop biomass is the producer's goal, a mix might be the safest choice. Although ecological research has found that a mix doesn't outperform the most productive monoculture, on average more diverse mixes outperform monocultures since which monoculture may be most productive could vary with annual weather differences. With the erratic precipitation events and frequent prolonged droughts Montana and the NGP experiences, a mix could offer resilience to the year to year variations. This research found that at one site, the more diverse mixes outperformed the less diverse mixes on average, when comparing aboveground biomass.

Chapter 3 found that cover crop mixes that include N fixers produce more biomass than mixes without nitrogen fixers. This study and literature on the subject show how variable cover crop biomass can be from year to year in semi-arid environments where precipitation events can be sporadic and few and far between. This highlights the potential benefits of multi-species cover crop mixes to create a more resilient system. This research in general highlights the importance of choosing cover crop species and mixes in direct association with the producer's goals. Perhaps the producer doesn't want to grow the most biomass possible, since that may use too much soil moisture, and instead want to focus on rapid early ground cover or atmospheric nitrogen fixation.

The varying termination field study found no major evidence that termination has an effect on soil biological parameters. This could be good news for producers looking to offset the cost of using cover crops. This research didn't find any negative correlations between the soil biological parameters measured and the removal of cover crop biomass in the form of haying or grazing. Therefore, producers could gain economic value from the cover crops without affecting the soil biological parameters any more than if the cover crops were chemically terminated. It is important to note that differences within the soil may still be happening during cover crop growth and in the short term after termination (this study measured nine months after termination). Soils were also only taken for one year for this study, whereas it is possible that years where cover crop biomass differences are present the previous year, there would be a soil parameter effect.

Soil biological parameters that were not measured in this study could also vary by termination or species and impact the quality of the soil.

Future Research

Soil enzymes are challenging to compare across studies. High variation in soil enzyme activity was measured in this study, making differences between and among treatments hard to detect. Future research to determine reference enzyme levels within certain soil types and a threshold at which values could be considered different and not associated with random variation and heterogeneity of the soil would make soil enzyme data much more reliable and comparable between site locations.

The lack of effects of termination strategy on the soil biological parameters measured in this experiment suggests that incorporating haying or grazing could be good from both an ecological and economic perspective. It is important to note that just because we didn't see differences in the parameters measured, other parameters could be changing. Additional economic analyses across varying moisture regimes in NGP, particularly drier areas such as semi-arid Montana, would be helpful for producers looking to adopt these relatively risky management practices. A cost analysis of expenditures needed to grow cover crops against the potential economic return from both quality and quantity of the cover crop forage would help a producer weigh the cost benefit to adopting this management technique.

This study was not specifically designed to examine species richness or functional richness, rather the mixes chosen allowed this topic to be explored; however, a more rigorous approach to studying these topics would produce data to more strongly make conclusions about diversity. An approach that could be used would be to randomly select single species and randomly select multiple species from a larger pool of potential species and compare the soil parameter or aboveground biomass averages within the diversity levels.

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APPENDICES

APPENDIX A

RANDOMIZED FOUR BLOCK DESIGN FOR THE AMSTERDAM FIELD SITE

Figure A1: Randomized four block design for the Amsterdam field site.

REP 4

Tap Roots 3	**Minus N Fixers 10**	**Fibrous Root 5**	**Minus Tap 7**	**Brassica 4**	**Fallow 11**	**Pea 2**	**Minus Fibrous 9**	**Minus Brassica 8**	**Full Mix 1**	**Nitrogen Fixers 6**

REP 3

Minus N Fixers 10	**Fallow 11**	**Minus Tap 7**	**Fibrous Root 5**	**Full Mix 1**	**Tap Roots 3**	**Brassicas 4**	**Minus Fibrous 9**	**Nitrogen Fixers 6**	**Pea 2**	**Minus Brassica 8**

REP 2

Fibrous Root 5	**Full Mix 1**	**Nitrogen Fixers 6**	**Minus Fibrous 9**	**Minus Tap 7**	**Brassicas 4**	**Tap Roots 3**	**Minus Brassica 8**	**Minus N Fixers 10**	**Fallow 11**	**Pea 2**

REP 1

Full Mix 1	**Minus Fibrous 9**	**Nitrogen Fixers 6**	**Pea 2**	**Minus Brassica 8**	**Minus N Fixers 10**	**Tap Roots 3**	**Fibrous Root 5**	**Minus Tap 7**	**Fallow 11**	**Brassicas 4**