

MULTI-SPECIES COVER CROPS IN THE NORTHERN GREAT PLAINS:
AN ECOLOGICAL PERSPECTIVE ON BIODIVERSITY
AND SOIL HEALTH

by

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CHAPTER ONE

INTRODUCTION TO THESIS

As the world faces climate change and a projected 40% increase in the human population by 2050 (United Nations, 2015), sustainable agricultural practices are essential to continue to produce food on soils that may have been previously degraded by unsustainable practices. Sustainable agriculture should especially focus on management that accumulates soil organic matter (SOM) and enhances soil biological processes, which in combination benefit soil structure, soil water holding capacity, and the supply of plant available nutrients to crops. One contribution to sustainable agriculture in Montana is to replace conventional summer fallow-wheat rotations with cover crop-wheat rotations.

Summer Fallow and the Search for a Management Replacement

Wheat growers of Montana produce some of the highest quality and most valuable wheat crop (*Triticum aestivum* L.) in the nation. In 2014, wheat was grown on approximately 5.5 million acres in Montana (USDA–NASS, 2014). Introduced in the 1900s, summer fallow leaves land plant-free in an interannual cycle with winter wheat, spring wheat, or barley as a means to accumulate soil water and nutrients. Increased understanding of soil processes has led the scientific community to conclude that fallow increases potential for erosion and nitrate leaching (Campbell et al., 1991), saline seepage (Tanaka et al., 2010), decreased soil organic matter (Campbell et al., 2000), and lower

biological activity (Acosta-Martinez et al., 2007). Because growers in the Northern Great Plains (NGP) have adapted their practices to producer- and researcher-led evidence, summer fallow has consequently decreased from 17M ha in 1971 to under 4M ha today in the NGP (Tanaka et al., 2010).

Continuous cropping is not a viable option in many parts of Montana, and so producers are seeking a partial fallow replacement where more water is conserved than continuous cropping while also building soil quality. A knowledge gap exists concerning the best fallow replacement alternatives, and we aim to address the effects of partial season cover crops on cash crop production and soil properties. Montana State University and especially Dr. Perry Miller have made significant contributions to our understanding and management of pulse cover crop effects on N additions and soil water (Miller et al., 2006; McCauley et al., 2012; O'Dea et al., 2013; Burgess et al., 2014; Miller et al., 2015). Legume green manures (LGMs) that are most commonly grown in the NGP are spring pea or lentil grown during the peak precipitation season (Tanaka et al., 2010). Legumes supply additional nitrogen through N_2 fixation by symbioses with Rhizobia bacteria. It is proposed that a diversity of cover crop species can improve a wide array of soil quality indicators. While interest grows for CCMs based on producer initiatives, especially in North Dakota, the short- and long-term responses remain unknown in the semi-arid NGP.

Multispecies cover crops have the potential to improve physical, chemical, and biological soil quality indicators. Based on successes using cover crop mixtures (CCM) across the nation and especially in North Dakota, farmers in a more arid Montana

environment are adapting the practice backed by an economic incentive from the Natural Resources Conservation Service EQIP (environmental quality incentives program) (USDA--NRCS 2014). However, CCM successes are anecdotal and few studies documenting their effects on soil properties have been published to date.

Cover Crop Growth and Potential

The potential benefits of a cover crop depend on 1) the amount of biomass produced, which is limited by low rainfall and short cover crop growing season in the NGP; 2) the quality of that biomass (defined by C:N), which depends on the species included in the cover crop and the maturity of plants at termination; and ultimately 3) the water and nitrate stored in the soil for the subsequent crop. Biomass quantity and quality jointly influence the persistence of residues on the surface, which can stabilize soil (Blanco-Canqui et al., 2013), reduce evaporation (Russel, 1939; Todd et al., 1991), increase soil biological activity to promote nutrient cycling (McDaniel et al., 2014), and influence the timing of nutrient delivery to the cash crop (Vigil and Kissel, 1991). Soil water and nitrate storage influence the success of the next cash crop (Zentner et al., 2004; Nielsen and Vigil, 2005; O'Dea et al., 2013) and the potential of cover crops to supplement fertilizer N supply to reduce costs and environmental degradation.

Soil Properties Relevant to Sustainable Agriculture

Soil properties that are relevant to sustainable agriculture, commonly referred to as soil quality indicators, include physical, biological and chemical parameters that

improve current and long-term conditions for crop success (Parr et al., 1992). We selected indicators that we expected to be sensitive to short-term changes in management as affected by growth patterns and the introduction of supplemental biomass of cover crops. Biological parameters are the driving force of chemical and physical changes in the soil (Liebig et al., 2006) and may be the first to respond following a change in land management (Nannipieri et al., 2001). Chemical parameters are indicative of the soil's capacity to store and supply nutrients. Physical parameters can be described by the stability of soil structure and can improve water-holding capacity. No single indicator of soil quality can provide a basis for which success can be measured, but the careful analysis of relationships between key components and processes can provide a lens for current and projected status of soil quality.

Research Objectives

As summer fallow is replaced by cover crops, we aim to address how cover crops influence soil properties. Past studies conducted across the Northern Great Plains have investigated the short- and long- term effects of LGMs on water, nitrate, and carbon storage, soil parameters including potentially mineralizable nitrogen (PMN) and enzymatic activity, and wheat yield. Less knowledge exists regarding similar short- and especially long-term effects of CCMs in the NGP. This four-year study replicated at four sites in Montana allows us to investigate how site characteristics and annual weather patterns can influence the performance of cover crop growth and the subsequent effect on soil quality. Our research approach to building cover crop mixtures using functional

group composition rather than species composition aims to make the work more broadly applicable to other regions. Other regions may utilize more adapted legume or brassica species and could still use our results to estimate their effects on soil and nitrate use throughout the soil profile or their effects on soil biological parameters.

Chapter Two investigates the optimal functional group composition of CCMs. Ecological theory suggests that diverse plant mixtures produce greater biomass (Tilman et al., 2001), and similar agroecology research suggests that cover crop mixtures have the same advantage (Malezieux et al., 2009). In the short cover crop growing season of Montana, however, the amount of biomass produced could be limited regardless of composition. We investigated the quantity of biomass production based on diversity and the composition of functional groups within a mixture as well as the associated soil water and nitrate storage to evaluate their effect on the following cash crop.

Chapter Three compares the two most contrasting cover cropping alternatives: single-species LGM and eight-species CCM. Potential differences in quality and quantity of the residues generated by the two systems will dictate how belowground processes respond to cover crops. Soil biological communities will respond to the availability of resources (Hooper et al., 2000), and diverse cover crop residues can affect soil nutrient availability for the cash crop. A range of qualities in CCMs can provide the dual function of nutrient release, similar to low C:N residues of the LGM, but also SOM buildup from the contribution of intermediate to high C:N residues (Hendrix et al., 1990; Kuo and Sainju, 1998). We investigated the extent to which functional groups that are expected to fulfill the upper range of biomass C:N, such as cereals, actually differ from the legumes

given the limitations to maturity by the short growing season. Whereas measureable SOM accumulation generally occurs on a decadal scale and is therefore difficult to measure any changes after only two rotations in 4 years, we measured PMN at the time of wheat growth as a proxy for soil organic N that can be mineralized and made plant-available via a microbially-mediated process. Many of the promised benefits of cover crop mixtures over LGMs are associated with the variability of litter qualities, but few studies have addressed the quality residue inputs in the field under variable climate conditions.

Chapter Four addresses the biggest reason that most producers identify for using cover crops: soil quality. Soil quality has been defined as: “as the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans” by the NRCS-UDSA, which for several years has been incentivizing cover crop mixtures. Academics define soil quality as “the capacity to function within an ecosystem and sustain biological productivity, maintain environmental quality and promote plant, animal and human health” (Doran and Parkin, 1994). My thesis is part of a larger study that also investigated subsequent crop yields and physical and chemical parameters. In this chapter, I investigated soil biological parameters including microbial biomass, soil enzymatic activity, and arbuscular mycorrhizal colonization to investigate how the presence of cover crops compared to summer fallow and how the functional group composition of cover crops can influence soil parameters that could immediately benefit cash crops.

This study has been presented to growers throughout Montana thanks to the exposure of our research during field days and state-wide presentations from extension specialist Dr. Clain Jones. The information provided in this thesis aims to further the understanding of how to best construct a CCM based on their soil water and nitrate use, quantity and quality of biomass production and effects on soil biological parameters.

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CHAPTER TWO

SOIL WATER, SOIL NITRATE, AND RESIDUE QUANTITY ASSOCIATED WITH
COVER CROP MIXTURES IN THE NORTHERN GREAT PLAINS

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Soil water, soil nitrate, and residue quantity associated with cover crop mixtures in the NGP

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ABSTRACT

Cover crop mixtures as a means to increase cropping system diversity and associated ecosystem services must be tested regionally, due to differences in climate and cropping system conventions. In the Northern Great Plains (NGP) of Montana, where because of limited precipitation cover crops are grown for only two months during peak precipitation from May-July to limit water use. At four sites across the NGP with the same dryland wheat – summer fallow rotation but varying rainfall and soil characteristics, we compared nine cover crop treatments with one, three, or four functional groups and a summer fallow control. Functional groups, each represented by two species, were nitrogen fixers, fibrous roots, taproots and brassicas. In three of six site-years, mixtures with three functional groups produced 0.22 to 0.55 Mg ha⁻¹ greater biomass than single functional group mixtures. Total soil nitrate content at cover crop termination was 1.7 to 4.8 times greater in the summer fallow treatment than cover crop treatments in five of six site years. Among cover crop mixtures, nitrate was often highest in nitrogen fixer only treatments. Total soil water content at cover crop termination was 2.8 to 8.0 cm greater in the summer fallow treatment than cover crop treatments in all site years. Total cover crop biomass did not directly correlate to residual soil water at termination, except for one site year where they were positively correlated ($r = 0.35$), likely due to extremely low precipitation that growing season. Our study showed that biomass production increased with functional group richness (from one to three functional groups).

HIGHLIGHTS

- Functional group richness increased cover crop biomass production at wetter sites.
- Soil water and nitrate was higher following summer fallow than following cover crops.
- Cover crop mixtures did not use water differently.
- Soil water was not correlated to cover crop biomass.
- Soil nitrate was highest in soils following cover crops with greater legume content

1. Introduction

In natural systems perennial plant diversity can increase biomass production and subsequently affect ecological processes and ecosystem services (Tilman et al., 1996, 1997, 2001; Trenbath, 1999), but most agricultural systems in the United States operate on annual cash crop monocultures. Dryland agriculture in the Northern Great Plains (NGP) conventionally produces spring or winter wheat (*Triticum aestivum* L.) under no-tillage management in fields with summer fallow in alternate years to conserve water. This results in limited, low quality (high C:N) residue inputs and long plant-free periods. In tilled systems, summer fallow increased erosion and nutrient leaching, decreased soil organic matter (Campbell et al., 1991, 2000), and decreased soil biological activity (Acosta Martinez et al., 2007). Crop rotation and intensification can alleviate these symptoms by contributing higher temporal diversity and a greater quantity of biomass to soils, which may stimulate microbial activity (Biederbeck et al., 2005) and improve physical and chemical properties associated with increased soil organic matter (SOM; Drinkwater et al., 1998; McDaniel et al., 2014).

Crop rotation and intensification options in the NGP are limited due to low and notoriously variable precipitation. Current producer interest in cover crops has shifted from single-species legume green manures (LGM) to diverse cover crop mixtures (CCM), the latter of which has received limited research attention in the region. Species-rich cover crop mixtures are expected to increase the quantity and quality of residues, and hence contribute to soil health and system resilience (Sainju and Singh, 1997; Trenbath, 1999; Wortman et al., 2012). These expectations are predicated on theories of natural

systems in which complementary niches allow higher species richness to produce greater biomass with a range of stoichiometric and physiological characteristics (Tilman and Snell-Rood, 2014). Agricultural systems, however, are limited by a host of management demands and stressors, and so cover crop growing seasons in the NGP are short to accommodate producers' schedules for profitable production and also importantly to limit plant use of resources in the soil including water and nutrients. Cover crops may further reduce water availability for the subsequent cash crop (Pikul et al., 1997). Moisture use can be controlled by limiting the length of the cover crop growing season, but a shorter growing season results in less potential for cover crops to positively impact the soil (Balkcom et al., 2012). Thus the comparison to biodiversity of natural systems and biomass production will be reduced accordingly. Benefits of cover crop mixtures relative to single species cover crops are based on theory, but realistic management goals, i.e., managing for a short growing season may limit benefits of biodiversity to the system.

Ecological theory suggests that diverse perennial plant communities are more productive (Tilman et al., 2001), but in natural and agricultural systems, plant biodiversity effects on overall biomass are highly variable (can be positive, negative, or neutral), and may be confounded by the behavior of a dominant species (Bardgett and Wardle, 2010). Increased productivity may positively correlate to the number of functional groups, as groups with different growth patterns more fully exploit soil resources (Levine and HilleRisLambers, 2009) and capture more sunlight (Tilman and Snell-Rood, 2014). Multi-species cover crops have shown higher productivity than single species (Malezieux et al., 2009; Smith et al., 2014) where growing season is not limited

by precipitation, but conversely, no difference was seen between single species and a 10-spp mixture in the drier Central Great Plains (Nielsen et al., 2015). Because evaporative demand, and therefore biomass and seed production, varies within the Great Plains along a general north-south gradient (Nielsen et al., 2015), the question regarding diversity of cover crop mixtures affecting total biomass production remains unanswered for producers in the NGP.

Soil nitrate use and storage is critical, as the grain protein content of wheat is correlated with soil N and fertilizer N inputs (Grant et al., 2002). Legumes as cover crops provide N through mineralization of residues and may alleviate demands on N fertilizer, which is non-renewable and highly energy intensive, with negative environmental impacts (Smil, 1999) such as N leaching to groundwater in Montana (Bauder et al., 1993). Cover crops can reduce nitrate leaching (Tonitto et al., 2006) by taking up N from soils, and then N stored in residues is released via mineralization during the subsequent season (i.e. “catch and release”), potentially when needed by cash crops. Plant N uptake is influenced by soil type, environmental conditions including water availability (Abreu et al., 1993), and the identity of their plant neighbors’ (Miller et al., 2007). Functional groups that efficiently store and return N to the soil may be more beneficial to the following wheat crop than those that either fix their own N and leave higher N in the soil susceptible to leaching or those that have too high C:N and decompose too slowly to release N.

Soil water management is crucial to the success of any cover crop in the NGP. Cover crop water use will reduce water available for the following cash crop. In previous

studies of cover crop effects in NGP, water-use-efficiency was negatively correlated with total biomass produced, but did not vary with species richness (Nielsen et al., 2015). Net water losses may be mitigated by early termination of CCMs similar to termination of LGMSs at early bloom (Aase et al., 1996; Allen et al., 2011; Biederbeck et al., 1998, 1998; Biederbeck and Bouman, 1994; Pikul et al., 1997; Zentner et al., 2004); by increased residue stubble for snow-catch (Zentner et al., 2004); and by introducing a crop to reduce soil evaporation (Russel, 1939; Todd et al., 1991).

The overall objective of this study was to compare the biomass productivity, along with water and nitrate use of cover crop mixtures, based on their functional group composition. Although previous studies measured cover crop biomass and water-use-efficiencies of cover crops, Nielsen et al. (2015) highlights the stark difference in cover crop water-use-efficiency and also cash crop yields between the central and northern Great Plains, where precipitation and solar radiation varies markedly. Our study is replicated across three years and four sites within the NGP and compares both functional group richness and composition. Cover crop mixtures in this study are combinations of four functional groups: nitrogen fixers, included for their fertility inputs; species with fibrous roots, for their high C inputs to soils; species with taproots, for their effects on soil structure and infiltration; and brassicas, due to their unique chemistry and their rapid ground cover.

To address our first objectives of comparing biomass across treatments, we designed the study so that we were able to 1) to identify functional groups that could produce greater biomass within the limitations of time and water specific to the NGP, 2)

to determine whether one of the four functional groups could be excluded from the FULL mixture without reducing total biomass in comparison to the FULL mixture, 3) to determine whether a higher number of functional groups would increase biomass, and finally 4) to determine whether these patterns are consistent across sites and years. We expected that a higher number of functional groups would not increase biomass due to precipitation limitations and that there would be few differences in biomass production between functional groups. We expected that the ranking of treatments based on biomass production would remain the same between sites years.

Our second objective was to investigate how soil nitrate remaining in the soil at cover crop termination differed following treatments. Soil nitrate will likely continue to undergo physical, biological, and chemical processes, but an available nitrate ‘snapshot’ at termination provides information on soil N levels that have been immediately affected by cover crop presence. We hypothesized that soil nitrate would be highest after summer fallow and that soils under CCMs that include N fixers would have higher nitrate concentrations compared to other functional groups. Furthermore, we expected that concentrations would vary with depth among treatments due to variable rooting depths among functional groups, likely with taproots reducing NO_3 the most at lower depths.

Our final objective was to investigate in-season effects and not over-winter effects of residues and how functional group differences in structure and physiology will influence their water use. For example, fibrous roots with limited canopy cover may not reduce soil evaporative processes, resulting in lower water available at cover crop termination, whereas tap rooted species may access water lower in the soil profile, also

resulting in lower total water content at depth. Specifically, we wanted to determine 1) whether the presence of a cover crop would reduce soil water content at termination compared to summer fallow as expected, and 2) whether within cover crop treatments, any functional group would conserve more soil water for a similar amount of biomass produced. If so, we aimed to identify the depths at which moisture was most conserved. We expect that overall water use would be directly correlated to the amount of biomass produced regardless of functional groups present.

2. Materials and Methods

2.1 Site characterization

The study was conducted at four locations (Table 1) in Amsterdam, Conrad, Bozeman, and Dutton, MT, all of which had been under at least three years of no-till management in fallow-wheat rotations prior to the study. Long-term average (LTA) annual temperatures range from 6.2 to 7.4 °C and precipitation from 303 to 469 mm (Table 2). Soil type at the two northern sites in MLRA 52 are clay loams classified as frigid, Aridic Argiustolls. At the two southern sites located in the Gallatin Valley, Bozeman is a loam to clay loam soil classified as a frigid, Typic Argiustoll and Amsterdam is a silt loam soil classified as a frigid, Aridic Calciustoll. Experimental plots at Amsterdam, Conrad, and Dutton were located on commercial wheat farms. The Bozeman site occurred on university-owned land with a history of hay/pasture in a high rainfall location, and was included as a deliberate contrast to the other low rainfall, low SOM sites to investigate whether cover crop effects would differ importantly in a more

well-endowed environment. Temperature and precipitation data were collected on-site with automated gauges at each site.

2.2 Experimental design and management

Cover crops were first implemented in 2012 at Amsterdam and Conrad and in 2013 at Bozeman and Dutton, with eleven cover crop treatments randomly assigned to plots (8 x 12 m) in each of four blocks in a randomized complete block design. The eleven treatments include: a summer fallow control (SF); a sole pea (disregarded in this analysis due to redundancy with the nitrogen fixer group); a FULL four-functional group cover crop mixture; four treatments with each functional group grown singly; and four treatments with one of the groups removed from the FULL for effective three-functional group treatments (Table 3).

Cover crops were planted in the last week of April or the first two weeks of May (details in Appendix) with a common target seeding rate of 120 plants m⁻², so that, for example, individual species in an eight species mix were seeded at a rate of fifteen plants m⁻². Seedling counts suggest that cover crops averaged 8% above the goal of 120 plants m⁻² (95% confidence interval: 4% - 12%) from 2013-2015. All species were planted in the same row to a depth of 1 to 2 cm. Termination by a glyphosate mixture coincided with the first-bloom of pea, as per McCauley et al. (2012). Biomass data in 2012 are excluded from this study because biomass production was extremely low (Tallman, 2014) due to dry conditions and downy brome (*Bromus tectorum*) pressure at the Conrad site, and also because of early seeding and termination prior to June 15 to meet a summer

fallow cutoff deadline established by USDA-Risk Management Agency (USDA-FCIC 2012). Plant growth in summer fallow treatments was controlled with herbicide.

In spring of odd years, following cover crop production at Amsterdam and Conrad, spring wheat (cv. *Duclair*) was seeded in rows perpendicular to cover crop rows with three levels of fertilizer [zero (0 kg N ha^{-1}), low (67 kg N ha^{-1}), and high (135 kg N ha^{-1})] in split plots ($3.6 \times 7.6 \text{ m}$). In fall of 2013 and 2015 following cover crops at Bozeman and Dutton, winter wheat (cv. *Warhorse*) was sown in the same split-plot design. A second rotation of cover crop treatments was implemented in the spring in 2014 at Amsterdam and Conrad and in 2015 at Bozeman and Dutton.

2.2 Cover crop sampling

Aboveground cover crop biomass was sampled from quadrats ($1 \times 0.3 \text{ m}$) in mid to late July based on first-bloom of spring pea. Plants were cut at the soil surface, separated by species, dried at 50°C until they reached constant mass, and weighed. In 2014 and 2015, after fertilizer treatments were initiated in the wheat stands, cover crop data were collected in both high and zero fertilizer treatments to account for possible carryover effects of the previous year's wheat fertilization. Cover crop biomass between high and zero fertilizer treatments were not significantly different, with the exception of Amsterdam in 2014. In that site-year, the cash crop was hailed out just days prior to planned harvest, so wheat residues remained on site without a harvest. As a result, soil nitrate levels were elevated in high fertilizer treatments at spring wheat seeding, and so within that site-year cover crop biomass was greater in high fertilizer plots with two of

the 11 treatments, the single functional group treatments BC and TR (Appendix). Data shown are the average of high and zero N treatments within each treatment.

2.3 Soil water and nitrate

We measured soil water and nitrate content one to four days after cover crop termination to compare among functional groups and mixtures. Two composite soil cores (3 cm diam. x 90 cm) were collected within each plot (medium N rate after one cycle) using a hydraulic probe. Within each 30-cm increment, the two samples were composited, homogenized, and stored cold until processing. Samples were dried for one week at 50 °C and gravimetric soil water was divided by soil bulk density for each depth volumetric water content. Soils were then ground using Dynacrush (Custom Laboratory Equipment, INC., Orange City, FL), and inorganic N content was determined using a 2 M KCl extraction method (Bundy and Meisinger, 1994) and analyzed on a Lachat flow injection analyzer (Lachat Instruments, Loveland, CO). Soil nitrate data were measured in only two treatments in 2013: summer fallow and FULL four functional group CCM, and measured in soils of all treatments in 2014 and 2015.

2.4 Statistical Analyses

All data were checked for normality and homogeneity of variance using residual plots and Q-Q plots. Two-way ANOVA models were used to test the effects of treatments on total biomass production. Analyses were performed in R package *stats* (version 2.15.3), with cover crop treatment and block as independent variables. Individual sites and years were analyzed separately due to large differences in site

conditions and slight differences in management. Statistically significant differences in response variables were determined using Fisher's LSD ($\alpha=0.05$) in *agricolae*. We used pre-planned orthogonal contrasts to compare cover crop biomass from one and three functional group treatments because there were an equal four treatments in each of those groups (as opposed to only a single treatment in four-functional groups in each block), and also preplanned contrasts between the FULL mixture vs each three-functional group treatment. The same two-way ANOVA model with cover crop treatment and block as independent variables was used to test for treatment effects on soil water and soil nitrate at individual depths. For soil water and nitrate, preplanned contrasts were also used to compare SF and the presence of a CC. Correlation analyses within site-years were conducted using *stats* package and Pearson's coefficients (r) are reported.

3. Results

3.1 Aboveground Biomass

Two months of cover crop growth resulted in aboveground biomass ranging from 1.6 Mg ha⁻¹ in 2015 at Dutton with 98 mm rainfall to 3.9 Mg ha⁻¹ in 2013 at Bozeman in 2013 with 196 mm rainfall. Within years, the site that received 30 - 45 mm higher rainfall produced higher biomass. Thus, the two northern sites in the MLRA 52 produced 24 to 28 % lower biomass in all years. Mean biomass production across cover crop treatments in 2013 was 3.9 and 2.9 Mg ha⁻¹ at Bozeman and Dutton, respectively. In 2015, a drier year, Bozeman and Dutton produced as much as 1 Mg ha⁻¹ less at each site than in 2013:

only 2.3 and 1.6 Mg ha⁻¹, respectively. In 2014, mean biomass production was 3.1 and 2.4 Mg ha⁻¹ biomass, averaged across treatments, respectively.

Our first objective was to identify functional groups that would produce higher biomass in the limited growing season of the NGP. In four of six site years, there were no differences in biomass production among the single functional group treatments. Where differences occurred, the highest producing functional group was FR, and lowest producing functional group differed (Figure 1). BC was the lowest producer at the driest site-year of the study (Dutton in 2015; Figure 1; $F = 15.4$, $p < 0.01$), and TR produced the lowest biomass in the wettest site-year of the study (Bozeman in 2013; Figure 1; $F = 8.6$; $p < 0.01$). However, BC tended to be one of the biggest contributors to total biomass in the FULL mix and in the three minus treatments in which it occurred in all site-years (Figure 1). Conversely, the FR treatment did not produce a high quantity of biomass within the three and four functional group CCMs except where brassicas were absent.

Our second question was whether including all four functional groups would be necessary for maximum biomass production by comparing the FULL treatment to three-functional group treatments. Removing a single functional group from the FULL mixture did not decrease biomass. At both sites in 2013 and 2014, the three-functional group treatments produced the same quantity of biomass as the FULL mixture (Table 5). In 2015, biomass in one of the three-functional group treatments was greater than the FULL mixture at both sites. Specifically, at Dutton in 2015, the MBC treatment averaged 0.50 Mg ha⁻¹ more biomass than the FULL treatment ($p = 0.04$). At Bozeman in 2015, the MTR treatment produced 0.47 Mg ha⁻¹ more ($p = 0.03$).

Our third question was whether a higher number of functional groups would increase biomass. Treatments with three-functional groups produced on average 0.33 Mg ha⁻¹ more biomass ($p < 0.05$) than treatments with one-functional group in three out of six site years (Table 5). In site-years with the highest biomass production, there were weak to moderate positive correlations between the number of species and total biomass production at Bozeman in 2013 ($r = 0.41$, $p < 0.01$) and Amsterdam in 2014 ($r = 0.32$, $p = 0.05$).

3.2 Soil nitrate at termination

Our assumption is that soil nitrate was similar across treatments within sites at cover crop seeding in the first rotation as well as the second rotation. Cover crop growth two years prior could confound soil nitrate results in rotation 2, but treatments have received equivalent N fertilization and wheat has been harvested, likely equilibrating soil N levels. Measurements in a subset of seven treatments including summer fallow indicated that soil nitrate at seeding at Bozeman and Dutton in 2015 was not different among treatments (Table 10). Soil nitrate levels reflect microbially-, chemically- and physically-mediated processes, and measures of soil nitrate after the growing season can approximate nitrate sequestered by cover crops and provide an estimate of soil N susceptible to leaching (Tonitto et al., 2006).

Our first question regarding soil nitrate was whether soils under cover crops would consistently have lower soil nitrate levels than soils under summer fallow and at which depths these results were most pronounced. At cover crop termination, nitrate content was 1.7 to 4.8 times greater in soils under summer fallow treatment than with a

cover crop in all site-years (Table 6) except Dutton in 2015, where weed biomass in the summer fallow treatment was comparable to total biomass in cover crop treatments (Table 4). The effect of greater soil nitrate under summer fallow in other site-years was consistently evident in the upper depths (0-30 cm and 30-60 cm) but was less pronounced or not present at the 60-90 cm depth, likely due to variability of cover crop rooting depths. The greatest differences between summer fallow and nitrate depletion by a cover crop were in the top 30 cm with an average difference of 27 kg NO₃-N ha⁻¹. At 30-60 cm, the trend was still strong but the difference was not as great (average difference of 18 kg NO₃-N ha⁻¹). Among sites, the difference in soil nitrate between summer fallow and cover crop treatments at Bozeman was the greatest with an average difference of 41 kg NO₃-N ha⁻¹ in the top 30 cm and 66 kg NO₃-N ha⁻¹ in the whole 90 cm profile, likely due to the abundance of nitrate in the soil initially. The difference between soil nitrate content following cover crop and summer fallow was not as great at Amsterdam, likely due to overall low nitrate concentration and high weed growth in that fallow treatment (Table 4). In 2013, nitrate levels were only measured in FULL and SF treatments, and nitrate levels in soils following SF tended to have greater NO₃ content at termination at 0-30 cm and 30-60 cm depths ($p < 0.08$, data not shown).

Our second question was whether soil nitrate differed among cover crop treatments and if soils under NF had greater soil nitrate than soils under other CCMs. In contrast to the comparison of soils following SF vs CC, most differences in soil nitrate content among cover crop treatments occurred at the mid-depth (30-60 cm) in all four site-years where soil nitrate data for all 9 CCMs are available (2014 and 2015). At mid-

depth, soils following NF treatments contained the most nitrate in all site years ($p < 0.05$) except at Amsterdam where soils following MBC had the most ($F = 3.5$, $p < 0.01$), and differences among the remaining treatments at this depth were minimal. NF treatments at mid-depth averaged $5 \text{ kg NO}_3\text{-N ha}^{-1}$ greater than all other CC treatments at Conrad in 2014 ($\text{LSD}_{(0.05)} = 2.9$), and 10 and $14 \text{ kg NO}_3\text{-N ha}^{-1}$ greater at Bozeman and Dutton ($\text{LSD}_{(0.05)} = 3.9, 6.3$), respectively in 2015. There were differences among cover crop treatments in the shallowest depth (0-30 cm) in three of four site years, but results among treatments were highly variable (Table 6). Soils following BC had the greatest nitrate at Amsterdam but were not different than TR or MFR (Table 6; $F = 2.6$, $p = 0.03$), and no differences were seen at Conrad. Soil nitrate content following NF was the highest at both sites in 2015 but at Bozeman was not different from MBC (Table 6; $F = 4.1$, $p < 0.01$) and at Dutton was not different than TR (Table 6; $F = 3.9$, $p < 0.01$).

We expected that soils following CCMs including nitrogen fixers would have greater soil nitrate content than CCMs without nitrogen fixers. Next to the NF treatment, MBC has the greatest proportion of total biomass represented by nitrogen fixers in all site-years (Figure 1) and was often among treatments with greatest soil NO_3 at termination. Other treatments in which NFs did not occur had highly variable results. In some site-years, soil nitrate following FR, BC, or TR was either among the highest or among the lowest. Soils following minus nitrogen fixers (MNF) contained 17-70% less nitrate at termination than that of soils following NF treatments.

3.3 Cover crop water use

The final objective of the study was to identify whether the presence of a cover crop would reduce water at termination compared to summer fallow as expected, and if among cover crop treatments, any functional group would maximize soil water content at termination in a relationship that was not explained only by the amount of biomass produced. If so, we aimed to identify at what depths was the water mostly conserved. Soil water content differed between summer fallow and cover crop treatments in the top two depths in all site-years (Table 7 and 8; $p < 0.05$) except Dutton in 2013 where it was only different in the upper depth (0-30 cm; Table 7; $p < 0.01$). Soil water use was greater under a cover crop below 60 cm only in three of six site years ($p < 0.05$). Total soil water to a 90 cm depth was on average 4.5 cm greater in SF than CC.

Our second objective was to identify whether any functional group would maximize soil water content at termination in a relationship that was not explained only by the amount of biomass produced, and so we first investigated the direct relationship between total biomass and soil water content. In five of six site years, soil water content was not correlated with cover crop biomass including weeds. There was a positive correlation at Dutton in 2015 (Table 9, $r = 0.35$, $p = 0.036$), which is the opposite of what would be expected. It is not as simple as more biomass used more water, and relative differences among treatments were variable among cover crop mixtures across site-years.

In 2013, post-season differences among CCMs occurred above 60 cm at both Bozeman and Dutton. Soils from the FR held the highest amount of total water (0-90 cm) among CCMs and approximately only 1 cm less water than summer fallow at Bozeman

(Appendix; $F = 3.4$, $p < 0.01$) and at Dutton (Appendix; $F = 2.2$, $p = 0.01$) despite high CC biomass production (Figure 1). Soils from the MFR treatment also had among the highest water content at termination at Dutton despite high CC biomass production MFR treatments that year (Figure 1). This suggests that linking functional groups to water use will be problematic at best.

In 2014 and 2015 there were no soil water differences at any depth among CCM treatments at the drier northern sites (Conrad and Dutton). At Bozeman, differences occurred only in the 30-60 cm depths (Table 8; $F = 4.3$, $p < 0.01$), and soils from NF treatments had the greatest water content at termination compared to soils under other cover crops and only 1.5 cm less than soils under summer fallow. At Amsterdam, differences occurred at all depths. Among cover crop treatments, soils following FR, MNF, and BC had the greatest water content in total (Table 8; $F = 13.8$, $p < 0.01$), all of which had at least 3 cm less water content than soils following summer fallow in that site-year. Different patterns emerged within each depth. Soils following BC and TR interestingly had the greatest shallow water content (0-30 cm; Table 8; $F = 14.9$, $p < 0.001$) but the lowest water content at the lowest depth (60-90 cm; Table 8; $F = 5.2$, $p < 0.001$) among soils following CC treatments. Alternatively soils under FR had low shallow water content and high water content deeper. Although this trend was strongest in soil under the FR treatment, it was also seen in other treatments within that site-year that are among the lowest water content at the surface and oppositely among the highest at depth (FULL, NF, MFR, and MBC). Soil water content following MNF and MTR was medial at all depths.

4. Discussion

As cover crops and more specifically cover crop mixtures become more prevalent in the NGP and elsewhere, research is needed that will better inform the development of mixtures based on their performance specific to a region and their effect on storage of water and nutrients in the soil. Here we have investigated the contribution of cover crop mixtures based functional groups inclusion or exclusion. Results are highly variable among years and cover crop productivity is highly dependent on growing season rainfall.

4.1 *Aboveground Biomass*

Fluctuations in cover crop biomass were explained by precipitation in our study and similar cover crop studies in the region. Across the region, LGMs have produced 1.0 Mg ha⁻¹ in the MLRA 52 (O'Dea et al., 2013) and 0.4 to 1.7 Mg ha⁻¹ across greater Montana (Miller et al., 2006) in relatively dry years and 2.8 Mg ha⁻¹ in average precipitation years (Burgess et al., 2014). Cover crop mixtures have fewer regional comparisons, the closest of which occur in the Central Great Plains. A 10-spp mixture produced between 2.0 and 4.8 Mg ha⁻¹ in 2012 and 2014 at two sites in Nebraska and Colorado (Nielsen et al., 2015). On average, the results from this study are lower than regional comparisons in the wetter and warmer Central Great Plains. Discrepancies in biomass production among functional groups within a mixture are not due to seeding rate or number of individuals (Appendix B.1.1-B.1.4; seedling counts).

Results from cover crop studies in other regions indicate that water limitation is important when adopting cover crops for their intended productivity and subsequent effects on soil quality. For example, cover crops in North Carolina produced 1.4 to 4.8

Mg ha⁻¹ of legume biomass and 3.9 to 8.8 Mg ha⁻¹ of grass biomass in annual rotations (Creamer and Baldwin, 2000). These productivity levels are similar to results in Mississippi (Dabney et al., 2001) and Pennsylvania (Mirsky et al., 2012). Cover crop mixtures of a legume and grass in Michigan produced up to 28 Mg ha⁻¹ (Snapp et al., 2004). Furthermore, because these cover crops are often grown annually during the shoulder season, systems receive two-, three- or four-fold increase in labile organic matter additions compared to agroecosystems of the NGP.

Functional group richness explained some of the differences in total biomass production in this study. Three functional group CCMs on average produced 0.22 to 0.55 Mg ha⁻¹ greater biomass than single functional group CCMs, but only at the two southern sites located in Gallatin Valley. In years presumably less limited by water there was a direct correlation with number of functional groups and total biomass produced but not when rainfall was below average. Dry years are common in the NGP, and so producers cannot be expected to manage as if there will always be plentiful rainfall.

In 2013 and 2014, no three-functional group treatment biomass was ever different than FULL. In 2015, differences emerge, and in some cases, the three-functional group treatment, which excluded the functional group that produced the lowest biomass alone at that site, resulted in greater biomass than FULL, suggesting that planting all of four functional groups in a mixture was not necessary. This could be because at Bozeman, TR, and at Dutton, BC, were the lowest biomass producers as single functional group treatments at each site, respectively, and when excluded from the mixture allowed the remaining mixtures (MTR and MBC) to produce greater total biomass. BC and TR

functional group biomass production was highly variable within and among years, seemingly most susceptible to changes in rainfall, weather, and competition, and so including these in a mixture but not relying on them for biomass production in an unpredictable environment could have advantages. Brassicas and/or taproots are more variable in biomass production and could be dropped from the mixture without detriment to the overall biomass production. There may be other benefits to these functional groups however.

Further research should focus on the influence of the quantity of biomass on belowground ecology and crop yields in these systems. CC residues in dryland Kansas reduced water and wind erosion of soil, but authors suggested the extent of this soil stability depended on the amount of residue inputs during fallow periods (Blanco-Canqui et al., 2013). Our study also includes research that suggests initial belowground biological responses correlate to the amount of cover crop biomass produced the previous year (Housman, 2015).

4.2 Soil nitrate and water

In regions where nitrogen leaching is an environmental and health problem, it may be advantageous for cover crops to use or ‘catch’ N, and store it as biomass, where it can be mineralized when cash crops are growing. If this is the case, we find the presence of a cover crop reduces the total N content in the soil between cash crops compared to summer fallow. However, previously observed lower NO₃ in soils immediately following early-termination LGM in the NGP had equalized by fall and the following spring (Biederbeck et al., 1996; Miller et al., 2006; Tanaka et al., 1997). Because no evidence of

N leaching was observed in these studies, it was speculated that the lack of differences between soil nitrate following LGM and summer fallow in the fall was from gaseous losses. ‘Catch’ crops would keep N in the system relative to the N loss from the summer fallow. Nitrogen fixers alone do not provide the service of temporarily removing N from the soil and storing it in plant biomass as efficiently as other CCMs. In the NGP’s cold, dry climate, slower decomposition will affect the timing of mineralization, and further work needs to be done to elucidate whether N will be released from diverse cover crop residues in a timely manner for the subsequent crop.

The semiarid NGP has a relatively low risk of nitrate leaching to groundwater with the exception of some watersheds such as the Judith Basin, MT due to coarse soils, a shallow depth to gravel, and shallow depth of the water table (Bauder et al., 1991). In this region, cover crops could alleviate N leaching where summer fallow is the principal anthropogenic cause of nitrate leaching to groundwater (Bauder et al., 1993). At lower depths, where N is most susceptible to entering groundwater, soil nitrate levels differed among CCMs at only two sites and the presence of a cover crop only differed from summer fallow at one site, perhaps because cover crop roots may not reach these depths in the short growing season. Alternatively, if N does not leach and the goal is to reduce chemical fertilizers for the following cash crop, nitrogen fixers use less soil N and ultimately supplement N to the soil whether directly through lower use of soil N and/or indirectly via breakdown of high N content in their residues. Previous studies show that soil N increases from LGMs may take five years to manifest when tillage is implemented

(Hoyt and Leitch, 1983), or after the second or third rotation (Allen et al., 2011; Zentner et al., 2004).

Soils from summer fallow treatments were unsurprisingly higher in water content at the time of cover crop termination, but producers are adopting cover crops in favor of other ecosystem services after generations of placing water storage priorities first. We investigated the critical issue of water storage among possible CCM options based on species richness and composition. Among CCMs there are few and inconsistent differences in water at termination. At the lowest depths (60-90 cm), variability was especially high, and this may be explained in part by rooting depth of cover crops. The water use limitation in lower depths was similar to other studies of early-terminated LGM in the region (Biederbeck and Bouman, 1994; Miller et al., 2006). When differences occurred at all depths at Amsterdam, soil from the FR treatment had the highest water content at depth, but among the lowest near the surface. Although FR plots produced high biomass, soil temperatures were higher in FR plots than other CCM treatments, suggesting that minimal canopy cover could result in increased evaporation from the surface (unpublished data). Alternatively, it could be the high density of roots in the shallow zone. BC and TR, on the other hand, possibly prevented high rates of evaporation at the surface (had high surface water content) and because of the deep roots of these functional groups, used water lower in the profile. This could be good support for using a mix, as both provide essential services to conserving water during cover crop growth, but this results of different functional groups using water at different depths was only apparent at one of six site-years.

It is possible that differences in water content at termination between summer fallow and cover crops would be alleviated during the period following termination and before wheat seeding, either 1.5 or 10 months depending on winter or spring wheat, respectively. Differences seen at cover crop termination may be alleviated by snow-catch by residues in the short-term and increase in organic matter's contribution to water storage in the long-term. The upper depths may be the most easily replenished by winter precipitation, and differences may dissipate as illustrated by Biederbeck and Bouman's (1994) findings that soil water to 0.6 m could be equal by spring, but lower depths could still show effects of depletion under similar full-bloom LGM termination. Therefore, among CCM treatments, differences that occur lower in the soil profile may not dissipate as sufficiently before wheat seeding. There were generally no differences at wheat planting when comparing soils following different species of legume-only cover crops but stark differences when comparing summer fallow and a LGM (Nielsen and Vigil, 2005).

The relationship between biomass production and water use is affected by species-specific factors (photosynthetic efficiency of species), seasonal patterns (timing of precipitation and water stress at particular stages of development), site conditions (limited soil fertility, soil physical conditions) and numerous other factors that can affect the relationship between biomass production and water use. Many of these factors associated with seasonal patterns and site conditions are not explicitly addressed here, but we rely on the power of replication that captures variability of these factors within the six site-years. The positive correlation between biomass production and water content at

termination at Dutton in 2015 was likely a result of variability in landscape across that site, as some areas are able to hold more water and allow for greater biomass production rather than areas that are consistently drought stressed.

Differences in water content in soils with different cover crop species within site-years may have been due to varying amounts of time to maturity across species. First-bloom of pea is the signal for termination to reduce water use while optimizing nitrogen fixation based on many previous studies under both tillage (Zentner et al., 2004), and no-till management (Miller et al., 2006), but there are no guidelines for how the other species use water. Radish bloomed prior to pea, lentil and cereals were often flowering at termination, but safflower and turnip had not flowered by the time of termination. Further studies are needed to identify the best timing for termination in a mixture for optimum water storage.

Based on soil nitrate at termination alone, we suggest that nitrogen fixers be coupled with other functional groups to remove more soil N from the soil to reduce the risk of leaching. Based on soil water at termination, a cover crop mixture should include functional groups that use the least water lower in the profile where water is not as easily replenished. Fibrous roots are especially pronounced at producing high biomass without using water deep in the profile. However, if water reduction from the presence of any cover crop occurs throughout the profile and is not sufficiently replenished by winter precipitation, subsequent grain yields will be affected the following year and cover crop mixtures may introduce an economic hurdle that many producers will not be willing to overcome for longer-term benefits.

Results of cover crop mixture effects on wheat yields are forthcoming in 2017 after two full rotations of the study have been completed. It has been reported previously in the NGP that the first rotation of cash crop following LGM produce similar or lower yields than summer fallow (Burgess et al., 2014; Miller et al., 2006; O'Dea et al., 2013; Pikul et al., 1997). In some studies, reduced yields have been attributed to lower soil N rather than soil moisture (O'Dea et al., 2013), and LGM's negative effects on yields can be particularly high in years with low precipitation (Nielsen and Vigil, 2005). Increases in crop yields were observed after six (three rotations) years of LGM cover crop rotation in the NGP (Miller et al., 2011).

Our study presents the first published effort to determine functional groups' roles in cover crop mixtures in the NGP, and underscores the need for further work on long-term effects of cover crops on soil water and nitrogen storage. Cover crop biomass can be increased with functional group richness greater than one but only in southern, wetter sites, and biomass is not increased from three to four functional groups. Soil water and nitrate are lower following cover crop than following summer fallow, but producers are implementing cover crops to avoid summer fallow known to have these benefits. And so the differences between cover crops would be informative, but there are few consistent differences except that fibrous root treatments tend to produce greater biomass without using more total water.

Table 1. Soil characteristics sampled within 30 days before first cover crop seeding at each of the four sites.

	Amsterdam	Conrad	Bozeman	Dutton
Location	45°43'6.74"N 111°21'52.37"W	48°12'47.55"N 111°29'41.09"W	45°40'11.91"N 110°58'38.62"W	47°59'49.96"N 111°34'8.27"W
Texture	Silt loam	Clay loam	Loam to Clay loam	Clay loam
pH	8.2	6.5	7.0	6.7
Soil Organic Carbon (g kg ⁻¹)	14	14	33	19
NO ₃ -N (mg kg ⁻¹)	6.0	8.5	7.3	7.5
Olsen P (mg kg ⁻¹)	13	28	32	43

Table 2. Climate Patterns at each of four sites. Cover crop growing season precipitation for HOBO weather stations is April - June. GDD calculated from day after seeding to day of herbicide termination. Long-term average (LTA) is calculated from 1981-2010, Western Regional Climate Center, from station nearest individual sites no more than 26 km away.

	Amsterdam		Conrad		Bozeman			Dutton		
	LTA	2014	LTA	2014	LTA	2013	2015	LTA	2013	2015
Annual Precipitation (mm)	358		303		469			303		
Annual Temperature (°C)	7.4		6.2		7.0			6.2		
Growing Season Rainfall (mm)	162	166	140	139	216	196	145	140	166	98
GDD (0° C)		860		881		899	878		931	1089

Table 3. Species included in cover crop mixture treatments

Treatment	Plant Species
Nitrogen Fixer (NF)	Spring pea (<i>Pisum sativum</i> L. cv. Arvika) Indianhead lentil (<i>Lens culinaris</i> Medik. cv. Indianhead)
Fibrous Root (FR)	Oat (<i>Avena sativa</i> L. cv. Oatana) †Canaryseed (<i>Phalaris canariensis</i> L.)
Taproot (TR)	Purple top turnip (<i>Brassica rapa</i> L.) Safflower (<i>Carthamus tinctorius</i> L. cv. MonDak)
Brassica (BC)	Radish (<i>Raphanus sativus</i> L. var. <i>longipinnatus</i>) Winter canola (<i>Brassica napus</i> L. var. <i>napus</i> cv. Dwarf Essex)
Full Mix (FULL)	NF + FR + TR + BC
Minus Nitrogen Fixers (MNF)	FR + TR + BC
Minus Fibrous Roots (MFR)	NF + TR + BC
Minus Taproots (MTR)	NF + FR + BC
Minus Brassicas (MBC)*	NF + FR + TR

†The fibrous root species selection in 2013 was millet (*Panicum miliaceum* L. sp.), which competed poorly and was replaced by canaryseed in 2014 and 2015.

*MBC treatment is only five species as taproot species purple top turnip is also a brassica and therefore excluded from this mixture.

Table 4. Weed biomass (Mg ha⁻¹) in the summer fallow treatment only by site year.

	-----2013-----		-----2014-----		-----2015-----	
	Bozeman	Dutton	Amsterdam	Conrad	Bozeman	Dutton
Weed Biomass	1.30	0.13	1.20	0.09	0.03	1.40

Table 5. Results of ANOVAs testing the effects of cover crop mixture on total biomass production.

		-----2013-----		-----2014-----		-----2015-----	
		Bozeman	Dutton	Amsterdam	Conrad	Bozeman	Dutton
Among single	p-value	0.005	0.817	0.102	0.099	0.113	<0.001
functional groups	$F_{(3,9)}$	8.57	0.31	2.79	2.83	2.64	15.43
FULL							
vs MNF		0.777	0.225	0.984	0.948	0.457	0.893
vs MFR		0.534	0.678	0.144	0.752	0.727	0.339
vs MBC		0.870	0.476	0.235	0.879	0.092	0.044
vs MTR		0.299	0.277	0.773	0.853	0.034	0.822
3 vs 1 functional	p-value	0.014	0.468	0.021	0.061	0.046	0.827
groups	$t_{(3>1)}$	2.62	0.73	2.44	1.95	2.08	-0.221

Table 6. Soil nitrate content (kg NO₃-N ha⁻¹) at cover crop termination at four sites 2014-2015. Treatments with different letters were significantly different within depth increments. Soil samples were collected from medium fertilizer application subplots only.

	-----2014-----						-----2015-----					
	Amsterdam			Conrad			Bozeman			Dutton		
Depth	0-30	30-60	60-90	0-30	30-60	60-90	0-30	30-60	60-90	0-30	30-60	60-90
SF	19.4	6.3	11.0	30.8	22.5	9.0	50.2	21.5	26.4	15.8	12.7	11.4
CC	4.1	1.2	10.1	4.6	3.6	4.1	8.8	4.9	18.2	12.1	6.7	10.3
p-value	<0.001	<0.001	0.831	<0.001	<0.001	0.024	<0.001	0.001	0.140	0.078	0.066	0.833
F _(1,35)	117.7	59.7	0.05	234.5	157.7	5.5	278.1	49.6	2.3	3.3	3.6	0.05
FULL	3.8 ^b	1.0 ^b	10.1	4.8	2.8 ^{bc}	2.7	7.3 ^c	2.8 ^b	23.7	12.6 ^{bc}	5.5 ^b	6.4 ^b
NF	3.5 ^b	1.3 ^b	17.6	6.6	8.1 ^a	4.7	13.5 ^a	14.0 ^a	25.7	18.4 ^a	19.3 ^a	26.4 ^a
FR	3.4 ^b	1.2 ^b	7.1	5.6	5.5 ^{ab}	8.2	9.1 ^{bc}	5.2 ^b	21.6	11.6 ^{bcd}	6.8 ^b	14.4 ^{ab}
TR	4.5 ^{ab}	1.0 ^b	8.0	3.4	2.6 ^{bc}	1.8	6.7 ^c	2.4 ^b	13.6	14.1 ^{ab}	3.9 ^b	6.4 ^b
BC	6.1 ^a	1.0 ^b	7.2	4.1	2.4 ^c	3.9	7.6 ^c	2.1 ^b	6.5	8.8 ^{cd}	4.8 ^b	7.5 ^b
MNF	4.1 ^b	0.9 ^b	2.8	3.9	2.7 ^{bc}	2.6	6.0 ^c	3.2 ^b	13.4	8.0 ^d	3.3 ^b	5.5 ^b
MFR	4.8 ^{ab}	1.1 ^b	12.7	2.2	1.9 ^c	4.7	9.2 ^{bc}	3.9 ^b	15.4	11.9 ^{bcd}	5.5 ^b	5.5 ^b
MTR	3.7 ^b	1.0 ^b	5.1	4.7	2.6 ^{bc}	2.8	7.4 ^c	5.4 ^b	24.9	12.8 ^{bc}	6.3 ^b	9.5 ^b
MBC	3.3 ^b	2.9 ^a	19.9	6.6	3.6 ^{bc}	5.2	12.4 ^{ab}	5.0 ^b	18.9	10.7 ^{bcd}	5.2 ^b	10.8 ^b
p-value	0.033	0.008	0.059	0.260	0.004	0.330	0.003	<0.001	0.118	0.004	<0.001	0.060
F _(9,27)	2.6	3.5	2.3	1.4	4.0	1.2	4.1	7.6	1.8	3.9	5.1	2.2
LSD	1.6	1.0	ns	ns	2.9	ns	3.7	3.9	ns	4.5	6.3	ns

Table 7. Soil water content (cm) at cover crop termination in rotation one at Bozeman and Dutton. Treatment with different letters were significantly different within depth increments.

-----2013-----						
	Bozeman			Dutton		
Depth	0-30	30-60	60-90	0-30	30-60	60-90
SF	7.39	8.13	8.55	9.34	9.24	9.10
CC	6.03	6.82	8.02	7.99	8.05	8.37
p-value	0.005	0.024	0.252	<0.001	0.130	0.420
F _(1,35)	9.01	5.5	1.4	15.6	2.4	0.7
FULL	5.64 ^{bc}	6.11 ^b	7.68	7.21 ^c	7.12 ^{bc}	7.31 ^b
NF	5.11 ^c	6.80 ^b	8.72	8.46 ^a	9.60 ^a	10.27 ^a
FR	6.56 ^{ab}	9.08 ^a	8.73	8.56 ^a	8.69 ^{ab}	9.84 ^a
TR	5.87 ^{bc}	6.43 ^b	8.60	8.03 ^{ab}	7.94 ^{abc}	7.23 ^b
BC	6.50 ^{ab}	6.76 ^b	7.07	7.97 ^{abc}	7.37 ^{bc}	7.16 ^b
MNF	6.19 ^{abc}	6.32 ^b	7.88	8.04 ^{ab}	6.65 ^c	7.24 ^b
MFR	7.01 ^a	6.73 ^b	7.67	8.32 ^a	9.62 ^a	10.42 ^a
MTR	5.80 ^{bc}	6.88 ^b	7.89	7.87 ^{abc}	7.91 ^{abc}	7.83 ^b
MBC	5.59 ^{bc}	6.30 ^b	7.92	7.46 ^{bc}	7.58 ^{bc}	8.04 ^b
p-value	0.052	<0.001	0.109	0.033	0.022	<0.001
F _(9,27)	2.3	6.3	1.9	2.6	2.9	5.4
LSD	1.1	1.0	ns	0.8	1.8	1.8

Table 8. Soil water content (cm) at cover crop termination in rotation two at four sites 2014-2015. Treatments with different letters were significantly different within depth increments. Soil samples were collected from medium fertilizer application subplots only.

	-----2014-----						-----2015-----					
	Amsterdam			Conrad			Bozeman			Dutton		
Depth	0-30	30-60	60-90	0-30	30-60	60-90	0-30	30-60	60-90	0-30	30-60	60-90
SF	6.41	6.24	6.24	8.54	8.87	6.49	7.55	8.53	8.71	6.70	7.66	6.43
CC	4.66	4.33	5.15	5.09	5.67	5.23	4.90	6.00	7.56	5.39	6.17	7.24
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.012	<0.001	0.032	0.375
F _(1,35)	45.3	51.2	26.9	127.4	86.0	13.0	69.7	56.4	6.9	21.4	5.0	0.81
FULL	4.52 ^d	4.25 ^b	5.34 ^{ab}	5.42	5.76	5.62	5.12	6.08 ^b	7.62	4.98	5.56	6.88
NF	3.98 ^e	3.47 ^c	5.27 ^{ab}	4.57	5.04	4.43	4.69	6.99 ^a	7.64	5.67	7.55	8.43
FR	4.59 ^{cd}	4.91 ^a	5.62 ^a	5.07	6.19	5.23	4.94	5.98 ^{bc}	8.03	5.39	6.44	7.72
TR	4.89 ^{bc}	4.32 ^b	4.66 ^c	4.84	5.72	5.01	4.67	5.88 ^{bc}	7.50	5.42	6.39	7.00
BC	5.55 ^a	4.91 ^a	4.94 ^{bc}	5.45	5.86	5.47	4.94	5.71 ^{bc}	7.18	5.53	5.26	6.34
MNF	5.05 ^b	4.76 ^a	5.24 ^{ab}	5.19	5.70	5.22	5.17	5.85 ^{bc}	7.51	5.49	5.33	5.87
MFR	4.38 ^d	4.14 ^b	5.27 ^{ab}	4.79	5.29	5.44	5.11	5.36 ^c	7.10	5.34	7.19	8.58
MTR	4.63 ^{cd}	4.12 ^b	4.66 ^c	5.39	6.08	5.36	4.88	6.35 ^b	8.09	5.23	6.20	7.55
MBC	4.35 ^d	4.11 ^b	5.35 ^{ab}	5.05	5.87	5.28	4.57	5.81 ^{bc}	7.37	5.45	5.58	6.79
p-value	<0.001	<0.001	<0.001	0.270	0.244	0.327	0.881	0.002	0.801	0.876	0.097	0.406
F _(9,27)	14.9	11.7	5.2	1.3	1.4	1.2	0.5	4.3	0.6	0.5	2.0	1.1
LSD	0.3	0.4	0.4	ns	ns	ns	ns	0.64	ns	ns	ns	ns

Table 9. Correlation matrix of cover crop biomass (Mg ha⁻¹) with soil water content at termination (cm).

	-----2013-----		-----2014-----		-----2015-----	
	Bozeman	Dutton	Amsterdam	Conrad	Bozeman	Dutton
Cover crop biomass	0.09	-0.19	0.13	-0.07	0.18	0.35*

Asterisks indicate significance differences ($\alpha = 0.05$).

Table 10. Soil water (cm) and nitrate (kg NO₃-N ha⁻¹) measured pre cover crop seeding in rotation two at all sites.

		-----2014-----						-----2015-----					
		Amsterdam			Conrad			Bozeman			Dutton		
Depth (cm)		0-30	30-60	60-90	0-30	30-60	60-90	0-30	30-60	60-90	0-30	30-60	60-90*
Soil nitrate (kg NO ₃ -N ha ⁻¹)													
All CCMs	p-value	--	--	--	--	--	--	0.55	0.32	0.27	0.23	0.23	na
(Pre cover crop)	F _(6,18)	--	--	--	--	--	--	0.8	1.3	1.4	1.5	1.5	na
Soil Water (cm)													
All CCMs	p-value	0.33	0.077	0.31	0.56	0.09	0.31	0.37	0.86	0.80	0.45	0.47	na
(Pre cover crop)	F _(6,18)	--	--	--	0.8	2.2	1.3	1.2	0.4	0.5	1.0	1.0	na
	F _(2,6)	1.4	4.0	1.5	--	--	--	--	--	--	--	--	--

*Soils at Dutton in 2015 were too dry pre-cover crop seeding to sample to 90 cm.

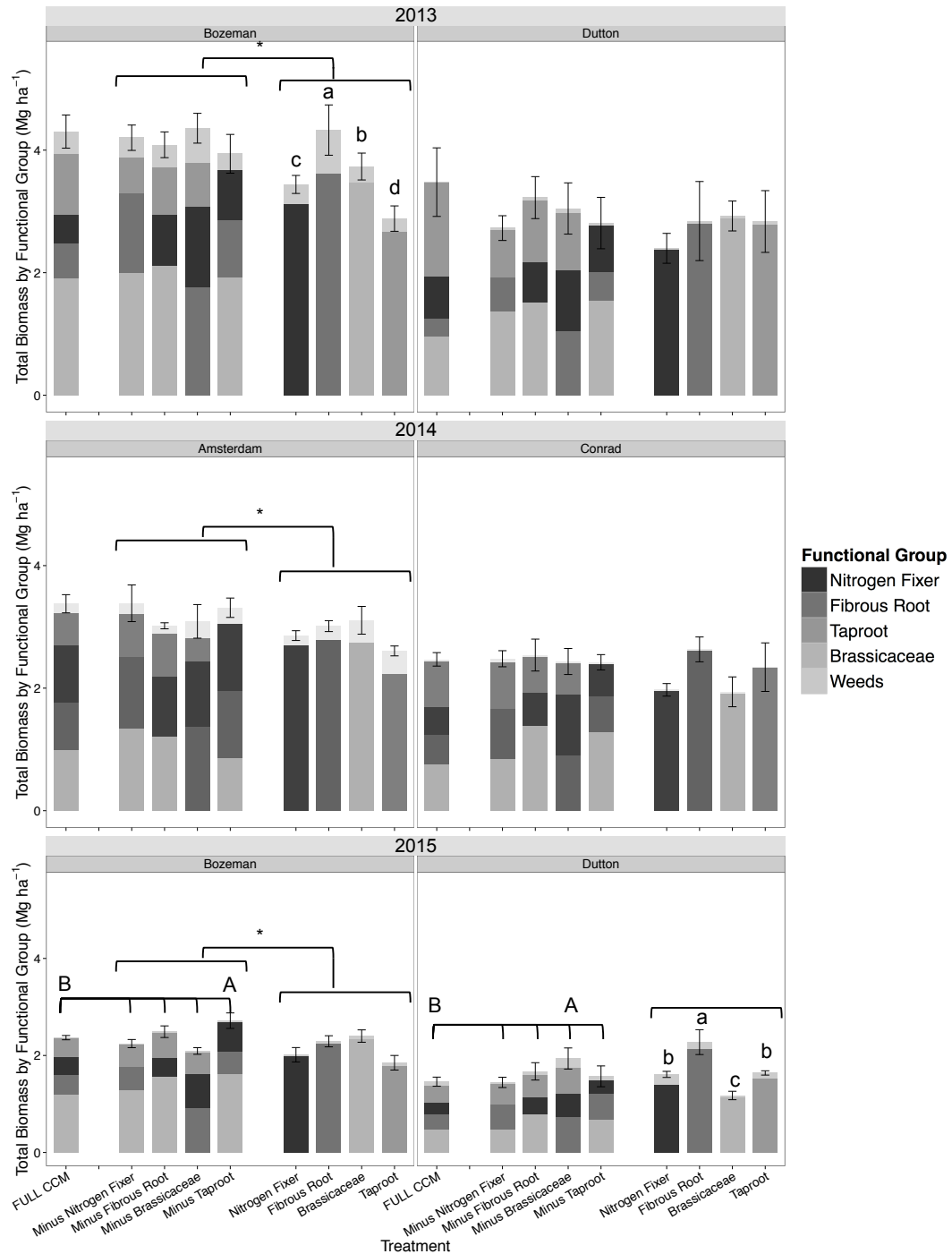


Figure 1. Total cover crop biomass by treatment in each of six site-years. Shaded bars within totals are average functional group contribution to the total biomass. Differences in total biomass within single functional group treatments are denoted with small letters. Differences between the FULL and the three functional group treatments are denoted by upper case letters. Differences between one- and three-functional groups are denoted with asterisk.

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ABSTRACT

Cover crop research in Montana within no-till farming systems has historically focused on single-species legume green manures (LGMs) to increase soil N, diversify and intensify cropping systems, and build soils. Recently, focus has shifted towards cover crop mixtures (CCMs), which may be able to further exploit ecosystem services, similar to the effects of increases in biodiversity in natural systems. Both legume-only cover crops and cover crop mixtures introduce high quality biomass inputs to enhance soil organic matter and C and N cycling. Ecosystem services associated with cover crop mixtures are based on increased productivity and variability of residue C:N, but little is known about these factors in the short, water-limited cover crop growing seasons of NGP. At four sites in Montana, we compared cover crop treatments to compare residue input quantity and quality. Single species LGM and an eight-species CCM did not differ in quantity of biomass produced in six of eight site-years. Cover crop quality (defined by the ratio of carbon-to-nitrogen, C:N) was greater (lower C:N) for LGMs than CCMs in two of six site years and not different in four of six site years. Individual functional groups within a CCM contributed a variable quality of biomass in which cereals produced relatively low quality (high C:N) biomass, brassicas and taproots produced intermediate quality biomass, and legumes produced the highest quality biomass within a mixture. The range of cover crop quality was narrower where growing season precipitation limited biomass production. Biomass N content (kg N ha^{-1}) was 33-40% greater in the single species LGM than CCM in only two of eight site-years and not different in the other six site-years. The presence of a cover crop (CCM, single species LGM, or both depending on site) increased potentially mineralizable nitrogen (PMN) compared to summer fallow. PMN was not correlated to biomass N produced the previous year but was positively correlated to C:N of the cover crop biomass in two of four site years ($r = 0.41$, $r = 0.69$). Managing cover crops for residue quality has potential to help producers maximize ecosystem services.

CHAPTER THREE

RESIDUE QUANTITY AND QUALITY FROM SINGLE SPECIES
LEGUME GREEN MANURE AND EIGHT-SPECIES COVER CROP MIXTURESIntroduction

Cropping systems of the northern Great Plains (NGP) are dominated by cereal crops, predominately spring wheat, winter wheat or barley in rotation with summer fallow, and alternatively, cover crops in rotation are used to infuse periodic diversity to improve long-term agroecosystem health (Liebig et al., 2006). Crop rotation can enhance soil functions such as supporting more diverse microbial communities, and beneficially altering belowground biogeochemical processes for nutrient delivery to the cash crop (Fornara and Tilman 2008; Halvorson and Schelgel, 2012; McDaniel et al., 2014a). Researchers and producers are especially interested in the potential for managing cover crops to improve soil C and N dynamics. Cover crop residues can provide plant available nutrients to the subsequent cash crop via mineralization, depending on the N content of residues based on functional group and termination timing (Vigil and Kissel, 1991; Sullivan and Andrews, 2012). Cover crops are also a source of organic C and N to rebuild critical soil organic matter (SOM) pools (Janzen et al., 1992; Orwin and Wardle, 2005). The potential benefits of a cover crop depend on: 1) the amount of biomass produced; 2) the quality (defined by carbon-to-nitrogen ratio; C:N; Hobbie, 1992; Chapin et al., 2002); and 3) the N content of that biomass, which depend on the species included in the cover crop and the maturity of plants at termination (Abbas, 2013; Sullivan, 1991).

SOM pools have been reduced after decades of tillage and limited OM inputs in the NGP (DeLuca and Keeney, 1993; DeLuca and Zabinski, 2011) although no till has become the majority practice in the NGP in the past two decades (Watts et al., 2009). Producers rely on the microbial recycling of stored energy and nutrients from organic matter to supply crops with plant-available nutrients in addition to chemical fertilizers (Tu et al., 2006; Seiter and Horwath, 2004; Fließbach and Mader, 2000). Management that promotes short and longer term C and N pools tends to be more productive and ecologically stable (i.e., less dependent on N fertilizer; Sanchez et al., 2004). Cover crop residue C:N dictates the rate of SOM turnover and formation, as litter C:N correlates to the rate of litter mass-loss (Agren et al., 2013). In general, management that increases mineralization consequently degrades the pool of organic matter (Seiter and Horwath, 2004), and so Hendrix et al. (1990) suggests that a diversity of C:N litter inputs is ideal, because rapidly mineralizing low C:N residues add to the pool of plant available N while high C:N residues concurrently build SOM.

Both LGM and CCM systems introduce a pulse of high quality residues in rotation with low quality wheat residues, but just how the two cover crop options differ in productivity and residue quality remains unknown. LGMs have been well studied in the region, but few CCM studies have been reported. Legume green manures, which have a low C:N due to their symbiotic N-fixing capabilities, increase biological activity, improve water filtration, increase soil N content, and increase grain protein and yield, but it may take at least four to six years before these changes are observed in the NGP (Allen et al., 2011; Biederbeck et al., 1998, 2005; Miller et al., 2015; Zentner et al., 2004).

Consistent with the idea that residues with a low C:N will increase nutrient release but offer little contribution to stable SOM, LGM did not increase soil organic carbon (SOC) after ten years compared to summer fallow, but continuous wheat cropping did, which supplies consistently lower quality residues than LGM (Engel, unpublished).

The benefits of cover crop mixtures have been elucidated in other regions and are associated with cover crop biomass quantity and quality. A meta-analysis of diverse cover crop mixtures shows that diversity can increase biomass production in agricultural systems (Malezieux et al., 2009). Similarly, higher biodiversity in natural systems increases biomass because plant diversity provides a broader exploitation of the soil matrix increasing biomass via niche complementarity (Tilman and Snell-Rood, 2014). A mixture of low and high C:N crop residues can promote nutrient cycling and soil stability (McDaniel et al., 2014b; Tiemann et al., 2015) and supply higher quantities of mineralized N in the long-term (Sanchez et al., 2001). However, there is a threshold (C:N = 30) above which N immobilization may occur (Paul and Clark, 1989) with negative effects on subsequent cash crop. Incorporating high quality hairy vetch residue with increasingly greater proportions of young cereal rye and annual ryegrass residues slowed the relative rate of N mineralization, and a minimum of 40% legume in a mixture was suggested to prevent N immobilization in soils following cover crops (Kuo and Sainju, 1998). This study was based on highly controlled, greenhouse-grown residues, and residue quality under field conditions may differ depending on water availability and preexisting site conditions.

Management practices specific to the NGP, namely short growing seasons and early termination of cover crops, may limit the quantity and quality of residue inputs and therefore the intended results. Short growing seasons limit the amount of biomass produced. Due to differences in physiology and morphology, species included in a CCM will likely vary in substrate quality, with cereals contributing the upper range of C:N and legumes at the lower range. But due to the limited growing season, cover crop plants will be young and typically have a lower C:N than they would as fully mature plants (Sullivan et al., 1991; Reiter et al., 2008; Balkcom et al., 2012), limiting the C:N range of the residues compared to their mature counterparts.

Whereas C:N gives information about subsequent ecological processes such as how microbial communities are able to process resources, residue data presented as total N content (kg N ha^{-1}) can represent the total N returned to the system and what can be made available for the next crop via mineralization (Vigil and Kissel, 1991). Plant N uptake does not represent a loss but reallocation within the system as cover crops use soil N (and atmospheric N in case of legumes), but then return it to the soil as residues. Biomass N of legumes can be negatively affected by droughty conditions via reduction of N fixation (Bremer et al., 1988; McCauley et al., 2012), and low and or untimely precipitation has been shown to reduce total biomass N in the NGP (Tanaka et al., 1997; O'Dea, 2013). The N contribution of LGMs has been well studied in the region, but the introduction of CCMs and its influence on N contributions is unknown.

Increasing soil organic carbon is a long-term goal that will take multiple rotations to detect in the soils of the NGP (McDaniel et al., 2014b), but N availability for cash

crops is an immediate demand. Potentially mineralizable nitrogen (PMN) is a measure of soil organic N that can be mineralized and made plant-available via a microbially-mediated process. At the time of wheat seeding PMN is a proxy for the amount of N available from organic matter and residues throughout the growing season of wheat (Canali and Benedetti, 2006). In the spring PMN was greater following LGM than summer fallow in the NGP after one or two rotations (Pikul et al., 1997; O'Dea, 2013), but CCMs with variable quality litters have not been investigated for their influence on PMN in the region. PMN is positively correlated to plant biomass N and negatively correlated with plant C:N (Lupwayi et al., 1999; Lupwayi, 2006).

Our objective was to compare the quantity and quality of biomass produced in LGM and CCM treatments and their effect on PMN. We expected that total biomass production and C:N would be greater in the CCM than the single species mix. We also compared C:N among functional groups within CCMs to investigate the range of biomass quality within the mixture. To better understand how nutrients were returned to the system from residues, we investigated whether total biomass N differed between CCM and LGM treatments, and how single functional groups contributed to the biomass N of the CCM. In response to cover crop treatments, we expected that differences would occur in PMN based on the quality of cover crop residues. We expected 1) that cover cropped soils would have greater PMN than summer fallowed soils; and 2) that among cover crop mixtures, those with a higher proportion of legumes would have the highest PMN because of higher total biomass N (kg N ha^{-1}) as well as the low C:N of those residues.

We also compared biomass quality and PMN following single functional group treatments after two rotations.

Materials and Methods

Site Characterization

The study was conducted at Amsterdam, Conrad, Bozeman, and Dutton, MT (Table 1), all of which had been under at least three years of no-till fallow-wheat rotations prior to the study. Long-term average (LTA) annual temperatures range from 6.2 to 7.4 °C and precipitation from 303 to 469 mm (Chapter 2, Table 2). Soil type at the two northern sites in MLRA 52 are clay loams classified as frigid, Aridic Argiustolls. At the two southern sites located in the Gallatin Valley, Bozeman is a loam to clay loam soil classified as a frigid, Typic Argiustoll and Amsterdam is a silt loam soil classified as a frigid, Aridic Calciustoll. Experimental plots at Amsterdam, Conrad, and Dutton were located on commercial wheat farms. The Bozeman site was on university-owned land with a long history of pasture/hay in a high rainfall location, and was included as a deliberate contrast to the other low rainfall, low SOM sites to investigate whether cover crop effects would differ importantly in a more well-endowed environment. Temperature and precipitation data were collected on-site with automated gauges (HOBO, Onset Computer Corp., Bourne, MA) at each site.

Experimental Design

Ten cover crop treatments and a summer fallow control were randomly assigned to main plots (8 m x 12 m) in four blocks in a split plot design. In 2012, Amsterdam and

Conrad were seeded at rates suggested by seed providers, adjusted to their proportional contribution to the mix. The following spring, spring wheat (cv *Duclair*) was seeded in rows perpendicular to cover crop rows with three levels of fertilizer [(zero (0), low (44 kg ha⁻¹), and high (88 kg ha⁻¹)] in split plots (8 m x 4 m). In 2014 and 2015 these fertilizer rates were increased to 67 kg ha⁻¹ (low) and 135 kg ha⁻¹ (high). In 2013, at Bozeman and Dutton, seeding rates were instead based on a common target of 120 plants m⁻², divided by the number of species in the mix. In the fall, winter wheat (cv *Warhorse*) was sewn. However at Dutton in 2015, winter wheat was replanted to spring wheat in mid May.

Treatments include: summer fallow (SF) for a control, legume green manure consisting of spring pea only (LGM), an eight-species/four-functional group cover crop mixture (CCM), and four treatments consisting of a single functional group. The selected functional groups are: nitrogen fixers (NF), included for their fertility inputs; species with fibrous roots (FR), for their high C inputs to soils; species with taproots (TR), for their effects on soil structure and infiltration; and brassicas (BC), due to their unique chemistry and their rapid ground cover. Due to severe downy brome (*Bromus tectorum*) infestation at Conrad in 2012, the CCM treatment, which contained oat and perennial ryegrass, was treated with glyphosate while the plots which did not include these monocot species were treated with a selective graminicide (see Tallman, 2014 for rates). Because we included four three-functional group treatments in the original experimental design, we sampled instead a treatment that was the full mixture excluding the fibrous root functional group (CCM*; Table 2). Cover crop treatments at Conrad in 2013 will be referred to as CCM but represent only three functional groups (six species) rather than four.

Cover Crop Sampling and Analysis

Aboveground cover crop biomass was sampled within four transects (1.0 x 0.3 m) at herbicide termination in early July, timed with first-bloom of spring pea. Plants were cut at the soil surface, separated by species, dried at 50 °C until they reached constant mass, and weighed. In rotation two, after fertilizer treatments were initiated in the intervening wheat year, cover crop data were collected in both high rate and zero N treatments to account for possible carryover effects of the previous year's wheat fertilization. Cover crop biomass between high and zero fertilizer treatments did not differ, with the exception of Amsterdam in 2014 where the cash crop was hailed out just days prior to planned harvest. As a result, cover crop biomass was greater in high fertilizer plots in two of the eleven treatments, BC and TR (Appendix; B.2.1). However, at all sites, data shown are the average of high and zero N treatments within each treatment.

Biomass by species was analyzed for C and N content from LGM and CCM in all site-years and from single functional group treatments in 2014 only. Complete samples were ground with a Wiley Mill (Thomas Scientific, Swedesboro, NJ) followed by grinding of a homogenized subsample with a Udy Cyclone Mill (UDY Corp., Fort Collins, CO) to 1 mm prior to analysis with a LECO Combustion Analyzer (LECO Corp., St. Joseph, MI) from a 0.1 g subsample. Biomass C:N is a ratio of carbon and nitrogen within a sample. For cover crop treatment average C:N, each species' C:N was multiplied by its proportion of total biomass of the mixture.

Soil Sampling

Soils were sampled in early April just prior to spring wheat seeding or spring growth of winter wheat (i.e. about nine months after CC termination) to assess the environment in which the cash crop would be sewn. Corers were flame-sterilized after an ethanol rinse between subplots to avoid contamination between treatments, and a composite of six cores (10-cm depth, 2-cm dia) was taken from each SF, LGM, and CCM treatments following rotation one and from treatments SF, LGM, CCM, NF, FR, TR, and BC treatments in low fertilizer treatments after rotation two. Soils were sieved to 2 mm and stored at 4 °C for less than 30d before lab analyses were performed.

Potentially Mineralizable Nitrogen (PMN)

As adapted from Keeney (1982), PMN was calculated as the difference between plant available N at time zero and after incubation. Three lab replicates were analyzed immediately for ammonium using a 1 M KCl extraction (shaken 1h, 250 rpm) and another three lab replicates were placed in moist, dark, anaerobic conditions at 25 °C for 14d. Ammonium N was determined by 1 M KCl extractions and analyzed on a Lachat flow injection analyzer (Lachat Instruments, Loveland, CO).

Statistical Analyses

All data were checked for normality and homogeneity of variance using residual and Q-Q plots. Two-way ANOVA models were used to test the effects of treatments on average C:N cover crop biomass and biomass N content. Analyses were performed in R package *stats* (version 2.15.3) with cover crop treatment and block as independent

variables. Individual sites and years were analyzed separately due to large differences in site conditions and small differences in management. Statistically significant differences in response variables were determined using Fisher's LSD ($\alpha = 0.05$) in *agricolae*.

Functional group C:N ratios within CCM mixture were analyzed using the same two-way ANOVA model with functional group and block as independent variables within each site-year. Correlation analyses within site-years were conducted using *stats* package and Pearson's coefficients (r) are reported.

Results

Biomass Quantity

Our first objective was to compare the quantity of biomass produced in LGM and CCM treatments. In 2012, aboveground biomass production differed between LGM and CCM, but relative production differed between sites. At Amsterdam, CCM produced on average 0.18 Mg ha^{-1} greater biomass than LGM (Table 3, $t = -2.4$, $p = 0.02$), and at Conrad CCM produced 0.18 Mg ha^{-1} less biomass than LGM ($t = 2.1$, $p = 0.05$). Total biomass production was low in 2012 at both sites ($<1 \text{ Mg ha}^{-1}$). In all other sites and years, there were no differences in aboveground biomass production between CCM and LGM.

Biomass Quality

Our objective was to determine whether the C:N ratio differed between cover crop treatments in early-terminated cover crops. Cover crop quality, as defined by C:N ratio, never exceeded 26 when all constituents of CCM treatments were averaged based on

weighted proportions of biomass. Average C:N ratio of cover crop biomass tended to be greater in a CCM than in the monoculture LGM, but the two treatments were different only in two of eight site-years (Table 4).

Functional groups within the CCM mix differed in C:N in six of eight site-years. Fibrous roots produced aboveground biomass with the highest C:N (18-37) and N fixers had the lowest C:N (13-20) in all site-years (Table 5). Brassicas and taproots produced biomass with an intermediate C:N (13-25), depending on the site. The greatest range in C:N among functional groups occurred at the two northern sites with a range of about ~15, whereas the average range of C:N among functional groups of all sites was ~11.

Taking a closer look at each site-year, C:N of biomass tissues varied with site and apparently with rainfall. At Bozeman, the site with the highest organic matter content, total C:N was consistently lower compared to other sites and the range of C:N was narrower among functional groups' C:N in CCM (Table 3 and 4). The C:N across functional groups were higher at the two northern sites, except when biomass production was low as in 2012 and in 2015. In contrast to consistently low C:N at Bozeman, sites that varied in rainfall and therefore biomass production – i.e., Dutton from 2013 to 2015 – produced variable quality biomass. In the drier year, C:N had a lower and narrower range but was still different among functional groups ($F=12.8$, $p<0.01$).

Data were available for single functional group treatments in 2014 (Table 6). At Conrad, NF treatment had the lowest C:N and the other three treatments were similar ($F = 8.5$, $p < 0.01$). At Amsterdam the fibrous roots treatment produced biomass with the highest C:N and the other treatments were similar ($F = 28.8$, $p < 0.01$).

Biomass Nitrogen Content

We expected that biomass N would be greater in the LGM treatment than CCM due to legumes' ability to access N from both the soil and the atmosphere and provide additional N to the system. Biomass N content was greater in the LGM treatment compared to CCM in only two of eight site-years (Figure 1) and not different in the other six site-years. At Conrad in 2014, LGM introduced 40% more N in total biomass than the CCM treatment ($F = 11.8$, $p < 0.01$). At Bozeman in 2015, LGM treatments had 33% more N in total biomass than CCM ($F = 20.9$, $p = 0.02$). The greatest functional group N contributor to the CCM, by proportion, was consistently brassicas (Figure 1). In 2014 at Amsterdam, when comparing single functional group treatments, there were no differences among single functional group treatments (Table 6; $F = 2.2$, $p = 0.16$). In 2014 at Conrad, differences occurred among the four functional groups ($F = 12.2$, $p < 0.01$), and the NF treatment produced more total biomass N than any other treatment. Although FR had the highest C:N ratio, fibrous species produced greater total biomass N than the brassica treatment when grown alone. Interestingly, the brassica treatment was the largest contributor of biomass N when grown in a mix but among the lowest when grown alone.

Potentially Mineralizable Nitrogen

We predicted that cover crops should affect PMN levels compared to SF, and in particular, cover crops with a higher proportion of legumes should have the highest PMN. In four of six site years, the presence of a cover crop (either CCM or LGM) increased PMN compared to summer fallow (Figure 2). At Conrad in 2013 and 2015, PMN did not

differ among the three treatments (2013, $F = 4.0$, $p = 0.08$; 2015, $F = 0.2$, $p = 0.87$). At Amsterdam, PMN following both cover crop treatments was higher than summer fallow in 2013 ($F = 6.5$, $p = 0.03$) and also in 2015 ($F = 6.2$, $p = 0.04$). At Bozeman following only one rotation, PMN was higher in the CCM treatment than SF, and PMN in the LGM treatment was intermediate ($F = 18.7$, $p < 0.01$). At Dutton following only one rotation, PMN following LGM was higher than that following SF and CCM ($F = 11.6$, $p = 0.02$). There were no differences when comparing PMN following individual functional group treatments at Amsterdam and Bozeman in 2015 (Table 6; Amsterdam, $F = 0.25$, $p = 0.86$; Conrad, $F = 0.1$, $p = 0.99$). PMN was not correlated with Biomass N produced the previous year at any site in any year (Table 7), but PMN was positively correlated with average treatment cover crop biomass C:N in two site years (Table 7).

Discussion

Benefits associated with the introduction of cover crops, including persistence of residues to stabilize soils, promoting soil biological activity, providing plant available nutrients, and building SOM rely on the quantity and quality of biomass introduced to the system. Summer fallow and LGMs have been well studied in the region, and so we had clear expectations about how these systems would perform relative to biomass quantity, quality, and PMN. However, cover crop mixtures are a new technique, and I provide insights into their impacts relative to LGMs and suggestions for future research.

Biomass Quantity and Quality

Cover crop biomass yields from this study were similar to or slightly above average for other studies seen in the region. Across the region, LGM cover crops have produced 1.0 Mg ha^{-1} in the MLRA 52 (O'Dea et al., 2013) and 0.4 to 1.7 Mg ha^{-1} across greater Montana (Miller et al., 2006) in relatively dry years and 2.8 Mg ha^{-1} in average precipitation years (Burgess et al., 2014). Biomass produced in LGM in this study ranged from 0.61 to 3.85 Mg ha^{-1} .

LGM and CCM produced similar quality biomass in six out of eight site-years, but average C:N varied with site and year. At sites for which plant growth was less limited by water or nutrients, C:N of both LGM and CCM was lower in comparison to when that was not the case. Soils at the Bozeman site have high organic matter and high nitrate, and so the soil provided abundant N to plants as they accumulated C from the atmosphere, resulting in consistently the lowest biomass C:N at that site. Average C:N was also lower when total biomass production was low due to low rainfall in 2012 at Conrad and in 2015 at Dutton. Regardless, in every site-year, average C:N was below a threshold of 30:1 designated as the general C:N above which residues will net immobilize N during early decomposition (Paul and Clark, 1989), but the critical value may vary slightly depending on site conditions and the chemical form of C and N in plant tissues (Reinertsen et al., 1984). Labile, low C:N residues are optimal for N mineralization to provide plant-available nutrients to the following cash crop, but build less SOM (Curtin et al., 2000).

Cover crop mixtures contain species with a range of C:N regardless of the short growing season, but did so to a greater extent when plant growth was less limited by water and/or nutrients. Cover crop mixture studies often couple legumes and cereals to achieve a certain C:N range and its associated benefits (Kuo and Sainju, 1998; Brennan et al., 2011). N fixers provide the lower bound of the range of CCMs and fibrous roots supply the upper bound. Introducing two other functional groups in the mixture altered the average treatment C:N depending on the year. In drier years, brassicas and taproots provided biomass more similar to N fixers, but in wet years they provided C:N of intermediate quality. We suggest that the effect of limited water on lower C:N is not due to stage of plant growth across years, as pea's first-bloom termination timing was applied in all years. Soil nutrient uptake is lower in water-limited soils because nitrogen, like most nutrients, are taken up by roots driven by transpiration (Dubey and Pessarakli, 2001; Hu et al., 2007), and because photosynthesis is dependent on N-containing enzymes and chlorophyll, overall biomass and C content is reduced as well (Bänziger et al., 2000; Wu et al., 2008). Water stress also reduces N fixation in legumes (Pimratch et al., 2008). If producers rely on N credits and attempt to build SOM via cover crop residues, they must consider temperature, moisture and existing soil C:N of their system (Agehara and Warncke, 2005).

A mixture of high and low C:N residues can potentially preserve surface residues for soil stability as high C:N residues decompose more slowly (Hendrix et al., 1990; Blanco-Canqui et al., 2013), while low C:N residues decompose more quickly and provide resources to a diversity of soil organisms (Neher, 1999; McDaniel et al., 2014a).

Cover crop-wheat rotations, relative to wheat-fallow rotations, inherently introduce a wider range of C:N to the system, although the diversity is across time, rather than spatial diversity (McDaniel et al., 2014b). Wheat straw residues provide a C:N near 80:1 in alternate years, and those tissues may have more of an effect on SOM accretion than cover crop residues (Drinkwater et al., 1998; Gregorich et al., 2001). However, legume systems are capable of maintaining soil C and N levels similarly to continuous wheat (Biederbeck et al., 1994, 1998; Campbell et al., 1997, 2001). Soil organic carbon accumulation had a strong relationship with biomass, regardless of species identification (Shrestha et al., 2012). Longer-term research should also investigate root biomass, as although it can be relatively small in semi-arid cover crops, root C may persist in the soil longer than that of highly labile shoots and contribute to SOM and soil organic N (Puget and Drinkwater, 2001).

Potentially Mineralizable Nitrogen

In four of six site years, PMN was higher in one of the cover crop treatments than summer fallow. O'Dea (2013) and Biederbeck et al. (1998) observed differences in PMN following continuous cropping (either continuous wheat, pea-wheat, or LGM-wheat) compared to fallow-wheat but no difference among annually cropped systems. These findings are consistent with our study in that PMN in soils following summer fallow was consistently among the lowest of the three treatments, but inconsistent in that the two systems of LGM and CCM differed from one another in one of six site-years.

Although there was no correlation between PMN and total biomass N in any site-year, there was a positive correlation between PMN and C:N. Perhaps the more easily

mineralized fraction of low C:N residues was mineralized before spring sampling but the residues with higher C:N (although still below the $C:N > 30$ immobilization threshold) were just starting to break down to inorganic N. To the contrary, O'Dea et al. (2015) found that low C:N residues of legume systems (either pea crop or LGM) resulted in sustained, steady release of mineral N compared to high C:N residues of continuous wheat.

The management of residues to optimize the synchronization of N release from residues with crop uptake demands is essential (Crews and Peoples, 2005; Gardner and Drinkwater, 2009). Our research suggests that residue quantity and average quality do not consistently differ between CCM and LGM. However, the spatial mixture of low and intermediate C:N residues within the CCM compared to the low C:N of the single species LGM could be advantageous to timing of N release. SOM accumulation cannot be accurately measured or estimated after only two rotations and may take several decades to detect (Shrestha et al., 2012). Because so many ecosystem services that producers rely on depend on SOM, modeling techniques and long-term cover crop mixture studies are needed.

Table 1. Soil characteristics sampled within 30 days before first cover crop seeding at each of the four sites.

	Amsterdam	Conrad	Bozeman	Dutton
Location	45°43'6.74"N 111°21'52.37"W	48°12'47.55"N 111°29'41.09"W	45°40'11.91"N 110°58'38.62"W	47°59'49.96"N 111°34'8.27"W
Texture	Silt loam	Clay loam	Clay loam	Clay loam
pH	8.2	6.5	7.0	6.7
Soil Organic Carbon (g kg ⁻¹)	14	14	33	19
NO ₃ -N (mg kg ⁻¹)	6.0	8.5	7.3	7.5
Olsen P (mg kg ⁻¹)	13	28	32	43

Table 2. Species included in cover crop treatments

Treatment	Plant Species
NF	Spring pea (<i>Pisum sativum</i> L. cv. Arvika) † Indianhead lentil (<i>Lens culinaris</i> Medik. Cv. Indianhead)
FR	Oat (<i>Avena sativa</i> L. cv. Oatana) †† Canary seed (<i>Phalaris canariensis</i> L.)
TR	Purple top turnip (<i>Brassica rapa</i> L.) Safflower (<i>Carthamus tinctorius</i> L. cv. MonDak)
BC	Radish (<i>Raphanus sativus</i> L. var. <i>longipinnatus</i>) Winter canola (<i>Brassica napus</i> L. var. <i>napus</i> cv. Dwarf Essex)
Legume Green Manure (LGM)	Spring pea
Full Mix (CCM)	NF + FR + TR + BC
Minus Fibrous Roots (CCM*)	NF + TR + BC

† The N fixer accompaniment to spring pea was common vetch in 2012 (*Vicia sativa* L.)

†† The fibrous root species selection in 2012 was ryegrass (*Lolium perenne* L. spp *multiflorum*) and in 2013 was millet (*Panicum miliaceum* L. sp.) which competed poorly.

Table 3. Cover crop biomass (Mg ha⁻¹) in two rotations at four sites.

Treatment	Amsterdam		Conrad		Bozeman		Dutton	
	2012	2014	2012	2014	2013	2015	2013	2015
LGM	0.76	3.15	0.61	2.39	3.85	2.42	2.66	1.64
CCM	0.94	3.38	*0.43	2.47	4.31	2.37	3.48	1.47
p-value	0.023	0.40	0.053	0.76	0.20	0.77	0.17	0.45
t _(9,27)	-2.4	-0.9	2.1	-0.3	-2.5	0.3	-1.4	0.8

*CCM is a three functional group treatment excluding fibrous roots. The t-statistics are reported from a pre-planned orthogonal contrast from a two-way ANOVA.

Table 4. Average C:N of cover crop by treatment at four sites, 2012-2015.

Treatments with different letters were significantly different within site-years. In 2014 and 2015, C:N was averaged between high and zero N rate applications within each plot.

Treatment	Amsterdam		Conrad		Bozeman		Dutton	
	2012	2014	2012	2014	2013	2015	2013	2015
CCM	14.0	23.1	*22.0	22.6	14.1	18.4	25.9	14.3
LGM	15.4	25.6	19.5	17.2	13.3	13.6	18.0	12.3
p-value	0.39	0.19	0.24	0.008	0.15	0.008	0.097	0.065
F _(1,3)	1.1		2.2		3.8	32.7	5.7	8.1
t _(6,18)		1.4		-3.0				

*CCM is a three functional group treatment excluding fibrous roots.

Table 5. Average C:N of cover crop by functional group within CCM treatment at four sites, 2012-2015. Functional groups with different letters were significantly different within site-years. In 2014 and 2015, C:N was averaged between high and zero N rate applications within each plot.

Treatment	Amsterdam		Conrad		Bozeman		Dutton	
	2012	2014	2012*	2014	2013	2015	2013	2015
N Fixers	15.2 ^b	19.5 ^c	19.7 ^b	18.8 ^c	14.2 ^{ab}	14.1 ^c	20.4 ^c	13.4 ^b
Fibrous Roots	18.6 ^a	32.6 ^a	--	34.5 ^a	18.7 ^a	23.0 ^a	36.9 ^a	18.4 ^a
Taproots	14.3 ^b	23.4 ^b	24.1 ^a	25.3 ^b	15.6 ^{ab}	17.9 ^b	26.9 ^b	15.0 ^b
Brassicas	15.7 ^b	22.6 ^{bc}	21.4 ^{ab}	26.2 ^b	11.8 ^b	18.6 ^b	24.6 ^{bc}	12.7 ^b
p-value	<0.001	<0.001	0.089	<0.001	0.069	<0.001	<0.001	0.003
F _(3,25)	12.0	26.7	2.9	24.3	2.7	13.9	12.8	6.2
LSD (0.05)	1.6	3.2	ns	3.8	ns	2.8	5.0	3.0

*CCM is a three functional group treatment excluding fibrous roots.

Table 6. Biomass quality (C:N) and total N content of single functional group treatments at Amsterdam and Conrad in 2014 and mean PMN (standard error) the following spring in 2015.

Treatment	Biomass C:N		Biomass N (kg N ha ⁻¹)		PMN (kg NH ₄ -N ha ⁻¹)	
	A	C	A	C	A	C
NF	23.9 ^b	14.9 ^b	52.9	59.2 ^a	43.7 (9.0)	17.8 (2.0)
FR	29.1 ^a	27.3 ^a	44.2	43.9 ^b	42.0 (4.7)	18.9 (4.5)
BC	21.9 ^b	27.9 ^a	56.9	29.3 ^c	36.9 (8.7)	18.5 (1.4)
TR	21.7 ^b	27.0 ^a	47.2	36.7 ^{bc}	45.3 (5.0)	19.5 (5.2)
p-value	0.006	<0.001	0.16	0.002	0.87	0.99
F-stat _{5,15}	8.5	28.8	2.2	12.2	0.3	0.04

Table 7. Correlation matrix of soil PMN with the characteristics of previous year's aboveground cover crop biomass.

	-----Rotation 1-----		-----Rotation 2-----	
	Bozeman 2014	Dutton 2014	Amsterdam 2015	Conrad 2015
Biomass N (kg N ha^{-1})	-0.17	-0.61	0.07	0.07
Biomass C:N	-0.11	0.69**	0.41**	0.11

Asterisks indicate significance level. ***<0.01; **<0.05; *<0.1

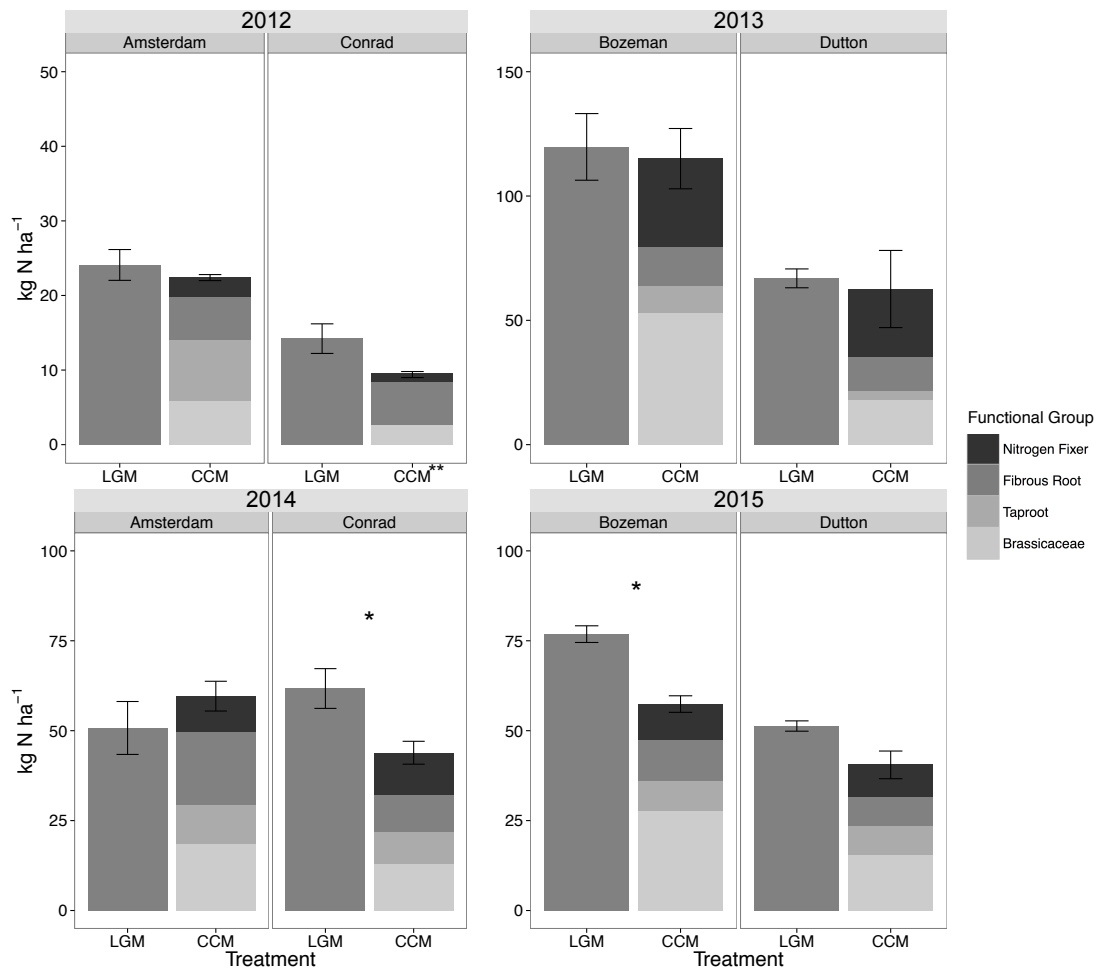


Figure 1. Biomass N content by functional group contribution. Note that scale on y-axes differ between years. Asterisks denote significant differences between treatments within site-years ($p < 0.05$). ** CCM is a three functional group treatment excluding fibrous roots.

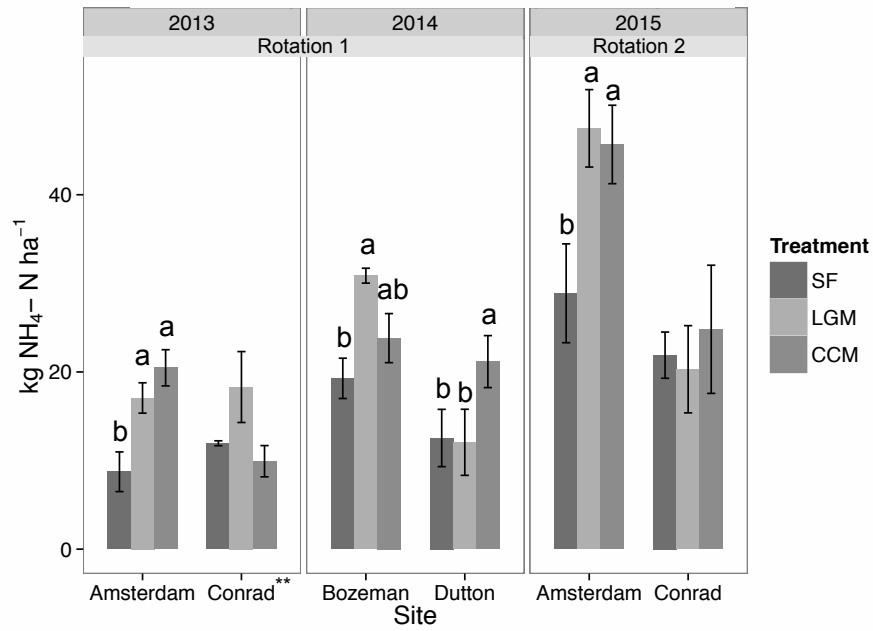


Figure 2. Mean PMN (kg NH₄-N ha⁻¹) and standard error bars following one and two rotations of SF, LGM, and CCM at four sites. **CCM was the three functional group treatment excluding fibrous roots.

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CHAPTER FOUR

MULTI-SPECIES COVER CROPS: EFFECTS ON SOIL BIOLOGY AFTER ONE AND TWO ROTATIONS IN THE SEMI-ARID NORTHERN GREAT PLAINS

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MULTI-SPECIES COVER CROPS: EFFECTS ON SOIL BIOLOGY AFTER ONE AND TWO ROTATIONS IN THE SEMI-ARID NORTHERN GREAT PLAINS

ABSTRACT

Farmers in Montana are experimenting with cover crop mixtures (CCMs) as partial summer fallow replacement, largely to promote ecosystem services such as improved nutrient cycling, resiliency to erosion, and overall improved soil quality. Summer fallow has been crucial for soil nutrient storage and moisture recharge in the water-limited region, but also increases erosion and nitrogen leaching, while reducing plant residues returned to soil. Legume green manure (LGM) and CCMs as fallow replacement introduce high quality (low C:N) biomass that decomposes quickly, which may promote biological activity. We compared soil biological activity following cover crops at four locations in Montana in three treatments—conventional summer fallow, single species LGM, and an eight-spp CCM—after both one and two rotations. An additional four treatments of two-spp single functional groups were compared at two of the four locations following rotation two. The four functional groups include: nitrogen fixers, fibrous roots, taproots, and brassicas. Following the first rotation, soil enzymatic activity of β -glucosidase, β -glucosaminidase, arylsulfatase, and acid and alkaline phosphatases showed few differences between the two cover crop treatments and summer fallow, but many enzymes showed positive correlation with total aboveground cover crop biomass produced the previous year at two site-years. After two rotations, enzyme activity was 1.3 times greater and microbial biomass was 1.4 times greater where a cover crop was present the previous year at one of two sites. Microbial biomass did not differ among treatments but was positively correlated to cover crop biomass from the previous year at one site-year ($r = 0.74$) and at another site-year after two rotations ($r = 0.38$). Mycorrhizal colonization of wheat roots after rotation one increased by 15% following either cover crop treatment compared to summer fallow at one site, but among all site-years results were inconsistent. Effects may have been cumulative, as belowground activity following two rotations showed more differences among treatments.

1. Introduction

Cover crop mixtures have been well studied in some regions of the United States, but agroecosystems of the Northern Great Plains (NGP) introduce particular benefits and also challenges, which require regional-specific consideration. Introduced in the 1900s, summer fallow leaves the soil plant-free in an inter-annual cycle with winter wheat, spring wheat, or barley, as a means to accumulate soil water and nutrients, but summer fallow also increases potential for erosion and nitrate leaching (Campbell et al., 1991) and decreases soil organic matter (Campbell et al., 2000) and soil biological activity (Acosta-Martinez et al., 2007). Alternatively, cover crops introduce diversity and contribute additional above- and below-ground biomass, which may stimulate biological activity (Biederbeck et al., 2005), stabilize soils and therefore reduce erosion (Blanco-Canqui et al., 2013), and increase nutrient cycling and soil organic matter (Reddy et al., 2003; Teasdale et al., 2007). Cover crop mixtures moreover introduce biodiversity and its associated ecosystem services such as increased productivity (Malezieux et al., 2009; Smith et al., 2014), resilience, and increased carbon and soil organic matter storage (Snapp et al., 2004; Tonitto et al., 2006; Schipanski et al., 2014). NGP agriculture is water-limited, and so the proper management of cover crops is essential.

Despite research that suggests initial wheat yields in the NGP following cover crops are not improved compared to summer fallow (either do not change or are reduced by up to 6%; Miller et al., 2006; O'Dea et al., 2013; Burgess et al., 2014), producer interest is increasing in part due to an increasing focus on soil health combined with USDA-NRCS incentive programs. Results of a Montana-wide survey reports that 90% of

respondents currently using cover crops will continue to use them, and 50% of all producers in the region acknowledge ‘soil health’ as the reason they do or would consider using cover crops (Jones et al., 2015). Given the potential negative impacts on cash crop productivity in the NGP and potential positive impacts on soil quality, we ask three questions: 1) can the presence of a cover crop change soil biological parameters compared to summer fallow; 2) if so, do those changes vary with species richness of the cover crop; and 3) does the functional group composition of the cover crop affect soil biological parameters?

In the cold, semi-arid climate of the NGP, a short (two- to three- month) cover crop growing season would occur at most only every other year in which a cash crop is not produced, the season being so short due to water limitation. Legume green manures (LGM), most commonly spring peas or lentil, are generally grown during the peak precipitation season from May to June. Compared to summer fallow, LGMs increased biological activity, improved water filtration, and increased labile soil organic matter and soil N storage after four to six years (Biederbeck et al., 1998, 2005; Zentner et al., 2004; Allen et al., 2011; Miller et al., 2006; McCauley et al., 2012; O’Dea et al., 2013; Miller et al., 2015). While interest in cover crop mixtures (CCMs) is growing in the NGP (Jones et al., 2015), successes are anecdotal and few studies have been published in the region.

We measured soil biological parameters to address producers’ interest in using cover crops to increase soil quality, but also because soil biological parameters should respond rapidly and are the driving force of chemical and physical changes in the soil following a change in land management (Nannipieri et al., 2001; Liebig et al., 2006). Soil

microbial activity is limited by labile C and energy (Schimel and Weintraub, 2003), and so carbon inputs from cover crop residues should increase microbial activity. Compared to LGM, more diverse CCMs could further influence belowground activity as a result of variation in rooting depth and phenology, in litter quantity and quality (C:N; Hooper et al., 2000), species-specific root exudates (Somers et al., 2004), and indirect effects due to a change in associated microbial communities (Brussard, 1998; Eisenhauer et al., 2010). Multi-species cover crops show greater productivity than single species alone (Malezieux et al., 2009; Smith et al., 2014) but not in the central Great Plains (Nielsen et al., 2015). Due to variation in morphology and stoichiometry, the residues of different cover crops influence soil biogeochemical cycles, and those diverse litter inputs provide energy sources for a wider base of organisms (Tiemann et al., 2015). For example, fungal biomass increases relative to bacterial biomass where higher C:N litters decompose slowly to create structural and compositional diversity (Wardle 2002) or where rotational diversity is introduced to agricultural systems (Bunemann et al., 2004; Gonzalez-Chavez et al., 2010; Suzuki et al., 2012).

Enzymes are the catalysts for soil metabolic processes, and so management methods that increase enzyme activity may also increase agricultural fertility and productivity (Shi, 2011; Bowles et al., 2014). Because exoenzymes, produced by plant roots, fungi, and bacteria, can long outlive their original source (Kivlin and Treseder, 2013), they may have long-term effects on the soil environment. Cover crops can result in an increase in microbial and enzymatic activity from above- and below-ground organic inputs compared to summer fallow, as enzymes are synthesized from amino acids

requiring C and N (Allison and Vitousek, 2005) provided by residues. Investigators reported an almost two to three times increase in activity of dehydrogenase, phosphatase, and arylsulfatase in a rotation of LGM cover crop in a six-year study in Saskatchewan (Biederbeck, 2005), and a 1.2 or 1.3 times increase in arylsulfatase and β -glucosidase in soils under a cover crop of winter rye after only three years in semiarid Texas (Acosta-Martinez et al., 2011). Cover crop diversity may further increase enzyme activity indirectly if species richness is accompanied by increased plant biomass, which introduces more residues but also creates higher demand for soil nutrients including phosphorus (Tilman et al., 1996; Oelmann et al., 2011). Increased demand for P results in increased exudation of phosphatase enzymes (Tarafdar and Jungk, 1987; Olander and Vitousek, 2000). Similar patterns may appear in enzymes involved with N and S cycling.

Arbuscular mycorrhizal fungi (AMF), which form a symbiotic relationship with plants in which they expand the surface area exploited in soils through a hyphal network, and thereby increase net plant uptake of nutrients, especially phosphorus (Smith et al., 2001), have often been ignored in agricultural systems (Barker et al., 2003; Drinkwater and Snapp, 2007). Root colonization by AMF is altered with chemical fertilization (Dekkers and Van der Werff, 2001; Ryan and Ash, 1999; Corkidi et al., 2002), because AMF become less essential for nutrient uptake. AMF also provide ecosystem services; they contribute to soil structure (Brundrett, 2002; Rillig, 2004) by promoting aggregation as they introduce organic biomass and excrete glomalin (Smith and Read, 2008) and can influence plant-water relations in winter wheat and other species (Allen and Boosalis, 1983; Auge, 2001). In place of a fallow period, cover crops provide carbohydrates for

mycorrhizal fungal survival between cash crops (Kabir and Koide, 2002). Colonization of AMF increased in an organic corn crop following both LGM and a seven-species mix, with species composition of CCMs more important than species diversity alone (Njeru et al., 2014). Brassicas, which are included in many CCMs, are characteristically non-mycorrhizal, and produce isothiocyanates that have a negative effect on soil fungal communities, so that they may actually reduce AMF potential (Pellerin et al., 2007).

Soil parameters, especially biological and chemical parameters, are highly variable in space and time (Parkin, 1993; Heuvelink and Webster, 2001). Although the soil may be directly influenced by the presence of a cover crop during active growth, it remains unknown whether changes in soil biota and/or function persist long enough to influence the growth of the subsequent year's cash crop. Physical parameters, such as soil stabilization, are expected to persist, but were only observed for the nine months following cover crops in the semi-arid plains of Kansas (Blanco-Canqui et al., 2013). In our study, cover crops are grown May-July and any substantial wheat growth begins at snowmelt in April when we analyze soil parameters, providing a nine to ten month window for biological parameters to possibly subside.

We investigated the effects of cover crops on belowground soil biological activity following one and two rotations of LGM, a four-functional group CCM, and a control treatment of conventional no-till summer fallow. The four functional groups include nitrogen fixers, included for their fertility inputs; species with fibrous roots, for their potential to add C to soils; species with taproots, for their effects on soil structure and infiltration; and brassicas, due to their unique rhizosphere chemistry and their

contribution to ground cover. After two rotations of cover crop, we also investigated single functional group treatments to address how each functional group contributes to responses seen in the full mixture. We investigated microbial biomass and expected that it would increase with cover crop biomass, which would increase availability of belowground resources. We expected that 1) the presence of a cover crop compared to summer fallow would increase soil enzyme activity, with differences being greater after two rotations than only a single rotation; and 2) among cover crop treatments, a more functionally diverse mixture would support greater enzyme activity due to greater cover crop biomass production and increased demand for soil nutrients. We hypothesized that AMF colonization of the following cash crop would increase after a cover crop compared to summer fallow, and the extent of colonization would depend on the functional groups included in the mixture.

2. Materials and Methods

2.1 Site characterization

The study was conducted across Montana in Amsterdam, Conrad, Bozeman, and Dutton (Table 1), all of which had been under at least three yr of no-till management in fallow-wheat rotations prior to study. Long-term average (LTA) annual temperatures range from 6.2 to 7.4 °C and annual precipitation from 300 and 469 mm (Chapter 2, Table 2). Soil type at the two northern sites in MLRA 52 are clay loams classified as frigid, Aridic Argiustolls and at the two southern sites located in the Gallatin Valley are silt loam (Amsterdam: frigid, Aridic Calcicustoll) and clay loam (Bozeman: frigid, Typic

Argiustoll). The Amsterdam, Conrad, and Dutton sites are located on commercial wheat farms, and the Bozeman site is on university-owned land, which had a long history in pasture prior to recent farming and cover crop implementation and has high organic matter.

2.2 Experimental Design

In 2012, ten cover crop treatments and a summer fallow control were randomly assigned to main plots (8m x 12m) in four blocks in a split plot design at Amsterdam and Conrad. In 2012, seeding rates were as suggested by seed providers, adjusted to their proportional contribution to the mix. The following April (see Appendix for dates), spring wheat (cv *Duclair*) was seeded in rows perpendicular to cover crop rows with three levels of fertilizer [(zero (0), low (67 kg ha⁻¹), and high (135 kg ha⁻¹)] in split plots (8m x 4m). In 2013, the same experimental design was implemented at Bozeman and Dutton, but seeding rates were changed to a common target of 120 plants m⁻² divided by the number of species in the mix. The following September winter wheat (cv *Warhorse*) was sewn.

Treatments include: summer fallow (SF) for a control, legume green manure consisting of spring pea only (LGM), an eight-species/four-functional group cover crop mixture (CCM), and four treatments consisting of a single functional group (NF, FR, TR, and BC). Due to downy brome (*Bromus tectorum*) pressure at Conrad in 2012, the full four-functional group treatment (CCM), which contained fibrous roots, was treated with herbicides to contain the spread of the weed. Because we included four three-functional group treatments in the original experimental design, we were able to sample a CCM

treatment that is the full mixture excluding the fibrous root functional group at Conrad in 2012 and the biological responses in 2013 (Table 2).

2.2. Plant sampling

Aboveground cover crop biomass was sampled and separated by species on the day of herbicide termination in mid to late July. In rotation two, data were collected in both high and zero N rate treatments to test for layover effects of the previous year's wheat fertilization scheme. Plants were dried at 50 °C until they reached constant mass, and weighed directly out of the oven. Samples were ground and analyzed for C and N on a LECO Combustion Analyzer (LECO Corp., St. Joseph, MI). Weed biomass from the summer fallow treatment is not available for Amsterdam and Conrad in 2012.

2.3. Soil sampling

Soils were sampled in early spring just prior to spring wheat seeding or spring growth of winter wheat (i.e. about nine months after CC termination) to assess biological activity and the environment in which the cash crop was sewn. Corers were flame-sterilized after an ethanol rinse between subplots to avoid contamination between treatments, and a composite of six cores (10 cm depth, 2 cm dia) was taken from each of SF, LGM, and CCM treatments following rotation one and from treatments SF, LGM, CCM, NF, FR, TR, and BC in medium fertilizer treatments only after rotation two. Soils were sieved to 2 mm and stored at 4 °C for less than 30d before lab analyses were performed.

2.4. Microbial biomass and extracellular enzyme activity

Microbial biomass activity was assessed by substrate-induced respiration (Fierer, 2003). A yeast solution was added to five g of field moist soil (adjusted to dry weight equivalent for subsequent calculations) and shaken for four h at room temperature. Headspace CO₂ concentrations were analyzed pre-incubation, and two and four h after yeast addition with gas chromatography (Varian CP 3800 gas chromatograph) described by O'Dea (2011). Soil extracellular enzyme activity was measured in one g field moist soil in duplicate and one control as outlined by Dick (1996; 2011) and Parham and Deng (2000). Samples were incubated with pNP-labeled enzyme-specific substrate for 1 h at 37 °C, and activity was quantified spectrophotometrically. Enzymes analyzed include: β -1,4,-glucosidase (EC 3.2.1.21; cleaves cellobiose from cellulose), β -1,4,-N-acetyl glucosaminidase (EC 3.2.1.30; cleaves N-acetyl glucosamine from chitin), arylsulfatase (EC 3.1.6.1; hydrolyzes organic sulfate esters from a phenolic group), and acid and alkaline phosphatases (EC 3.1.3.1/2; cleaves phosphates from organic phosphorus compound). Geometric means were calculated as the fifth root of the product of all enzymatic activity and used as an index of soil quality (Garcia-Ruiz et al., 2008).

2.5. Mycorrhizal colonization

At wheat anthesis, single plants were harvested for mycorrhizal colonization from the same treatments as soil sampled above. Roots were cleared in KOH and stained with trypan blue (McGonigle et al., 1990). Mycorrhizal structures in root segments were counted by a gridline intersection method on a compound microscope.

2.6. Statistical analyses

All data were checked for normality and homogeneity of variance using residual and Q-Q plots. Two-way ANOVA was used to test for treatment effects on soil microbial biomass, individual soil enzymes and their geometric mean, and AMF colonization. Each site-year was analyzed separately due to small changes in management and large differences in site characteristics. Analyses were performed in R package *stats* (version 2.15.3) with cover crop treatment and block as independent variables. Significant differences in response variables were determined using post-hoc tests and Fisher's LSD ($\alpha=0.05$). Correlations between cover crop biomass and biological response variables were analyzed using *stats* package and Pearson's coefficients (r) are reported. Correlations are not presented for 2013 biological responses because weed biomass from summer fallow treatments was not available from Amsterdam and Conrad in 2012.

3. Results

3.1 Microbial biomass and extracellular enzyme activity

We expected that the presence and quantity of cover crop biomass would increase microbial biomass, with greater differences after two rotations. Following rotation one, microbial biomass increased only at two sites where cover crop biomass exceeded 1 Mg ha⁻¹. In 2012 at Amsterdam and Conrad, there was low biomass production (Tallman, 2014) and no differences in microbial biomass in soils following summer fallow, single species LGM, or CCM treatments. Cover crop biomass was > 2 Mg ha⁻¹ in 2013, and microbial biomass differed in soils measured nine months after cover crop treatments at

one of two sites. At Dutton in 2014, microbial biomass following the CCM mixture was 1.5 and 1.7 times greater than the single species LGM or summer fallow, respectively (Table 3; $F = 6.8$, $p = 0.03$).

Following two cover crop rotations, microbial biomass differed among the three treatments at Amsterdam in 2015. At Amsterdam, the presence of a cover crop, either CCM or LGM, resulted in an increase in microbial biomass by 1.4 or 1.3 times as compared to soils with the summer fallow treatment (Table 3; $F = 6.5$, $p = 0.03$). There was a positive correlation between the previous summer's aboveground cover crop biomass and the following spring's soil microbial biomass at Amsterdam in 2015 ($r = 0.53$, $p < 0.01$). Among single functional group cover crop treatments, there were no differences at either Amsterdam or Conrad (Table 6). At Conrad in 2015, there were no differences in soil microbial biomass among summer fallow, LGM, or CCM (Table 3; $F = 0.4$, $p = 0.36$), nor differences among single functional group treatments (Table 6; $F = 0.7$, $p = 0.61$).

We expected that the same factors that affected microbial biomass (i.e. presence of a cover crop and abundance of cover crop biomass) would also affect enzyme activity. Following the first cover crop rotation, individual enzyme activities did not differ among SF, LGM, or CCM regardless of year or site, except for acid and alkaline phosphatases at Dutton and arylsulfatase at Bozeman (Figure 1). Similarly to microbial biomass, enzymatic activity did not differ among cover crop treatments in 2012, likely due to low cover crop biomass production. In 2014 at both Bozeman and Dutton, β -glucosidase and β -glucosaminidase activities did not differ following cover crops compared to summer

fallow ($p > 0.10$). At Dutton, acid phosphatase activity was 1.2 to 1.3 times greater in CCM than either LGM or SF, and alkaline phosphatase activity was 1.8 times greater in the LGM treatment than SF but the CCM treatment was not different than either (acid phosphatase $F = 3.7$, $p = 0.09$; alkaline phosphatase $F = 3.8$, $p = 0.09$). Arylsulfatase activity was 1.2 times greater following both CCM and LGM than when following SF at Bozeman ($F = 5.6$, $p = 0.04$) with a similar trend at Dutton ($F = 3.0$, $p = 0.12$).

Among enzymes measured in rotation two, differences only occurred among SF, LGM, and CCM in one individual enzyme at each site. β -glucosaminidase activity at Conrad was 1.4 and 1.5 times greater following CCM than LGM and SF, respectively ($F = 5.9$, $p = 0.04$), and acid phosphatase activity at Amsterdam was 1.3 times greater following a cover crop than summer fallow ($F = 15.8$, $p < 0.01$). There were no differences in any individual enzyme assays involved in C, N, S or P cycling between single functional group CCMs (Table 4).

The geometric means following rotation one were 30% greater after the CCM and single species LGM treatments compared to summer fallow at Dutton in 2014 (Figure 1; $F = 5.8$, $p = 0.04$) but not different among the three treatments at Bozeman in 2014 ($F = 1.7$, $p = 0.26$) or Amsterdam or Conrad in 2013 (Amsterdam $F = 0.02$, $p = 0.98$; Conrad, $F = 2.6$, $p = 0.16$). Following two rotations, the geometric mean of five enzymes showed that cover crops have an influence on soil enzyme activity (Figure 1). At Amsterdam in 2015, the presence of either LGM or CCM resulted in a 30% increase in the geometric mean of enzyme activity compared to summer fallow ($F = 8.6$, $p = 0.02$), but among single functional group cover crop mixtures there were no differences (Table 4; $F = 0.08$,

$p = 0.97$). At Conrad in 2015 the geometric mean was 1.4 and 1.5 times greater following CCM than LGM or SF ($F = 21.7$, $p < 0.01$) but again no differences were seen in the geometric mean following single functional group CCMs (Table 4; $F = 1.0$, $p = 0.43$). The geometric mean was significantly correlated to previous year cover crop biomass in two of four site-years where data are available (Table 5; Figure 2; Dutton, 2014: $r = 0.74$, $p < 0.01$; Amsterdam, 2015: $r = 0.38$, $p < 0.01$).

At Dutton, four of five soil enzymes had a positive correlation with cover crop biomass (Table 5; $p < 0.10$), while at Bozeman, only arylsulfatase was positively correlated with cover crop plus weed biomass ($r = 0.69$, $p < 0.05$). Correlation analysis did not elucidate an association between the previous year's cover crop biomass and extracellular enzyme activity following two rotations at Conrad (Table 5; $p > 0.1$) but did at Amsterdam in three of five individual enzymes (Table 5; $p < 0.05$).

3.2 Mycorrhizal Colonization

We expected that AMF colonization would be greater following a cover crop than summer fallow but that the extent of colonization would depend on the functional groups included in the mixture. AMF colonization differed among the three treatments following the first rotation at two of four sites, but the trend was apparent at all sites (Figure 4). Mycorrhizal colonization of wheat at Conrad, which is a site with adequate to excessive Olsen P (28 ppm), increased from 11 to 20-22% following CCM or LGM when compared with summer fallow ($p = 0.15$). At Amsterdam where Olsen P is much lower (13 ppm), and overall AMF colonization was greater, CCM tended to induce greater AMF colonization than LGM or SF treatments ($p = 0.15$). Bozeman had the highest AMF

colonization of all sites and following one rotation, AMF colonization after summer fallow was 16-17% lower than CCM or LGM ($F = 6.5$, $p = 0.03$). At Dutton, CCM resulted in colonization 1.5 times greater than or equal to SF but greater than LGM ($F = 6.2$; $p = 0.04$). Following two rotations, there were differences between the three treatments at Conrad, where AMF in wheat growing in soils with the LGM treatment was only 1.1 times greater than in CCM or SF treatments ($F = 15.0$, $p = 0.01$). AMF colonization levels did not differ between treatments at Amsterdam ($F = 2.4$, $p = 0.19$). When comparing AMF colonization in wheat following individual functional group treatments at Amsterdam and Conrad in 2015, there were no differences (Table 6).

4. Discussion

The replication of this study across sites that vary in soil type and climate enable us to consider the extent to which our results can be generalized, and in fact biological responses are highly variable between sites. Soil type and landscape characteristics affect the belowground response (Garcia-Ruiz et al., 2008). The two sites range from silt to clay loams, which may influence enzyme activity, especially in semi-arid systems. Dry soils limit the movement and/or diffusion of enzymes and therefore increase interaction with soil particles, which force microorganisms to increase extracellular enzyme production and also protect enzymes from being degraded and increase their half-life (Burns, 1982). Soil particle surfaces are greater and more positively charged in clay loam soils of the northern sites, and may therefore amplify this effect. Organic matter can behave similarly as it provides protection on its positively charged surfaces (Stemmer et al., 1998), but also improves water holding capacity and provides a nutrient supply. Further research is

needed, as the four sites representing two soil types are confounded by other environmental factors.

We expected that absolute differences would occur among sites, as enzyme activity is often influenced by regional and micro-environmental conditions (Sinsabaugh et al., 1991). For example, arylsulfatase, along with total microbial activity, decreases with an aridity gradient (Li and Pariente, 2003). This helps to explain why at Bozeman — the site with the highest growing season precipitation — enzymatic activity, microbial biomass, and AMF was consistently greater than the other three sites. Similarly, variable belowground responses among the other three sites may be attributable to differences in cover crop biomass produced the year prior, which corresponded to growing season precipitation and GDD (Chapter 2). Given a lower overall response at the other three sites, we expected a similar enhancement in biological activity with the addition of cover crops. Instead the belowground response to cover crops differed among sites.

Our research indicates that within site-years, biomass quantity associated with the previous year's cover crop biomass was the best indicator of belowground activity. Because there were few differences in cover crop biomass production between CCM and LGM across site-years (Chapter 3), there were no distinct differences in belowground activity between treatments and instead we saw a linear trend with biomass and biological activity. The immediate belowground response to the presence of cover crops may be due to a priming effect rather than the direct breakdown of fresh cover crop residues, although enzymes are functionally redundant in both decomposition pathways (Fontaine et al., 2003). The increase in the rate of SOM decomposition after fresh organic

matter input is the result of an increase in microbial activity due to metabolic energy obtained from decomposition of fresh organic matter (Kuzyakov et al., 2000; Fontaine et al., 2003). The size of the priming effect increases with the amount of added organic material (Dumontet et al., 1985; Mary et al., 1993; Asmar et al., 1994), consistent with our results showing increased microbial activity correlated with cover crop biomass. This may have overall negative impacts on agroecosystems if cover crops further reduce stable soil organic carbon that is only partly offset by direct C inputs of fresh organic material, rarely producing a positive priming effect (Bingeman et al., 1953; Kuzyakov et al., 2000).

Single functional group treatments allowed us to investigate how plant identity rather than richness alone affects belowground responses. Cover crops based on functional group diversity differ in many factors that we do not address here including their associated exudates (Somers et al., 2004; Ehrenfeld et al., 2005), microbial communities (Burns et al., 2015), root morphology (Fang et al., 2011), and changes to microenvironment abiotic factors such as temperature and moisture (Eviner et al., 2006). Temperature and moisture differed among single functional group cover crop treatments, as fibrous roots treatments had warmer temperatures and lower moisture content in surface soils due to low canopy shading (Neff et al., unpublished; Chapter 1). However, there were no differences in microbial biomass, enzymatic activity, or AMF colonization among the four single functional group treatments at Amsterdam or Conrad in 2015. The similarity of biomass production among treatments (Chapter 2) explains the corresponding similarity in belowground activity measured nine to ten months after cover crop growth. Similarly, Hacker et al., (2015) reported that the relationship between

increased plant species richness and increased phosphatase activity was highly coupled to substrate availability (C) and soil microbial biomass, but there was no direct relationship with richness or identity of functional groups and phosphatase activity.

We investigated enzyme activity as a response to management, as research suggests that quantifying hydrolytic soil enzyme activity estimates degradability of organic matter and availability of associated nutrients for the cash crop throughout the season (Visser and Parkinson, 1992; Gil-Sotres et al., 2005). We measured potential activity in the lab with unlimited substrates, indicative of greater presence and potential activity of enzymes rather than increased *in situ* activity (Geisseler, 2010). While plants may be directly responsible for some enzyme production, it is likely an insignificant contribution (Sticklen, 2008) and more likely of microbial origin (Caldwell, 2005) in response to substrates. Unlike the response at Amsterdam and Dutton, there were few, if any, correlations of microbial biomass or enzyme activity with previous year cover crop production at Bozeman and Conrad. Bozeman has high rainfall and also high organic matter, which provides a background level of nutrients and enzyme-related activity, so that the cover crops do not add as much compared to a limited system with low organic matter. Conrad, on the contrary, is often limited by water, and annual fluctuations in precipitation potentially drive changes more significantly than treatments applied.

Aside from a correlation to cover crop biomass, there were no consistent patterns with responses of enzymes involved with C, N, S, and P cycling between cover crop treatments among site-years. Trasar-Cepeda et al., (2008) suggest that varied behavior of enzymes could be due to their association with different stages of degradation and that

their utility as soil quality indicators may have been over estimated due to the complexity of their response. A single enzyme's activity cannot represent soil quality (Nannipieri et al., 1990; Garcia-Ruiz et al., 2008), and in calculating the geometric mean as a combined index of soil quality, we found it to be the most responsive to change in management.

Following rotation two, geometric means differed between summer fallow and the presence of a cover crop, but enzymatic activity at Amsterdam was greater in both CC treatments than SF, and at Conrad was greater in CCM than either LGM or SF.

AMF colonization was independent of cover crop identity, including surprisingly single functional group brassicas, which does not directly support AMF symbioses (Pellerin et al., 2007). One of the cover crop treatments, either CCM or LGM, performed better than summer fallow in three of six site-years, and so perhaps it was just the shortened fallow period (Lekberg and Koide, 2005). It also suggests that low weed biomass in summer fallow systems can sustain AMF propagules, or that the movement of agronomic machinery transports propagules between neighboring plots even with low disturbance agricultural practices. The greater colonization rates across treatments at Amsterdam and Conrad in 2015 compared to the same sites in 2013 could be due to timing of wheat root harvest, as AMF colonization continues to increase during active growth but when conditions become less favorable for root growth, AMF switch to vesicle and or spore production (Garcia and Mendoza, 2008; Liu et al., 2009) and root colonization decreases (Kabir et al., 1997). Further research should also be done to investigate whether wheat-AMF symbioses can improve yields in the presence and/or absence of P fertilizers. In some soil and environmental conditions (i.e., arid Australia)

AMF colonization can become parasitic rather than mutualistic and actually decrease subsequent wheat yields (Ryan et al., 2005; Ryan and Kirkegaard, 2012). Direct effects of AMF on soil quality benefits such as soil aggregation and resilience to drought should be addressed but may take longer than two rotations to observe.

One recurring response is that with low biomass production, there will be likely be less immediate soil biological response, which stresses the importance of choosing cover crop mixtures with high biomass production (Chapter 2). However, cover crop biomass is influenced mostly by growing-season precipitation and growing-degree days rather than species selection (Chapter 2). Long-term research in the NGP suggests that after the adoption of LGM cover crops, significant changes to soil parameters are not evident until after three or four rotations, and so we can expect that it will take as long or even longer to elucidate how the two cover crop systems differ from one another. As cover crop options are explored and adapted to semi-arid regions, interest is gravitating towards forage opportunities as a supplemental source of income to implement crop rotations with cash crops. Our research suggests that cover crop biomass is essential for subsequent effects on belowground ecology. Forage practices would remove all or most residues from the surface, and so future research should consider the relative contribution of aboveground and belowground cover crop biomass.

Table 1. Site characteristics sampled within 30 days of first cover crop seeding at each of the four sites.

	Amsterdam	Conrad	Bozeman	Dutton
Location	45°43'6.74"N 111°21'52.37"W	48°12'47.55"N 111°29'41.09"W	45°40'11.91"N 110°58'38.62"W	47°59'49.96"N 111°34'8.27"W
Texture	Silt loam	Clay loam	Silt loam	Clay loam
pH	8.2	6.5	7.0	6.7
Soil Organic Carbon (g kg ⁻¹)	14	14	33	19
Olsen P (mg kg ⁻¹)	13	28	32	43

Table 2. Species included in experimental treatments

Treatment	Plant Species
Summer fallow (SF)	--
Pea only (LGM)	Spring pea (<i>Pisum sativum</i> L. cv. Arvika)
Full Mix (CCM)	NF + FR + TR + BC
Nitrogen Fixer (NF)	Spring pea † Indianhead lentil (<i>Lens culinaris</i> Medik. Cv. Indianhead)
Fibrous Root (FR)	Oat (<i>Avena sativa</i> L. cv. Oatana) †† Canary seed (<i>Phalaris canariensis</i> L.)
Taproot (TR)	Purple top turnip (<i>Brassica rapa</i> L.) Safflower (<i>Carthamus tinctorius</i> L. cv. MonDak)
Brassica (BC)	Radish (<i>Raphanus sativus</i> L. var. <i>longipinnatus</i>) Winter canola (<i>Brassica napus</i> L. var. <i>napus</i> cv. Dwarf Essex)
Minus Fibrous Roots (CCM*)	NF + TR + BC

† The nitrogen fixer accompaniment to spring pea was common vetch in 2012 (*Vicia sativa* L.)

†† The fibrous root species selection in 2012 was ryegrass (*Lolium perenne* L. spp *multiflorum*) and in 2013 was millet (*Panicum miliaceum* L. sp.) which competed poorly.

Table 3. Microbial biomass ($\mu\text{g CO}_2 \text{ g soil}^{-1} \text{ hr}^{-1}$) means and standard error from six site-years.

	-----Rotation 1-----				-----Rotation 2-----	
	-----2013-----		-----2014-----		-----2015-----	
Treatment	Amsterdam	Conrad	Bozeman	Dutton	Amsterdam	Conrad
CCM	445 (20)	*257 (38)	574 (18)	361 ^a (46)	260 ^a (26)	294 (29)
LGM	403 (30)	354 (69)	591 (38)	237 ^b (20)	263 ^a (8)	212 (23)
SF	369 (38)	341 (35)	550 (50)	341 ^b (35)	193 ^b (20)	261 (46)
p-value	0.24	0.17	0.76	0.029	0.032	0.34
F-stat _{2,6}	1.9	2.5	0.3	6.8	6.5	1.3
LSD (0.05)	ns	ns	ns	102.7	54.0	ns

* In CCM treatment at Conrad 2013, enzymes were measured in the three functional group treatment that excludes fibrous roots rather than the four functional group treatment (CCM).

Table 4. Mean enzymatic activity (standard error) of five enzymes and geometric mean following rotation two at Amsterdam and Conrad in 2015 for single functional group treatments.

Treatment	β -glucosaminidase		β -glucosidase		Alkaline Phosphatase		Acid Phosphatase		Arylsulfatase		Geometric Mean	
	A	C	A	C	A	C	A	C	A	C	A	C
Nitrogen fixers	20.8 (2.2)	38.9 (9.6)	156.4 (15.5)	129.8 (17.5)	68.6 (1.9)	78.3 (12.5)	87.2 (8.0)	350.8 (26.9)	75.5 (5.61)	41.8 (11.8)	67.8 (4.2)	87.9 (14.2)
Fibrous roots	19.1 (2.0)	40.5 (3.1)	152.2 (2.2)	113.3 (9.9)	73.1 (2.8)	79.5 (8.6)	82.9 (7.2)	377.4 (18.7)	80.1 (8.2)	28.7 (6.4)	67.3 (4.3)	82.1 (6.8)
Brassicas	23.6 (3.1)	48.3 (5.2)	167.6 (8.8)	147.1 (18.7)	73.0 (2.2)	157.9 (53.7)	83.5 (1.9)	354.8 (72.6)	70.7 (7.2)	71.9 (27.0)	69.8 (3.7)	114.5 (15.2)
Taproots	21.1 (2.5)	42.9 (4.3)	156.1 (6.4)	138.5 (16.7)	68.7 (6.2)	112.8 (20.2)	86.0 (4.9)	317.3 (24.2)	71.0 (7.5)	45.2 (14.1)	67.0 (4.0)	96.3 (12.2)
p-value	0.62	0.75	0.73	0.59	0.78	0.31	0.97	0.81	0.82	0.47	0.97	0.43
F-stat _{5,15}	0.6	0.4	0.5	0.7	0.4	1.4	0.1	0.3	0.3	0.9	0.1	1.0
LSD (0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 5. Correlation matrices (r) of soil biological response with the previous year's aboveground cover crop biomass production (Mg ha^{-1}).

Soil biological response	-----Rotation 1-----		-----Rotation 2-----	
	Bozeman 2014	Dutton 2014	Amsterdam 2015	Conrad 2015
Microbial biomass	0.10	0.44	0.53***	0.07
β -glucosidase	0.44	0.68**	0.27	0.22
β -glucosaminidase	0.21	0.13	0.39**	0.26
Arylsulfatase	0.69**	0.55*	0.23	0.04
Acid Phosphatase	0.20	0.68**	0.57***	0.05
Alkaline Phosphatase	0.48	0.53*	0.31	0.07
Geometric Mean	0.48	0.74***	0.38**	0.09

*** <0.01; **<0.05; *<0.1

Table 6. Mean AMF colonization and MB (standard error) following two rotations of single functional group treatments at Amsterdam and Conrad in 2015.

Treatment	AMF (% roots colonized)		Microbial Biomass ($\mu\text{g CO}_2 \text{ g soil}^{-1} \text{ hr}^{-1}$)	
	A	C	A	C
Nitrogen fixers	72 (3)	47 (2)	221 (20)	266 (34)
Fibrous roots	75 (3)	49 (4)	220 (12)	233 (26)
Brassicas	75 (2)	44 (3)	267 (10)	297 (34)
Taproots	79 (4)	40 (5)	222 (11)	255 (47)
p-value	0.18	0.27	0.12	0.61
F-stat _{5,15}	2.0	1.6	2.6	0.7

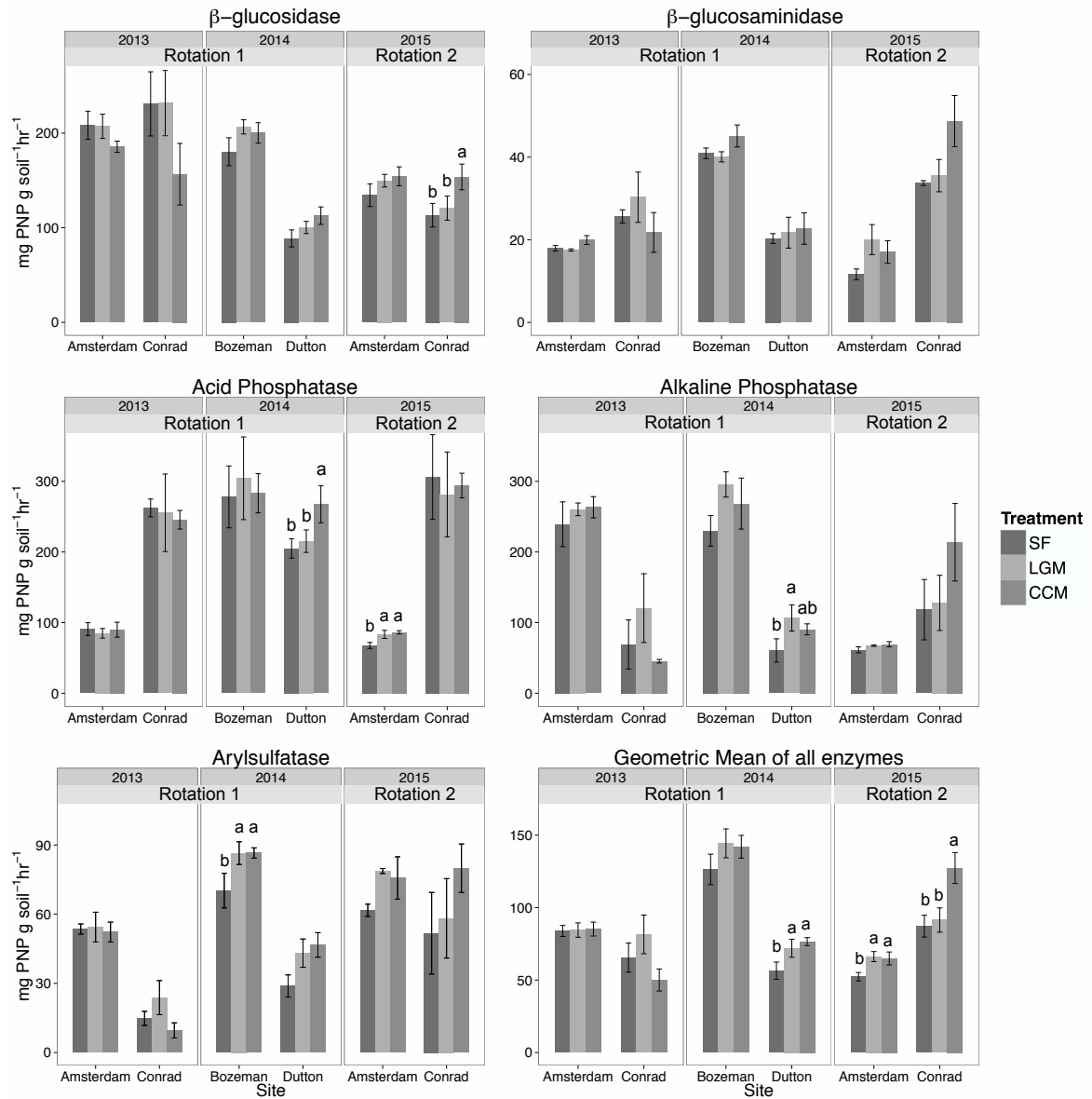


Figure 1. Mean enzymatic activity ($\text{mg PNP g soil}^{-1} \text{hr}^{-1}$) and standard error bars for β -glucosidase, β -glucosaminidase, acid and alkaline phosphatases, arylsulfatase, and the geometric mean of five enzymes following one and two rotations of SF, LGM, and CCM. Different letters indicate differences among treatments within site years ($\alpha = 0.05$) In CCM treatment at Conrad 2013, enzymes were measured in the three functional group treatment that excludes fibrous roots.

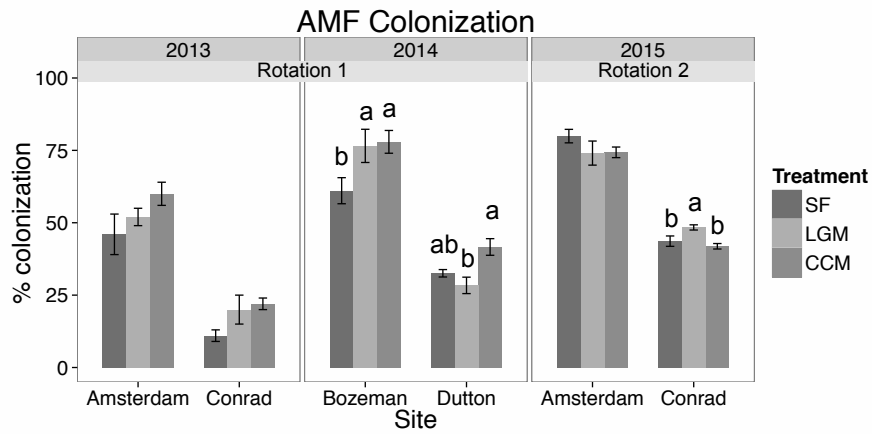


Figure 4. Arbuscular mycorrhizal colonization (%) with standard error bars for summer fallow, eight-spp CCM, and one-spp LGM following one rotation at four sites and two rotations at two sites.

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CHAPTER FIVE

CONCLUSION

My research explored an option for increasing the sustainability of agriculture in Montana and especially focused on methods to accumulate soil organic matter (SOM) and enhance soil biological processes. Introducing partial season cover crops could reduce the negative impacts associated with current conventional summer fallow practices and build SOM to make food production possible into the future.

The objectives of my thesis were: 1) to determine what combination of functional groups within cover crop mixtures could maximize biomass production and minimize water use; 2) to compare the quality of biomass production between an eight-species (four functional group) cover crop mixture with single species legume green manure; and 3) to investigate the effects of a multi-species cover crop on plant available N and soil biological factors, relative to summer fallow and a single-species cover crop. The project's objectives also included analysis of wheat yield and protein following cover crop implementation, and although results are preliminary, the findings give insights into the drivers of cash crop success.

We determined that growing season precipitation was critical for producing substantial cover crop biomass. When biomass production was less water-limited, a three functional group mixture produced higher biomass than a single functional group mix 50% of the time, and a four functional group mixture never produced more biomass than a three functional group mixture. When considering water and nitrate use, higher biomass

production did not use more water, given termination of CCMs in early July, but a more detailed assessment of differences between functional groups relative to their water use at different depths due to rooting depths, and the reduction in evaporation due to shading is warranted.

Average litter quality did not consistently differ between a single species legume cover crop and a mixture of high and low quality residues in a cover crop mixture, but the range of litter quality within the cover crop mixture depended on site and growing conditions, especially rainfall. Soil biological responses were highly variable between treatments and in some site years correlated to previous year cover crop biomass production, possibly due to a priming effect in soils. The priming effect is the increase in the rate of SOM decomposition after fresh organic matter input, and is the result of an increase in microbial activity due to metabolic energy obtained from decomposition of fresh organic matter (Kuzyakov et al., 2000; Fontaine et al., 2003). The priming effect could ultimately lead to the degradation of SOM at a faster rate than CCMs can contribute to this pool (Bingeman et al., 1953; Kuzyakov et al., 2000).

Wheat Yields Following Early Cover Crop Adoption

As farmers in Montana begin to adopt cover crop cocktails to improve soil health as lead by incentives from the National Resources Conservation Service, questions remain about how the following cash crop will be affected. With focus on soil and crop sustainability, the widespread adoption of these management practices depends on the farmer's ability to remain agronomically and economically productive. We have preliminary data on spring and winter wheat yields following cover crop cocktail

implementation at four locations across Montana (Miller et al., 2016), and a full data set will be completed after the harvest of 2016. Data from wheat yields in 2013 are not reported here (Tallman, 2014). Crop yields following summer fallow produced the highest dry grain yields in two of four site years (at the two northern sites). When comparing cover crop treatments with only nitrogen fixers (LGM and NF) to treatments excluding any nitrogen fixer (FR, BC, TR, and MNF), protein content was higher following the nitrogen fixers in all four site years, but there were no differences in total grain yield between these two groups. Wheat protein is important in deciding the price of grain, and so having a legume in a cover crop boosts grain quality.

Considerations for Future Research

Knowing what we know now, there may be better approaches to building cover crop mixtures for the region such as optimizing species composition within functional groups. Within functional groups, there was consistently one species that outcompeted the other with the exception of the taproots in which biomass production was fairly evenly distributed between the two species. Perhaps in Montana we do not have the luxury of a high number of species that are well adapted to the cold, dry climate and short growing season. Future studies on cover crop mixtures could pare down the number of species and still include all four functional groups. Because there were no consistent differences in soil enzymatic activity or microbial biomass between treatments but rather responses to the quantity of biomass, it could be that a simple mix of nitrogen fixers and fibrous roots could accomplish the goals of cover crop management to improve soil biological health. Based on our study, we expect that this mixture would not use more

water compared to other mixtures and would also create the greatest range of quality of cover crop residues. Legume and cereal mixtures are common in cover crop mixtures across the nation, and should be evaluated for implementation in Montana.

Biodiversity in natural systems has associated benefits that were not explicitly measured in this study, which could falsely lead us to believe that a less diverse cover crop could be sufficient. Additional parameters associated with plant biodiversity could include more belowground growing season characteristics of functional groups such as root exudates and rooting structure, depth, and density. Additionally, microbial community composition may respond to plant diversity. Whereas we only studied total microbial biomass, the fungal to bacterial ratio will respond to resource quality (Wardle, 2002) and microbial species composition may be associated with plant species (Hooper et al., 2000; Kowalchuk et al., 2002; De Deyn et al., 2011). These parameters may be more sensitive to management changes and give us better insight into the ability to influence belowground activity, but our understanding of how these parameters affect long-term goals such as soil organic matter accumulation and crop yields may be limited

Post growing season, decomposition of residues is at the core of the ability of cover crops to provide long-term resources to the soil, and we have addressed the importance of understanding microbially mediated mechanisms that regulate decomposition and nutrient cycling (Bradford et al., 2013). Decomposition in agroecosystems, however, incurs additional drivers not considered in natural systems. Herbicide application influences the chemical breakdown of residues. Photodegradation, through which ultraviolet light induces a direct loss of C to the atmosphere and a change

in the chemical composition of remaining litter to a lower molecular mass (Austin and Vivanco, 2006), may be a dominant control over decomposition in arid and semi-arid environments (Parton et al., 2007) like Montana. Mechanical breakdown from the movement of farm equipment over residues is likely increasing the rate of decomposition, as compared to fracturing of organic materials from fauna, known to be a driver of decomposition in natural systems (Bardgett and Wardle, 2010). These factors should be considered in the future when addressing the decomposition and contribution of residues to agricultural soils, and a potential method to better follow residues through decomposition pathways could be through isotope labeled residues.

Challenges in Soil Health Research

The term ‘soil health’ is defined “as the continued capacity of soil to function as a vital *living* ecosystem that sustains plants, animals, and humans” by USDA- NRCS. Current attention on soil health, addressed by governmental agencies and the research community alike, is illustrated by posters and talks at the joint 2015 meeting of the Agronomy Society of America/Soil Science Society of America/Crop Society of America, which produced 365 talks/posters using the term ‘soil health’. Despite the excitement and desire to share knowledge, there is little agreement on how to quantify and monitor biological, chemical, or physical changes to soil health in agroecosystems. Firstly, soil’s response to change in management is highly variable, making it difficult to determine which parameters should be monitored. For example, in this study average soil enzyme activity had such high standard error in some site-years that differences were difficult to detect. Secondly, the response of crops to changes in soil parameters is mostly

unknown, making it difficult to determine what is a positive or negative change. For example, microbial biomass was positively correlated with the quantity of cover crop biomass produced the year prior, but this pattern could be a result of the priming effect that actually induces the degradation of native soil organic matter. Soils exist in great spatial and temporal heterogeneity, making it a challenge to determine how a single factor influences the system within the context of many covarying factors (Colman and Schimel, 2014). An increase in sample size to account for high variability will increase likelihood of finding significant differences among treatments.

Potential benefits to agroecosystems associated with soil quality are mostly associated with the accumulation of soil organic matter, which takes decades to centuries to accumulate. This experimental agricultural study that lasted only four years (or two rotations) may not be sufficient to document changes in soil health, but a modeling study suggested a biomass input threshold of $2.5 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, above which SOM would increase and below which it would decrease (Shrestha et al., 2012). Some assays are being developed such as permanganate oxidizable organic carbon that can detect early changes to soil organic carbon pools (Culman et al., 2011). But even so, Kravchenko and Robertson (2011) warn of inferring too much on soil carbon change from too little data and insufficient statistical tools.

The completion of two rotations at all four sites will conclude in 2016 and provide further information for the cumulative effects of cover crops on soil biological parameters and also wheat yields. Thus far, we investigated two rotations of cover crop growth at four sites across Montana and learned about management techniques specific to the

region. We have adjusted species composition due to competition and adjusted critical seeding and termination timing. Although results are highly variable, we have made suggestions on which functional groups are essential for increasing cover crop biomass, reducing soil water use, regulating soil nitrate levels, and producing variable quality biomass (defined by C:N). Site-specific conditions should be considered in all cases. We have evaluated chemical and biological responses to cover crop treatments at all four sites following one rotation and at two sites following two rotations. Due to the high variability of belowground parameters, we do not have definitive suggestions for the composition of CCMs to increase belowground activity, but suggest that optimizing the quantity of biomass produced in a CCM is the best way to increase activity and build SOM. This study contributes to management of early adoption of CCMs in the region and directs future studies in CCM functional group selection and parameters to measure changes in soil health.

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APPENDICES

APPENDIX A

AGRONOMIC FIELD MANAGEMENT INFORMATION

Table A.1 Agronomic field management for cover crop mixture study at Amsterdam, Conrad, Bozeman, and Dutton, 2014-2015.

	Amsterdam		Conrad		Bozeman		Dutton	
	2014	2015	2014	2015	2014	2015	2014	2015
Soil Sample	5 May	30 Mar	8 May	31 Mar	--	29 Apr	--	22 Apr
Pre cover crop herbicide	25 ^{&} Apr, 2 ^{&&} May	--	8 May ^{\$}	--	--	--	--	--
Cover crop seeding date	8 May	--	9 May	--	--	30 Apr	--	27 Apr
Cover crop stand counts	29 May	--	4,5 June	--	--	29 May	--	4 June
Cover crop Insecticide	29 May [%]	--	--	--	--	4 June [%]	--	--
Cover crop biomass harvest	9 July	--	7,8 July	--	--	30 June	--	1 July
Cover crop termination	9 July*	--	7,8 July*	--	--	30 June*	--	1 July*
Post cover crop herbicide	14 [†] , 17 ^{††} July	--	--	--	--	10 Aug [†]	--	--
Soil Sample	16 July	--	8 July	--	--	1 July	--	8,9 July
Wheat cultivar	--	Vida sw	--	Duclair sw	--	Yellow-stone ww	Duclair sw	Warhorse ww
Winter wheat termination	--	--	--	--	--	--	12 May	--
Wheat seeding	--	3 Apr	--	19 Apr	--	9 Sept	13 May	22 Sept
Urea application	--	3 Apr [#]	--	19 Apr	--	9 Sept [#]	13 May [#]	22 Sept [#]
Wheat herbicide	--	--	--	17 Apr [†]	--	--	--	--
Wheat harvest	--	11 Aug	--	20 Aug	12 Aug	--	15 Sept	--

[†]1.68 kg ha⁻¹ glyphosate + AMS

^{††}0.98 kg ha⁻¹ glyphosate + AMS + Helfire

*2.24 kg ha⁻¹ glyphosate + 1.5lb/10g AMS + 12.8 oz/10 g MSP + 2 oz/a Sharpen & 0.84 kg ha⁻¹ Roundup RT3

&&0.98 kg ha⁻¹ Roundup RT3

[%]0.14 kg ha⁻¹ Warrior II

^{\$}1.68 kg ha⁻¹ Roundup Powermax[®]

[#]Urea (0, 67, 135 kg N ha⁻¹)

Table A.2.1 Precipitation and temperature data from four sites in the cover crop year (2012 and 2014 at Amsterdam and Conrad, 2013 and 2015 at Bozeman and Dutton).

	Amsterdam			Conrad			Bozeman			Dutton		
	LTA	2012	2014	LTA	2012	2014	LTA	2013	2015	LTA	2013	2015
Precipitation (mm)												
September - March	137	83	151	98	69	131	212	134	131	98	92	112
April	39	68	24*	26	60	42	57	24	21*	26	25	3*
May	61	43	39*	50	48	16	80	85	60*	50	71	53*
June	62	34	103*	64	83	81	79	102	19*	64	80	6*
July	30	27	10	35	24	18	38	10	38	35	26	33
August	27	13	74	32	10	41	35	30	19	32	42	14
TOTAL	358	278	395	303	319	335	501	385	320	303	356	267
Growing Season Total	162	147	166*	140	154	139*	216	196	145	140	166	98
Average Temperature (° C)												
April	7.3	7.5	5.8	5.9	8.1	6.7	6.3	4.6	7.9*	5.9	4.3	7.4
May	12.0	9.1	11.0	11.1	10.8	11.4	10.9	11.2	9.8*	11.1	10.9	9.9
June	16.4	15.6	13.9	15.2	15.5	14.3	15.2	15.7	18.6*	15.2	15.3	18.3
July	20.8	21.1	20.2	18.8	19.6	20.1	19.5	20.5	19.2*	18.8	20.0	19.5
August	20.1	18.9	17.9	18.1	18.0	18.9	18.9	19.8	18.7	18.1	20.0	19.3
Annual Average Temperature	7.4	7.3	5.8	6.2	7.0	6.0	7.0	6.9	7.8	6.2	6.8	7.7
GDD† (0° C)	--	832	860	--	716	881	--	899	878	--	931	1089

†GDD calculated from day after cover crop seeding to day of herbicide termination.

Table A.2.2 Precipitation and temperature data from four sites in 2014 and 2015.

	Amsterdam			Conrad			Bozeman			Dutton		
	LTA	2014	2015	LTA	2014	2015	LTA	2014	2015	LTA	2014	2015
Precipitation (mm)												
September - March	137	151	104	98	131	112	212	211	131	98	131	112
April	39	24*	46	26	42	4	57	36	21*	26	42	3*
May	61	39*	85	50	16	68	80	47	60*	50	16	53*
June	62	103*	15	64	81	4	79	71*	19*	64	81	6*
July	30	10	45	35	18	33	38	7*	38	35	18	33
August	27	74	19	32	41	14	35	70*	19	32	41	14
TOTAL	358	395	329	303	335	267	501	396	320	303	335	267
CC growing Season Total	162	166*	--	140	139*	--	216	--	145	140	--	98
Average Temperature (° C)												
April	7.3	5.8	5.5	5.9	6.7	7.4	6.3	6.1	7.9*	5.9	6.7	7.4
May	12.0	11.0	10.3	11.1	11.4	9.9	10.9	11.1	9.8*	11.1	11.4	9.9
June	16.4	13.9	18.1	15.2	14.3	18.3	15.2	13.3*	18.6*	15.2	14.3	18.3
July	20.8	20.2	19.2	18.8	20.1	19.5	19.5	20.9*	19.2*	18.8	20.1	19.5
August	20.1	17.9	18.5	18.1	18.9	19.3	18.9	17.7*	18.7	18.1	18.9	19.3
Annual Average Temperature	7.4	5.8	7.2	6.2	6.0	7.7	7.0	6.0	7.8	6.2	6.0	7.7
GDD (0° C) †	--	860	--	--	881	--	--	--	878	--	--	1089

†GDD calculated from day after cover crop seeding to day of herbicide termination.

APPENDIX B

SUPPLEMENTAL COVER CROP INFORMATION

Table B.1.1 Cover crop stand count (m⁻²) at Amsterdam in 2014. Target rate was 120 plants m⁻² for all treatments. Percentages are variation from target.

Treatment	Total	Pea	Lentil	Oat	Canary seed	Turnip	Safflower	Radish	Canola
LGM	108 (-10)	108	--	--	--	--	--	--	--
FULL	109 (-9)	15	16	17	15	8	11	19	8
NF	135 (+13)	63	72	--	--	--	--	--	--
FR	133 (+11)	--	--	74	58	--	--	--	--
TR	111 (-7)	--	--	--	--	43	50	--	--
BC	108 (-10)	--	--	--	--	--	--	64	44
MNF	114 (-5)	--	--	25	22	9	19	24	15
MFR	108 (-10)	16	24	--	--	10	18	25	16
MTR	113 (-6)	19	16	27	24	--	--	16	11
MBC	122 (+2)	21	24	38	26	--	14	--	--

Table B.1.2 Cover crop stand count (m⁻²) at Conrad in 2014. Target rate was 120 plants m⁻² for all treatments. Percentages are variation from target.

Treatment	Total	Pea	Lentil	Oat	Canary seed	Turnip	Safflower	Radish	Canola
LGM	70 (-42)	70	--	--	--	--	--	--	--
FULL	72 (-40)	10	11	11	8	10	9	8	5
NF	84 (-30)	34	50	--	--	--	--	--	--
FR	99 (-18)	--	--	55	44	--	--	--	--
TR	77 (-36)	--	--	--	--	35	31	--	--
BC	80 (-33)	--	--	--	--	--	--	45	35
MNF	78 (-35)	--	--	19	10	13	9	13	14
MFR	78 (-35)	13	15	--	--	10	13	13	14
MTR	77 (-36)	12	14	13	11	--	--	14	12
MBC	63 (-47)	17	13	11	10	--	11	--	--

Table B.1.3 Cover crop stand count (m⁻²) at Bozeman in 2015. Target rate was 120 plants m⁻² for all treatments. Percentages are variation from target.

Treatment	Total	Pea	Lentil	Oat	Canary seed	Turnip	Safflower	Radish	Canola
LGM	146 (+21)	146	--	--	--	--	--	--	--
FULL	119 (-1)	14	15	16	17	13	13	16	17
NF	136 (+13)	66	70	--	--	--	--	--	--
FR	136 (+13)	--	--	70	66	--	--	--	--
TR	81 (-32)	--	--	--	--	41	40	--	--
BC	92 (-23)	--	--	--	--	--	--	52	40
MNF	117 (-2)	--	--	21	20	17	17	23	19
MFR	107 (-11)	14	21	--	--	19	14	23	17
MTR	138 (+15)	24	22	21	25	--	--	25	21
MBC	131 (+9)	26	24	29	27	--	25	--	--

Table B.1.4 Cover crop stand count (m⁻²) at Dutton in 2015. Target rate was 120 plants m⁻² for all treatments. Percentages are variation from target.

Treatment	Total	Pea	Lentil	Oat	Canary seed	Turnip	Safflower	Radish	Canola
LGM	180 (+50)	180	--	--	--	--	--	--	--
FULL	141 (+17)	21	16	19	24	12	15	21	14
NF	135 (+13)	78	56	--	--	--	--	--	--
FR	166 (+38)	--	--	88	78	--	--	--	--
TR	68 (-44)	--	--	--	--	27	41	--	--
BC	96 (-20)	--	--	--	--	--	--	34	63
MNF	123 (+3)	--	--	24	21	16	20	24	18
MFR	111 (-7)	24	21	--	--	14	17	24	11
MTR	141 (+18)	26	19	25	27	--	--	25	21
MBC	134 (+12)	35	23	28	28	--	20	--	--

2014 and 2015 Cover Crop Biomass by Species and Including Weeds

Our questions in Chapter 2 regarded the contribution of functional group biomass to cover crop mixtures, but when constructing cover crop mixtures, species selection is critical. For those considering species within mixtures, the data is included here. Details on the collection of this data are in Chapter 2.

In all of the single functional group treatments, there is a species that outcompetes the other, although the tap-rooted species represented by turnip and safflower are the most evenly distributed between species and dominance of one or the other changes between sites and years. The dominant contributors in the FULL mix, by species, are radish followed by pea and oat. The lowest contributor is consistently the fibrous root complement to oat, which were ryegrass, millet, or canary seed depending on the year. Lentil is also persistently low competitor when grown with pea. Not too much attention should be paid to the disproportionate growth of the two species in a mix, as the combination of the two species still contributes the intended benefit of including that functional group. For example, Pea/vetch mix while they fix N at variable rates provide the cumulative function of adding N to the system as can be evaluated in the lower nitrate use of that treatment.

Weed biomass varied among site-years. It is highest in summer fallow in 2015 at Dutton as a result of poor timing of herbicide management. Overall average weed biomass was also high at Bozeman in 2013 and Amsterdam in 2014 where overall target species biomass was high. Among cover crop treatments weed biomass was similar except at Amsterdam in 2014 where BC and TR had the highest weed biomass followed by MBC, MTR, and FR ($F = 2.2$, $p = 0.05$).

Species selection of functional groups within a cover crop mixture is critical. Our data supports that within each functional group, one of the selected species outcompetes. Although number of plants is similar at emergence, total biomass production is consistently higher in one of the species. Within the nitrogen fixer functional group, spring pea outperforms lentil. Within the fibrous root group, oat outperforms each of the cereals it has been paired with throughout the 4-year study. Within the Brassica group,

tillage radish outcompetes winter canola. Within the brassicas group, species biomass production is more similar between the two species. I suggest that a future study could reduce the number of species from 8 to perhaps if total biomass production is the ultimate goal.

Weed biomass in conventional summer fallow years is controlled with management via herbicides, but CCMs that include forbs and cereals disable the use of these chemicals. And so, if some cover crop mixtures based on their functional groups were to allow for the proliferation of a noxious weed like downy brome it would be unpalatable to producers and in reverse functional groups that do exclude weeds may be desirable. Among site-years, weed biomass varied mostly due to management diligence and timing of herbicide application. However within a site-year, herbicide management was consistent and in the three years of study, weed biomass did not notably differ among CCM treatments. Brassicas did not significantly reduce weeds as it was selected to do.

Table B.2.1 Cover crop biomass total and by species (Mg ha⁻¹) from all 10 CC treatments at Amsterdam in 2014.

Treatment	Total	Pea	Lentil	Oat	Canary seed	Turnip	Safflower	Radish	Canola	Weeds
LGM	3.15	2.97	--	--	--	--	--	--	--	0.17 ^b
FULL	3.38	0.77	0.17	0.62	0.16	0.18	0.35	0.87	0.12	0.15 ^b
NF	2.86	2.34	0.36	--	--	--	--	--	--	0.16 ^b
FR	3.02	--	--	2.36	0.43	--	--	--	--	0.23 ^{ab}
TR	2.61	--	--	--	--	0.61	1.63	--	--	0.37 ^a
BC	3.11	--	--	--	--	--	--	2.42	0.33	0.36 ^a
MNF	3.39	--	--	1.01	0.17	0.19	0.50	1.14	0.21	0.17 ^b
MFR	3.02	0.75	0.24	--	--	0.20	0.49	1.05	0.17	0.13 ^b
MTR	3.31	0.92	0.18	0.89	0.20	--	--	0.69	0.17	0.26 ^{ab}
MBC	3.09	0.87	0.20	1.20	0.17	--	0.37	--	--	0.28 ^{ab}
p-value	0.18									0.051
F	1.6									2.2
LSD	ns									0.17

Table B.2.2 Cover crop biomass total and by species (Mg ha⁻¹) from all 10 CC treatments at Conrad in 2014.

Treatment	Total	Pea	Lentil	Oat	Canary seed	Turnip	Safflower	Radish	Canola	Weeds
LGM	2.39 ^{abc}	2.38	--	--	--	--	--	--	--	0.02
FULL	2.47 ^a	0.37	0.08	0.41	0.07	0.54	0.21	0.62	0.14	0.03
NF	1.98 ^{bc}	1.68	0.28	--	--	--	--	--	--	0.02
FR	2.63 ^a	--	--	2.24	0.38	--	--	--	--	0.02
TR	2.35 ^{abc}	--	--	--	--	1.72	0.61	--	--	0.01
BC	1.94 ^c	--	--	--	--	--	--	1.40	0.53	0.02
MNF	2.48 ^a	--	--	0.72	0.10	0.59	0.17	0.63	0.22	0.06
MFR	2.54 ^a	0.46	0.08	--	--	0.34	0.26	1.04	0.34	0.02
MTR	2.42 ^{abc}	0.45	0.09	0.49	0.08	--	--	1.04	0.25	0.03
MBC	2.43 ^{ab}	0.88	0.12	0.72	0.18	--	0.50	--	--	0.03
p-value	0.080									0.42
F	2.0									1.1
LSD	0.47									ns

Table B.2.3 Cover crop biomass total and by species (Mg ha⁻¹) from all 10 CC treatments at Bozeman in 2015.

Treatment	Total	Pea	Lentil	Oat	Canary seed	Turnip	Safflower	Radish	Canola	Weeds
LGM	2.42 ^{ab}	2.38	--	--	--	--	--	--	--	0.04
FULL	2.37 ^{ab}	0.32	0.04	0.33	0.09	0.22	0.17	0.87	0.32	0.01
NF	2.02 ^{bc}	1.77	0.23	--	--	--	--	--	--	0.02
FR	2.30 ^{abc}	--	--	1.79	0.46	--	--	--	--	0.04
TR	1.89 ^c	--	--	--	--	1.00	0.78	--	--	0.07
BC	2.40 ^{ab}	--	--	--	--	--	--	1.73	0.60	0.06
MNF	2.25 ^{bc}	--	--	0.41	0.07	0.26	0.22	0.99	0.30	0.01
MFR	2.42 ^{ab}	0.34	0.06	--	--	0.31	0.20	10.07	0.48	0.02
MTR	2.72 ^a	0.55	0.05	0.39	0.09	--	--	1.33	0.28	0.04
MBC	2.10 ^{bc}	0.63	0.08	0.73	0.18	--	0.45	--	--	0.02
p-value	0.001									0.39
F	3.7									1.1
LSD	0.32									ns

Table B.2.4 Cover crop biomass total and by species (Mg ha⁻¹) from all 10 CC treatments at Dutton in 2015.

Treatment	Total	Pea	Lentil	Oat	Canary seed	Turnip	Safflower	Radish	Canola	Weeds
LGM	1.64 ^{bc}	1.51	--	--	--	--	--	--	--	0.12
FULL	1.47 ^{cd}	0.23	0.01	0.24	0.07	0.08	0.26	0.26	0.12	0.09
NF	1.62 ^{bcd}	1.30	0.11	--	--	--	--	--	--	0.20
FR	2.28 ^a	--	--	1.67	0.48	--	--	--	--	0.14
TR	1.64 ^{bc}	--	--	--	--	0.25	1.28	--	--	0.12
BC	1.17 ^d	--	--	--	--	--	--	0.88	0.27	0.02
MNF	1.44 ^{cd}	--	--	0.44	0.06	0.08	0.36	0.37	0.11	0.03
MFR	1.68 ^{bc}	0.32	0.02	--	--	0.18	0.30	0.58	0.22	0.06
MTR	1.52 ^{bcd}	0.28	0.003	0.44	0.09	--	--	0.52	0.17	0.08
MBC	1.94 ^{ab}	0.44	0.03	0.53	0.21	--	0.53	--	--	0.19
p-value	0.004									0.49
F	3.7									1.0
LSD	0.45									ns

Table B.3. Soil water and nitrate content following single species legume green manure at both sites in 2014 and 2015.

Depth (cm)	0-30	30-60	60-90	Total	0-30	30-60	60-90	Total
		<u>Amsterdam 2014</u>				<u>Conrad 2014</u>		
Soil water (cm)	4.9	3.4	5.1	12.6	5.0	5.3	5.0	15.3
Soil nitrate (kg NO ₃ -N ha ⁻¹)	4.4	1.4	20.2	26.1	7.3	7.2	4.2	18.6
		<u>Bozeman 2015</u>				<u>Dutton 2015</u>		
Soil water (cm)	4.9	6.3	8.6	19.7	5.3	6.6	8.7	20.5
Soil nitrate (kg NO ₃ -N ha ⁻¹)	12.6	20.9	52.5	86.0	15.5	20.1	15.2	50.8

2014 and 2015 Cover Crop Biomass by High and Low Fertilizer Treatment

Cover crop biomass data was collected from high and low fertilizer treatments in 2014 and 2015 to account for N fertilization with the previous cash crop but reported as the average in Chapter 2. The results and analyses of biomass in high and low fertilizer treatments are included for reference.

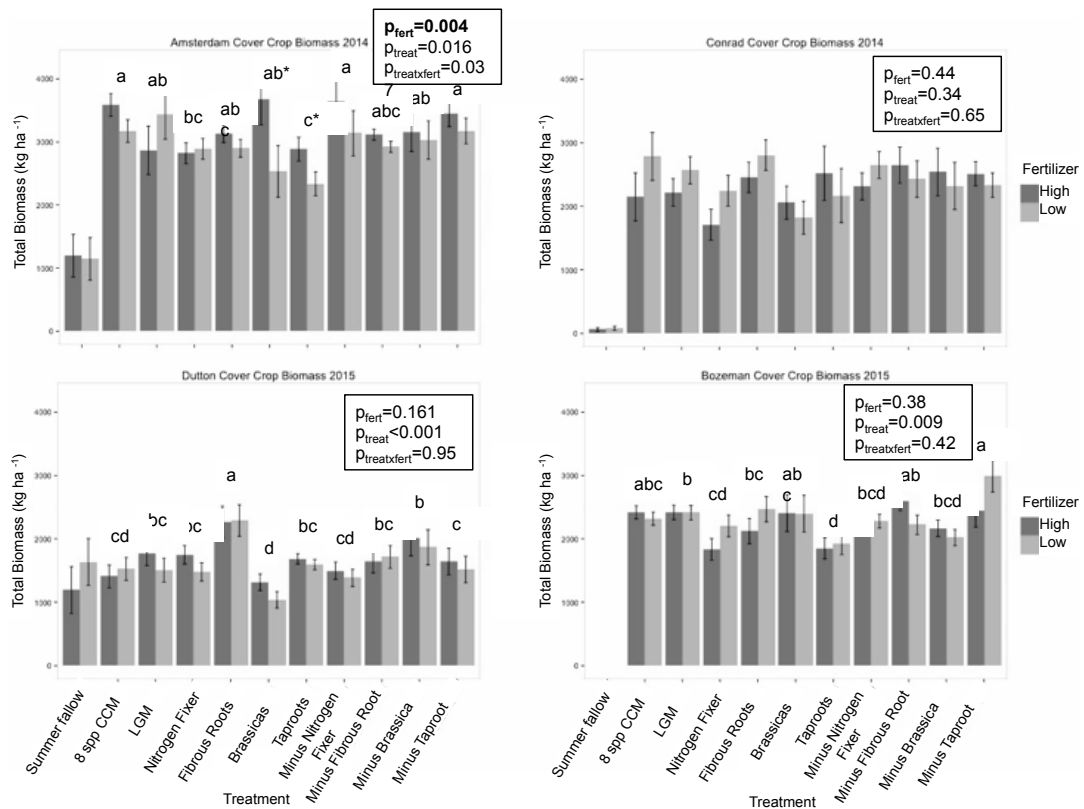


Figure B.1. Cover crop biomass (kg ha⁻¹) by high and low fertilizer treatments applied previous year at wheat seeding at four sites, 2014-2015. Treatments with different letters were significantly different ($p < 0.05$). Letters with asterisks were statistically different ($p < 0.05$) between high and low fertilizer within treatment.

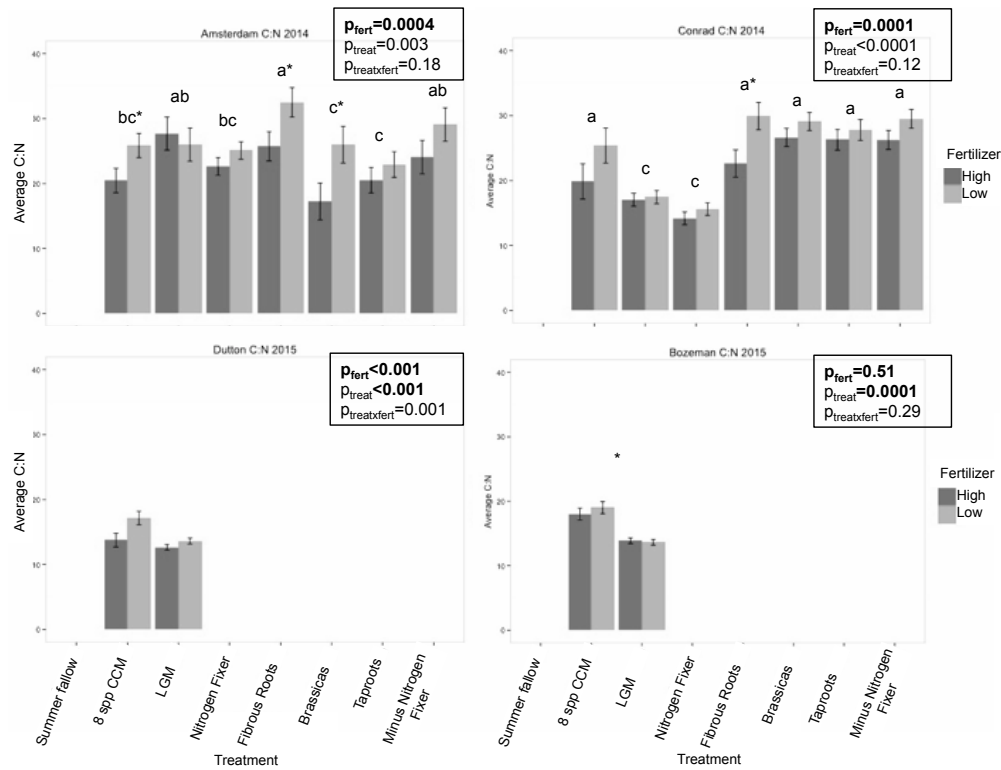


Figure B.2. Cover crop C:N by high and low fertilizer treatments applied previous year at wheat seeding at four sites, 2014-2015. Treatments with different letters were significantly different ($p < 0.05$). Letters with asterisks were statistically different ($p < 0.05$) between high and low fertilizer within cover crop treatments.

Spring pea C:N grown alone and as part of a cover crop mixture

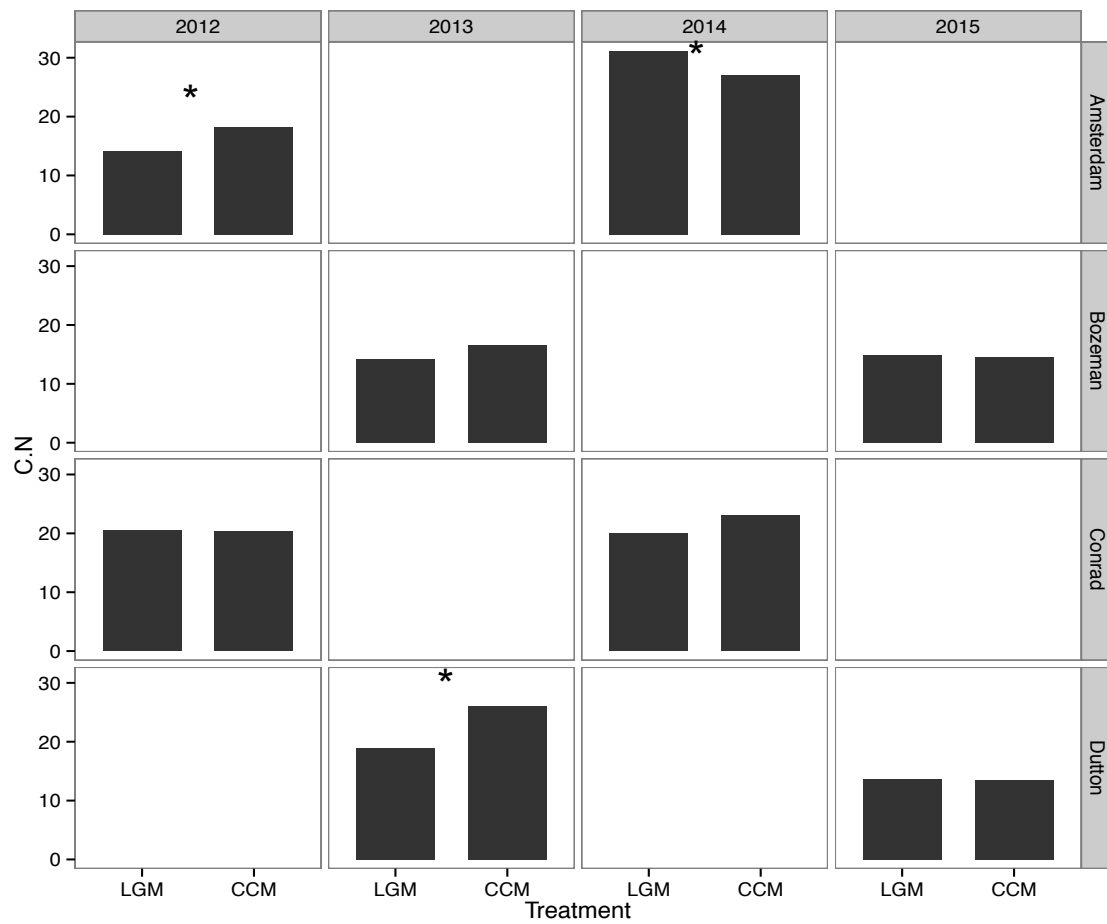


Figure B.3. C:N of pea grown in alone (LGM) versus grown in a mixture (CCM). Asterisks denote differences between treatments ($\alpha = 0.05$).